ABSTRACT • Introduction: The classification of chronic rhinosinusitis with nasal polyposis (CRSwNP) into eosinophilic and non-eosinophilic has prognostic and therapeutic implications. It was never reported in a Middle Eastern population. Methods: Retrospective study in a tertiary care center located in Lebanon. Records of 76 patients with CRSwNP treated by endoscopic sinus surgery were reviewed. Two pathologists independently counted the number of eosinophils and neutrophils in the three fields containing the greatest degree of cellular infiltration. Eosinophilic CRSwNP was defined by five or more eosinophils per high-power field. Results: The mean number of eosinophils and neutrophils in the population were respectively 106 and 10 per high-power field. Sixty-eight patients (89.5%) had eosinophilic CRSwNP while eight (10.5%) had non-eosinophilic CRSwNP. In the asthmatic subgroup (14.5%), all patients had an eosinophilic profile. The proportion of eosinophilic CRSwNP in the non-asthmatic subgroup was 88%. The interobserver agreement between the two pathologists was very good. Conclusion: The majority of the studied population had an eosinophilic CRSwNP. The histopathological profile of CRSwNP in a Middle Eastern population seems to be similar to the reported profile of Western populations.

Keywords: chronic rhinosinusitis; polyposis; eosinophils; neutrophils

INTRODUCTION

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nasal and paranasal mucosa that persists for more than 12 weeks. It represents the second most common chronic disease in the USA afflicting 16% of the population [1]. CRS is classified into two main groups based on the presence or absence of polyps: CRSsNP (without nasal polyposis) and CRSwNP (with nasal polyposis). CRSwNP represents 40% of CRS [2]. Nasal polyps are defined as an inflammatory outgrowth of sinonasal epithelium that manifests as an edematous semitranslucent lobular mass in the nasal and paranasal cavities. A number of conditions may usually be associated with the development of CRSwNP like asthma, aspirin exacerbated respiratory disease (AERD), cystic fibrosis, Kartagener syndrome, Young syndrome and Churg-Strauss syndrome [3]. It seems that the inflammation in CRSwNP results from dysfunctional host-environment interaction involving various exogenous agents. Several pathophysiological hypotheses have been proposed: biofilm, microorganisms, staphylococcus superantigens, fungi, immune barrier, and excessive T helper 2 cell response [4]. Respiratory epithelium dysfunction is a major component of polyp formation [4]. Moreover, polyps are classified according to the preponderance of submucosal eosinophils or neutrophils. This histopathological classification of CRS into eosinophilic and non-eosinophilic reflects a different underlying inflammatory process associated with variable disease severity [5]. Eosinophilic CRSwNP is the predominant group in the
Western population (USA and Europe) representing 65 to 90% of all CRSwNP [6-8]. However, in the Asian population the eosinophilic group is less common with higher proportion of the neutrophilic group [9]. A therapeutic implication for this classification has been reported with the efficacy of macrolides in the Asian neutrophilic group [10].

There is no current data in the literature reporting the histological profile of CRSwNP in a Middle Eastern population. We propose to conduct the first study that evaluates this profile with possible prognostic and therapeutic conclusions.

MATERIAL AND METHODS

Patients’ selection
This is a retrospective study of patients with CRSwNP treated by functional endoscopic sinus surgery (FESS) between 1999 and 2014 at Hôtel-Dieu de France University Hospital located in Beirut, Lebanon. Patients’ charts were reviewed for date of admission, age, sex, prior surgery, asthma, aspirin sensitivity, related pathologies and pathology report.

Exclusion criteria were: age < 18 years, unilateral polyposis, CRSsNP, aspirin sensitivity in Samter’s triad, Kartagener syndrome, Churg-Straus disease and cystic fibrosis.

Histologic analysis
To evaluate the degree of cell infiltration, two pathologists counted independently the number of eosinophils and neutrophils determined by H&E stains in the three fields containing the greatest degree of cellular infiltration using light microscopy (x 400 magnification). The total number of eosinophils and neutrophils present with a 10 x 10-mm reticulate present in the eyepiece was determined as the count per high-power field (HPF).

For each patient the number of eosinophils and neutrophils was defined as the mean of the three fields for each pathologist [11]. Eosinophilic CRSwNP was defined by five or more eosinophils per HPF. The proportion of patients with eosinophilic and non-eosinophilic CRSwNP was then calculated [5].

Statistical analysis
Agreement between the two observers beyond that expected by chance was calculated for each clinical variable, using the kappa (κ) statistic for weighted kappa for ordinal variables. Variables with a lower 95% confidence limit (LCL) of κ > 0.4 were considered to have acceptable agreement. A subgroup analysis included patients with or without asthma.

RESULTS

Patients’ characteristics
Between 1999 and 2014, 76 patients underwent FESS for bilateral CRSwNP after applying the exclusion criteria. The age of the selected population ranged from 18 to 86 years with a mean of 41.9 years. The sex ratio was 1:1 (38 males and 38 females). Asthma was found in 11 patients (14.5%) while 65 patients (85.5%) were not asthmatic. Fifty-four patients (71%) underwent a primary FESS while 22 (29%) had a history of previous sinus surgery (Table I).

Histopathological profile
The number of eosinophils in the population ranged from 0 to 522 with a mean of 106 (SD = 117.3) while the number of neutrophils ranged from 0 to 132 with a mean of 10 (SD = 8.8). Sixty-eight patients (89.5%) had 5 or more eosinophils per HPF defining the eosinophilic group. Eight patients (11.5%) had less than 5 eosinophils per HPF defining the non-eosinophilic group. In the non-eosinophilic group, the mean neutrophils count was 9.5: 4 patients (50%) had less than 5 neutrophils, 2 (25%) had 5 to 10 neutrophils and 2 (25%) had more than 10 neutrophils. The histologic profile was concordant between the two pathologists in 74 cases (97.3%) and discordant in 2 cases (2.7%). The interobserver agreement was very good with a variable kappa of 0.86 (95% confidence interval: 0.67-1).

Subgroup analysis
In the asthmatic subgroup (14.5%), all the patients had an eosinophilic profile. In the non-asthmatic subgroup (85.5%), 57 patients (88%) had an eosinophilic profile while 8 (12%) had a non-eosinophilic profile (Figure 1).

DISCUSSION

CRS is not considered as a single pathology but rather as a syndrome with the necessity to define subgroups of disease [12].

Phenotypes are defined by an observable characteristic or trait [1]. The most widely phenotype classification of CRS is based on the presence or absence of polyposis. Other factors include disease severity, recurrence and response to conventional therapy. However, they do not reveal the diversity of the underlying molecular process of CRS. These different molecular or biological subtypes of CRS are defined as endotypes [13]. By identifying these endotypes, we can define subgroups of CRS in relation to response to different treatment regimens [2].
for CRS found no clinically significant improvement in patient-oriented quality of life measures with long-term macrolide therapy for CRS. However, there may be an effect among the subgroup of patients with low serum IgE [22]. This meta-analysis included three randomized controlled trials in three different populations: Iran [23], Australia [24] and Netherland [25]. The efficacy of macrolide in the treatment of CRS seems to be due to its anti-inflammatory effect. Macrolide inhibits neutrophil chemotaxis and adherence by inhibiting the release of IL-8 [26]. Thus its efficacy seems to concern the Asian population with a characteristic neutrophilic profile. This may explain the divergence of results marked by geographical variation. We report the first study in a Middle Eastern population in order to define its histopathological profile.

Defining the histopathological profile of CRS is crucial. However the definition of mucosal eosinophilia is still controversial. The most commonly used cut-off is 5 eosinophils per HPF as defined by Kountakis et al. in 2004 [5]. They found that the patients in the eosinophilic group had more severe disease, as noted by a higher incidence of asthma, higher CT scores, and higher preoperative and postoperative endoscopy scores compared with those in the non-eosinophilic group. Moreover, when the number of mucosal eosinophils was fewer than or equal to five cells/HPF, none of these cells stained for EG2, which is a marker for activated eosinophils. Conversely, when tissue eosinophilia was present (≥ 5 cells/HPF), the majority of eosinophils (78%) were activated.

The same cut-off was used by Soler et al. based on in vivo evidence of eosinophil activation [27]. Moreover they observed the impact of eosinophilia on quality-of-life outcome above 10 eosinophils/HPF [28]. On the other hand, the correlation with the disease severity defines cut-offs as high as 70 [19] and 100 [11] eosinophils per HPF in the Asian population. Ikeda et al. defined a cut-off for neutrophilic CRCwNP of 20 neutrophils per HPF [11].

In our study, we found a predominant eosinophilic profile of CRSwNP in 89.5% of the studied population. This proportion was still high – 87% – in the non-asthmatic subgroup. These values are consistent with those reported in North America and in Europe [6-8]. A recent survey by the Global Allergy and Asthma European Network (GA2LEN) found a strong association between asthma and CRS [29]. In general, asthma occurs in 15 to 50% of patients with CRS that is characterized by a polyposis phenotype and an eosinophilic endotype [30]. We found this association in our series with a rate of 14% and an eosinophilic profile in all the patients.

The non-eosinophilic subgroup represents 10.5% of the studied population. We did not find in this subgroup a predominant neutrophilic profile. The mean of neutrophils was 9.5 per HPF and only 50% had more than 5 neutrophils per HPF. There may be a subgroup that is non-eosinophilic non-neutrophilic with a different major inflammatory cell that was not reported in our study.

The main endotyping of CRSwNP and CRSsNP is the histopathological classification into eosinophilic and neutrophilic subtypes [5]. Eosinophilic CRSwNP mainly found in Western countries is characterized by a T helper 2 cell response, a decrease in regulatory T-cell function, an increase in interleukin-5 (IL-5) and a ECP/MPO* ratio superior to 1 [14-16]. On the other hand, neutrophilic CRSwNP found mainly in Asian countries is characterized by a T helper 1 cell response shift with high expression of IL-8 and IL-17 receptor D [17]. Zhang et al. compared 26 Belgian patients having CRSwNP to 29 south Chinese with the same condition [18]. The ECP/MPO ratio was 2.08 in the Belgian group compared to 0.25 in the south Chinese group showing an eosinophilic predominance in the former and a neutrophilic predominance in the latter. Moreover, the Belgian group had higher expression of T helper 2 cell response markers like eotaxin, IL-5 and total serum immunoglobulin E (IgE) whereas the south Chinese group had higher expression of T helper 1 cell response markers like interferon gamma (IFNg), IL-1β, IL-6 and IL-17.

Eosinophilic CRSwNP is considered as a poor prognostic factor associated with more severe disease and a higher incidence of asthma, higher CT scores, a higher recurrence rate and higher preoperative and postoperative endoscopy scores [11,19,20]. Beside the prognosis value of the current classification of CRSwNP into eosinophilic and neutrophilic, a therapeutic implication has been established by many studies. In 1984, Kudoh et al. reported the remarkable improvement of symptoms in erythromycin treated Japanese patients suffering from diffuse panbronchiolitis [20,21]. These patients had concomitant CRS. An improvement of symptoms was also observed for this patients’ group. Long-term low-dose erythromycin therapy was used primarily in Japan and the first report with an English abstract was published as late as 1991 [10]. A meta-analysis on macrolide therapy

*ECP/MPO: eosinophil cationic protein/myeloperoxidase

Figure 1. Histopathological profile in asthmatic and non-asthmatic subgroups in percent

Asthma 0 Non-asthma 12 Eosinophil 100 Non-eosinophil 88
This may be one of our study limitations that also include its retrospective type, the medium size sample and the lack of correlation to disease severity.

In conclusion, our study is the first to report the histopathological profile of CRSwNP in a Middle Eastern population that seems to be consistent with the Western population. Middle Eastern patients may therefore follow the same therapeutic guidelines. Future studies in the same population could analyze other molecular features with a correlation to disease severity. Also, considering the medium sized sample, further multicentric national and even regional studies are necessary to confirm our findings.

REFERENCES