International session 2

IS2-1   Multi-instance learning for eosinophil quantification of sinonasal histopathology images

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Introduction: Chronic rhinosinusitis (CRS) is a heterogeneous disease and defining eosinophilic or non-eosinophilic subtype is critical to understand the pathophysiological mechanism and poses a great impact on treatment decision makings and prognosis prediction. A microscopic study of the number of eosinophils distributing in the sinonasal tissue relies on manual counting and is time-consuming, labor-intensive, and subjective. Therefore, we aim to develop an autonomic system for eosinophil quantification of sinonasal histopathology images via deep learning approach.

Method: Retrospective pathologic images of sinonasal tissues from subjects of bilateral CRS were obtained, and the number of eosinophils was counted and labeled by medical researchers. These images were thereafter randomly divided into 2 groups: the training data set and the validation data set. Candidate images were selected from each whole slide after filtering the noise area and background. Each candidate image was divided into patch images, and these patch images were appraised by criterion of intensity and colors. Through multi-instance learning, the training data was processed for model construction, and then the model was verified by test data.

Results: Twenty patients of bilateral CRS undergoing endoscopic sinus surgery were recruited, and their pathological images were retrieved to a total of 43 whole slide images. These images were processed, and there were 15153 training patches and 1894 validation patches. The validation performance was 86.99% in the mean of baseline and 88.84% by method of confusion. We compared the experimental results and pathologic reports, and the testing performance was 90.90% for the diagnosis of tissue eosinophilia and 91.67% for that of non-eosinophilia.

Conclusion: A computer-aided eosinophil quantification of the sinonasal histopathology images was established. This autonomic quantification system can aid in determining the immunotypes of CRS and making treatment strategies for CRS.
Vertebral artery stenosis predicts cerebrovascular diseases following radiotherapy for nasopharyngeal carcinoma

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Introduction: Radiotherapy for nasopharyngeal carcinoma (NPC) may induce cerebrovascular diseases including ischemic stroke and transient ischemic attack (TIA), which can cause severe disability. However, information on the incidence and predictors of cerebrovascular diseases is scarce. This study aimed to estimate the incidence of cerebrovascular diseases following NPC, and attempts to ascertain the predictors of cerebrovascular diseases to facilitate early prevention.

Method: We performed a retrospective cohort study on 655 NPC patients who received radiotherapy between 2006 and 2018 in a medical center. This study analyzed the incidence, clinical and imaging presentation of patients with cerebrovascular diseases. Cox proportional hazard model was used to identify risk factors associated with cerebrovascular diseases following radiotherapy.

Results: There were 14 patients who developed an ischemic stroke, and 3 patients developed a TIA after a mean follow-up of 5.8 years. Most ischemic events were from large-artery atherosclerosis (76.5%), and the most common symptom of ischemic stroke was unilateral limb weakness (57.1%). The cumulative incidence of ischemic stroke or TIA 15 years after radiotherapy was 9.1% (95% confidence interval [CI] = 4.7-17.2%). Multivariate Cox regression identified vertebral artery stenosis (HR: 18.341; 95% CI = 3.907-86.100; P < 0.001), atrial fibrillation (HR: 13.314; 95% CI = 1.306-135.764; P = 0.029), and hypertension (HR: 7.511; 95% CI = 1.472-38.320; P = 0.015) as independent predictors of ischemic stroke or TIA.

Conclusion: Our study found that NPC patients with vertebral artery stenosis, atrial fibrillation, or hypertension carry a higher risk for ischemic stroke or TIA. Regular assessment of vertebral artery after radiotherapy was suggested.
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IS2-3 Distinct gene set enrichment profiles in eosinophilic and non-eosinophilic chronic rhinosinusitis with nasal polyps.

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Introduction: Chronic rhinosinusitis with nasal polyps (CRSwNP) can be classified as eosinophilic CRS (ECRS) and nonECRS according to the Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis (JESREC) criteria. This study performed a detailed transcriptomic analysis in CRSwNP classified as ECRS, nonECRS, and a group of ECRS with comorbid aspirin intolerant asthma (Asp).

Method: Gene expression profiles of nasal polyps; ECRS (N=9), nonECRS (N=8), and Asp (N=3), and the uncinate process; control (Ctrl) (N=6), were generated by bulk RNA barcoding and sequencing (BRB-seq). A differentially expressed genes (DEGs) analysis was performed using DESeq2 software in iDEP. Phenotype-specific pathways of expressed genes were identified with A Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using the Database for Annotation, Visualization, and Integrated Discovery (DAVID).

Results: A total of 3004 genes were identified by DEGs analysis to be associated with ECRS vs Ctrl (ECRS-Ctrl), nonECRS vs Ctrl (nonECRS-Ctrl), and Asp vs control (Asp-Ctrl), but no DEGs were identified with ECRS vs nonECRS (ECRS-nonECRS). Hierarchical cluster analysis of the DEGs revealed no distinct segregation among the three CRSwNP groups. Separation into two clusters using hierarchical cluster analysis with all CRSwNP revealed that these two clusters can be classified as clusters with severe or mild type 2 inflammation, regardless of ECRS, nonECRS, and Asp.

Conclusion: All nasal polyps may have a common pathological background as an endotype with severe or mild type 2 inflammation, regardless of the phenotype of ECRS, nonECRS, and Asp.

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**IS2-4  Expression and localization of the bitter taste receptor in patients with chronic rhinosinusitis.**

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Introduction: Taste receptors are present not only in the tongue but also in the nose. Especially, the bitter taste receptor (T2R) and the sweet taste receptor (T1R) play roles in innate immunity of the upper respiratory tract. There are 25 subtypes of T2Rs. Gram-negative bacteria activate T2R38 in sinonasal ciliated cells, initiating calcium (Ca2+) signals that activate constitutive nitric oxide synthase (NOS)-dependent nitric oxide (NO) production. NO increases ciliary beating and mucociliary transport and diffuses directly into the airway surface liquid layers, where it directly permeabilizes and kills bacteria (Cohen NA. Laryngoscope, 2017). T2R38 functionality is altered by single nucleotide polymorphism in the TAS2R38 gene (Bachmanov AA et al. Curr Pharm Des. 2014). The functional T2R38 contains proline (P), alanine (A), and valine (V). Homozygous PAV/PAV individuals are “supertasters” that perceive T2R38-specific agonists as intensely bitter. It has been reported that PAV/PAV “supertasters” had a lower frequency of gram-negative sinonasal infection (Lee RJ et al. J Clin Invest. 2012). Previous study showed that patients with CRS rated the bitter compounds as less intensely bitter than did control subjects. Therefore, the expression and function of T2Rs as a part of innate immunity may be a promising candidate in CRS pathophysiology.

Method: We investigated the expression level of T2Rs mRNA, localization and fractional exhaled NO (oral and nasal FeNO) in CRS with different phenotypes. RT-PCR was performed using primers targeting related genes from the ethmoid sinus mucosa and nasal polyp to measure T2Rs mRNA levels. We also extracted genome DNA from blood and genotyped a single nucleotide polymorphism (SNP) of T2R38. The distribution of T2R38 in the tissue was confirmed by immunostaining. We also performed qualitative taste tests for each enrolled patient.

Results: In CRS patients, the T2R38 mRNA levels of nasal polyps were detected lower levels. In non ECRS patients, the T2R38 mRNA levels of ethmoid sinus mucosa showed significant reduction. Interestingly, they accompanied lower nasal FeNO levels compared to the control patients. In those non-ECRS patients, the reaction to bitterness taste appeared insensitive. Immunohistorical images indicate T2R38 was localized in the sinus ciliated epithelium.

Conclusion: Bitter taste receptors reflect the difference in the phenotype and endotype of CRS. Specific analysis of bitter taste receptors may provide useful information for predicting the type of CRS.

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IS2-5 Inflammatory endotyping based on cytokines and prostaglandins and their related receptors in chronic rhinosinusitis using macroarray.

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Introduction: Chronic rhinosinusitis (CRS) can be divided into phenotypes by clinical finding and into endotypes by functional or pathophysiological findings. The aim of this study is to characterize inflammatory endotypes based on mRNA expression of cytokines, synthases for prostaglandins (PGs) and their receptors in CRS using macroarray.

Method: Nasal polyps (NPs) and uncinate tissues (UTs) were collected from 90 patients who underwent endoscopic sinus surgery. The patients included 75 CRS and 15 non-CRS. Thirty genes were selected to make our original DNA array plate to analyze the expressions of 10 cytokines: IFN-γ, IL-4, IL-5, IL-10, IL-13, IL-17A, IL-22, IL-25, IL-33 and TSLP, 4 PG synthases: HPGDS (PGD2), PTGES (PGE), PTGS1 (COX-1) and PTGS2 (COX-2), and their 16 receptors: IFNGR1, IL4R, IL5RA, IL10RA, IL13RA1, IL17RA, IL22RA1, IL17RB (IL-25 receptor), IL1RL1 (ST2: IL-33 receptor), CRLF2 (TSLP receptor), DP, CRTH2, EP1, EP2, EP3 and EP4. Clustering analysis was performed according to the expression results and clinical findings of patients from each cluster were investigated.

Results: The samples were divided into 3 clusters. Cluster 1 showed elevated expressions of IL4, IL5, IL13, TSLP, IL1RL1, HPGDS and GPR44, cluster 2 showed elevated expressions of IL17A and PTGES, cluster 3 showed elevated expression of IL25. Regarding clinical features, the main component of each cluster is NP from eosinophilic CRS (eCRS) patient for cluster 1, NP from Non-eCRS and UT from CRS without NP patient for cluster 2 and UT from non-CRS patient for cluster 3.

Conclusion: The results showed that classification according to gene expression precisely reflected clinical findings in CRS, and suggested that there were associations between type 2 inflammation and PGD2, and also between type 3 inflammation and PGE2 in CRS.

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IS26 Nasal Mycology by Nanopore Sequencing in Patients with Chronic Rhinosinusitis

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Introduction: Nanopore sequencing (NS) is a third-generation sequencing technology capable of generating reads of long sequences. This study investigates nasal mycology in patients with chronic rhinosinusitis (CRS) using NS.

Method: Thirteen CRS patients who underwent functional endoscopic sinus surgery were enrolled in the study. The unilateral nasal cavity was rinsed with 20 ml of sterile distilled water. The collected nasal irrigant was loaded into a centrifuge tube and sent to the laboratory for processing. One part of the specimen was inoculated for fungal culture, and the other part was sequenced for total DNA. Lysozyme was added to the cell pellets to release the total genomic DNA and extract nucleic acid from the specimen. When NS output DNA Fastq files, the sequenced data were uploaded to the EPI2ME WIMP workflow for quantitative and real-time species analysis and comparison.

Results: Using Ponikau et al.’s method, fungi grew from the specimens of 11 of 13 (84.6%) patients. Among them, Aspergillus sp. and Penicillium sp. were cultured in 4 patients, Cladosporium sp. in 3 patients, and Candida albicans in, Mucor sp. and Chaetomium sp. in 1 patient. Using NS, the detected fungi abundance (OTU) ranged from 81 to 2226 and Shannon species diversity ranged from 1.094 to 1.683 at the genus level. Malassezia sp. was sequenced in 13 patients, Aspergillus sp. in 12 patients, Candida albicans in 11 patients, and Penicillum sp. and Chaetomium sp. in 10 patients.

Conclusion: It took about 7 to 14 days for fungal culture in CRS patients, and only up to 3 fungal species were detected in each patient. On the other hand, NS took 1 to 2 days to detect fungi, and OUT ranged from 81 to 2226. Our results showed that the NS was more sensitive and faster in detecting the fungi from the nasal irrigant of CRS patients.