

Periostin and receptor activator of nuclear factor κ -B ligand expression in allergic fungal rhinosinusitis

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Background: Allergic fungal rhinosinusitis (AFRS) is a disease demonstrating substantial eosinophilic inflammation and characteristic radiographic bony erosion/expansion. Periostin is an extracellular matrix protein associated with eosinophil accumulation in eosinophilic esophagitis, allergic asthma mucus production, and chronic rhinosinusitis (CRS) polyp formation. Receptor activator of nuclear factor κ -B ligand (RANKL) is an osteoclast activator present in osteoporosis and periodontal disease. We sought to evaluate periostin and RANKL expression in AFRS and correlate these levels with radiographic scales of disease severity.

Methods: Thirty sinus tissue specimens were intraoperatively collected from 3 patient groups: AFRS; CRS without nasal polyps (CRSsNP); and controls ($n = 10$ per group). Specimens were analyzed by semiquantitative reverse-transcription polymerase chain reaction (sq-RT-PCR) and immunofluorescence (IF) labeling/confocal microscopy for the presence of both periostin and RANKL. Immunofluorescence staining intensity was quantified by pixel density analysis. Preoperative computed tomography (CT) scans from each patient were scored using both the Lund-Mackay and CT bone erosion scoring systems.

Results: Periostin was significantly elevated in AFRS sinus tissue compared to CRSsNP and controls, as demonstrated

by IF ($p < 0.001$) and PCR ($p = 0.011$). RANKL was not detected in sinus tissue by IF or PCR. Periostin levels positively correlated with radiographic indices of disease severity for both soft tissue and bone, using Lund-Mackay ($r = 0.926$ [PCR] and $r = 0.581$ [IF]) and CT bone erosion ($r = 0.672$ [PCR] and $r = 0.616$ [IF]) scoring systems, respectively.

Conclusion: Periostin is increased in AFRS tissue compared to CRSsNP and controls. Periostin levels positively correlate with radiologic disease severity scores. The increased levels of periostin in AFRS are possibly tied to its intense eosinophilic inflammatory etiology. © 2014 ARS-AAOA, LLC.

Key Words:

periostin; RANKL; allergic fungal sinusitis; bone erosion; radiographic; chronic rhinosinusitis; nasal polyp; inflammation

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Allergic fungal rhinosinusitis (AFRS) is a relatively common disease state in certain geographic regions. AFRS has been classically defined by Bent and Kuhn to include 5

major criteria: (1) type I hypersensitivity; (2) nasal polypoid; (3) characteristic radiographic findings; (4) eosinophilic mucin without fungal invasion; and (5) positive fungal stain.¹ Studies have shown that AFRS can constitute up to 30% of patients undergoing functional endoscopic sinus surgery (FESS) for sinonasal inflammatory disease, with a particularly high preponderance in the Southeastern United States.² It can be surmised that AFRS made up a substantial percentage of the nearly 30 million Americans diagnosed with rhinosinusitis in 2009.³ Surgical intervention is currently the main treatment option for these patients, supplemented by nasal rinses, oral and topical steroids, and sometimes allergen-specific immunotherapy in the postoperative setting. However, despite the prevalence of this disease and the relative void of effective medical therapies, little is known regarding its cause or the cellular mechanisms behind its pathophysiologic features.

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Periostin is an extracellular matrix protein involved in cell adhesion with a recently identified role in multiple organ systems, including cardiac remodeling, colorectal cancer, eosinophilic esophagitis, and allergic airway diseases.^{4–6} Periostin messenger RNA (mRNA) expression has been shown to increase in cardiac myocytes and fibroblasts secondary to mechanical stretch; this may be similar to the mechanical stretch that has been theorized as the potential cause for AFRS bone erosion and expansion.⁷ More recently, periostin has been implicated directly in regulating eosinophil accumulation and T helper 2 (Th-2) mucosal inflammation in allergic eosinophilic esophagitis.⁴ Additionally, initial work in asthmatic airway samples has shown periostin to be a potent stimulator of the transforming growth factor β (TGF β) signaling pathway with a prominent role in collagen fibrillogenesis, as well as an important component in a negative feedback loop to help reduce mucus production initiated by other Th-2 cytokines.⁸

The role of periostin in the sinonasal cavity has also recently been further delineated. Using polymerase chain reaction (PCR) and immunohistochemistry techniques, periostin levels were found to be significantly elevated in polypoid tissue in patients with CRS and nasal polyposis with and without aspirin sensitivity when compared with controls.⁹ These increased periostin levels were found to be specific to the polypoid tissue sample itself and not universally elevated in samples of adjacent “healthy” mucosa from the same patient.¹⁰ However, despite periostin’s significant role in Th-2-mediated pathways, eosinophil recruitment, mucus production, and sinonasal inflammatory disease, it has yet to be evaluated in patients with AFRS.

The receptor activator of nuclear factor κ -B (RANK) belongs to the tumor necrosis factor (TNF) superfamily and is present on the surface of osteoclasts involved in bone breakdown.¹¹ It is activated by RANK ligand (RANKL) and inhibited by osteoprotegerin (OPG), which acts as a direct inhibitor by binding to RANKL and thereby preventing osteoclastogenesis.¹² The upregulation of RANKL and the downregulation of OPG have been implicated in a range of bone-resorption diseases including, osteoporosis, rheumatoid arthritis, and periodontal diseases.¹³ Recently, levels of RANKL expression have also been shown to positively correlate with progressive bone erosion in patients with rheumatoid arthritis based on objective analysis of radiographic imaging.¹⁴ Furthermore, RANKL is expressed in cell types other than osteoclasts, including breast, prostate, and odontogenic epithelium.¹⁵ However, RANKL has yet to be examined in relation to the bony erosion and expansion that is frequently seen in AFRS. Therefore, the purpose of this study was to examine if periostin and RANKL are elevated in AFRS sinus tissue compared to chronic rhinosinusitis without nasal polyposis (CRSsNP) and controls. Additionally, we sought to further understand the specific correlation between periostin and RANKL expression and the radiographic severity of sinus disease using soft tissue and bony radiographic scales.

Patients and methods

Study population and tissue collection

Sinonasal tissue was collected from 30 patients (10 per group) with 3 distinct sinonasal disease phenotypes. The first group contained patients undergoing ESS for suspected AFRS based on their perioperative fulfillment of the Bent and Kuhn AFRS criteria. The second group consisted of patients undergoing ESS for CRSsNP as part of their treatment algorithm for their disease process. These patients all met criteria for CRS defined by the Rhinosinusitis Task Force Committee of the American Academy of Otolaryngology–Head and Neck Surgery (AAO-HNS) but did not have sinonasal polyps on clinical, radiographic, or endoscopic exam.¹⁶ The third group was comprised of noninflammatory controls—patients with no symptomatic, endoscopic, or radiographic evidence of inflammatory sinonasal disease. These patients were typically undergoing sinonasal approaches for skull base pathology. Additionally, all patients had to have preoperatively obtained CT imaging of their sinuses to be included in the study. Exclusion criteria were: previous sinus surgery, cystic fibrosis, immune deficiency, solid organ or bone marrow transplant, autoimmune conditions, ciliary disorders, granulomatous disorders, and vulnerable patient populations. Patients who had used certain medications within 7 days prior to surgery were also excluded. Patients on calcitriol, rapamycin, bisphosphonates, and fish oil, which have been shown to alter RANKL systemic soluble levels were excluded.^{17–20} Patients on nasal or systemic steroids, antihistamines, antileukotrienes, or immunosuppressive medications, all of which could theoretically affect the inflammatory status of our sinonasal tissue samples were also excluded.

Approval was obtained from the Emory University Institutional Review Board (IRB) prior to initiation of research, and all patients donating tissue for this study gave written, informed consent. After Emory University IRB approval was obtained, patients eligible for inclusion were consented preoperatively for intraoperative tissue procurement. During the endoscopic procedure, sinus tissue specimens were obtained from the nasal polyps or diseased sinuses for the AFRS and CRSsNP groups. For the controls, sinus tissue was obtained from the most easily accessible sinus cavity, typically the sphenoid sinus. All experimental tissue was obtained from the sinus mucosa; no experimental tissue was obtained from the nasal cavity, turbinates, or nasal septum. Tissue specimens were rinsed in normal saline to remove blood and mucus, divided in half, and placed in either RNAlater[®] (Life Technologies, Grand Island, NY) (for PCR processing) or in Tissue-Tek[®] OCT (Fisher Scientific, Pittsburgh, PA) (for immunofluorescence processing) and then snap frozen at -80°C .

RNA extraction

Samples were removed from RNAlater[®] and homogenized in 1 mL of Trizol[®] (Life Technologies, Grand Island, NY). The organic and aqueous phase was separated by adding

200 μ L chloroform, vortexing, and then centrifuging at 10,000g for 15 minutes at 4°C. The aqueous phase was then transferred to a sterile Eppendorf tube and total RNA was isolated using RNeasy spin columns (Qiagen, Valencia, CA) according to the manufacturer's protocol. During RNA purification all specimens were treated with RNase-free DNase I to remove contaminating DNA (Qiagen, Carol Stream, IL). The RNA samples were then tested for purity and quantity using a Nanodrop spectrophotometer, and RNA integrity was determined by running 500 ng of purified total RNA on a 1.2% Tris-acetate-ethylenediamine tetraacetic acid (EDTA) (TAE) agarose gel containing 2 μ g/mL ethidium bromide and visualizing the ribosomal RNA on an ultraviolet (UV) transilluminator. This typically revealed both 28S and 18S mRNA bands, a finding indicative of an intact, nondegraded, and therefore, viable specimen. Alternatively, a smeared product at that bottom of a lane without intact bands implied a degraded or contaminated sample which was subsequently discarded and not included in analysis. Only samples that yielded good 260/280 ratios (>1.8) and had observable intact ribosomal RNA bands based on gel electrophoresis were used. The RNA was then reverse-transcribed using Revertaid Kit (Fisher Scientific, Atlanta, GA) and Oligo dT primer according to the manufacturer's instructions. One microgram of total RNA was used as a starting template.

RT-PCR quantification

Real-time quantitative reverse-transcription polymerase chain reaction (RT-qPCR) was performed using primers previously described and synthesized by IDT (Integrated DNA Technologies, Chicago, IL): periostin: GTCTTTGA GACGCTGGAAGG and AGATCCGTGAAGGTGGTT TG; RANKL: ATACCCTGATGAAAGGAGGA and GGG GCTCAATCTATATCTCG; and GAPDH: AGAAGGCT GGGGCTCATTTG and AGGGGCCATCCACAGTCTT C.^{19,21,22} PCR was optimized to use the equivalent of cDNA generated from 10 ng of total RNA. Semiquantitative PCR was performed using iQTM SYBR[®] green Supermix, the equivalent complementary DNA (cDNA) generated from 10 ng total RNA, and 200 nm of primer in a 25- μ L reaction. Real-time PCR was run on a Bio-Rad[®] MyiQTM PCR machine with the following cycling conditions; initial denaturing: 2 minutes at 95°C, 37 cycles with denaturing at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds. Data was analyzed using the Livak method.²³

Immunofluorescence staining and confocal microscopy

Tissue samples were sectioned on a Leica cryostat (Leica Biosystems, Richmond, IL) at 6- μ m thickness. Slides were fixed with precooled acetone (−20°C) for 5 minutes at 25°C and rinsed 3 times in Dulbecco's phosphate buffered saline (DPBS). Nonspecific binding was blocked by incubating slides in 2% goat serum in DPBS for 1 hour at 25°C, and

the slides were again rinsed 3 times with DPBS. Slides were then incubated at 4°C for 18 hours with primary antibodies: rabbit anti-human periostin antibody (Abcam Inc., Cambridge MA; 1:500 in blocking buffer) or rabbit anti-human RANKL antibody (Abcam, Inc., Cambridge, MA; 1:500 in blocking buffer). Following primary antibody incubation, slides were again washed in DPBS 3 times and incubated at 25°C with Alexa Fluor[®] 488-labeled goat anti-rabbit (Life Technologies, Grand Island, NY; 1:500 in blocking buffer) and Alexa Fluor[®] 546 phalloidin (actin stain; Life Technologies, Grand Island, NY; 1:500 in blocking buffer) secondary antibodies for 60 minutes in a dark environment. Specimens were again washed 3 times in DPBS and incubated with To-Pro[®] nuclear stain (Life Technologies, Grand Island, NY; 1:1000 in blocking buffer) at 25°C for 5 minutes. Specimens were washed again 3 times in DPBS, dried, and then mounted with p-phenylenediamine anti-quench reagent and sealed. To prevent any fading of fluorescent stain, slides were immediately visualized with a Zeiss LSM510 laser scanning confocal microscope (Zeiss Microimaging Inc., Thornwood, NY) coupled to a Zeiss 100M Axiovert with a 40 $\times$ Pan-Apochromat oil lens. Fluorescent dyes were imaged sequentially to eliminate cross talk between channels. Images were processed with Zeiss LSM5 image browser software. Representative images were selected for each specimen stained for either periostin or for RANKL and saved for further analysis. Images for analysis were chosen based on overall quality of the image rather than degree of periostin or RANKL staining. Things that were considered when choosing an image included an intact epithelial-subepithelial junction, consistent staining for all 3 stains on an image, etc. This standard was kept consistent among all images and across all groups.

Image analysis

A representative image was obtained for each specimen immunostained for either periostin or RANKL. An area 50- μ m-deep was then drawn from the most superficial aspect of the subepithelium (where periostin and RANKL are primarily located) along the entire length of the image. The ImageJ software program (NIH; <http://imagej.nih.gov/ij/>) was used to obtain a numerical value for the pixel density per cross-sectional area for each tissue specimen. These numerical values were used as an objective measure for the amount of periostin or RANKL staining for a given sample.

Radiographic analysis

Each patient's preoperatively obtained CT scan of the sinuses was reviewed in a blinded fashion by 1 of the authors (A.L.) and graded using both the Lund-Mackay CT staging system and the AFRS bone erosion staging system.^{24,25}

Statistical analysis

Because of the small sample sizes in each group, initial analyses used nonparametric (ie, Mann-Whitney *U* and

Kruskal-Wallis) tests to examine for group differences. Follow-up parametric tests (ie, independent samples *t* test, analysis of variance [ANOVA] with Tukey post hoc testing) were conducted to determine if patterns of significance differed across analytic techniques. Because the pattern of significance did not differ between nonparametric and parametric analyses, only the parametric analyses are reported. Parametric statistical tests are more powerful and afford greater ability to probe for significant differences among groups. To examine the strength and direction of the relationship between variables, Pearson correlations were also conducted. Statistical significance was set at *p* < 0.05.

Results

Study patients

Ten patients were recruited to donate sinus tissue from each of the 3 disease-specific groups. Demographic data among subjects was consistent across groups except that the average age in the AFRS group was notably younger when compared to the CRSsNP and controls (Table 1). This is likely due to the fact that the inclusion criteria required that this be the subject’s first surgery and AFRS typically presents at relatively young age of onset. There is no evidence to suggest that periostin levels are related to age and therefore we think that this difference should not have affected the validity of our data.

TABLE 1. Patient demographics

Patient group	n	Men (%)	Age, years (mean, range)	Smoker (%)
AFRS	10	70	34.2 (24–49)	10
CRSsNP	10	50	60.4 (30–73)	10
Control	10	60	52.7 (27–79)	10

AFRS = allergic fungal rhinosinusitis; CRSsNP = chronic rhinosinusitis without nasal polyps.

Immunofluorescence staining

Figure 1 shows an example of tissues section labeled to detect periostin from an AFRS patient. The periostin (green) was localized primarily to the subepithelial layer, as previously demonstrated. F-actin is presented in red as a counterstain to delineate cell structure. The To-Pro® nuclear stain (blue) highlights the individual epithelial cells. Figure 2 demonstrates that the intensity of periostin was relatively similar among subjects within 1 group but visibly varied in intensity across disease groups. As seen in Figure 2, the most intense periostin staining was present in the subepithelial layer of the AFRS group, with less intense periostin staining in the CRSsNP and noninflammatory control groups.

Image analysis was performed to evaluate the staining intensity and presented as mean ± standard deviation (SD)

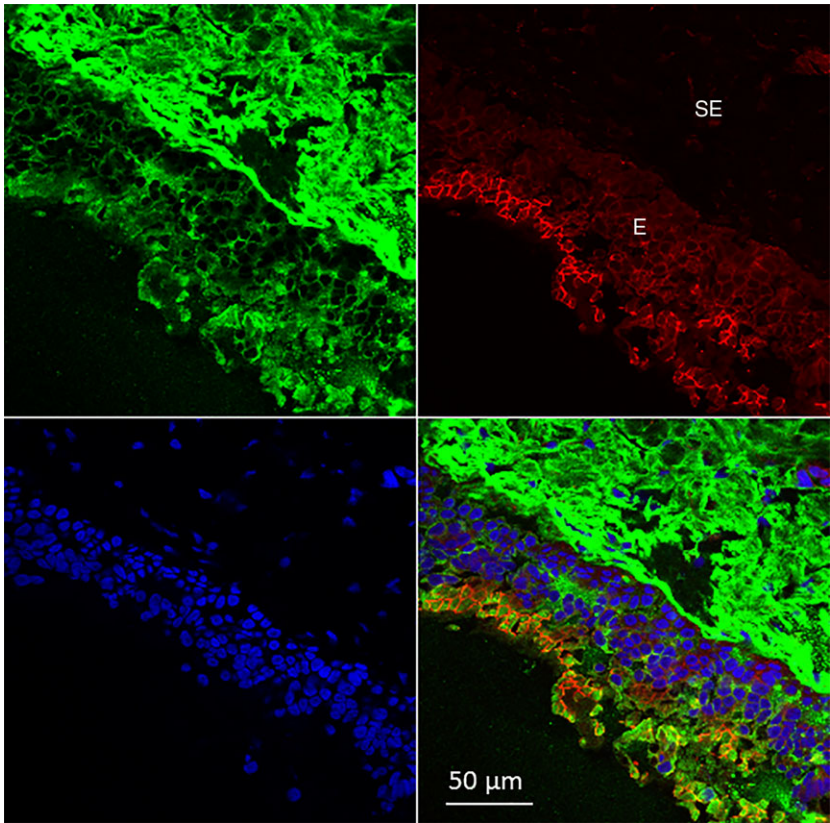


FIGURE 1. Tissue from an AFRS patient showing the periostin immunostain uptake in the subepithelial layer (green) vs actin in the epithelium (red) and To-Pro® nuclear stain in the epithelium (blue). AFRS = allergic fungal rhinosinusitis; E = epithelium; SE = subepithelial layer.

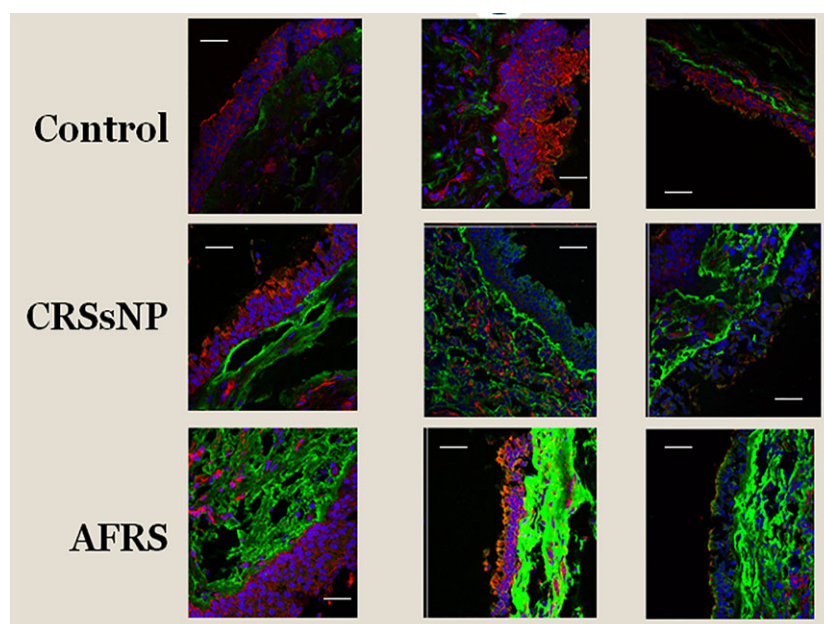


FIGURE 2. Tissue specimens which demonstrate that the intensity of periostin was relatively similar among subjects within 1 group but visibly varied in intensity across disease groups. Periostin = green, Actin = red, To-Pro® nuclear stain = blue. Scale bar = 50 μ m. AFRS = allergic fungal rhinosinusitis; CRSsNP = chronic rhinosinusitis without nasal polyps.

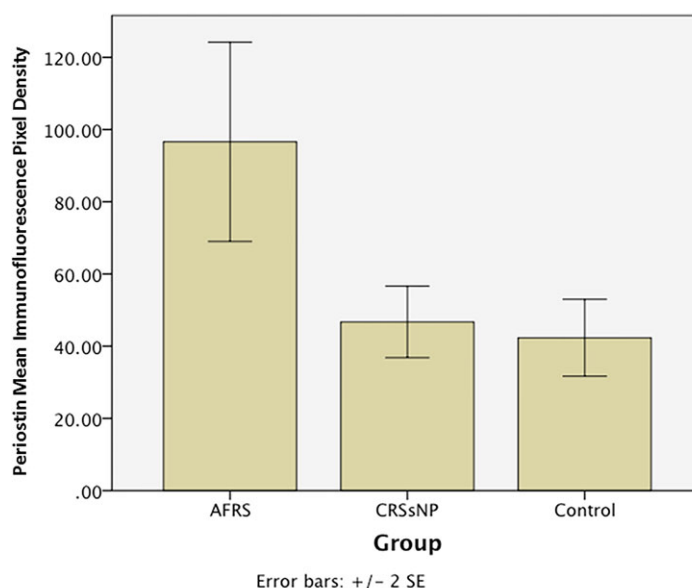


FIGURE 3. Graph demonstrating the mean pixel density per area differences for periostin immunofluorescence between patient groups. AFRS = allergic fungal rhinosinusitis; CRSsNP = chronic rhinosinusitis without nasal polyps; SE = standard error.

of the pixel density per cross-sectional area for each group. There was a significant difference across all 3 disease groups for periostin staining ($F[2,27] = 11.21$, $p < 0.001$, $\eta^2 = 0.45$). Tukey post hoc analysis revealed that the AFRS group ($M = 96.56$, $SD = 43.62$) evidenced significantly higher pixel density than the control group ($M = 42.31$, $SD = 16.86$, $p = 0.001$) and the CRSsNP groups ($M = 46.70$, $SD = 15.64$, $p = 0.002$). The CRSsNP and control groups were not significantly different from one another

(Fig. 3). RANKL was not readily identified by immunofluorescence labeling of tissue sections from the respective patient groups, regardless of disease group (Fig. 4).

PCR

Periostin transcripts were identified in all tissue samples by PCR. Periostin levels in the control tissue were normalized to 1 and used as a baseline value, as required for

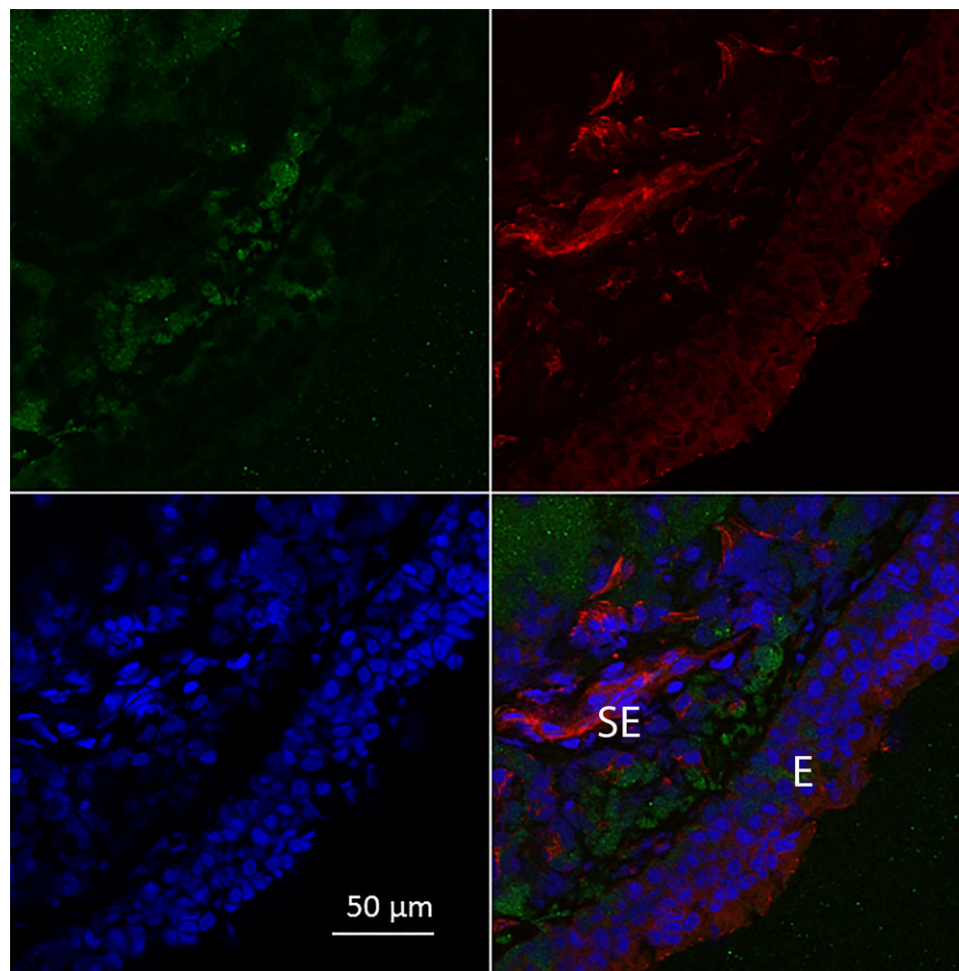


FIGURE 4. Tissue from an AFRS patient showing the RANKL immunostain (green) uptake in the SE vs actin (red) in the epithelium (E) and To-Pro® nuclear stain in the epithelium (blue). Notably, there is no substantial uptake of RANKL immunostain in the epithelial or SE layers. AFRS = allergic fungal rhinosinusitis; E = epithelium; RANKL = receptor activator of nuclear factor κ -B ligand; SE = subepithelial layer.

quantitative comparison by the Livak method. Periostin mRNA transcript levels were significantly higher in the AFRS group ($M = 3.19$, $SD = 1.29$) compared to the CRSsNP ($M = 1.65$, $SD = 1.28$), $t(18) = 2.67$, $p = 0.02$, $d = 1.19$ (Fig. 5). RANKL showed no substantial amplification over the water control across all 3 patient groups.

Radiographic correlation

Periostin immunofluorescence pixel density and PCR data values for each patient, inclusive of all 3 study groups, were then compared to their corresponding Lund-Mackay and AFRS CT bone erosion scores to determine if a correlation was present. Figure 6 shows the positive correlation across all 3 groups between the periostin immunofluorescence pixel density and CT scoring systems for both the Lund-Mackay scale, $r(28) = 0.58$, $p = 0.001$, and the CT bone erosion scale, $r(28) = 0.62$, $p < 0.001$. It also depicts the positive correlation between the periostin PCR data and CT scoring systems for both the Lund-Mackay scale, $r(18) = 0.93$, $p < 0.001$, and the CT bone erosion scale,

$r(18) = 0.67$, $p = 0.001$ (Fig. 6). These correlations indicate that the periostin level, as measured by immunofluorescence or PCR, is positively associated with radiographic measures of disease severity in CRS by soft tissue (Lund-Mackay) and bony (CT bone erosion) measures. Lund-Mackay and CT bone erosion scores were also compared across all patients. This demonstrated a positive correlation of $r(28) = 0.81$ and $p < 0.001$ between the 2 radiographic disease severity scales.

Finally, correlation analyses were individually performed for PCR and IF values for each of the 3 study groups in comparison to the 2 radiographic measures. In the AFRS group, the Lund-Mackay and CT bone erosion scores show a very strong remaining correlation ($r = 0.899$, $p < 0.001$), indicating that the relationship between these 2 radiologic staging systems is substantially carried by the AFRS group in this study. In the control group, the immunofluorescence scores and Lund-Mackay scores retain a significant correlation ($r = 0.671$, $p = 0.034$), although the significance level is not as strong as in the original analysis that included all groups. The loss of other significant correlations with

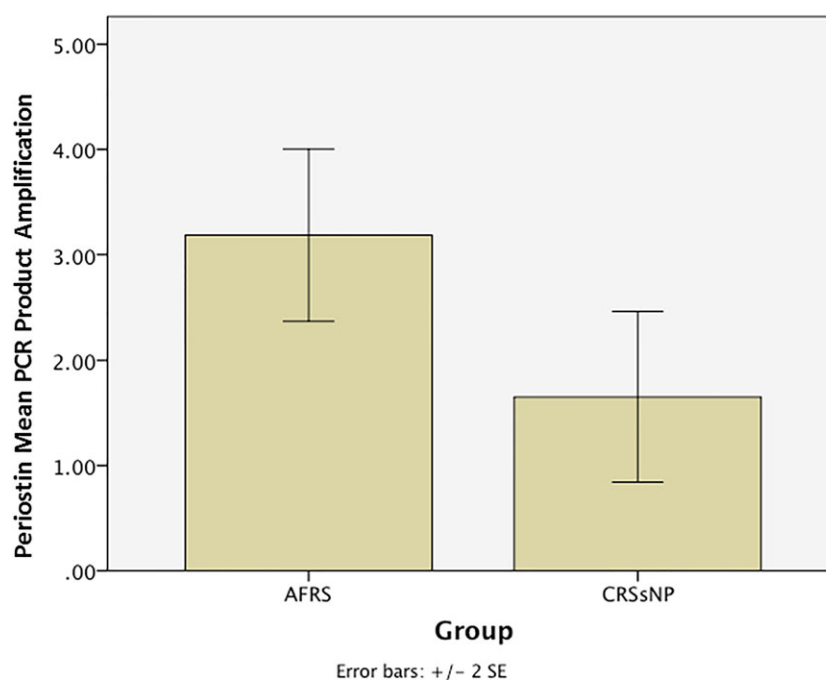


FIGURE 5. Graph demonstrating the relative amount of periostin transcripts using PCR between the AFRS group and the CRSsNP group compared to the control (normalized to 1). AFRS = allergic fungal rhinosinusitis; CRSsNP = chronic rhinosinusitis without nasal polyps; PCR = polymerase chain reaction; SE = standard error.

individual group analysis may be due to either periostin representing a true marker of CRS disease state across various categories of disease, or the power of the study dropping from 30 specimens to only 10, making it more difficult to find a significant correlation.

Discussion

Although periostin has previously been shown to be present in Th-2-mediated disease states, including CRS with nasal polyps (CRSwNP), this is the first study to demonstrate the existence of periostin specifically in AFRS. This was confirmed through the implementation of 2 distinct identification techniques, sq-RT-PCR and immunofluorescence. Although sq-RT-PCR has been widely used in past studies,^{9,10} this is also the first time, to our knowledge, that immunofluorescence labeling and confocal microscopy has been used to detect periostin protein in sinonasal tissue. This allowed for another method by which to validate periostin's presence in sinonasal tissue and as well as relatively quantify it across tissue samples. Both tests indicated that periostin levels were nearly 3 times higher in the AFRS specimens compared to controls and nearly double in the AFRS specimens compared to CRSsNP. This is particularly interesting in that it suggests a clear difference in the presence of periostin in a Th-2-mediated (AFRS) vs Th-1-mediated (CRSsNP) disease process.

The findings of the present study in AFRS tissue further support previous studies that have identified periostin as an integral component in Th-2 mucosal inflammation in eosinophilic esophagitis and allergic asthma. Previous

studies on eosinophilic esophagitis have shown that periostin knockout mice exhibit a significant decrease in eosinophil recruitment to the lungs and an increase in blood eosinophilia after intranasal inhalation of allergens.⁴ Therefore, along with this new knowledge that periostin is significantly increased in AFRS tissue, we hypothesize that periostin may play a role in the inherent finding of exuberant eosinophilic infiltration present in AFRS patients.

RANKL was not identified in any sinonasal tissue samples. Although its inherent role in bony remodeling led to the initial hypothesis that RANKL may have a role in AFRS bony expansion and erosion, we failed to identify it in any sinonasal tissue samples, diseased or control, via reverse-transcription PCR (RT-PCR) or immunofluorescence. At this time, it does not appear that RANKL plays a substantial role in inflammatory sinonasal disease.

This study is also unique in that it found a positive correlation between a specific protein in AFRS tissue and the CT bone erosion scoring system. Several studies have identified correlations between various protein levels in sinonasal tissue from CRS patients and their corresponding Lund-Mackay scores.^{26,27} However, considering the unique qualities of classic AFRS and the substantial bony remodeling found on imaging, a previously-reported CT bone erosion scoring system was also used in conjunction with the Lund-Mackay system.²⁵ In this study, both radiologic scoring systems showed a positive correlation with periostin levels that were identified via both sq-RT-PCR and immunofluorescence. However, the periostin and CT bone erosion correlations were largely carried by the AFRS patients, with almost all other patients from both the CRSsNP and

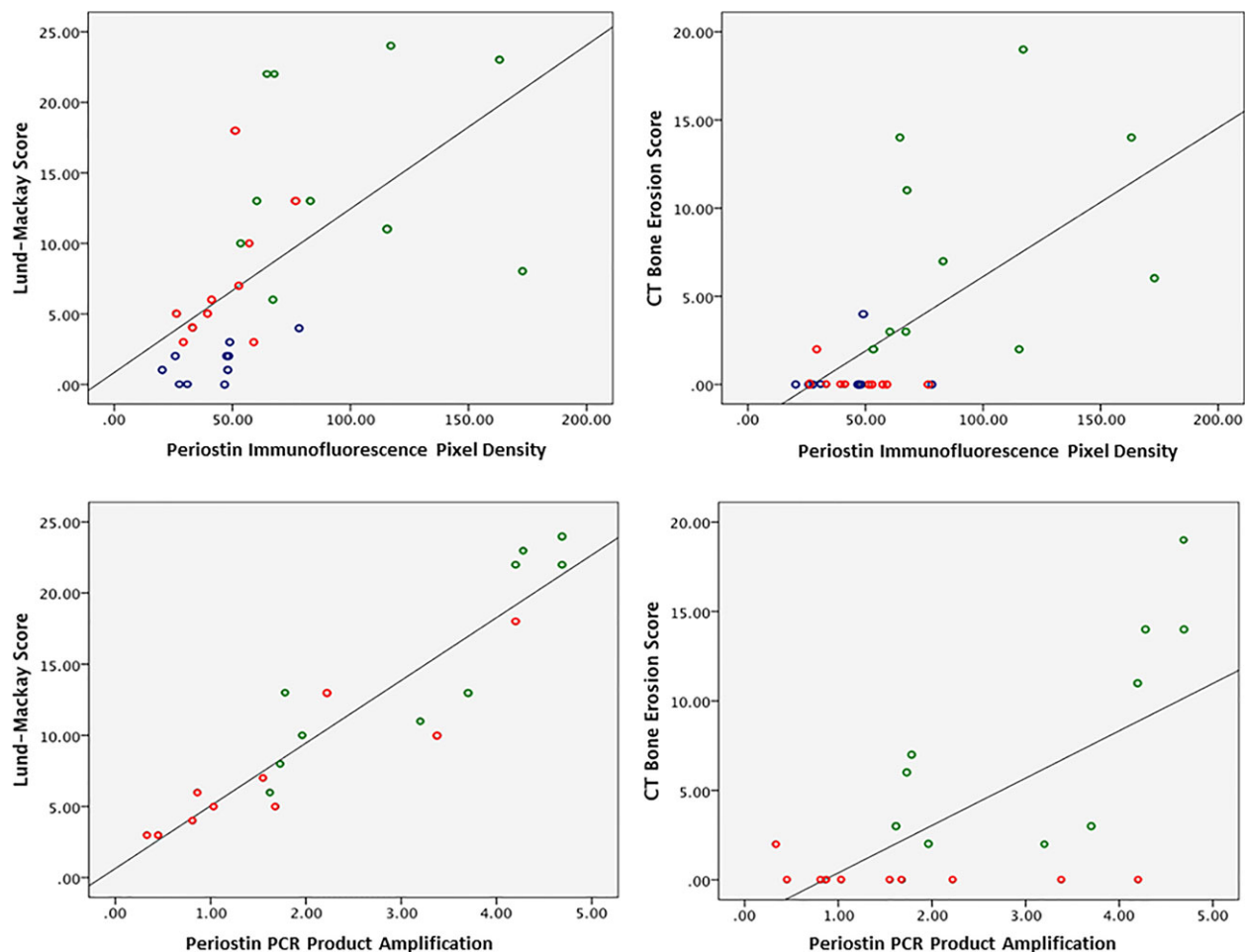


FIGURE 6. Graphs demonstrating the positive correlations between periostin immunofluorescence values and Lund-Mackay and CT bone erosion scores, as well as the positive correlations between periostin PCR values and Lund-Mackay and CT bone erosion scores. AFRS (green), CRS (red), control (blue). AFRS = allergic fungal rhinosinusitis; CRS = chronic rhinosinusitis; CT = computed tomography; PCR = polymerase chain reaction.

control groups scoring near zero on the CT bone erosion system. In contrast, the strong positive correlation identified between the periostin levels and Lund-Mackay CT scores was seen across both sinonasal inflammatory groups. Finally, the Lund-Mackay scores and CT bone erosion scores were compared to one another, and once again a positive correlation was identified, carried predominantly by the AFRS patients; this is rather expected, given the substantial sinus opacification and bony changes seen in the AFRS group.

This study has certain limitations. First, some of the control subjects had mildly increased CT scores secondary to pituitary tumors that partially eroded the posterior-superior aspects of the sphenoid sinus. Although it would have been ideal for the entire control group to be free of any radiologic abnormalities affecting the paranasal sinuses, this study also required harvesting surgical tissue from the sinuses of control patients; therefore, patients with skull base pathology and only mild radiologic abnormalities were recruited to fulfill the control group. Additionally, the sample size was relatively small; stronger positive cor-

relations may have been more easily identified then if larger samples were included.

Potential future studies stemming from this work include comparing periostin levels in various etiologies of polypoid disease, such as AFRS, CRSwNP with and without associated atopic disease, CRSwNP in aspirin-exacerbated respiratory disease, and CRSwNP in cystic fibrosis. This would lay the groundwork for determination of periostin's role in nasal polyps from patients with different inflammatory etiologies and its precise function in sinonasal inflammatory disease.

Currently, there are relatively few treatment options for patients with AFRS. These efforts in detecting specific proteins elevated AFRS and understanding its pathophysiology are important as we move toward better diagnostic capabilities in sinonasal inflammatory disease and targeted therapies to medically control AFRS. This study has identified periostin as a protein which is not only significantly increased in AFRS, but also whose presence has been shown to directly correlate with disease severity. In turn, this study substantiates periostin's promising new role in the

pathophysiology of AFRS as well as its potential use in future treatment regimens.

Conclusion

This study shows that periostin is elevated in AFRS tissue compared to CRSsNP and control sinus tissues via both sq-RT-PCR and immunofluorescence. Additionally, the levels of periostin appear to correlate with the degree of disease severity identified on CT imaging as graded by both the Lund-Mackay and CT bone erosion scoring systems. RANKL was not identified in any paranasal sinus

tissue in any specimen. Overall, periostin appears to play a role in the pathophysiology of AFRS, possibly in the propagation of its inflammatory cascade and eosinophil accumulation. 

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