

SUPPLEMENTS

Methods of nasal cytology comparison-1

We compared nasal cytology between nasal smear with cotton stick, nasal smear with glass stick (NSGS), nasal brush with swab, nasal scraping with curette, and nasal lavage. Except for NSGS, the procedure followed for each sampling method was according to the specifications noted by Pipkorn *et al.* [1] (Table S1).

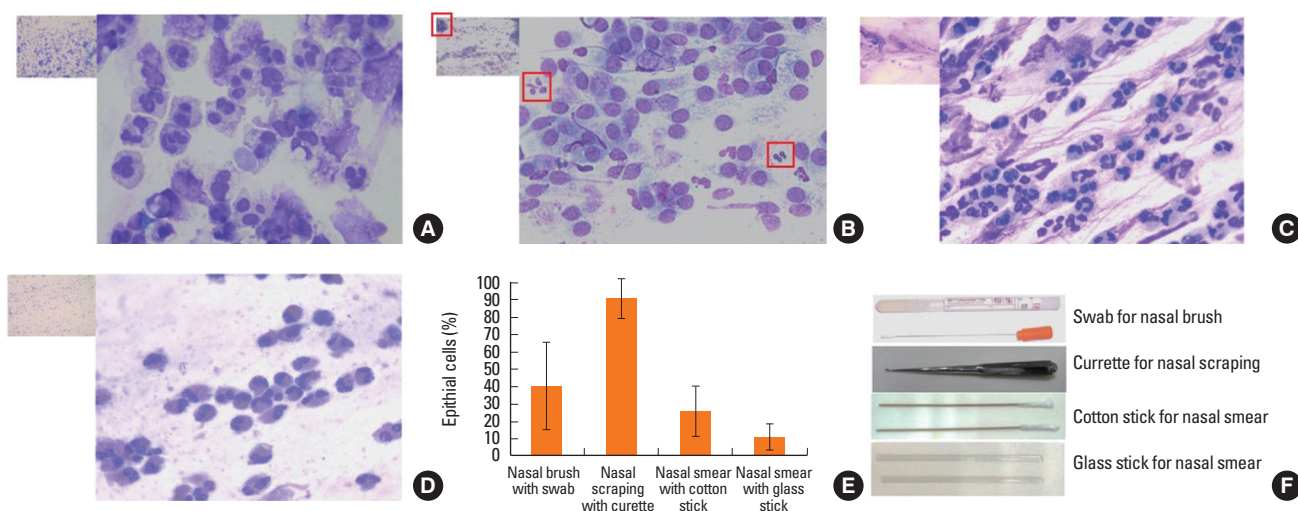
Supplementary Table 1. Description of the methods of nasal cytology

Methods	Description
Nasal scraping	First, absorb any excess secretion on the surface using a cotton stick. Then scrape the anterior part of inferior turbinate with a curette and smear over a slide.
Nasal brush with swab	Introduce a swab (with fiber on the top) into the nasal cavity, rotate the swab on the surface of nasal mucosa, then put the swab into buffer solution and cell centrifuge.
Nasal lavage	Drip 5 mL saline into each nostril, collect the liquid, and cell centrifuge.
Nasal smear with cotton stick	Move a cotton stick over the nasal mucosa from the anterior to the posterior part of the nasal cavity, then smear over a slide.
Nasal smear with glass stick (NSGS)	First, ask the patient to blow their nose, even if they have no obvious secretions. Then, place a thin glass stick into the nasal vestibule or anterior part of nasal cavity if necessary gently rotate the stick, and let the secretions attach. Avoid rubbing the surface of the nasal mucosa as much as possible. Take the stick out and smear a thin layer on a slide, covering 2/3 of it. If the secretion is not enough to cover the demanded area, sampling can be repeated. If the patient has no secretions at all, the smear should be done another time.

Method of nasal cytology comparison-2

20 AR patients were included for each collection method of nasal cytology. The results are shown in Fig. S1. For nasal brush with swab, the percentage of epithelial cells was $40.14\% \pm 26.6\%$. The cells were edematous (Fig. 1A). For nasal scraping, the percentage of epithelial cells was $91.15\% \pm 13.71\%$ and resulted in some cell clumps on the slides (Fig. 1B). For nasal smear with cotton stick, the percentage of epithelial cells was $26.1\% \pm 16.31\%$ and resulted in fibers among the cells on the slides (Fig. 1C). For NSGS, the percentage of epithelial cells was $10.87\% \pm 7.33\%$. The shapes of the cells were well preserved and cells were well distributed (Fig. 1D).

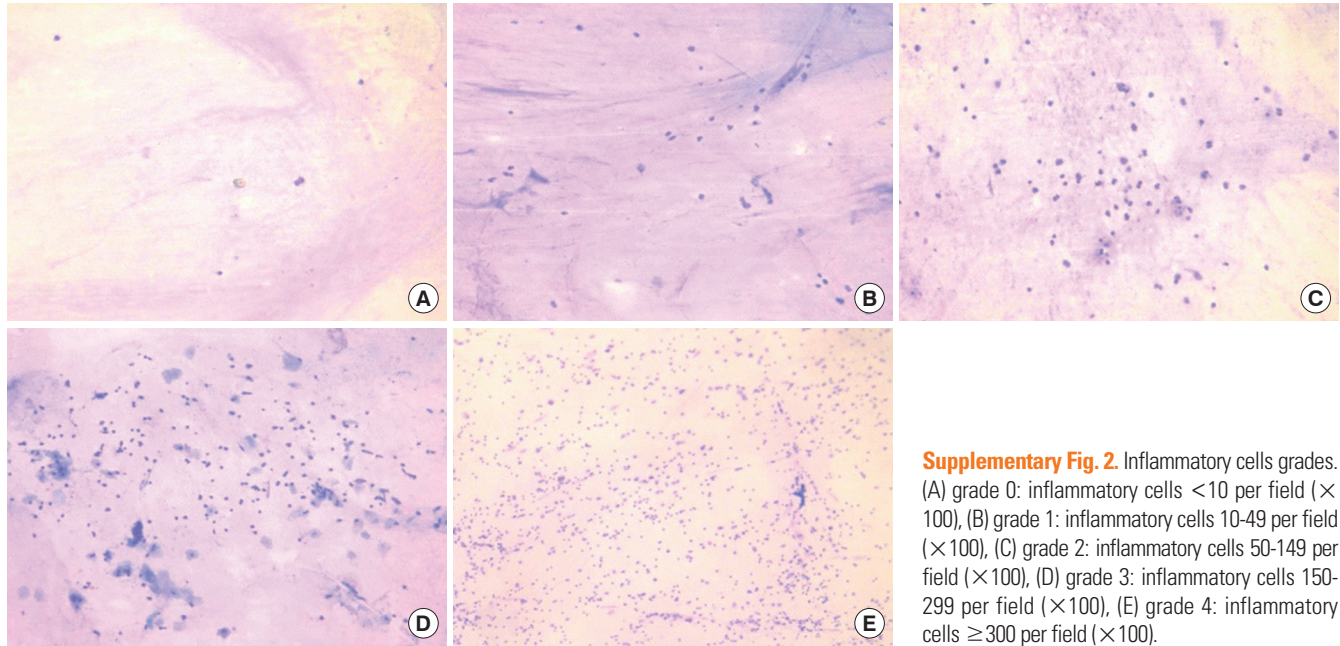
The epithelial cell distribution of the different methods is shown in Fig. 1E. The instruments used in nasal cytology collection are shown in Fig. 1F. Nasal lavage was attempted, but was poorly tolerated by half of the adult and most pediatric patients. The data from this method are not shown.



Supplementary Fig. 1. Comparison of different methods of nasal cytology. (A) nasal brush with swab, (B) nasal scraping with curette (the red box at the left corner showed cell clump, other 2 boxes showed neutrophils), (C) nasal smear with cotton stick, (D) nasal smear with glass stick, (E) the epithelial cells distribution of different methods, (F) the instruments of the nasal cytology.

Grading system for nasal cytology using NSGS

The grading system for subtyping the different groups was developed in accordance with other reports and the expertise of the investigators as described in the text. The typical pictures of the grading were shown in Fig. S2.



Supplementary Fig. 2. Inflammatory cells grades. (A) grade 0: inflammatory cells <10 per field ($\times 100$), (B) grade 1: inflammatory cells 10-49 per field ($\times 100$), (C) grade 2: inflammatory cells 50-149 per field ($\times 100$), (D) grade 3: inflammatory cells 150-299 per field ($\times 100$), (E) grade 4: inflammatory cells ≥ 300 per field ($\times 100$).

Stability of NSGS study

We studied the stability of NSGS by comparing results from the following 4 scenarios:

1. Same time & same side: 2 samplings from the same nostril at the same time.
2. Same time & different side: 2 samplings from different nostrils at the same time.
3. Different time & same side: 2 samplings from the same nostril at different times (6 to 24 hours between the sampling, no medicine use during the interim).
4. Different readers: 2 skillful technicians read the same slide.

A comparison between the percentage of neutrophils, eosinophils, and inflammatory cell quantity grades for the different NSGS collection scenarios is shown in Table S2.

There was excellent consistency between the same side & same time and different side & same time sampling scenarios ($r > 0.9$ of Eos% and Neu%, $r > 0.85$ of inflammatory cell grade under both conditions). Comparisons between samples collected at different

Supplementary Table 2. Stability of nasal smear with glass stick (NSGS)

		Number	Eos%*	r value	Neu%*	r value	Inflammatory cell grade	r value
Same time & same side	Slide 1	48	27.87 \pm 39.16	0.92	49.21 \pm 45.09	0.99	2.40 \pm 0.96	0.9
	Slide 2		27.59 \pm 39.26		47.41 \pm 45.24		2.38 \pm 1.02	
Same time & different side	Left side	48	38.31 \pm 41.76	0.91	40.85 \pm 42.4	0.9	2.42 \pm 0.96	0.85
	Right side		38.99 \pm 44.13		38.1 \pm 43.9		2.35 \pm 0.96	
Different time & same side	First slide	24	36.41 \pm 38.77	0.86	46.92 \pm 41.06	0.74	2.46 \pm 1.06	0.82
	Later slide		39.5 \pm 38.13		39.67 \pm 38.18		2.38 \pm 1.10	
Different reader	Reader 1	21	29.05 \pm 39.44	0.96	61.08 \pm 43.18	0.97	2.76 \pm 0.94	0.87
	Reader 2		35.66 \pm 41.18		54.82 \pm 43.45		2.81 \pm 0.98	

*The percentages of eosinophils and neutrophils were reported as 0 if the inflammatory cells grade was 0 or 1 (the same as below).

times were less consistent ($r=0.86$ of Eos%, $r=0.74$ of Neu% and $r=0.82$ of inflammatory cell grade). Different readers had very similar results of Eos% ($r=0.96$), Neu% ($r=0.97$) and inflammatory cell grade ($r=0.87$).

NSGS in normal people

21 healthy volunteers (no nasal symptoms and negative skin prick tests) were also evaluated to verify the applicability of NSGS.

Nasal cytology smears by NSGS were obtained from 21 healthy volunteers (13 males, age 26.55 ± 16.35 years old), 16 of which provided enough nasal secretions for analysis. No eosinophils were found in any smears. The inflammatory cell quantity grade was 1.05 ± 0.97 (grade 0=8; grade 1=7; grade 2=4; grade 3=2).

Discussion of the nasal cytology method study

The optimal method of collecting nasal cytology should be minimally invasive, reliable, well tolerated by most patients, easy to perform in clinic, and produce enough inflammatory cells for analysis.

The biggest problem with nasal lavage was the lack of acceptance and cooperation by the patients. Half of adults and most pediatric patients could not tolerate sample collection. Nasal scraping sampled both mucosal tissue and overlying secretions. Additionally, the quantity of inflammatory cells fluctuated with the amount of secretions collected [2]. When the surface secretion was removed before scraping, there were 91.15% epithelial cells. Epithelial cell clumps and overlapping cells made the slides difficult to read. When using nasal lavage or a brush, cell damage during centrifuge is an important consideration [3]. Some cells were edematous or broken after centrifuge in this study. Nasal smear with a cotton stick was poorly reproducible and lacked quantitative information [4]. Friction against the nasal mucosa and cells sticking to the cotton stick caused variable results.

NSGS was the most reliable method. The smooth glass stick had very little friction against the nasal mucosa and secretions adherent to the glass were easy to smear. The cells were evenly distributed and cell shapes were well preserved. According to Pipkorn U *et al.*, the disadvantages of nasal smear are the limited quantities of nasal secretions and unclear origin of the cells obtained [1]. During sampling with NSGS, the secretions on the surface of inferior turbinates were obtained by glass stick and smeared. Even when patients could not blow out any secretions, enough were obtained for analysis using NSGS. In this study, 468/493 AR patients and 16/21 healthy volunteers could be successfully sampled and analyzed. Although the exact origination of the inflammatory cells is unknown, NSGS provides adequate information to assess local nasal inflammation.

The stability of NSGS was evaluated by comparing different sampling scenarios and interpretations by different readers. We found excellent consistency between the same side & same time and different side & same time sampling scenarios. Comparisons between samples collected at different times were less consistent, which may be due to slight changes in local nasal inflammation over time. Different readers interpreted the slides similarly because the slides contained well preserved and well distributed cells.

References

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3. Piacentini GL, Kaulbach H, Scott T, Kaliner MA. Evaluation of nasal cytology: a comparison between methods. *Allergy*. 1998;53:326-8.
4. Whelan CFA. Problems in the examination of nasal smears in allergic rhinitis. *J Laryngol Otol*, 1980; 84:399-404.