特集　味覚・嗅覚診療の最前線

嗅覚障害の疫学と臨床像

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キーワード●嗅覚障害，異嗅症，疫学，加齢変化

はじめに

嗅覚障害は視覚障害，聴覚障害などと共に感覚器障害の１つである．しかし，視覚，聴覚と比較すると，感覚低下者数は引けを取らないが，嗅覚障害を訴えて医療機関を受診する患者は少ない．そのため医療サイドからの関心が低く，対応も遅れているのが現状である．本稿では，嗅覚障害の臨床像を述べるとともに，嗅覚障害に関する疫学的な調査結果について報告する．

Ⅰ 嗅覚障害の分類

嗅覚障害は量的障害と質的障害に分けられる（**表１**）．量的障害には嗅覚低下と嗅覚脱失が含まれており，前者はにおいの感じ方が弱くなった状態であり，後者は全くにおいがしなくなった状態である．病院や医院を受診する患者の大部分は量的障害に含まれる．

一方，質的障害とは，においの感じ方に変化が生じる状態で，代表的なものは異嗅症である．異嗅症は，刺激性異嗅症と自発性異嗅症に分けられる．刺激性異嗅症とは，もののにおいを嗅いだときに，「従来のにおいと異なって感じる」，「何のにおいを嗅いでも同じにおいに感じる」などの，においを嗅いだときに感じる異常である．一方，自発性異嗅症とは，「常に鼻や頭の中ににおいを感じる」，「何もにおいがないはずなのに，突然においを感じる」など，実際ににおい刺激が与えられていない状況でにおいを感じる状態を指す．これらの異嗅症は，その症状単独で生ずることは少なく，ほとんどは量的障害に合併して起こることが多い．

表１　嗅覚障害の分類

- 量的嗅覚障害（quantitative olfactory disorder）
  - 嗅覚低下（hyposmia）
  - 嗅覚脱失（anosmia）
- 質的嗅覚障害（qualitative olfactory disorder）
  - 異嗅症（dysosmia）
    - 刺激性異嗅症（parosmia）
    - 自発性異嗅症（phantosmia）
  - 嗅盲（olfactory blindness）
  - その他
    - 嗅覚過敏（hyperosmia）
    - 悪臭症（cacosmia）
    - 自己臭症（egorrhea symptom）
    - 幻臭（phantosmia）
    - 鉤発作（uncinate epilepsy）

嗅盲とは，ある特定のにおいのみが分からない状態を指す．いくつかのにおい物質が指摘されているが，有名なのは青酸（シアン化水素）で，この甘酸っぱいにおいを感じない人が20％ほどいるといわれている．人間に存在する約400種類のにおい分子受容体のうち，一部の受容体が遺伝子変異により発現しないために生じるものと推測されている．

その他の質的障害には，実際の嗅覚の異常で

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はないが，患者により嗅覚の異常と捉えられて受診する疾患が含まれている．嗅覚過敏は，語句からは一見，量的障害に思われるが，患者の訴えを聞くと嗅覚閾値の低下ではなく，その感じ方による不快な症状が前面に出ているため，質的障害に含めるのが妥当である．悪臭症とは，副鼻腔炎や扁桃炎など，主に上気道の炎症性疾患あるいは腫瘍性疾患により，病巣が悪臭を放つもので，患者は常に悪臭を感じると訴える．

一方，自己臭症とは，実際にはないにもかかわらず，自分が口臭，鼻臭あるいは体臭を放っていると思い込んでいる状態であり，心因性あるいは精神疾患がその背景に存在することが多く，対応に苦慮する疾患である．幻臭とは統合失調症の一症状であり，異嗅症との区別は難しいが，基礎に統合失調症が存在すれば，この病態と判断できる．鉤発作は嗅覚中枢における自発的発火による症状で，側頭葉てんかん発作の前兆（aura）として現れることもあるが，原因が分からないことも多く，明確な分類は困難であるため，その他の質的障害に含めた．

II 嗅覚障害の病態別および原因別分類

嗅覚障害のうち，量的障害すなわち嗅覚低下と嗅覚脱失は，鼻腔から嗅覚野である眼窩前頭皮質までのあらゆる嗅覚路の異常によって生じうる．しかし，障害を受ける部位により病態ならびに原因が異なるため，**表2**に示される4つの病態に分類される．また，呼吸性嗅覚障害は伝導性嗅覚障害と呼ばれ，呼吸性以外の3つの嗅覚障害はまとめて神経性嗅覚障害とも呼ばれている．

1. 呼吸性嗅覚障害

鼻腔の病変により，におい分子が嗅粘膜まで到達しないために生ずる嗅覚障害である．鼻をつまんで塞ぐことにより，容易にこの状態が作り出せる．主な原因としては，慢性副鼻腔炎，アレルギー性鼻炎，鼻中隔弯曲症など，鼻副鼻腔の疾患が挙げられる．適切な治療によってほとんどは改善が可能である．

表2　量的嗅覚障害の部位別分類と原因疾患

分類	障害部位	原因疾患
呼吸性嗅覚障害	鼻副鼻腔	慢性副鼻腔炎 アレルギー性鼻炎 鼻中隔弯曲症
嗅粘膜性嗅覚障害	嗅粘膜 （嗅神経細胞）	感冒罹患後嗅覚障害 薬物性嗅覚障害
末梢神経性嗅覚障害	嗅神経軸索	頭部外傷
中枢性嗅覚障害	嗅球～ 嗅覚中枢	頭部外傷 脳腫瘍，頭蓋内手術 神経変性疾患 パーキンソン病 アルツハイマー病 脳血管性認知症 Kallmann 症候群

2. 嗅粘膜性嗅覚障害

鼻腔深部に存在する嗅粘膜における嗅細胞が傷害を受けて嗅覚の低下を来す状態である．原因として最も多いのは感冒であり，感冒罹患後嗅覚障害と呼ばれる．原因ウイルスは特定されていないが，嗅細胞が傷害されるため，感冒治癒後も嗅覚障害が持続する．嗅細胞は新生能力を有しているため，改善例も少なくないが，治癒までは長期間を要する．薬物性嗅覚障害の一部も嗅粘膜性嗅覚障害に含まれる．また，前記の慢性副鼻腔炎による嗅覚障害でも，罹病期間が長期に及ぶ場合は，炎症による嗅細胞の変性が起こるため，嗅粘膜性嗅覚障害を合併することがあり，混合性嗅覚障害と呼ばれている．慢性副鼻腔炎で適切な治療を行い，嗅粘膜への気流を改善しても嗅覚が回復しない症例では，混合性嗅覚障害が疑われる．

3. 末梢神経性嗅覚障害

第1脳神経である嗅神経は双極細胞であり，細胞体が嗅粘膜に存在し，その軸索は頭蓋底篩板の小孔を貫いて頭蓋内に入り，嗅球で上位ニューロンとシナプスを形成する．その軸索が頭蓋内に入った部分で断裂して起こるのが，末梢神経性嗅覚障害である．大部分が頭部打撲，

特に前頭部あるいは後頭部の打撲により発生するが，開頭手術によっても起こりうる．頭部外傷により前頭葉あるいは嗅球が傷害を受けている場合は中枢性嗅覚障害とされ，MRIなど画像検査で診断は可能であるが，純粋な末梢神経性嗅覚障害では画像検査でも異常が現れないことが多い．そのため，嗅覚障害の診断とその証明に苦慮するところであり，なおかつ病変範囲が小さいにもかかわらず，治療による改善度が良くないのが特徴である．

4. 中枢性嗅覚障害

嗅球を含めた頭蓋内の嗅覚路の異常による嗅覚障害である．発生頻度としては頭部外傷による外傷性嗅覚障害が最も多い．近年，パーキンソン病やアルツハイマー病などの神経変性疾患の発症前に嗅覚障害が出現することが判明し[1,2]，嗅覚検査がこれらの疾患のバイオマーカーとして用いられるようになっている．中枢性嗅覚障害では，嗅覚自体の低下と共に，その認知能力および識別能力の低下が特徴とされている．

5. 嗅覚障害の原因別頻度と特徴

表3に金沢医科大学耳鼻咽喉科嗅覚外来における原因別頻度を示す．最も多いのは慢性副鼻腔炎によるものであり，アレルギー性鼻炎も含めると嗅覚障害患者の半数以上を占めている．次いで感冒罹患後，頭部顔面外傷と続くが，原因不明の嗅覚障害も少なからず存在する．それ以外の少数の原因として，先天性，薬物性，中枢性がある．

各原因の割合は施設により異なり，問診の時点で厳格に嗅覚障害の有無を問えば，診療所においては慢性副鼻腔炎，アレルギー性鼻炎の頻度がきわめて高くなることが想像されるが，頻度は変わっても原因別の順位は変わらないものと思われる．したがって，嗅覚障害を訴えて患者が受診した場合には，これらの原因疾患を念頭に置く必要がある．また，異嗅症を単独の症状として受診する患者は少ないが，このような場合は心因性を疑う必要がある．味覚障害では心因性の障害が少なからず存在するが，嗅覚低下および嗅覚脱失では心因性はきわめて少ないのが特徴である．

表3　嗅覚障害の原因別頻度と性差，年齢

原因	総数	女性	男性	平均年齢
慢性副鼻腔炎	195（48%）	75	120	55.2
アレルギー性鼻炎	23（ 6%）	11	12	44.6
その他の鼻疾患	8（ 2%）	2	6	50.1
感冒罹患後	71（18%）	59	12	54.2
頭部顔面外傷	25（ 6%）	15	10	49.2
中枢性	11（ 3%）	5	6	69.5
先天性	5（ 1%）	3	2	26.0
薬剤/化学物質	4（ 1%）	1	3	56.7
不明	60（15%）	33	27	61.6
その他	2（ 0%）	2	0	34.5
総計	404	206	198	54.0

（金沢医科大学耳鼻咽喉科嗅覚外来，2009～2012年）

これらの原因のなかで，顕著な特徴を示すのが感冒罹患後嗅覚障害である．発生頻度は女性に多く男性の約5倍であり，40～50歳代の発症がほとんどである．これは嗅覚外来をもつ多くの施設からの報告で共通しているが，その理由はいまだ分かっていない．

外傷性嗅覚障害は，1980年代までは男性に多いとされていたが，現在はその傾向はなくなっている．これは女性の社会進出ならびに車を運転する女性人口の増加によるものであり，実際に，外傷の内訳として転倒，転落によるものと交通外傷が多い．

先天性嗅覚障害は生まれつきの嗅覚障害であるが，患者の平均年齢をみると，無嗅覚を自覚するのが必ずしも幼少期でないことが分かる．

原因不明の嗅覚障害患者の平均年齢は，他の原因と比べると高くなっているが，これは加齢による低下が相当数含まれているためと思われる．しかし，このようななかにはパーキンソン病やアルツハイマー病の患者が含まれている可能性があり，注意が必要である．

III 嗅覚障害の疫学

他の感覚と同様，嗅覚も加齢と共に低下する．

視覚が低下すると新聞や本を読むのに難渋するし，聴覚が低下すると他人との会話，テレビ，ラジオの視聴などに支障を来すため，比較的多くの患者が受診するが，嗅覚の場合，他人に気付かれることはなく，徐々に低下が進む場合には本人も気付いていないことが多い．また，多少の嗅覚低下では，視覚や聴覚と比較して，生活に支障を来すことが少ない．したがって，嗅覚の場合，嗅覚低下＝嗅覚障害とはいえないことが，視覚や聴覚との大きな違いといえる．そのため，嗅覚障害患者に関する統計学的報告は多くなされているが，嗅覚低下に関する疫学調査は，わが国ではいまだなされていない．近年，欧米でいくつかの疫学調査が報告されているので，本稿では欧州および米国での研究結果について述べる．

スペインのカタロニア地方において2003年に行われたOLFACAT studyでは，5～91歳までの10,783名に簡易な嗅覚検査とアンケート調査を行い9,348名から有効回答を得ている．その結果，全体の19.4%に検知の低下を認め，48.8%に同定能の低下を認めた．また，検知，同定の脱失を認めたのはそれぞれ0.3%，0.8%であった．嗅覚低下はすべての年代において女性よりも男性に多く，検知脱失のリスクファクターとして性別（男性）が，同定能消失のリスクファクターとして高齢と頭部外傷の既往が指摘された．興味深いことに，検知能力がすべての年代において加齢と共に低下するのに対して，同定能力は加齢と共に一旦上昇し，40～60歳代でプラトーとなり，それ以後，低下すると報告されている[3]．

米国において1993年から現在まで継続的に行われているBeaver Dam Offspring Studyでは，2,838名の成人に対する調査が行われ，そのうち3.8%に嗅覚低下を認め，65歳以上に限れば13.9%に嗅覚低下を認めたと報告されている[4]．また，リスクファクターとしては，高齢，鼻茸，鼻中隔弯曲症など鼻疾患の存在，血管病変，女性では喫煙が挙げられている．嗅覚検査開発のために行われた過去の研究でも，65歳以上で嗅覚が有意に低下することが報告されている[5,6]．したがって，嗅覚低下で問題になるのは，特定される疾患を除けば65歳以上の高齢者であるといえる．

わが国は2007年に65歳以上の高齢者人口が21%を超え，超高齢社会に突入している．今後も高齢者人口は増加するため，嗅覚低下者は年々増加が見込まれる．嗅覚障害患者が日常生活で最も困っていることは，食品の腐敗に気付かないことであり，それ以外にも，ガス漏れ，煙に気付かないなどの生活面での安全，味覚の変化による食欲の低下，食への関心の低下，調理の不具合も，半数以上の患者が日常の支障と感じている[7,8]．

おわりに

嗅覚障害の分類も交えて，その臨床像について概説した．疫学調査では加齢に伴う嗅覚低下が問題視され，さらに増加が見込まれる高齢者への対応の重要性を指摘した．嗅覚障害は他人も自身も気付きにくい疾患ではあるが，疾患による嗅覚障害患者のみならず，高齢者も含め，国民が安全かつ質の高い生活を送るために嗅覚は欠かせない感覚であり，医学，医療，福祉などさまざまな方面からの介入が今まで以上に求められるようになるものと思われる．

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Ann Allergy Asthma Immunol 114 (2015) 30–35

Contents lists available at ScienceDirect

Correlation of basophil infiltration in nasal polyps with the severity of chronic rhinosinusitis

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ARTICLE INFO

Article history:
Received for publication July 1, 2014.
Received in revised form August 19, 2014.
Accepted for publication September 22, 2014.

Introduction

Chronic rhinosinusitis (CRS) represents a heterogeneous disease group characterized by local inflammation of the sinonasal tissues.[1] It has been defined as symptomatic inflammation of the sinonasal mucosa that lasts more than 12 weeks as confirmed by computed tomography (CT) and nasal endoscopy.[2] Generally, CRS is divided into 2 subsets based on endoscopic findings: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP).[3]

In Europe and the United States, eosinophilia is evident in nasal polyps (NPs) from patients with CRSwNP.[4] In contrast, heterogeneity of CRSwNP has been reported in East Asian countries, such as Japan, Korea, and China, where the presence and extent of eosinophilia are variable and a significant proportion of NPs do not manifest local eosinophilia.[5] Although the pathogenesis of CRS remains controversial, eosinophilic inflammation is considered at least partly responsible.

Eosinophilic NPs contain an environment enriched for T_H2 cytokines, including interleukin (IL) 4, IL-5, and IL-13.[6] T_H2 cytokines are believed to contribute to the pathogenesis of eosinophilic CRSwNP, with IL-5 and IL-13 inducing eosinophil recruitment and promoting their activation and IL-4 promoting the switching of the immunoglobulin to the IgE isotype.[7]

Basophils are reportedly increased in the bronchial submucosa of asthmatic patients and the nasal submucosa of patients with allergic rhinitis.[8,9] Research is increasingly addressing the role of basophils in inducing T_H2-type responses.[10] Although CRSwNP is a highly T_H2-biased disease, making the involvement of basophils likely, there are few reports of the involvement of basophils in CRS.[11]

We performed a histologic analysis of eosinophils, basophils, and T cells in NPs. We also studied the association among the basophil count, clinical features, and severity on sinus CT.

Methods

Patients

We included 33 patients (26 men and 7 women) with CRSwNP who were surgically treated in the Department of Otolaryngology, The University of Tokyo Hospital, Tokyo, Japan, from September 1, 2009, through December 31, 2012. Written informed consent was obtained from each patient. The mean age was 58.1 years (range, 40–76 years) at the time of surgery. CRSwNP was diagnosed based on the criteria of the European Academy of Allergology and Clinical Immunology position article. It required endoscopic evidences of NPs and 2 or more of the following symptoms for at least 3 months: blockage or congestion, discharge (anterior or posterior drip), facial pain or pressure, and reduction or loss of smell.[12]

We excluded patients with CRSwNP associated with chronic obstructive pulmonary disease, diffuse panbronchiolitis, Churg-Strauss syndrome, cystic fibrosis, and any congenital mucociliary diseases. In addition, patients who had received systemic corticosteroids or other immunomodulating drugs within 1 month before surgery were also excluded.

The patients were divided into an eosinophilic CRS (ECRS) group and a noneosinophilic CRS (non-ECRS) group. Categorization was based on the eosinophil count in a microscopic field (original magnification ×400) of the subepithelial area of a hematoxylin-eosin (H&E)–stained NP section. The cutoff value for the ECRS group was an eosinophil count greater than 50 cells per field. The non-ECRS group did not fulfill this criterion.

Before surgery, each patient underwent CT of the sinuses, and the Lund-Mackay CT score was determined.[13] The score was

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Disclosures: Authors have nothing to disclose.

http://dx.doi.org/10.1016/j.anai.2014.09.017

calculated by assigning scores of 0, 1, or 2 for maxillary, anterior ethmoid, posterior ethmoid, frontal, and sphenoid sinuses and 0 or 2 for the ostiomeatal complex for right and left sides. The total score ranged from 0 to 24.

The local ethics committee of The University of Tokyo Hospital approved the study. Informed consent was obtained from each patient before collecting the samples.

Sampling and Immunohistochemical Analysis

The NP specimens were harvested during endoscopic sinus surgery. Each sample was fixed in 10% formalin, embedded in paraffin, sectioned at 4-μm thicknesses, mounted on Matsunami adhesive silane–coated slides (Matsunami Glass, Osaka, Japan), and used for H&E staining and immunohistochemical analysis.

After deparaffinization and rehydration, sections were subjected to heat-induced antigen retrieval using a citrate buffer solution (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). For endogenous peroxidase inhibition, 3% hydrogen peroxidase was used. Fetal bovine serum was used to block nonspecific binding. The following primary antibodies were used for identifying inflammatory cells in the specimens: antihuman proform of eosinophil major basic protein (proMBP-1; mouse monoclonal, clone J175-7D4; BioLegend, San Diego, California), anti-CD3 (rabbit monoclonal, clone SP7; Nichirei, Tokyo, Japan), anti-CD4 (rabbit monoclonal, clone SP35; Acris Antibodies Inc, San Diego, California), and anti-CD8 (mouse monoclonal, clone C8/144B; Nichirei). Color development was achieved with 3,3′-diaminobenzidine, which rendered positive cells brown. Finally, sections were counterstained with hematoxylin and mounted.

Cell Counting

To determine the degree of eosinophil infiltration in the tissues, 2 of the authors (R.K. and K.K.) independently and manually counted the number of infiltrated cells in 5 random fields. H&E sections under light microscopy at high magnification (original magnification ×400) were used in a masked manner. The numbers of basophils, T cells, $CD4^+$ T cells, and $CD8^+$ T cells were counted in a same manner using sections that were immunostained for each cell type.

Cytospin and May-Grünwald-Giemsa and Immunohistochemical Staining

To confirm the specificity of the antihuman proMBP-1 antibody for basophils, we prepared smears of blood basophils, blood eosinophils, NP eosinophils, and NP mast cells as follows.

Human peripheral blood was obtained from a healthy volunteer. Peripheral blood leukocytes were isolated using Polymorphprep (Axis-Shield PoC AS, Oslo, Norway) according to the manufacturer's protocol. NP specimens harvested from 2 patients were digested separately by Dispase (4 mg/mL; Gibco, Carlsbad, California) for 60 minutes at 37°C in Dulbecco modified Eagle medium (supplemented with 10% fetal calf serum, 100 U/mL of penicillin, and 100 U/mL of streptomycin). Contaminating erythrocytes were removed by hypotonic lysis. After washing, the cells were resuspended in complete RPMI 1640 medium.

Basophils ($CD45^+$, $CD3^-$, $CD14^-$, $CD16^-$, $CD19^-$, $CD20^-$, $CD34^-$, $CD56^-$, FcεRI high, and $CRTH2^+$) and eosinophils ($CD45^+$, $CD3^-$, $CD14^-$, $CD16^-$, $CD19^-$, $CD20^-$, $CD34^-$, $CD56^-$, $FcεRI^-$, and $CRTH2^+$) were obtained from the peripheral blood of a healthy volunteer and patients with NPs. Mast cells ($CD45^+$, $CD3^-$, $CD14^-$, $CD16^-$, $CD19^-$, $CD20^-$, $CD34^-$, $CD56^-$, FcεRI high, and c-kit+) were obtained from patients with NPs. The cells were sorted by a FACS Aria 3 flow cytometer (BD Biosciences, San Jose, California) and transferred to slides in a cytospin centrifuge. Some of the slides were immediately fixed in methanol for 5 minutes and stained with May-Grünwald-Giemsa following standard protocols. The remaining slides were fixed in methanol overnight. We then performed immunohistochemical staining with the antihuman proMBP-1 antibody for basophils. Images were captured using a Keyence microscope with a digital camera.

Statistical Analysis

Statistical analyses were performed using SPSS statistical software (SPSS Inc, Chicago, Illinois). The significance of the between-group differences in the age, Lund-Mackay score, blood eosinophil counts, and immune cell counts in NPs was determined using the Mann-Whitney test. In the ECRS group, simple linear regression analyses were performed to evaluate the association of each parameter with the Lund-Mackey score based on the assumption that the score reflected the severity of CRSwNP. After considering confounding variables, multiple linear regression analyses were performed to assess which parameter contributed most to the score. $P < .05$ was considered statistically significant.

Results

Clinical Characteristics of ECRS and Non-ECRS Patients

Of the 33 patients included in the study, 14 were classified into the ECRS group and 19 into the non-ECRS group. The ECRS group included 1 woman and 13 men, whereas the non-ECRS group included 6 women and 13 men. The mean (SE) values for key parameters were as follows. Mean ages in the ECRS and non-ECRS groups were 57.5 (9.3) years (range, 42–76 years) and 58.5 (9.8) years (range, 40–72 years), respectively. Mean percentages of eosinophils among total leukocytes in the peripheral blood of the ECRS and non-ECRS groups were 5.5% (1.3%) (range, 3.1%–7.1%) and 2.3% (1.9%) (range, 0.4%–7.4%), respectively. Mean Lund-Mackay scores in the ECRS and non-ECRS groups were 10.6 (5.2) (range, 4–19) and 9.2 (3.8) (range, 1–16), respectively. In the ECRS group, 3 patients had allergic rhinitis, 2 had asthma, and the remaining 9 had no relevant comorbidities. In the Non-ECRS group, 3 patients had allergic rhinitis, 1 had asthma, and the remaining 15 had no relevant comorbidities. The data are summarized in Table 1. Between the 2 groups, there were no significant differences in

Table 1
Patient characteristics

Characteristic	ECRS (n = 14)	Non-ECRS (n = 19)	Total (n = 33)
Sex, M/F	13/1	13/6	26/7
Age, mean (SD) [range], y	57.5 (9.3) [42–76]	58.5 (9.8) [40–72]	58.1 (9.5) [40–76]
Lund-Mackey score, mean (SD) [range]	10.6 (5.2) [4–19]	9.2 (3.8) [1–16]	9.8 (4.5) [1–19]
Blood eosinophil count, mean (SD) [range], %	5.5 (1.3) [3.1–7.1][a]	2.3 (1.9) [0.4–7.4][a]	3.7 (2.3) [0.4–7.4]
Eosinophil counts in NPs, mean (SD) [range]	135.2 (94.8) [53–385][a]	9.1 (12.8) [0–48][a]	61.6 (86.0) [0–385]
History of asthma, No.	2	1	3
History of AR, No.	3	3	6

Abbreviations: AR, allergic rhinitis; ECRS, eosinophilic chronic rhinosinusitis; non-ECRS, noneosinophilic chronic rhinosinusitis; NPs, nasal polyps.
[a] $P < .001$.

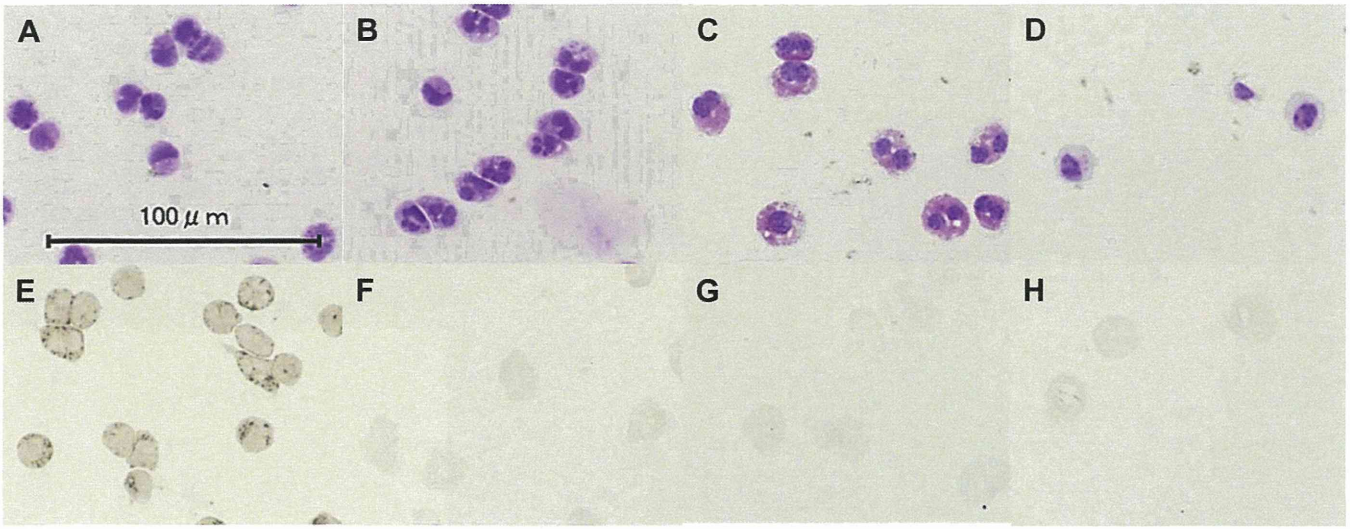

Figure 1. Hematoxylin-eosin staining (A–D) and antihuman proform of eosinophil major basic protein antibody immunohistochemical staining (E–H) smears. Blood basophils (A and E), blood eosinophils (B and F), nasal polyp eosinophils (C and G), and nasal polyp mast cells (D and H) are shown. Scale bar represents 100 μm.

either age or Lund-Mackay scores, but blood eosinophil counts were significantly higher ($P < .001$) in the ECRS group.

May-Grünwald-Giemsa and Immunohistochemical Staining

In the smear of blood basophils, most cells were positive for the proMBP-1 antibody. In contrast, blood eosinophils, NP eosinophils, and NP mast cells were negative for the antibody. Images of each smear are shown in Figure 1.

Immunohistochemical Analysis of the Basophils and T Cells in NPs

We counted the number of cells positive for proMBP-1 (basophils), CD3 (T cells), CD4, and CD8 using immunohistochemical staining in the NP sections from ECRS and pon-ECRS patients. Typical images of the H&E and immunohistochemical staining are shown in Figures 2 and 3.

Figure 4 shows that there were significantly more basophils in NPs of the ECRS group compared with the non-ECRS group. Conversely, there were significantly fewer $CD4^+$ T cells and CD8+ T cells in NPs of the ECRS group than in NPs of the non-ECRS group.

Association Between Immunohistochemical Analysis and Clinical Severity

In simple linear regression analyses, only the basophil count in NPs correlated with the Lund-Mackay score. Age, blood eosinophil

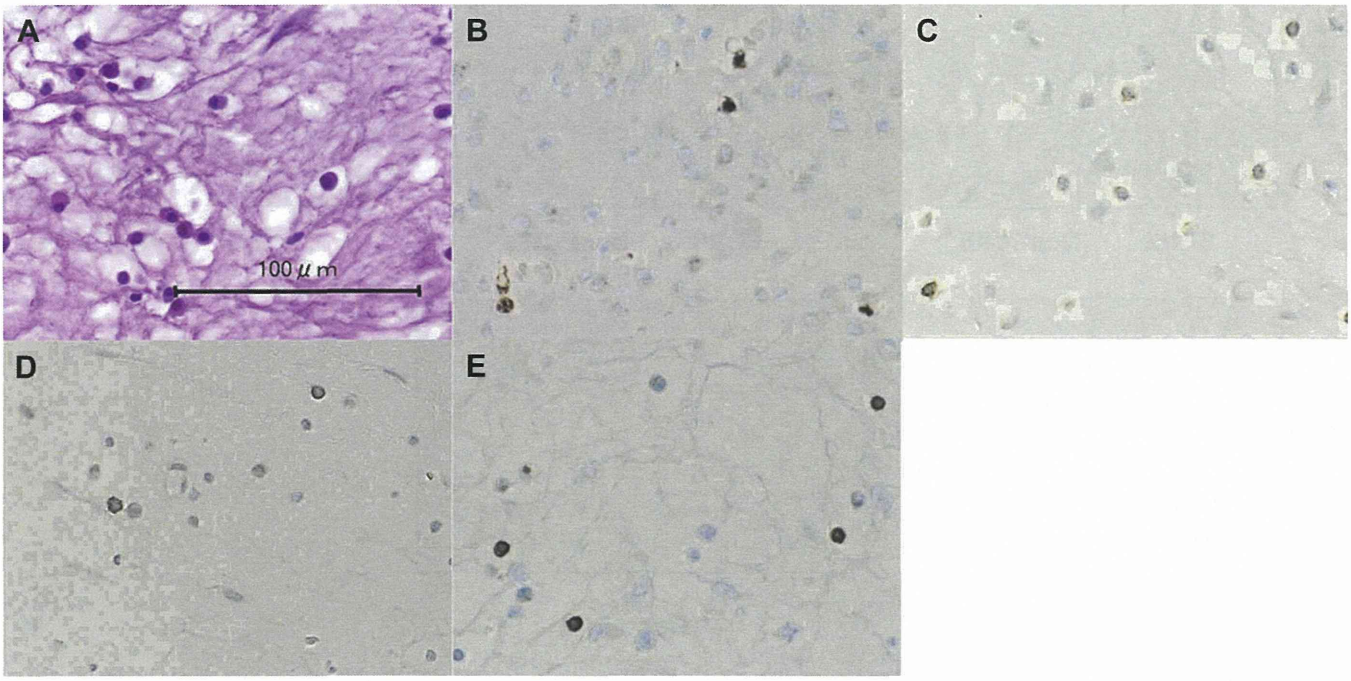

Figure 2. Images of nasal polyp sections from patients with eosinophilic chronic rhinosinusitis. Hematoxylin-eosin staining (A), immunohistochemical staining of basophils (B), T cells (C), $CD4^+$ T cells (D), and $CD8^+$ T cells (E). Scale bar represents 100 μm.

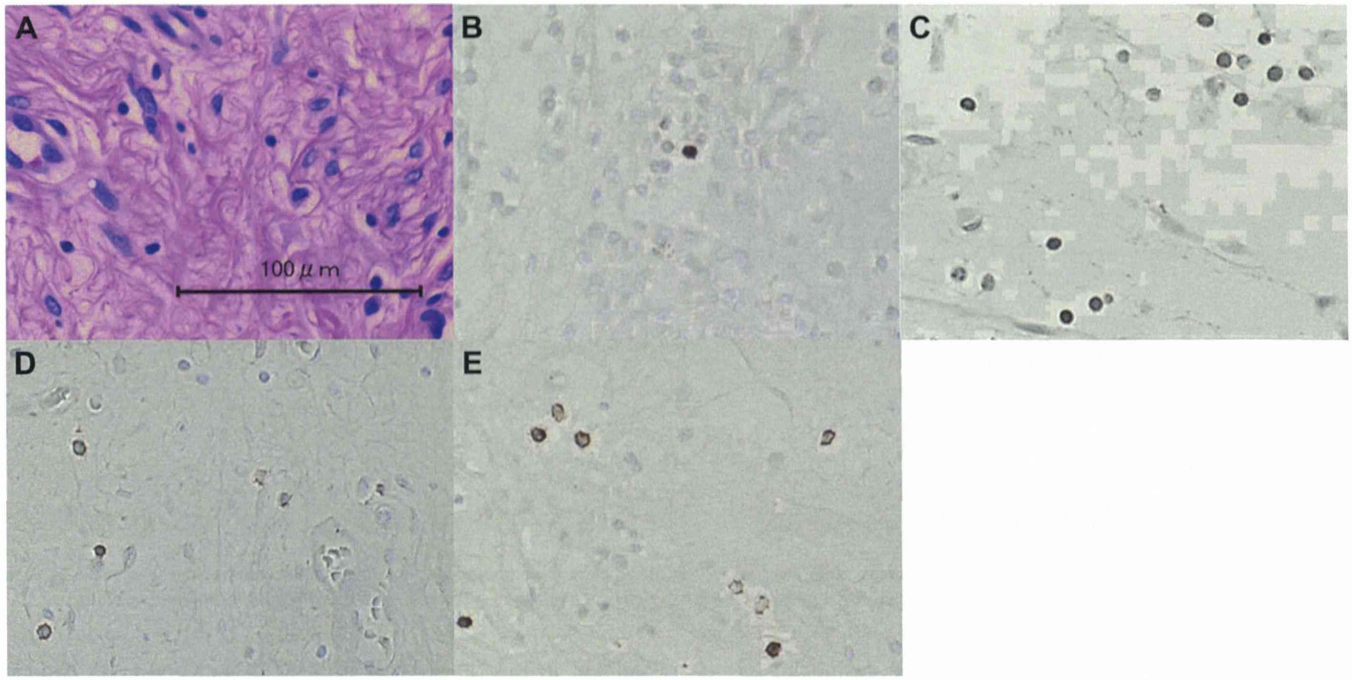

Figure 3. Images of nasal polyp sections from patients with noneosinophilic chronic rhinosinusitis. Hematoxylin-eosin staining (A) and immunohistochemical staining of basophils (B), T cells (C), $CD4^+$ T cells (D), and $CD8^+$ T cells (E). Scale bar represents 100 μm.

count, and immunohistochemical counts (T cells, $CD4^+$ T cells, and $CD8^+$ T cells) did not correlate with the score. The results of the simple linear regression analysis between the basophil count and the Lund-Mackay score are shown in Figure 4.

We then included the following items as explanatory variables in multiple linear regression analysis: age, blood eosinophil count, immunohistochemical counts (basophils, T cells, $CD4^+$ T cells, and $CD8^+$ T cells). The results are presented in Table 2. The basophil

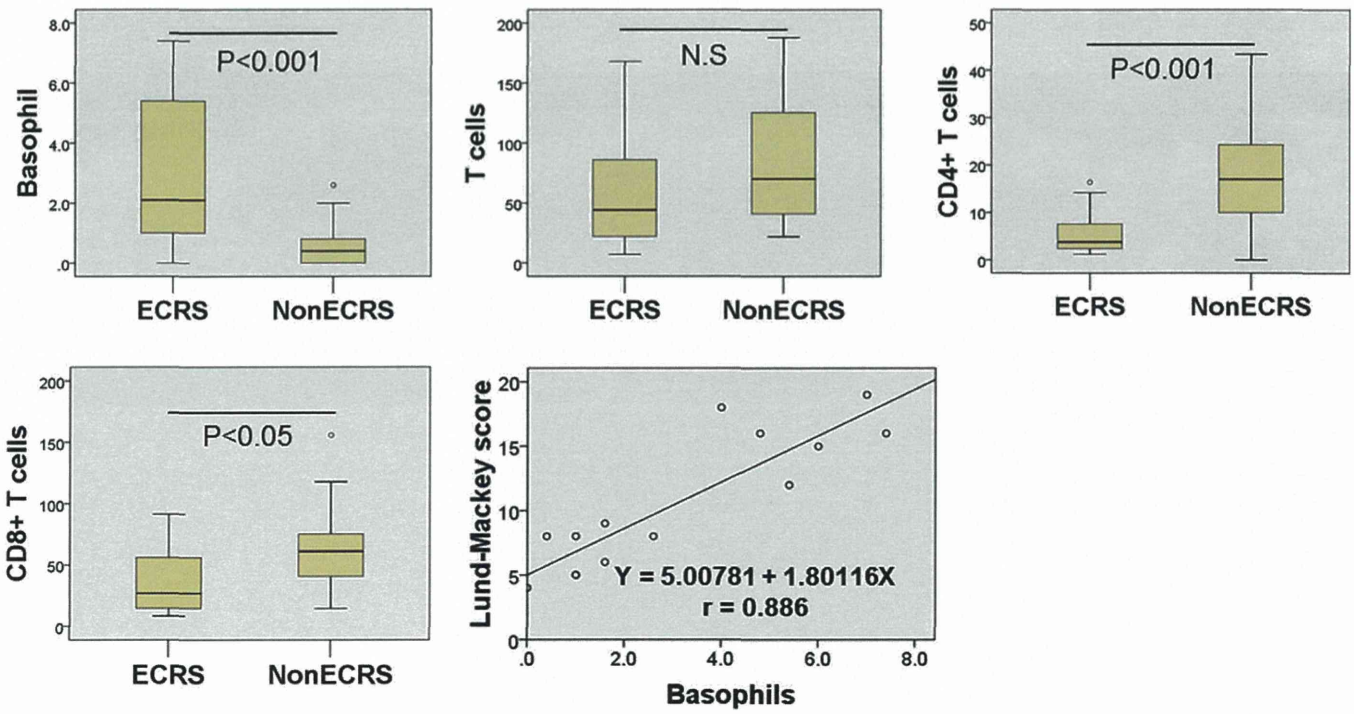

Figure 4. Comparison of the numbers of basophils, T cells, $CD4^+$ T cells, and $CD8^+$ T cells between patients with eosinophilic chronic rhinosinusitis and those with noneosinophilic chronic rhinosinusitis. Box and whisker plots represent the median, lower quartile, upper quartile, lower extreme, and upper extreme. The results of the simple linear regression analysis between the basophil counts and the Lund-Mackay score are shown.

Table 2
Multiple linear regression models with the Lund-Mackay Score as the dependent variable

Dependent variable	Partial regression coefficient (95% CI)	*P* value
Age	−0.1857 (−0.2228 to −0.1486)	<.005
Blood eosinophils	0.3599 (0.0761 to 0.6436)	.25
Eosinophils	−0.0095 (−0.0157 to −0.0033)	.17
Basophils	2.0651 (1.9245 to 2.2057)	<.001
T cells	0.0076 (−0.0049 to 0.0200)	.57
$CD4^+$ T cells	0.0309 (−0.0400 to 0.1019)	.68
$CD8^+$ T cells	0.0437 (0.0182 to 0.0691)	.14

Abbreviation: CI, confidence interval.

count was positively associated with the Lund-Mackay score. In contrast, there was negative correlation between age and the Lund-Mackay score.

Discussion

In this study, compared with NPs of the non-ECRS patients, basophil count was significantly increased in NPs of the ECRS patients. Conversely, compared with the non-ECRS patients, $CD4^+$ and $CD8^+$ T-cell levels were significantly decreased in ECRS patients. Furthermore, the basophil count in NPs was positively correlated with the severity of the Lund-Mackay score in the ECRS patients. These results suggest that basophils are involved in the recruitment of eosinophils into NP tissue and that basophil count in the tissue reflects the severity of eosinophilic inflammation.

Basophils account for only 0.1% to 1% of all peripheral blood leukocytes, and for a long time they were considered a redundant cell type.[10] Recently, basophils were reported to be involved in inducing and perpetuating T_H2 responses in eosinophilic diseases. Ono et al[14] reported that acute exacerbations of asthma were accompanied by increased expression of the CD203c activation marker on blood basophils. In addition, interactions between basophils and epithelial cell–derived cytokines, such as IL-33 and thymic stromal lymphopoietin (TSLP), are described as promoting T_H2 cytokine production. Noti et al[15] reported elevated TSLP expression and exaggerated basophil responses in the esophageal biopsy tissue of patients with eosinophilic esophagitis. Suzukawa et al[16] reported that, in vitro, IL-33 induced T_H2 cytokine production by basophil via its ST2 receptor. Although the involvement of these mechanisms has not been reported in ECRS, increases in both TSLP and IL-33 have been reported in NPs. Nagarkar et al[17] reported that TSLP messenger RNA and protein levels were increased in NPs when compared with uncinate process. Reh et al[18] reported that IL-33 messenger RNA expression in sinonasal epithelial cells was increased in patients with severe CRSwNP than in patients with mild CRSwNP. These reports suggest that basophils may have a key role in the pathogenesis of ECRS, as with other eosinophilic diseases. Our results are consistent with this understanding. To the best of our knowledge, this report is the first to evaluate the association between the basophil count in NPs and clinical characteristics. We found that basophils infiltrated NPs and that the basophil count was significantly higher in the ECRS group than in the non-ECRS group. We also found that the basophil count in NPs correlated with the Lund-Mackay score in ECRS patients. Perić et al[19] indicated a correlation between the Lund-Mackay score and the level of cytokines, such as IL-4, in the nasal secretions of CRSwNP patients. Our results agree with their results, revealing that biomarkers of T_H2 inflammation were associated with the severity of the Lund-Mackay score.

It is interesting that the $CD4^+$ T-cell count was significantly lower in the ECRS group than in the non-ECRS group. There are a few reports on the immunohistochemical analysis of T cells in NPs. Consistent with our results, Hao et al[20] and Stoop et al[21] reported the $CD8^+$ T-cell count was higher than $CD4^+$ T-cell count in NPs. Regarding $CD4^+$ T-cell counts, Stoop et al[21] reported no significant differences between NPs and the mucosa of the middle turbinate. Although we expected that numerous T_H2 cells infiltrated tissues with eosinophilia, these cells may not be the major source of T_H2 cytokines. Indeed, basophils and natural helper cells have recently received attention as major sources of T_H2 cytokines. Halim et al[22] reported that lung natural helper cells produced large amounts of T_H2 cytokines through TSLP or IL-33 stimulation. Although we cannot clarify the number of lymphocytes in this study, the few $CD4^+$ T cells do not necessarily conflict with the severity of T_H2 inflammation.

We also found a weak negative correlation between age and the severity of the Lund-Mackay score. There are no reports discussing the contribution of age to the severity of ECRS. In murine model of asthma, which also entails eosinophilic inflammation, contrasting results have been reported. For example, Kang et al[23] reported a positive correlation between age and the eosinophil count of bronchoalveolar lavage fluid, whereas Yagi et al[24] reported a negative correlation between age and eosinophil counts in the peribronchial tissue. Although our result suggests that the severity of ECRS may be reduced with age, we cannot exclude the possibility of selection bias toward milder cases in elderly patients, particularly because the patients enrolled were restricted to those who could undergo surgery with general anesthesia.

Some limitations in the present study must be considered before concluding that the basophil count in NPs correlates with the severity of ECRS. First, it is unclear whether the Lund-Mackay score strictly reflected the degree of sinusitis severity. Although it is difficult in practice, we need a long-term follow-up with a unified treatment protocol to compare severity among cases. Second, absolute basophil numbers were inherently low. Therefore, although we found an approximate correlation between local basophil distribution and clinical severity in ECRS, it is difficult to develop a helpful parameter for disease prognostication from these immunohistochemical results.

In conclusion, the basophil count was increased in NPs of ECRS patients, and the degree of basophil recruitment correlated with the severity according to the Lund-Mackay score. These results are consistent with current limited knowledge that basophils are related with the severity of eosinophilic inflammation.

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