

# Age and Expression of CD163 and Colony-Stimulating Factor 1 Receptor (CD115) Are Associated With the Biological Behavior of Central Giant Cell Granuloma



Adrian Kahn, DMD,\* Gavriel Chausbu, DMD, MSc,† Lana Ginene,‡  
and Marilena Vered, DMD§

**Purpose:** Central giant cell granulomas (CGCGs) are clinically classified as nonaggressive (nA-CGCGs) and aggressive (A-CGCGs). However, histopathologically, all lesions feature spindle mononuclear cells (MCs) and multinuclear giant cells (GCs) in a hemorrhage-rich stroma. We aimed to investigate the presence of cells with a monocyte- or macrophage-related phenotype and, together with clinical variables, to examine their predictive potential for the biological behavior of CGCGs.

**Patients and Methods:** For our investigation, we implemented a retrospective cohort study. Sections were immunohistochemically stained for colony-stimulating factor 1 receptor (CSF-1R) (CD115), CD163, CD68, and nuclear factor  $\kappa$ B. The clinical variables included age, gender, and location of lesions. Associations between immunostains, clinical variables, and CGCG aggressiveness were analyzed by the Wilcoxon (Mann-Whitney) exact test and *t* test. Significant variables were further analyzed by a logistic regression model followed by receiver operating characteristic (ROC) curve analysis for diagnostic sensitivity. Significance was set at  $P < .05$ .

**Results:** Patients with A-CGCGs ( $n = 36$ ) were younger than those with nA-CGCGs ( $n = 31$ ) ( $P = .002$ ). Logistic regression showed that CD163-GC ( $\beta = -0.870$ ,  $P = .031$ ) and CD115-MC ( $\beta = -0.783$ ,  $P = .027$ ) had a significant protection effect (odds ratio, 0.419 [95% confidence interval, 0.190 to 0.925], and odds ratio, 0.457 [95% confidence interval, 0.229 to 0.913], respectively). ROC curve analysis showed that CD163-GC and CSF-1R (CD115)-MC combined were the best predictor in distinguishing nA-CGCGs from A-CGCGs (area under ROC curve, 0.814;  $P < .001$ ). At the optimal cutoff value (0.408), sensitivity was 87% and specificity, 65%.

**Conclusions:** Increasing age and high expression of CD163-GC and CSF-1R (CD115)-MC can serve as significant predictors of nA-CGCGs. A functional link between CD163-GC and the characteristic areas of extravasation of erythrocytes is discussed.

© 2017 American Association of Oral and Maxillofacial Surgeons  
*J Oral Maxillofac Surg* 75:1414-1424, 2017

\*Lecturer, Department of Oral and Maxillofacial Surgery, School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel.

†Associate Professor and Head, Department of Oral and Maxillofacial Surgery, School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel; and Head, Department of Oral and Maxillofacial Surgery, Rabin Medical Center, Petah Tikva, Israel.

‡Student (as part of fulfillment of DMD degree), School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel.

§Associate Professor and Head, Department of Oral Pathology and Oral Medicine, School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel.

The study was supported by the Ernest and Tova Turnheim Clinical Research Fund in Dentistry, Tel Aviv University.

Conflict of Interest Disclosures: None of the authors have any relevant financial relationship(s) with a commercial interest.

Address correspondence and reprint requests to Dr Kahn: Department of Oral and Maxillofacial Surgery, School of Dental Medicine, Tel Aviv University, Tel Aviv 69978, Israel e-mail: [dr.adykahn@gmail.com](mailto:dr.adykahn@gmail.com)

Received October 6 2016

Accepted January 2 2017

© 2017 American Association of Oral and Maxillofacial Surgeons  
0278-2391/17/30003-4

<http://dx.doi.org/10.1016/j.joms.2017.01.001>

Central giant cell granulomas (CGCGs) of the jawbones (termed *central giant cell lesions* by the World Health Organization) are defined as localized, benign but sometimes aggressive osteolytic proliferations consisting of fibrous tissue with hemorrhage and hemosiderin deposits, presence of osteoclast-like giant cells, and reactive bone formation.<sup>1</sup> The histopathologic features are not predictive of the biological behavior, with similar findings observed in both aggressive CGCGs (A-CGCGs) and nonaggressive CGCGs (nA-CGCGs).<sup>2</sup> Moreover, the histopathologic features of A-CGCGs were found to be similar to those of giant cell tumors (GCTs) of the long bones, raising the possibility that these 2 entities belong to the same spectrum of lesions.<sup>3</sup> In contrast, cytogenetic studies have suggested that CGCGs and GCTs are distinct entities based on the identification of somatic mutations in the *H3F3A* gene in GCTs<sup>4</sup> but not in CGCGs.<sup>5</sup>

Other extragnathic lesions with histopathologic features similar to those seen in CGCGs include the diffuse-type giant cell tumor (DGCT, formerly known as *pigmented villonodular synovitis*), which is a rare tumor of the joints. Similarly to CGCG, DGCT also encompasses aggressive and nonaggressive lesions. Chromosomal translocations involving chromosome 1p13 have been reported in the synovial cells in DGCT and have shown that colony-stimulating factor 1 (CSF-1) is the gene at the breakpoint.<sup>6</sup> The *CSF1* translocation results in overexpression of its protein product, CSF-1; as a result, there is considerable recruitment to the lesion site of cells that express the CSF-1 receptor (CSF-1R [CD115]). The CSF-1R-positive (CSF-1R<sup>+</sup>) cells are of the bone marrow-derived monocytic lineage that may be further differentiated into cells with macrophage or osteoclast phenotypes.<sup>6,7</sup> Depending on the microenvironment, the CSF-1R<sup>+</sup>-derived macrophages can further evolve either into cells with an anti-inflammatory phenotype (CD163<sup>+</sup>) or into proinflammatory, mature macrophages (CD68<sup>+</sup>).<sup>8</sup> It has been shown that the CSF-1 presence drives macrophage differentiation into the CD163<sup>+</sup> route.<sup>9-11</sup> The CD163<sup>+</sup> macrophages are associated with maintenance of tumor aggressiveness.<sup>8,12,13</sup> In addition, it has been shown that CSF-1 is critical for osteoclast generation, maturation and survival.<sup>11,14</sup>

We aimed to investigate a panel of markers representing cells with a monocyte- or macrophage-related phenotype and, together with clinical variables, to examine their association with the biological behavior of CGCGs. We hypothesized that an abundance of cells expressing these markers found to be related to aggressive biological behavior in other conditions also will show an association with the aggressive variant of CGCG. More specifically, we expected

that the expression of CSF-1R, CD163, and nuclear factor  $\kappa$ B (NF- $\kappa$ B)—a key factor in both macrophage (especially of the CD163 phenotype) and osteoclast differentiation<sup>15,16</sup>—will be associated with A-CGCGs.

## Patients and Methods

To assess the research purpose, we designed and implemented a retrospective cohort study. The study was approved by the Ethics Committee of Tel Aviv University.

The study population was composed of all consecutive specimens of CGCGs stored in the departmental database (1995-2015). To be included in the study, the submitted biopsy request form had to contain the relevant clinical information. Recurrent lesions; cases with known previous treatment with calcitonin, corticosteroids, or any other agent; and cases with a diagnosis of hyperparathyroidism or other giant cell-containing type of lesion were excluded from the study.

CGCG cases were classified as nonaggressive (nA-CGCG) and aggressive (A-CGCG) based on the criteria established by Chuong et al<sup>17</sup> and validated by several other studies on the biological behavior of CGCGs.<sup>18-20</sup> The nA-CGCGs were characterized by minimal or no symptoms, slow growth, absence of root resorption or cortical perforation, and a low tendency to recur. The A-CGCGs met the criteria of pain, rapid growth, root resorption, cortical perforation, and recurrence.

Variables that were related to the biological behavior of CGCGs included age at diagnosis, gender, and lesion location (mandible or maxilla), as well as the scores of the immunohistochemical stains. Immunohistochemical stains (3- $\mu$ m-thick sections) were performed with antibodies against CSF-1R (CD115 [Acris, Herford, Germany]; 1:100; with lung carcinoma as positive control), CD68 (Zytomed, clone kp1 [BioGenex, San Ramon, CA]; 1:100; with tonsil as positive control), CD163 (clone k20-T [Acris]; 1:200; with liver as positive control), and NF- $\kappa$ B (p65, polyclonal [Alexis Biochemicals, San Diego, CA]; 1:50; with ovarian carcinoma as positive control). CD68 was used as a ubiquitous, highly expressed marker by human monocytes and tissue macrophages.<sup>21</sup> Regarding NF- $\kappa$ B, nuclear or nuclear-cytoplasmic staining was considered. Negative controls for all types of stains were performed by omitting the primary antibodies. The chromogen used to visualize the product of the immunoreaction was diaminobenzidine (ScyTek, West Logan, UT).

Staining assessment for each marker was performed separately for the multinucleated giant cells (GCs) and mononuclear cells (MCs). The overall percentage of stained cells was multiplied by the most common

**Table 1. ANALYSIS OF AGE, GENDER, AND LOCATION VERSUS STAINING SCORES**

|          | Spearman Rho (2 Tailed) |       |       |       |       |       |                |       |
|----------|-------------------------|-------|-------|-------|-------|-------|----------------|-------|
|          | NF-κB                   |       | CD163 |       | CD68  |       | CSF-1R (CD115) |       |
|          | GC                      | MC    | GC    | MC    | GC    | MC    | GC             | MC    |
| Age      | 0.1                     | -0.1  | 0.2   | 0.0   | -0.2  | 0.1   | 0.0            | -0.1  |
|          | .3047                   | .6476 | .0605 | .7688 | .1151 | .3183 | .8760          | .6265 |
| Gender   | -0.2                    | 0.1   | -0.3  | -0.1  | 0.1   | 0.0   | 0.0            | 0.0   |
|          | .0862                   | .5189 | .0332 | .4503 | .5027 | .7277 | .9515          | .8345 |
| Location | 0.0                     | 0.0   | -0.1  | 0.0   | -0.2  | -0.1  | 0.1            | 0.1   |
|          | .7380                   | .9477 | .3084 | .9475 | .1531 | .2855 | .3384          | .6218 |

Abbreviations: CSF-1R, colony-stimulating factor 1 receptor; GC, multinuclear giant cell; MC, mononuclear cell; NF-κB, nuclear factor κB.

*Kahn et al. Central Giant Cell Granuloma. J Oral Maxillofac Surg 2017.*

staining intensity (1, weak; 2, moderate; and 3, strong), with a maximum possible score of 3. The results were presented as the mean staining score for each type of cell.

**STATISTICAL ANALYSIS**

Because of the small sample size and non-normal distributions of staining scores, the associations between age, gender, and location and staining scores (MCs, GCs); the associations between staining scores and CGCG variants; and the differences in constant values of patients between CGCG variants were analyzed by the Wilcoxon (Mann-Whitney) exact test. A *t* test was used to analyze for differences in constant values between gender and location. Correlations between staining scores of MCs and GCs (all stains) within A-CGCGs and nA-CGCGs, as well as the correlation between age and staining scores, were analyzed by the Spearman Rho correlation test. Logistic regression

was then conducted to evaluate the risk regarding the associations between significant scores, clinical variables, and the aggressiveness of CGCGs, as assessed by the relative risk (RR) and 95% confidence interval (CI). This was followed by receiver operating characteristic (ROC) curve analysis for diagnostic sensitivity. Statistical analysis was performed with SPSS software (version 21; SPSS, Chicago, IL), and statistical significance was set at *P* < .05.

**Results**

Sixty-seven CGCG cases were included in the study and were classified as the A-CGCG group (n = 36) and nA-CGCG group (n = 31). There were 17 female and 19 male patients in the A-CGCG group and 16 female and

**Table 2. ANALYSIS OF AGE, GENDER, AND LOCATION VERSUS CGCG VARIANTS**

|                    | A-CGCG Group  | nA-CGCG Group | <i>P</i> Value |
|--------------------|---------------|---------------|----------------|
| Age, mean ± SD, yr | 26.74 ± 15.82 | 45.69 ± 20.84 | .0002          |
| Gender, n          |               |               |                |
| Female             | 17            | 16            | >.05           |
| Male               | 19            | 15            | >.05           |
| Location, n        |               |               |                |
| Maxilla            | 10            | 8             | >.05           |
| Mandible           | 26            | 23            | >.05           |

Abbreviations: A-CGCG, aggressive central giant cell granuloma; CGCG, central giant cell granuloma; nA-CGCG, nonaggressive central giant cell granuloma.

*Kahn et al. Central Giant Cell Granuloma. J Oral Maxillofac Surg 2017.*

**Table 3. STAINING SCORES VERSUS CGCG VARIANTS**

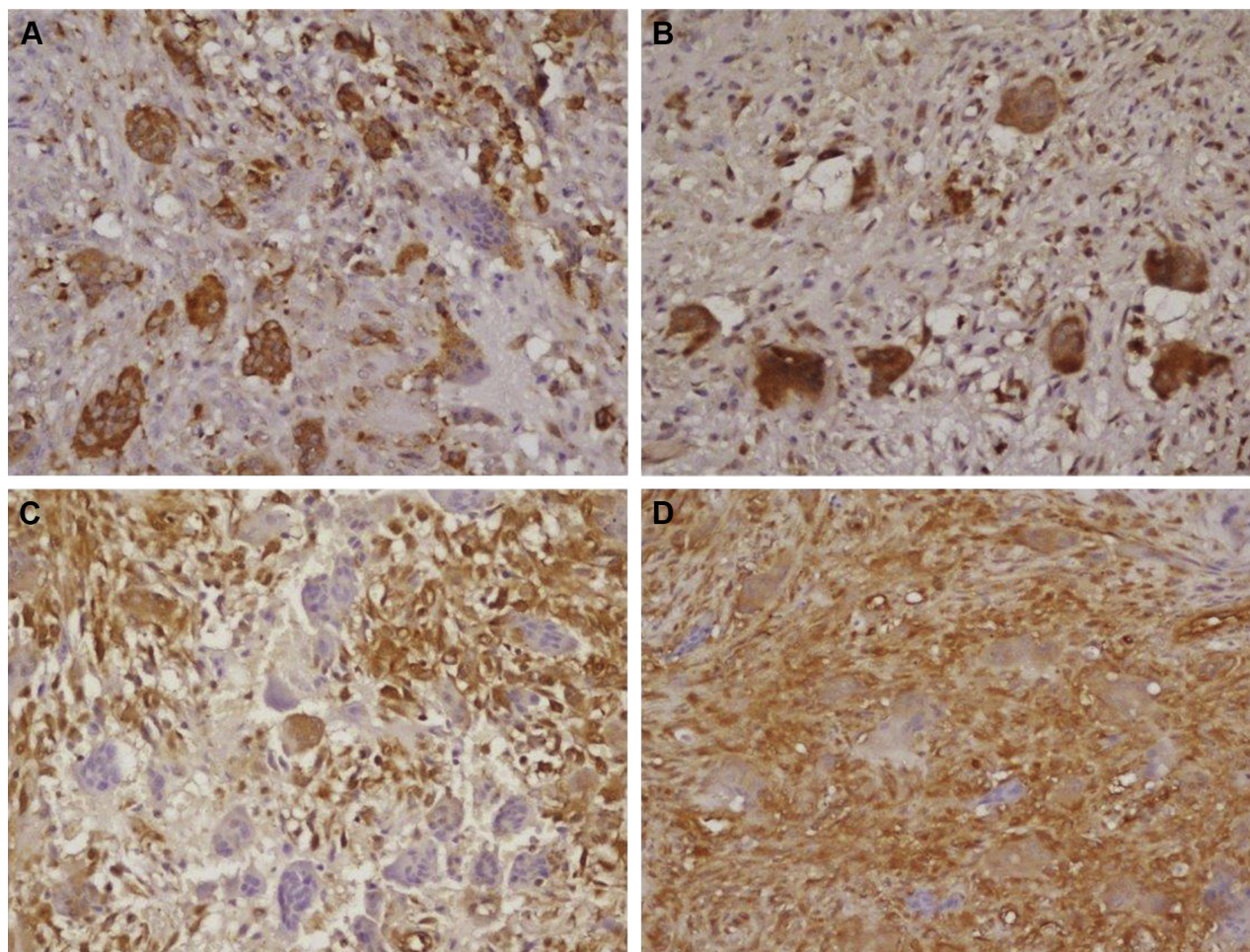
|                   | A-CGCG Group (n = 36) | nA-CGCG Group (n = 31) | <i>P</i> Value |
|-------------------|-----------------------|------------------------|----------------|
| CSF-1R (CD115)-GC | 1.26 ± 0.9            | 1.52 ± 0.9             | >.05           |
| CSF-1R (CD115)-MC | 1.56 ± 0.9            | 1.9 ± 0.75             | .061           |
| CD163-GC          | 1.39 ± 0.64           | 1.88 ± 0.75            | .005           |
| CD163-MC          | 0.64 ± 0.7            | 0.68 ± 0.68            | >.05           |
| CD68-GC           | 2.79 ± 0.33           | 2.78 ± 0.16            | >.05           |
| CD68-MC           | 0.69 ± 0.39           | 0.76 ± 0.45            | >.05           |
| NF-κB-GC          | 1.12 ± 0.81           | 1.31 ± 0.81            | >.05           |
| NF-κB-MC          | 1.02 ± 0.86           | 1.19 ± 0.69            | >.05           |

*Note:* Data are presented as mean ± standard deviation.

Abbreviations: A-CGCG, aggressive central giant cell granuloma; CGCG, central giant cell granuloma; CSF-1R, colony-stimulating factor 1 receptor; GC, multinuclear giant cell; MC, mononuclear cell; nA-CGCG, nonaggressive central giant cell granuloma; NF-κB, nuclear factor κB.

*Kahn et al. Central Giant Cell Granuloma. J Oral Maxillofac Surg 2017.*





**FIGURE 1.** Central giant cell granuloma sections immunostained with CD68 (A), CD163 (B), colony-stimulating factor 1 receptor (CSF-1R) (CD115) (C), and P65 (D) (original magnification  $\times 200$ ). One should note the abundance of CSF-1R (CD115) staining in the mononuclear cells compared with limited staining in the multinuclear giant cells.

Kahn et al. Central Giant Cell Granuloma. *J Oral Maxillofac Surg* 2017.

15 male patients in the nA-CGCG group. The A-CGCG group of lesions comprised 10 maxillary and 26 mandibular lesions, and the nA-CGCG group comprised 8 maxillary and 23 mandibular lesions.

Staining assessment was performed by 2 authors (M.V. and L.G.), with a  $\kappa$  coefficient of 0.84. In case of disagreement, the final score was defined based on revision of the stained sections and a common consensus.

#### CLINICAL VARIABLES (AGE, GENDER, LOCATION) VERSUS STAINING SCORES

Correlations were calculated between each cofounder (age, gender and location) and each staining score (Table 1). A significant correlation was found only between CD163-GC and gender ( $r = -0.3$ ,  $P = .0332$ ). Female patients showed a higher expression of CD163-GC than male patients (median, 1.8 vs 1.7;  $P = .0034$ , Wilcoxon 2-tailed test). No correlations

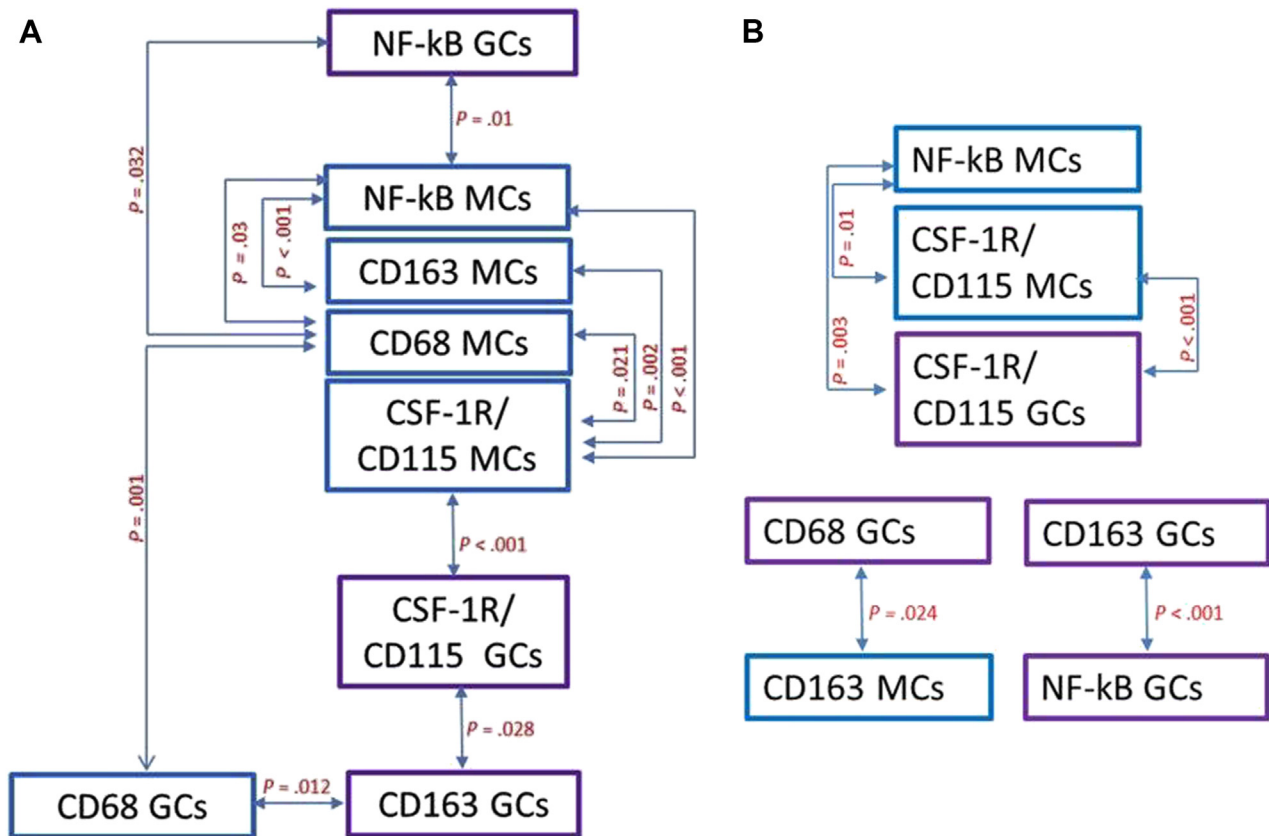
were found between age or location and staining scores.

#### CLINICAL VARIABLES (AGE, GENDER, LOCATION) VERSUS CGCG VARIANTS

Among the clinical variables, only age was different between the A-CGCG and nA-CGCG groups (Table 2). Patients with A-CGCGs were significantly younger than those with nA-CGCGs ( $P = .0002$ ).

#### STAINING SCORES VERSUS CGCG VARIANTS

Mean staining scores in terms of CGCG variants are summarized in Table 3. In both the A-CGCG and nA-CGCG groups, the most common phenotype of GCs was that of CD68, followed by CD163, CSF-1R (CD115), and NF- $\kappa$ B. In both the A-CGCG and nA-CGCG groups, the most common phenotype of MCs was that of CSF-1R (CD115), followed by NF- $\kappa$ B, CD68, and CD163. In both the A-CGCG and nA-CGCG



**FIGURE 2.** Correlations between cell types and selected markers in aggressive (A) and nonaggressive (B) central giant cell granulomas. CSF-1R, colony-stimulating factor 1 receptor; GCs, multinuclear giant cells; MCs, mononuclear cells; NF-κB, nuclear factor κB.

Kahn et al. Central Giant Cell Granuloma. J Oral Maxillofac Surg 2017.

groups, the staining scores were higher in GCs than in MCs with the exception of CSF-1R (CD115), for which MCs were more diffusely stained than GCs.

The mean staining score of CD163-GC was significantly higher in nA-CGCGs than in A-CGCGs ( $P = .005$ ). The mean staining score of CSF-1R-MC was borderline higher in nA-CGCGs than in A-CGCGs ( $P = .061$ ). Figure 1 shows representative sections of CGCGs stained with the various markers.

Analyzing correlations of the staining scores within each CGCG variant (Fig 2) yielded 11 significant correlations in A-CGCGs and only 5 in nA-CGCGs. The pattern of correlations also was different. CD163-GC had only 1 significant correlation in nA-CGCGs and 2 significant correlations in A-CGCGs. Within nA-CGCGs, there was a strong correlation between CD163-GC and NF-κB-GC ( $r = 0.7$ ,  $P = .0001$ ).

**Table 4. AGE AS FACTOR OF CGCG VARIANT, GENDER, AND LOCATION**

|         | CGCG Variant |         | Gender |        | Location |          |
|---------|--------------|---------|--------|--------|----------|----------|
|         | A-CGCG       | nA-CGCG | Female | Male   | Maxilla  | Mandible |
| n       | 36           | 31      | 33     | 34     | 18       | 49       |
| Age, yr |              |         |        |        |          |          |
| Mean    | 26.74        | 45.69   | 39.36  | 33.14  | 38.55    | 35.12    |
| SD      | 15.82        | 20.84   | 20.011 | 20.106 | 20.682   | 20.070   |
| P value | .0002        |         | .2023  |        | .5256    |          |

Abbreviations: A-CGCG, aggressive central giant cell granuloma; CGCG, central giant cell granuloma; nA-CGCG, nonaggressive central giant cell granuloma.

Kahn et al. Central Giant Cell Granuloma. J Oral Maxillofac Surg 2017.

**Table 5. LOGISTIC REGRESSION WITH MINIMAL MODEL CONTAINING AGE AND STAINING SCORES FOUND AS SIGNIFICANT PREDICTORS FOR A-CGCGS**

| Study Variable    | $\beta$<br>Coefficient | 95% CI         |                | <i>P</i><br>Value |
|-------------------|------------------------|----------------|----------------|-------------------|
|                   |                        | Lower<br>Bound | Upper<br>Bound |                   |
| CD163-GC          | -0.870                 | 0.190          | 0.925          | .031              |
| Age               | -0.048                 | 0.924          | 0.983          | .002              |
| CSF-1R (CD115)-MC | -0.783                 | 0.229          | 0.913          | .027              |
| Age               | -0.058                 | 0.913          | 0.975          | .001              |

Abbreviations: A-CGCGs, aggressive central giant cell granulomas; CI, confidence interval; CSF-1R, colony-stimulating factor 1 receptor; GC, multinuclear giant cell; MC, mononuclear cell.

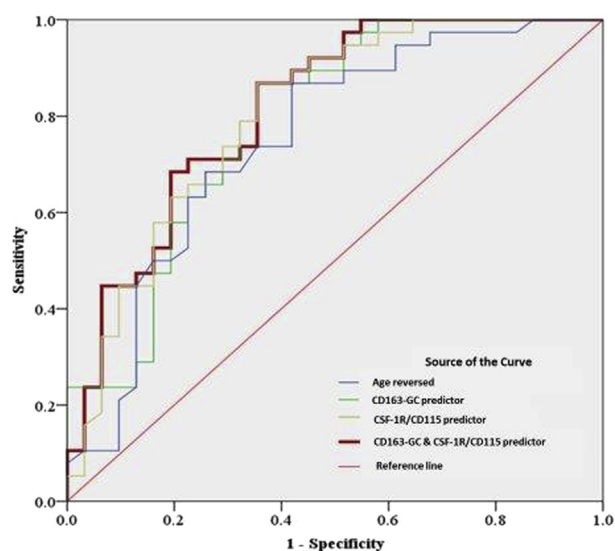
Kahn et al. *Central Giant Cell Granuloma. J Oral Maxillofac Surg* 2017.

#### MODEL FOR PREDICTION OF BIOLOGICAL BEHAVIOR OF CGCGS

##### Univariate Analysis

Univariate analysis was performed for all potential predictive variables (age, gender, location, and staining scores with A-CGCGs as the dependent variable and nA-CGCGs as the reference). This yielded 3 predictive variables: age ( $\beta = -0.051$ ;  $P = .001$ ; odds ratio (OR), 0.950 [95% CI, 0.923 to 0.979]); CD163-GC ( $\beta = -1.024$ ;  $P = .008$ ; OR, 0.359 [95% CI, 0.168 to 0.768]); and CSF-1R (CD115)-MC ( $\beta = -0.557$ ;  $P = .064$ ; OR, 0.573 [95% CI, 0.317 to 1.034]).

The mean age of patients with A-CGCGs was significantly lower than that of patients with nA-CGCGs



**FIGURE 3.** Receiver operating characteristic curve analysis. CSF-1R, colony-stimulating factor 1 receptor; GC, multinuclear giant cell.

Kahn et al. *Central Giant Cell Granuloma. J Oral Maxillofac Surg* 2017.

( $P = .0002$ ) (Table 4). Because age was found to be a predictor of the biological behavior of CGCGs, it was further analyzed as a factor of gender and location to seek for multicollinearity or a confounder effect. Age did not differ significantly as a factor of gender and location; therefore, it could be added to the logistic regression for predicting CGCG aggressiveness with no need to control gender and location.

##### Multivariate Logistic Regression

Because the independent variables did not have a normal distribution and age was found to be a strongly predictive variable, logistic binary analysis with a minimal model containing age with each staining score was performed. The dependent variable was A-CGCGs, and nA-CGCGs served as a reference. Among all staining scores, only CD163-GC and CSF-1R (CD115)-MC were significant (Table 5): CD163-GC ( $\beta = -0.870$ ,  $P = .031$ ) with a protection effect (RR, 0.419 [95% CI, 0.190 to 0.925]) and CSF-1R (CD115)-MC ( $\beta = -0.783$ ,  $P = .027$ ) also with a protection effect (RR, 0.457 [95% CI, 0.229 to 0.913]). Then, these variables together with age were combined into one multivariate regression model. The significance of these staining scores became borderline (CSF-1R [CD115]-MC,  $P = .076$ ; CD163-GC,  $P = .092$ ), but age remained significant ( $\beta = -0.056$ ,  $P = .001$ ) with a protection effect (RR, 0.946 [95% CI, 0.914 to 0.978]). Because we aimed to find the best predictor in this model, these scores were further examined separately and in combination using ROC curve analysis.

##### ROC CURVE ANALYSIS

The logistic regression showed the predictive potential of age and of the expression of CD163-GC and CSF-1R (CD115)-MC for the biological behavior of CGCGs. This was further supported by ROC curve analysis (Fig 3, Table 6). The area under the ROC curve (AUROC) for the 2 staining score models (CD163-GC and CSF-1R [CD115]-MC) was the best predictor (AUROC, 0.814) and conveyed a combined accuracy of these variables in distinguishing nA-CGCGs from A-CGCGs in terms of sensitivity and specificity ( $P < .001$ ).

CSF-1R (CD115)-MC was the second best predictor (AUROC, 0.802) and conveyed good potential to distinguish nA-CGCGs from A-CGCGs in terms of sensitivity and specificity ( $P < .001$ ). CD163-GC was the third best predictor (AUROC, 0.787) and was able to distinguish nA-CGCGs from A-CGCGs in terms of sensitivity and specificity ( $P < .001$ ).

Age was the basic predictor in each model. For the purpose of calculations, reciprocal age values (ie, 1 divided by age) were used. Age alone also



**Table 6. ROC CURVE ANALYSIS FOR MODELS OF STAINING SCORE PREDICTORS AND RECIPROCAL AGE VALUES (1 DIVIDED BY AGE)**

| Test Result Variable                     | Area  | SE*   | Asymptotic Significance <sup>†</sup> | Asymptotic 95% CI |             |
|--|-------|-------|--------------------------------------|-------------------|-------------|
|  |       |       |                                      | Lower Bound       | Upper Bound |
| Reciprocal age value (1 divided by age)  | 0.750 | 0.061 | $P < .0001$                          | 0.630             | 0.869       |
| CD163-GC predictor                       | 0.787 | 0.057 | $P < .0001$                          | 0.676             | 0.898       |
| CSF-1R (CD115)-MC predictor              | 0.802 | 0.055 | $P < .0001$                          | 0.695             | 0.909       |
| CD163-GC and CSF-1R (CD115)-MC predictor | 0.814 | 0.052 | $P < .0001$                          | 0.712             | 0.917       |

Abbreviations: CI, confidence interval; CSF-1R, colony-stimulating factor 1 receptor; GC, multinuclear giant cell; MC, mononuclear cell; ROC, receiver operating characteristic.

\* Under the nonparametric assumption.

† Null hypothesis: true area = 0.5.

Kahn et al. Central Giant Cell Granuloma. J Oral Maxillofac Surg 2017.

can be a good predictor (AUROC, 0.750) ( $P < .001$ ). The data regarding the optimal cutoff values calculated from the ROC curves with optimal sensitivity and specificity for all parameters are summarized in Table 7.

## Discussion

We have examined a panel of markers related to the differentiation of monocyte- or macrophage-derived cells in a series of CGCGs, and we aimed to find how, together with clinical variables, they can aid in distinguishing between A-CGCGs and nA-CGCGs. For this purpose, we assessed the expression of CSF-1R (CD115), CD163, and NF- $\kappa$ B, known as markers related to biological aggressiveness in various benign and malignant conditions, and hypothesized that their expression also will show an association with the aggressive variant of CGCGs. We found that CD163 and CSF-1R (CD115) were significant predictors of the biological behavior of CGCGs (specificity of 36% and 65%, respectively; 87% sensitivity for both markers), but in contrast to our hypothesis, high expression was associated with nA-CGCGs and not with A-CGCGs. Furthermore, we found that increasing

age was a significant predictor of nA-CGCGs (87% sensitivity, 58% specificity).

Among the selected markers, the expression of CSF-1R (CD115) and CD163 has not been previously examined in CGCGs. CD68 was examined in the context of comparison between CGCGs and their soft tissue counterpart<sup>22-24</sup> and GCTs.<sup>25</sup> The expression of NF- $\kappa$ B in CGCGs was examined in regard to the differentiation of the monocytic or macrophage-derived cells into osteoclasts<sup>26</sup> and, more recently, in regard to the biological behavior of CGCGs.<sup>27</sup> Our selection of markers was based on the findings from DGCT, an entity that histopathologically is characterized by GCs and clinically has aggressive and nonaggressive variants analogous to CGCGs. The current concept on the etiopathogenesis of DGCT is that a minority of neoplastic cells with *CSF1* translocation lead to abnormal accumulation of non-neoplastic CSF-1R (CD115)-expressing cells that ultimately form a tumorous mass.<sup>6</sup> Pharmacologic targeting of CSF-1R (CD115) in locally advanced and/or metastatic lesions resulted in an improvement in the clinical status of the patients.<sup>28</sup> Accordingly, we have assumed that the biological aggressiveness of CGCGs could be a factor of an increased cell population of CSF-1R (CD115)

**Table 7. SENSITIVITY AND SPECIFICITY OF PREDICTIVE VARIABLES: AGE, CD163-GC, AND CSF-1R (CD115)-MC**

|                                | Optimal Cutoff Point | Sensitivity | Specificity | P Value |
|--------------------------------|----------------------|-------------|-------------|---------|
| CD163-GC and CSF-1R (CD115)-MC | 0.408                | 87%         | 65%         | <.001   |
| CSF-1R (CD115)-MC              | 0.436                | 87%         | 65%         | <.001   |
| CD163-GC                       | 0.445                | 87%         | 36%         | <.001   |
| Age                            | 44 yr                | 87%         | 58%         | <.001   |

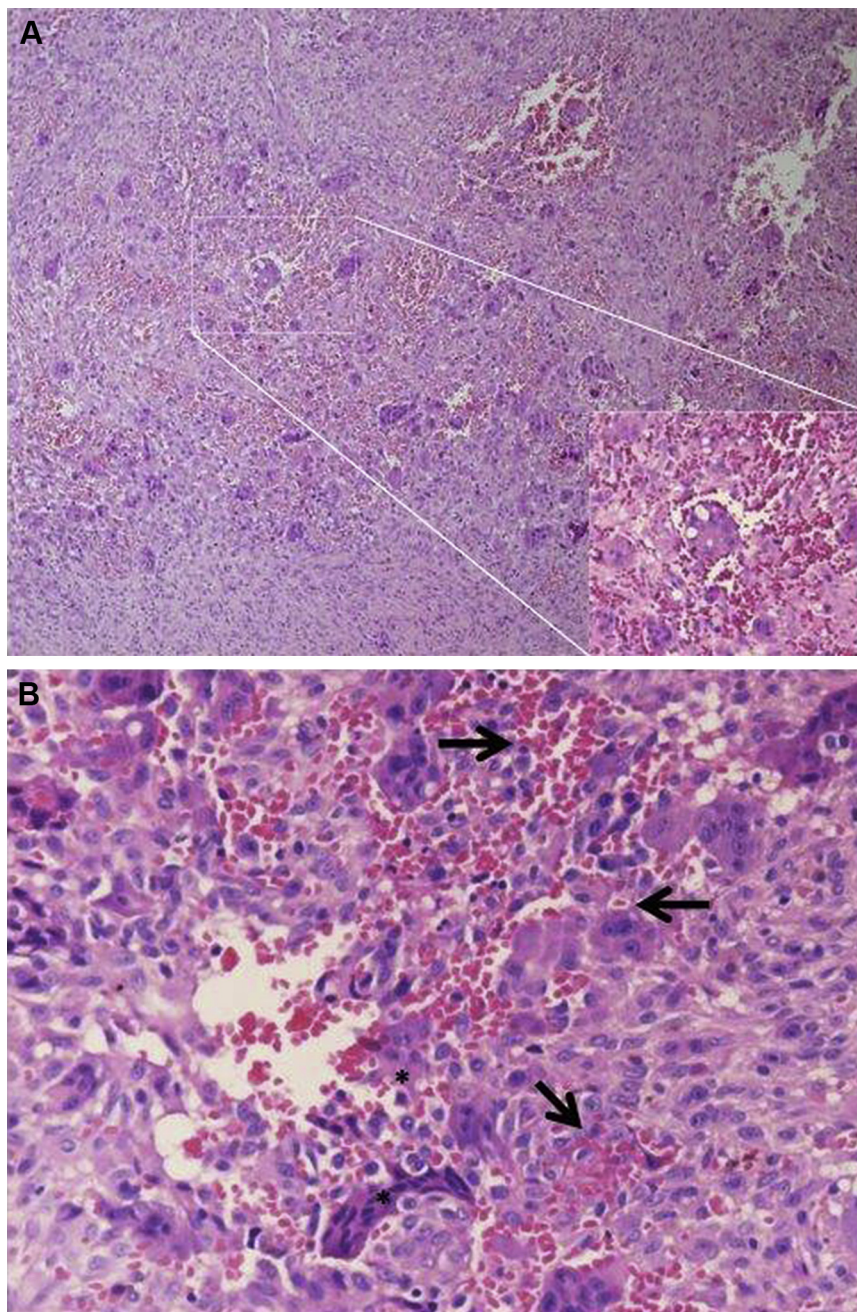
Abbreviations: CSF-1R, colony-stimulating factor 1 receptor; GC, multinuclear giant cell; MC, mononuclear cell.

Kahn et al. Central Giant Cell Granuloma. J Oral Maxillofac Surg 2017.

phenotype. However, contrary to our assumption, our results showed that expression of CSF-1R (CD115) in MCs was significantly associated with nA-CGCGs. To this end, it should be noted that CGCGs have not been investigated in regard to *CSF1* chromosomal translocations. In addition, CSF-1R-MC in nA-CGCGs was found to have only limited correlations with cells positive for other examined markers, whereas in

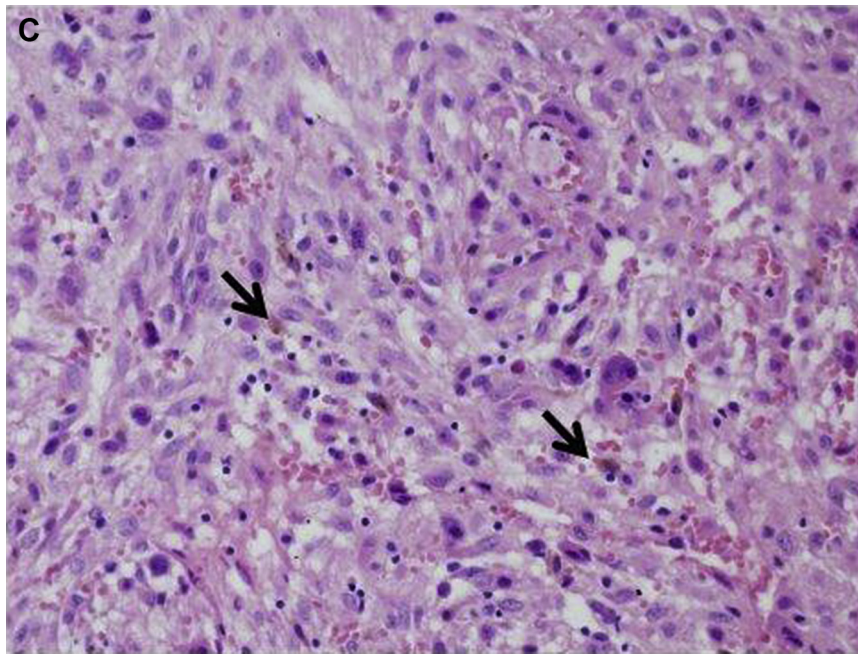
A-CGCGs, CSF-1R (CD115)-MC showed an extensive network of interactions with other types of lesion cells, possibly reducing their protective effect. Nonetheless, these findings should not entirely rule out the need to pharmacologically explore the efficacy of anti-CSF-1R (CD115) in CGCGs.

In regard to the expression of CD163, we designed this study having in mind its role as an



**FIGURE 4.** A, Giant cells (GCs) are predominantly associated with hemorrhagic areas (hematoxylin-eosin [H&E] stain, original magnification  $\times 40$ ). The *inset* shows a GC lined by erythrocytes. B, GCs and their cellular processes (*asterisks*) are surrounding and engulfing extravasated erythrocytes (*arrows*) (H&E stain, original magnification  $\times 200$ ). Areas with minimal hemorrhage are associated with a low number of GCs. (Fig 4 continued on next page.)





**FIGURE 4 (cont'd).** C, Hemosiderin deposits (arrows) are evidence of previous processing of the extravasated erythrocytes and metabolized hemoglobin by the GCs (H&E stain, original magnification  $\times 200$ ).

*Kahn et al. Central Giant Cell Granuloma. J Oral Maxillofac Surg 2017.*

immunomodulator, usually associated with anti-inflammatory, pro-tumorigenic activity.<sup>8,12,13</sup> However, CD163 originally was identified as a scavenger receptor for hemoglobin (Hb)-haptoglobin complexes.<sup>29,30</sup> Its expression is restricted to cells of the monocyte or macrophage lineage, predominantly those residing in tissues such as the red pulp in the spleen, Kupffer cells in the liver, and lymph nodes, as well as in a perivascular location. Metabolism of Hb is a main function of tissue macrophages because of their ability to engulf senescent erythrocytes (extravascular hemolysis) or take up Hb released from ruptured erythrocytes (intravascular hemolysis) and immature erythrocytes in the bone marrow. Unless cleared rapidly, the released Hb can be toxic. Within the CD163<sup>+</sup> macrophages, the Hb molecules are metabolized into bilirubin and iron.<sup>29</sup> The characteristic histopathologic elements of CGCGs are actually a reflection of this process, encompassing numerous foci of hemorrhage with extravasated erythrocytes, GCs closely located to these extravasation areas, and hemosiderin deposits (Fig 4).<sup>31,32</sup> Given the role of CD163 in the metabolism of Hb, we now can provide an explanation for the specific location of CD163-GC in these areas. In previous studies it has been shown that the vascular channels in CGCGs are modified and are characterized by gaps and incomplete endothelial lining,<sup>33,34</sup> thus facilitating the extravasation process, which will finally result in recruiting CD163 macrophages. The cause of the presence of the

extravasated erythrocytes in CGCGs has not been investigated so far, but rather accepted as an integral component in the landscape of this lesion.

The causative factor (or factors) for the increased permeability of the blood vessels in CGCGs leading to erythrocyte extravasation may be a result of altered pressure and concentration gradients, hemodynamic forces (blood flow, vascular area available for exchange), and the intrinsic permeability of the vascular wall.<sup>35</sup> It can be suggested that in the jawbones, processes such as eruption of teeth, dental trauma, and periodontal- and/or pulp-related inflammation also may be considered feasible factors for modifying intrabony blood flow. Yet an additional factor with an impact on the vascular component is parathyroid hormone-related protein (PTHrP), which essentially functions as parathyroid hormone but at a local level: it has an effect of vascular dilatation, increased blood flow, and decreased blood pressure.<sup>36</sup> The presence of PTHrP in CGCGs has been reported, albeit not in terms of the clinical behavior of the lesions.<sup>37</sup>

The reason that CD163-GCs convey a “protective effect” and are associated with nA-CGCGs may lie in the fact that once the hemorrhagic areas are formed, these cells are effectively acting to metabolize the released Hb. The possibility is raised that in A-CGCGs the expression of CD163 is downregulated or molecularly aberrant; therefore the accumulation of CD163 macrophages becomes ineffective. Alternatively, the underlying, as-yet-unknown factor for the formation of the hemorrhagic areas could act at a higher pace than

the ability of the CD163 macrophages to accumulate, resulting in A-CGCGs. The reason that most of the A-CGCGs develop in young patients, as was found in our study and in previous reports,<sup>17,38</sup> could be a factor of any of the aforementioned possibilities, assumedly enhanced by the process of tooth development and eruption.

Brown tumor of hyperparathyroidism and cherubism are other GC-containing lesions of the jawbones, histologically indistinguishable from CGCGs. In regard to hyperparathyroidism, the excessive parathyroid hormone and its effect on the vasculature may be a feasible explanation for the generation of the hemorrhagic areas that result in recruitment of CD163 macrophages in an identical manner to that proposed for CGCGs. Regarding cherubism, a mutation in the *SH3BP2* gene has been identified.<sup>39</sup> This induces dysregulated activity of PTHrP,<sup>39</sup> which in turn also may be related to the effect that it has on the vascular component at the lesion site, ultimately resulting in the accumulation of CD163-GC.

On the basis of our findings, we raise the possibility of shifting our assumption that the widely recognized, histologically characteristic cellular components of MCs and GCs necessarily constitute the etiopathologic factors of CGCGs and suggest opening new platforms of research in which these cells may rather be considered the outcome of and not the primary reason for the generation of CGCGs.<sup>6</sup> Yet, concomitant with the suggested concept on the role of CD163-positively stained cells, within the complex molecular milieu of CGCGs with numerous interactions among cells with various phenotypes, CSF-1R (CD115) also can act in promoting osteoclastogenesis with a resulting subpopulation of cells bearing a phenotype and functionality of osteoclast-like cells.<sup>11,26,30,31,40</sup> Nevertheless, studies based on larger series of CGCGs with extended methodologies are needed to confirm our initial findings.

In conclusion, we have conducted a molecular study on CGCGs using a panel of markers that are related to cells of a monocyte or macrophage phenotype. We report, for the first time, that high expression of CD163 and CSF-1R (CD115), as well as increasing age, can serve as significant predictors of the nonaggressive variant of CGCGs, with a sensitivity of 87% and a specificity of up to 65%. Furthermore, we suggest that giant cells that express CD163 may be engaged in the metabolism of Hb released by the extravasated erythrocytes, a characteristic histopathologic feature of CGCGs. On the basis of our initial findings, we may suggest new investigatory pathways in which the GCs and MCs, hitherto recognized as the main factors in the pathogenesis of CGCGs, should be regarded as outcomes of an as-yet-unidentified etiology for this lesion.

## References

- Jundt G: Central giant cell lesion. In: Barnes L, Evenson JW, Reichart P, et al. WHO Classification of Tumours. Pathology and Genetics. Head and Neck Tumours. Lyon, France, IARC Press, 2005, p 324
- Peacock ZS, Jordan RC, Schmidt BL: Giant cell lesions of the jaws: Does the level of vascularity and angiogenesis correlate with behavior? *J Oral Maxillofac Surg* 70:1860, 2012
- Resnick CM, Margolis J, Susarla SM, et al: Maxillofacial and axial/appendicular giant cell lesions: Unique tumors or variants of the same disease? A comparison of phenotypic, clinical, and radiographic characteristics. *J Oral Maxillofac Surg* 68:130, 2010
- Behjati S, Tarpey PS, Presneau N, et al: Distinct H3F3A and H3F3B driver mutations define chondroblastoma and giant cell tumor of bone. *Nat Genet* 45:1479, 2013
- Gomes CC, Diniz MG, Amaral FR, et al: The highly prevalent H3F3A mutation in giant cell tumours of bone is not shared by sporadic central giant cell lesion of the jaws. *Oral Surg Oral Med Oral Pathol Oral Radiol* 118:583, 2014
- West RB, Rubin BP, Miller MA, et al: A landscape effect in tenosynovial giant-cell tumor from activation of CSF1 expression by a translocation in a minority of tumor cells. *Proc Natl Acad Sci U S A* 103:690, 2006
- Cupp JS, Miller MA, Montgomery KD, et al: Translocation and expression of CSF1 in pigmented villonodular synovitis, tenosynovial giant cell tumor, rheumatoid arthritis and other reactive synovitides. *Am J Surg Pathol* 31:970, 2007
- Ries CH, Cannarile MA, Hoves S, et al: Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. *Cancer Cell* 25:846, 2014
- Geissmann F, Manz MG, Jung S, et al: Development of monocytes, macrophages, and dendritic cells. *Science* 327:656, 2010
- Martinez FO, Sica A, Mantovani A, Locati M: Macrophage activation and polarization. *Front Biosci* 13:453, 2008
- Fend L, Accart N, Kintz J, et al: Therapeutic effects of anti-CD115 monoclonal antibody in mouse cancer models through dual inhibition of tumor-associated macrophages and osteoclasts. *PLoS One* 8:e73310, 2013
- Dayan D, Salo T, Salo S, et al: Molecular crosstalk between cancer cells and tumor microenvironment components suggests potential targets for new therapeutic approaches in mobile tongue cancer. *Cancer Med* 1:128, 2012
- Mantovani A, Sozzani S, Locati M, et al: Macrophage polarization: Tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* 23:549, 2002
- Hodge JM, Collier FM, Pavlos NJ, et al: M-CSF potently augments RANKL-induced resorption activation in mature human osteoclasts. *PLoS One* 6:e21462, 2011
- Davignon JL, Hayder M, Baron M, et al: Targeting monocytes/macrophages in the treatment of rheumatoid arthritis. *Rheumatology (Oxford)* 52:590, 2013
- Komori H, Watanabe H, Shuto T, et al:  $\alpha(1)$ -Acid glycoprotein up-regulates CD163 via TLR4/CD14 protein pathway: Possible protection against hemolysis-induced oxidative stress. *J Biol Chem* 31:30688, 2012
- Chuong R, Kaban LB, Kozakewich H, Perez-Atayade A: Central giant cell lesions of the jaws: A clinicopathologic study. *J Oral Maxillofac Surg* 44:798, 1986
- O'Malley M, Pogrel MA, Stewart JC, et al: Central giant cell granulomas of the jaws: Phenotype and proliferation-associated markers. *J Oral Pathol Med* 26:159, 1997
- Ficarra G, Kaban LB, Hansen LS: Central giant cell lesions of the mandible and maxilla: A clinicopathologic and cytometric study. *Oral Surg Oral Med Oral Pathol* 64:44, 1987
- Whitaker SB, Waldron CA: Central giant cell lesions of the jaws. A clinical, radiologic, and histopathologic study. *Oral Surg Oral Med Oral Pathol* 75:199, 1993
- Holness CL, Simmons DL: Molecular cloning of CD68, a human macrophage marker related to lysosomal glycoproteins. *Blood* 81:1607, 1993
- Sarode SC, Sarode GS: Cellular cannibalism in central and peripheral giant cell granuloma of the oral cavity can predict biological behavior of the lesion. *J Oral Pathol Med* 43:459, 2014

23. Torabinia N, Razavi SM, Shokrolahi Z: A comparative immunohistochemical evaluation of CD68 and TRAP protein expression in central and peripheral giant cell granulomas of the jaws. *J Oral Pathol Med* 40:334, 2011
24. Flórez-Moreno GA, Henao-Ruiz M, Santa-Sáenz DM, et al: Cytomorphometric and immunohistochemical comparison between central and peripheral giant cell lesions of the jaws. *Oral Surg Oral Pathol Oral Radiol Endod* 105:625, 2008
25. Aragão Mdo S, Piva MR, Nonaka CF, et al: Central giant cell granuloma of the jaws and giant cell tumor of long bones: An immunohistochemical comparative study. *J Appl Oral Sci* 15:310, 2007
26. Itonaga I, Hussein I, Kudo O, et al: Cellular mechanisms of osteoclast formation and lacunar resorption in giant cell granuloma of the jaw. *J Oral Pathol Med* 43:459, 2003
27. Tobón-Arroyave SI, Hurtado-García P, García-Quintero OD, et al: Immunoexpression of NF- $\kappa$ B and their inhibitory subunits I $\kappa$ B $\alpha$  and I $\kappa$ B $\beta$  in giant cell lesions of the jaws: Implications for their clinical behavior. *J Oral Pathol Med* 44:752, 2015
28. Cassier PA, Gelderblom H, Stacchiotti S, et al: Efficacy of imatinib mesylate for the treatment of locally advanced and/or metastatic tenosynovial giant cell tumor/pigmented villonodular synovitis. *Cancer* 118:1649, 2012
29. Kristiansen M, Graversen JH, Jacobsen C, et al: Identification of the haemoglobin scavenger receptor. *Nature* 409:198, 2001
30. Van Gorp H, Delputte PL, Nauwynck HJ: Scavenger receptor CD163, a jack-of-all-trades and potential target for cell-directed therapy. *Mol Immunol* 47:1650, 2010
31. Pogrel AM: The diagnosis and management of giant cell lesions of the jaws. *Ann Maxillofac Surg* 2:102, 2012
32. de Lange J, van den Akker HP, van den Berg H: Central giant cell granuloma of the jaw: A review of the literature with emphasis on therapy options. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 104:603, 2007
33. Vered M, Buchner A, Dayan D: Giant cell granuloma of the jawbones—A proliferative vascular lesion? Immunohistochemical study with vascular endothelial growth factor and basic fibroblast growth factor. *J Oral Pathol Med* 35:613, 2006
34. Lim L, Gibbins JR: Immunohistochemical and ultrastructural evidence of a modified microvasculature in the giant cell granuloma of the jaws. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 79:190, 1995
35. Dvorak HF: Vascular permeability to plasma, plasma proteins, and cells: An update. *Curr Opin Hematol* 17:225, 2010
36. Suva JL, Freeman AN, Martin TJ: Parathyroid hormone-related protein. Gene structure, biosynthesis, metabolism and regulation. In: Bilezikian JP, Marcus R, Levine MA, et al: *The Parathyroids. Basic and Clinical Concepts* (ed 3). San Diego, CA, Elsevier, 2015, pp 45-64
37. Houpis CH, Tosios KI, Papavasileiou D, et al: Parathyroid hormone-related peptide (PTHrP), parathyroid hormone/parathyroid hormone-related peptide receptor 1 (PTHr1), and MSX1 protein are expressed in central and peripheral giant cell granulomas of the jaws. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 109:415, 2010
38. Vered M, Nasrallah W, Buchner A, Dayan D: Stromal myofibroblast in central giant cell granuloma of the jaws cannot distinguish between non-aggressive and aggressive lesions. *J Oral Pathol Med* 36:495, 2007
39. Hyckel P, Berndt A, Schleier P, et al: Cherubism—New hypotheses on pathogenesis and therapeutic consequences. *J Cranio-maxillofac Surg* 33:61, 2005
40. Haegel H, Thioudellet C, Hallet R, et al: A unique anti-CD115 monoclonal antibody that inhibits osteolysis and skews human monocyte differentiation from M2-polarized macrophages toward dendritic cells. *MAbs* 5:736, 2013