1**ABSTRACT**

2**Objectives:** The purpose of this study was the evaluation of any alterations in the 3microvascular network of the nasal mucosa in patients with pulmonary sarcoidosis 4and the investigation of potential correlations with olfactory acuity and serum levels 5of angiotensin-converting enzyme (sACE).

6**Design:** Patients' nasal mucosa was examined with contact endoscopy (CE). A novel 7classification scheme for the microvascular pattern at the anterior septal mucosa 8(Little's area) was introduced and implemented. Olfaction was tested using 9sniffin'sticks. Fifteen healthy subjects served as controls.

10**Participants:** 15 patients with pulmonary sarcoidosis and sinonasal symptoms.

11**Main outcome measures:** Microvascular pattern at the anterior septal mucosa 12(Little's area). Olfaction tested using sniffin'sticks.

13**Setting: Tertiary referral medical centre.**

14**Results:** The nasal microvascular network was disrupted under CE in most (14/15) 15patients, while in one patient no microvascular net could be detected. Moreover, 16hyposmia was documented in four patients and complete anosmia in one patient. In 17healthy subjects, a very strong correlation between vascular pattern of the mucosa and 18olfactory test results was found (r=0.93).

19**Conclusions:** Contact endoscopy findings show promise and should be further 20tested, to evaluate their validity as a surrogate marker of mucosal nasal inflammation 21in sarcoidosis patients with sinonasal symptoms. Vascular patterns of nasal mucosa 22and olfaction seem to be strong correlated.

Keywords: sarcoidosis; pulmonary; nasal; microvascular; contact endoscopy; sniffin' sticks

Keypoints

- Changes in the microvascular network of the nasal mucosa can be accompanied by changes in olfaction.
- The lack of a special classification of microvascular patterns of the nasal mucosa has led us to the introduction and use of a modified version of the Negoro's classification.
- . A major future research challenge is the validation of the use of the vascular patterns of nasal mucosa as a tool to predict nasal (and possibly, also pulmonary) disease activity and treatment response in patients with pulmonary sarcoidosis and symptoms of sino-nasal disease.
- Contact endoscopy using traditional and novel colorants emerges as a promising procedure for on-site diagnosis of both early and advanced or late inflammatory mucosal spots or generalized mucosal affection in sarcoidosis.
- The utility of sACE as a diagnostic and prognostic tool is restricted by its rather low sensitivity and specificity.

1 INTRODUCTION

Sarcoidosis is a chronic inflammatory disease, characterized by the formation of non-caseating granulomas. The lungs are affected in 90% of patients [1,2]. Extrapulmonary involvement is observed in more than 50% of cases, mainly involving the liver (65%), the bones and joints (33%), the skin (25%), the eyes (25%) and the central nervous system (15%) [1-3].

Otorhinolaryngological symptoms are frequent in sarcoidosis, thanks to a predilection for the upper and lower respiratory tract [4-6]. Sinonasal involvement varies widely, from 1% in isolated forms to 15% in pulmonary sarcoidosis [7-9]. Nasal symptoms than not are more often non-specific, with nasal obstruction being the commonest presenting symptom [7,8]. Histologically documented sarcoidosis of the upper respiratory tract (SURT) occurs in approximately 5% of patients [2,3] and diagnosis is predicated on clinical and radiological findings, combined with evidence of non-caseating granulomas on biopsy [10]. Due to the non-specific symptomatology, CT scan of the paranasal sinuses has been recently proposed as the gold standard for SURT diagnosis [11-14].

Our aim was to evaluate the potential pathological microvascular network alterations within the nasal mucosa and any concomitant deterioration in olfactory acquity in sarcoidosis patients with sinonasal symptoms. We have also investigated for correlations between the observed microvascular patterns and potential olfaction defects and serum ACE (sACE) levels.

2 MATERIALS AND METHODS

2.1 Study design

We analyzed retrospectively the cases of patients with pulmonary sarcoidosis, referred to our ENT Clinic by the treating pulmonologist for evaluation of symptoms of sinonasal disease between January 2017 and December 2019.

All subjects gave written informed consent prior to the study. According to the Greek legislation, an Ethic Committee approval was not required due to the retrospective design of our investigation and the pseudonymization of patient data. All of them were treated according to the guidelines for Management of sarcoidosis in clinical practice, as also reported in previous studies [3,4].

2.2 Subjects

Fifteen white patients (6 males and 9 females, Group A), with a mean age of 44.3 (SD=±4.86) years, sinonasal symptoms and previously histologically proven diagnosis of pulmonary sarcoidosis (typical granulomatous inflammation in tissue biopsy), were included in our study. The foremost frequent signs in physical examination were nasal polyps, epistaxis and nasal crusts. All of the patients had been previously investigated with a CT scan of the paranasal sinuses, revealing findings of chronic sinus inflammation, with mucosal thickening and opacification of one or more sinuses, in all of the cases. The involvement of the maxillary and the ethmoid sinuses was more frequent than the involvement of the frontal sinus. None of the patients showed signs or symptoms of acute Sarcoidosis (Lofgren's syndrome). All patients were under treatment with systemic (oral) corticosteroids, with a mean duration of

corticosteroid treatment of 4.2±2.2 years. All the patients, because of nasal congestion and crusting, were receiving intranasal corticosteroids (mometasone, as nasal spray, dose: twice per day) at the time of the examination. Patients' baseline characteristics are presented in **Table 1**. Fifteen healthy subjects (6 males and 9 females**, Group B)**, with mean age 45.5 years (SD=±6.66 years) were also studied.

Bearing in mind that, such diseases as primary biliary cirrhosis, alcoholic liver disease, hyperparathyroidism, hyperthyroidism, diabetes mellitus, multiple myeloma, amyloidosis, Gaucher's disease or leprosy, may cause higher levels of ACE, these conditions have been used as exclusion-criteria. Patients' findings were also compared to those of 15 healthy volunteers, which served as a control group. The data of those patients have been chosen randomly from a data base, used for previous studies of ours [15-17].

2.3 Examination Methods-Contact Endoscopy

Conventional fiber optic endoscopy, contact endoscopy, and olfaction test were all first performed by two of the authors (P.P and G.K), blinded for the underlying condition, with a contact endoscope (Karl Storz, 7215AA, 0°, 23 cm long, 4 mm in diameter; Tuttlingen, Germany) as an outpatient procedure. A standardized technique was employed in each patient. A contact endoscope (CE) was connected to a 150W xenon light source, a video camera, and an SVHS video recorder (Olympus, nCare medical recorder). The endoscope was slowly inserted through the nasal cavity to the nasopharynx, which was assessed for any abnormality. All findings were recorded digitally. All endoscopies and recordings were performed by an investigator blinded to the condition of the patients. A second examiner reached in all cases the same

conclusion. All pictures taken, came from the Little's area, where the vesicles more simple to be detected because of the Kiesselbach's plexus.

After careful suction of secretions, the anterior part of the nasal cavity was stained with methylene blue 1%. Then the mucosa was gently touched with the tip of the contact endoscope.. The whole procedure, including both sides of the nasal cavity, lasted between 5- and 10-min.

To our knowledge, there has not been any standardized scale describing the morphology of the blood vessels of the nasal mucosa to date. Based on previous experience and studies on the morphology and vascularization of the oral mucosa [18], we decided to use a modification of Negoro's classification for the description of the microvascular patterns of the nasal mucosa. Based on the accessibility of the Kiesselbach's area at the anterior septal mucosa that comprises four arterial anastomoses, therefore very easy to identify and describe with the use of the contact endoscope, the authors have chosen to modify and apply Negoro's Classification for the characterization of vascular morphology of nasal mucosa [17]. This classification describes with great accuracy the different vascular patterns [17]. According to the modified Negoro's Classification, there are 5 morphological types of nasal mucosa vascularization: **Type A** (clear loop and wooden branch shape), **Type B** (unclear loop and wooden branch shape), **Type C** (elongated blood vessels), **Type D** (granular shape or dotted shape) and **Type E** (unclear blood vessels). Type A represents the "healthiest" morphology and Type E corresponds to the "most pathological" morphology.

2.4 Sniffin' Sticks Test

For odor presentation, the cap was removed from the pen for approximately 3s, the pen's tip was brought in front of the patient's nose and thoroughly moved from left to right nostril and backwards. Individual scores can be related to standard values for (a) normosmia (normal olfactory function), (b) hyposmia (impaired olfactory function) or (c) functional anosmia (residual or absent olfactory function) [18].

2.5 Statistical analysis

The null hypothesis is that there is no correlation between the different microvascular patterns and the olfactory results or the sACE-levels. The results of Sniffin' Sticks have also been compared. To test if the recorded values were normally distributed, we applied a quantile-quantile plot (QQ plot), which proved that the distribution was not normal. As a result, non-parametric tests were applied. The level of statistical significance was set at $p < 0.05$. On each occasion, the findings between two groups were compared by using Kruskal-Wallis and Mann-Whitney tests. The Bonferroni correction was applied when necessary. Tukey's multiple comparison was used to detect differences significant at the 0.05-level in mean thresholds for both groups.

For analysis of the regression between smell tests and vascularization of the nasal mucosa, the Kendall rank correlation coefficient was applied. Kendall's tau measures the strength of the relationship between the two variables, assessing values between minus one and plus one. A positive correlation signifies that the ranks of both the variables are increasing, whereas a negative correlation signifies that as the rank of one variable is increased, the rank of the other variable is decreased. The null hypothesis was that the two variables examined on each occasion were independent.

The results were analyzed using SPSS software (Version 12 for Windows, SPSS Inc. Chicago, IL, USA).

3 RESULTS

Based on the findings obtained from the control group, contact endoscopy allowed us to identify squamous epithelium, ciliated epithelium, glandular ostia, mucus, and sub-mucosal vascular network. Squamous epithelium was usually present in the anterior tip and inferior border of the lower turbinate, and in the anterior septum and vestibule. Displacing the contact endoscope, ciliated epithelium was visible in the rest of the inferior turbinate and in other areas of the nasal cavity.

By using contact endoscopy, we also managed to observe the condition of the microvascular network correlated to the damage of the nasal mucosa. More specifically, 4 patients (27 %) had a **Type C** microvascular network pattern (elongated blood vessels), 10 patients (67%) had a **Type D** pattern (granular or dotted shape) and only 1 patient a **Type E** pattern (absence of any definitive vascular net). In the control group, 8 healthy (53%) subjects showed a **Type A,** and the rest 7 patients (47%) a **Type B** pattern. No septum perforation was observed in any subject of the study group. **Pictures 1A and 1B** depict the subject's septum's nasal mucosa, in comparison with normal findings of a subject from the control group that are presented in **Picture 2**.

With the use of the Sniffin' sticks we observed normosmia in the majority of the patients (n=10), hyposmia in other 4 patients (3 patients have identified 11 out of 16 odors and 1 patient 12 odours) and only 1 patient was found with anosmia (3 out of 16 odors identified). Interestingly, the 4 patients suffering from hyposmia had a **Type D**

vascular pattern, and the patient with anosmia has a **Type E** pattern. In this latter patient no pathological findings regarding microvascular structures were observed, either disrupted or diminished, apart from extremely edematous and friable mucosa. It should be also stressed, that all these patients suffering from olfaction disorders were not diagnosed with nasal polyps. All subjects of the control group showed a normosmia. Our findings are presented in detail on **Table 2**.

The application of Kendall's - tau criterion between the vascular pattern and the Sniffin' Sticks test results showed only a weak correlation for the patients of Group A (r=0.2). Stronger correlations have been found between vascularization patterns and sACE-levels, as well as sACE levels and the results of the olfactory test (r=0.3 and r=0.4, respectively). Interestingly, in healthy subjects a strong correlation between vascularization of the mucosa and olfactory test results was found (r=0.93).

Another interesting finding is the lack of direct association of ACE levels with olfaction. As depicted in Tables 1 and 2, the levels of sACE in patients suffering from hyposmia range between 34 and 189 nmol/mL By the anosmic patient the level of sACE was estimated at 36 nmol/mL.

4 DISCUSSION

In this study we demonstrated, that patients with sarcoidosis and sinonasal symptoms display significant alterations in the nasal mucosa, both macroscopic (crusts, necrotized tissue) and microscopic (disturbed microvascular patterns). We also showed that the latter finding may also be combined with a reduced olfactory acuity, or, in some patients, with complete absence of it. On the other hand, it has been shown that in healthy subjects a strong correlation exists between vascularization of

the mucosa and olfactory acuity. Till now, there is not enough data correlating the degree of the inflammation or alterations of the mucosa to the presence of microvascularization.

The lack of a special classification of the microvascular patterns of the nasal mucosa has led us to the introduction and use of a modified version of the Negoro's classification [17]. This classification has been previously successfully applied to describe alterations oral mucosa [15-17]. However, we did not use it aiming to explain any morphological variations, such as those of the mucosa's surface, but rather as an adjunctive tool in our attempt to provide a more accurate classification of the vascular network patterns, in both patients and healthy subjects. To our knowledge this is the first time that such an approach has been attempted.

The relative variability in the microvascular pattern observed in the group of healthy subjects deserves attention, as although they were never smokers, receiving intranasal medication and no medical history suggestive of sinonasal disease, they had displayed two different vascular patterns (**Type A** and **B**).

In case of chronic inflammation of the nasal mucosa, the cellular turnover is accelerated allowing the presence of less mature cells at the superficial layers [20,21]. The latter finding could explain the unclear microvascular net or even its lack in some of our patients. To our knowledge, the present study is one of the very few, if not the only one, to provide such information.

Another interesting finding of our study is the fact that 5 of the patients had impaired olfaction (4 with hyposmia, 1 with anosmia). These findings are in accordance with those reported in a previous study, in which the authors suggest that the coexistence

of chronic rhinosinusitis and the presence of at least 2 of the signs of nasal crusting, anosmia, or epistaxis are highly specific for sarcoid rhinosinusitis [5].

We have also investigated the potential correlation of sACE levels to vascularization of the nasal mucosa. Interestingly, a modest correlation between microvascular pattern on CE and sACE levels (r=0,3) was found. Although 30–80% of sarcoidosis patients have increased sACE levels with sensitivity ranging between 22% and 86% and specificity between 54% and 95% [22-23], we found no other study correlating sACE-levels to the severity of otolaryngological findings. ACE is an acid glycoprotein that converts angiotensin I into angiotensin II, produced by lung endothelial cells, mainly activated alveolar macrophages [19,20]. However, its utility as a diagnostic and prognostic tool is restricted by its rather low sensitivity and specificity [21-24], as it may be elevated in other granulomatous disorders (such as tuberculosis, borelliosis, histoplasmosis, Gaucher's disease, silicosis), hyperthyroidism, diabetes, and other diseases [24,25].

Our study has a few limitations. Patients were already under at the time of evaluation, therefore, our findings may have been affected by treatment with corticosteroid therapy, both systematically and locally. Furhermore, the retrospective analysis of their data did not allow the evaluation of microvascular patterns of the mucosa before and after treatment. Nonetheless, the evaluation (CE, Sniffin Sticks) was rather done prospectively. Another limitation of our study is its small sample size, however the relative rarity of the sinonasal involvement in pulmonary sarcoidosis should be considered. To ensure more valid comparisons between changes in the nasal mucosal microvascular network and olfaction, particularly in the different stages of the disease, a larger longitudinal prospective study should follow, that may allow the

establishment of vascular robust patterns, as well as the evaluation of treatment effectiveness on the associated sino-nasal symptoms in pulmonary sarcoidosis. Finally, we have not investigated nasal vascular morphology in sarcoidosis patients without nasal symptoms, because this was not possible at the time of drafting the protocol This could be a matter of further investigation.

5 CONCLUSIONS

In conclusion, our findings suggest that changes in the microvascular network of the nasal mucosa may be used as a surrogate marker of pulmonary sarcoidosis. These changes may also be accompanied by changes in olfaction. This is further supported by the very strong correlation between vascular pattern of the mucosa and olfactory test results in healthy individuals.

A major future research challenge is the validation of the use of the vascular patterns of nasal mucosa on CE as a tool to predict nasal (and possibly, also pulmonary) disease activity and treatment response in patients with pulmonary sarcoidosis and symptoms of sino-nasal disease.

LEGENDS

TABLE 1. Clinical characteristics of the 15 patients with biopsy proven sinonasal sarcoidosis, participating in the study. Abbreviations: CT, computed tomography, MSB: maxillary sinus bilateral; ES, ethmoidal sinus; ESB: ethmoidal sinus bilateral;

FS: frontal sinus, F: Female, M: Male, OCS: Oral steroids, INS: Intranasal steroids. The normal range for ACE is less than 40 nmol/mL/min.

TABLE 2. Findings on conventional fiber optic endonasal endoscopy, contact endoscopy and Sniffin' Sticks test of the study participants. **N=normosmia, H=hyposmia, A=anosmia.**

PICTURE1A. The picture depicts the microvascular network of a female patient (age 148), suffering from nasal sarcoidosis during the last 5 years. The vesicles seem to have an unclear form, they are rather thin and disrupted.

PICTURE 1B. The mucosa of the same patients seen macroscopically. The mucosa is obviously necrotized in multiple regions (the septum and under the turbinate).

PICTURE 2. The vascularization of nasal mucosa (septum) in a healthy subject of the control group.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Kunitake R , Kuwano K, Miyazaki H, Hagimoto N, Nomoto Y, Hara N. Apoptosis in the course of granulomatous inflammation in pulmonary sarcoidosis. Eur Respir J. 1999;13(6):1329-1337.

2. Coup AJ, Hopper IP. Granulomatous lesions in nasal biopsies. Histopathology 1980;4(3):293-308.

3. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. Lancet. 2003; 361(9363):1111–1118.

4. Baughman RP, Lower EE, Tami T. Upper airway. 4: Sarcoidosis of the upper respiratory tract (SURT). Thorax. 2010;65(2):181-186.

5. Fergie N, Jones NS, Havlat MF. The nasal manifestations of sarcoidosis: a review and report of eight cases. The Journal of Laryngology and Otology. 1999; 113:893-898. J Laryngol Otol. 1999;113(10):893-898.

6. Dessouky O. Isolated sinonasal sarcoidosis with intracranial extension: case report. Acta OtorhinolaryngolItal. 2008;28(6):306.

7. Ozdoğan A, Acioğlu E, Karaman E, Oz B, Müsellim B. A difficult case: sarcoidosis of the middle ear. Am J Otolaryngol. 2009;30(4):281-284.

8. Frederiksen LG, Jørgensen K. Sarcoidosis of the nose treated with laser surgery. Rhinology. 1996; 34(4):245-246.

9. Damuth TE, Bower JS, Cho K, Dantzker DR. Major pulmonary artery stenosis causing pulmonary hypertension in sarcoidosis. Chest. 1980; 78(6):888-891.

10. Whittemore KR, Jr., Dargie JM, Lally T. Sarcoidosis of the pinnas in a 17-year-old. Am J Otolaryngol. 2012; 33(5):631-633.

11. Mortaz E, Adcock IM, Barnes PJ. Sarcoidosis: Role of non-tuberculosis mycobacteria and Mycobacterium tuberculosis. International Journal of Mycobacteriology. 2014; 3(4):225-229.

12. Cardona PJ. Reactivation or reinfection in adult tuberculosis: Is that the question? International Journal of Mycobacteriology. 2016;5(4):400-407.

13. Krespi YP, Kuriloff DB, Aner M. Sarcoidosis of the sinonasal tract: a new staging system. Otolaryngol-Head and Neck Surg. 1995;112(2):221-227.

14. Kirsten AM, Watz H, and Kirsten D. Sarcoidosis with involvement of the paranasal sinuses - a retrospective analysis of 12 biopsy-proven cases. BMC Pulm Med. 2013; 13: 59. Pavlidis P, Gouveris H, Gorgulla H, Hast HJ, Maurer J. Electrogustometry and contact endoscopic findings in patients with head and neck malignancies treated with chemotherapy, radiotherapy or radiochemotherapy. Chem Senses. 2015; 40(3):165-1671.

15. Pavlidis P, Cámara RJA, Kekes G, Gouveris H. Bilateral taste disorders in patients with Ramsay Hunt syndrome and Bell palsy. Ann Neurol. 2018; 83(4):807-815.

16. Pavlidis P, Gouveris H, Kekes G. Electrogustometry Thresholds, Tongue Tip Vascularization, Density, and Form of the Fungiform Papillae Following Smoking Cessation. Chem Senses. 2017; 42(5):419-23.

17. Negoro A, Umemoto M, Fukazawa K, et al. Observation of tongue papillae by video microscopy and contact endoscopy to investigate their correlation with taste function. Auris Nasus Larynx. 2004; 31(3):255-259.

18. Valeyre D, Prasse A, Nunes H, et al. Sarcoidosis. Lancet. 2014; 383(9923):1155–1167.

19. Schriever V, Croy I, Haehner, A, Hummel T. Updated Sniffin' Sticks normative data based on an extended sample of 9139 subjects. Archives of Oto-Rhino-Laryngology. 2019;276:719-728.

20. Duchemann B, Lavolé A, Naccache J-M, Nunes, et al. Laryngeal sarcoidosis: a case-control study. Sarcoidosis Vasc Diffuse Lung Dis. 2014;31(3):227-34.

21. Aubart FC, Ouayoun M, Brauner M, et al. Sinonasal involvement in sarcoidosis: a case-control study of 20 patients. Medicine. 2006; 85(6):365-371.

22. Braun JJ, Gentine A, Pauli G. Sinonasal sarcoidosis: review and report of fifteen cases. Laryngoscope. 2004; 114(11):1960-1963.

23. Nakamura Y, Takeda T, Ishii M, et al. Elevation of serum angiotensin-converting enzyme activity in patients with hyperthyroidism. J Clin Endocrinol Metab. 1982; 55(5): 931–934.

24. Zorn SK, C. Stevens A, Schachter EN, Gee JB. The angiotensin converting enzyme in pulmonary sarcoidosis and the relative diagnostic value of serum lysozyme. Lung. 1980; 157(2): 87–94.

25. Gulati S, Krossnes B, Olofsson J, Danielsen A. Sinonasal involvement in sarcoidosis: a report of seven cases and review of literature. Eur Arch Otorhinolaryngol 2012; 269(3):891-896.