WPAS Symposium: Rhinitis : mechanisms, endotypes and management

Biomarkers in allergic rhinitis

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1. Biomarkers : definitions and criteria

A biological marker (biomarker) is a physical sign or laboratory measurement that can serve as an indicator of biological or pathophysiological processes or a response to a pharmacological intervention. There is an ongoing exploration of new biomarkers and initially, all biological compounds of the inflammatory cascade could be eligible candidates. Ideally, a biomarker should have the following characteristics:

1) Clinical relevance: indicating a clear relationship between the biomarker and the pathophysiological events in a disorder,causing a clinical endpoint.
2) Sensitivity and specificity for intervention effects.
3) Reliability and repeatability: the biomarker should be measured in a precise and reproducible way.
4) Simplicity of sampling methodology and measurement/detection technique to promote widespread use.

2. Sampling techniques of the upper airways

Several tools and techniques are available for sampling of the upper airway biomarkers. Similarly to the lower airways, there are 3 fractions that can be sampled for biomarkers: cellular, soluble and volatile fractions.

Soluble substances such as mediators and cytokines can be obtained by nasal lavage (NAL) techniques. Two methods are being used to obtain NAL fluid: first, the head-back method introduced by Naclerio. In this method, NaCl 0.9% is instilled into the nose while the subject is closing off the nasopharynx. Another NAL technique is the so-called ""head-forward"" method where a nasal pool device is used to instill saline into the nose. When comparing the methods, the first has been shown to yield more reproducible ECP levels, while the latter allows a higher and more reproducible recovery of cell counts. Overall, with the exception of IgE, NAL-biomarkers show substantial intra- and intersubject variability and most inflammatory

markers remain below the detection limit of the commonly applied quantification assays. Attempts to improve the biomarker yield have been undertaken by increasing the dwelling time of the lavage fluid in the upper airways by reducing the dilution factor using a filter paper or a synthetic absorptive matrix (SAM) for the absorption of nasal secretions/epithelial lining fluid or by optimizing the nasal fluid collection by a nasal secretion collector with polyurethane absorption foams and by the development of more sophisticated detection techniques including multiplex,mRNA analysis, metabolomics and proteomics. However all techniques have their specific limitations and most of them await further validation.

Although cells can be found in the NAL fluid, cellularity, cellular profiles including mRNA patterns can be more accurately assessed by nasal brushes (NAB) and nasal biopsies. Nasal brushing is a simple, relatively patient-friendly method to obtain cells from the nasal mucosa. And despite variability in the individual cell counts,

NAB may be particularly suitable for studies in children, large groups and pathophysiological or intervention studies requiring multiple samplings. Furthermore, NAB enables to pick up signals from inflammatory stimuli, including nasal allergen challenge, and may therefore be a valuable tool in the assessment of the effects of anti-inflammatory interventions.

Nasal biopsies provide more reproducible information than nasal brushings on the nasal epithelium and the mucosa, and additionally on the submucosa as well, however, the methodology does not allow frequently repeated samplings within one individual. Moreover, the methodology requires specialized centers with ample experience. In analogy to the lower airways, more recently attempts have been made to assess nasal inflammation by measuring nasal nitric oxide (nNO) .

3. Mast cell-derived markers

Histamine is the most prominent mediator released from mast cells and basophils during the early phase allergic reaction. This release is reflected by a peak in the NAL level of histamine which is maximal at 15-20 min after nasal challenge. A late peak can be found during the late phase reaction between 6 and 8 h post-challenge. Unfortunately, high baseline levels of histamine (along with substantial variability) preclude its use as a biomarker of disease severity. Therefore, pre-nasal allergen challenge, nasal washings are needed to remove pre-existent histamine.

Other mast cell-derived mediators present in nasal lavage during the early reaction include tryptase, PGD2, and leukotrienes. These mediators are probably more stable and hence more reliable markers of mast cell degranulation.

More recently, chymase along with its inhibitor, cleaved secretory leucocyte protease inhibitor (cSLPI), has been quantified in NAL fluid of allergic rhinitics with increased levels following nasal allergen challenge as compared to sham challenge. In this study, cSLPI appeared to reflect the activity of chymase

recovered from the NAL and sputum of patients with allergic rhinitis and asthma, respectively.

4. Eosinophil derived markers

Eosinophils can be found in the cell pellet of the NAL fluid. In addition, NAB and biopsies are a source of BMK13 positive (activated) eosinophils. Soluble markers of eosinophil activation are among other ECP and EPX. These mediators appear in the NAL fluid approximately 6-10 h post-nasal allergen challenge.

Despite a substantial inter-subject variability, the rise in ECP levels after nasal grass pollen challenge has been shown to correlate with nasal symptoms during pollen season. Moreover, ECP in the NAL fluid is increased in allergic patients during season compared with an out-season assessment. In addition, using

ECP post-challenge allows to study the efficacy of topical corticosteroids.

Treatment with intranasal fluticasone resulted in 76% reduction in the late phase nasal symptoms and 83% reduction in ECP levels in NAL of patients with allergic rhinitis. While an early increase in LTB4 and LTC4 in the NAL fluid reflects mast cell degranulation, a late increase in LTC4 points at activation of eosinophils and possibly basophils as well.

5. Markers of nasal permeability

Albumin and a2 macroglobulin are leakage markers indicative of nasal permeability following allergen challenge. Albumin has been used to characterize the early and late phase nasal response. However, albumin is also produced by nasal glands. Therefore, a2 macroglobulin might be a more specific leakage marker of the nasal allergic response. Plasma exudation or leakage is a result of inflammatory mediators promoting nasal permeability.

Efficacy of drugs targeting components of inflammation(including these mediators) can be evaluated by albumin and a2macroglobulin levels. Antihistamines effectively suppress the a2 macroglobulin peaks in NAL fluid following nasal allergen challenge. Topical corticosteroids reduce the recovery of a2 macroglobulin and albumin in NAL fluid during active disease and following nasal allergen challenge . In a more recent nasal allergen challenge study, vascular endothelial growth factor

(VEGF) has been found in the NAL during the early phase of the nasal allergic reaction . This growth factor is a potent inducer of endothelial cell growth and angiogenesis and is responsible for increased capillary permeability.

6. Nasal nitric oxide (nNO)

Similarly to exhaled NO in asthma, nasal NO (nNO) has been thought to be a useful marker of upper

airways inflammation in allergic rhinitis. Standard operation procedures have been established to measure NO in both upper and lower airways. More recently, nNO measurements by the portable NO-analyzer, MINO,were validated against the gold standard chemiluminescence NO analyzer in both healthy volunteers and patients with AR.

Hence, this totally non-invasive, simple, fast and repeatable upper airways sampling methodology could be added to the existing diagnostic and research tools.

Normal levels of nasal nNO range from approximately400-900 ppb. Paranasal sinuses substantially contribute to nNO measurements by a continuous production of high levels of nNO(up to 25 ppm) by inducible NO-synthases expressed in the epithelium. The role of NO in the sinuses is likely to increase local host defense by direct inhibition of pathogen growth and by stimulation of mucociliary activity. In contrast, conditions with a low nNO production, including cystic fibrosis and primary ciliary diskinesia (PCD), are associated with a high susceptibility to sinus infections. In addition, local application of an NO-synthase inhibitor to a healthy volunteer was found to be associated with a drop in nNO levels and the development of a maxillary sinusitis 3 days later .

Apart from the endogenous source, ambient NO may also substantially affect nNO measurements. Both endogenous and exogenous “”high-output”” nNO sources may interfere with the interpretation of nNO measurements. Overall (active) allergic inflammation induces higher NO production and several studies report increased nNO levels in both symptomatic and asymptomatic allergic rhinitics as opposed to non-allergic controls. In contrast, low(er) nNO levels may be found in conditions such as nasal blockage and nasal polyps.

In daily practice, nNO measurement seems a less attractive candidate for disease monitoring or treatment evaluation due to substantial variability in long-term intra-subject nNO levels (as a result of the aforementioned endogenous and exogenous factors) in combination with only a marginal effect of anti-inflammatory therapy reported by some researchers.

In clinical trials involving nasal allergen challenge, nNO levels can be reliably measured after the massive nasal congestion and rhinorrhoea present in the early phase have subsided.

In conclusion, apart from assessments of clinical signs and symptoms, various biomarkers can be obtained by several more or less non-invasive sampling methods to evaluate the nasal allergic response and disease activity in allergic rhinitis. So far, none of the assessment methods or biomarkers has been validated and both endogenous and exogenous factors introduce a substantial variability.

Presently, nasal biomarkers cannot be readily implemented in the daily clinical practice. However, some of these biomarkers may be useful for evaluation of the efficacy of novel treatment modalities in early clinical studies of allergic rhinitis. Nasal lavage and nasal brushings can be relatively easily implemented in nasal provocation studies. The applicability and long-term reproducibility of nNO await further investigation.

Reference

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