Journal of Immunological Methods 404 (2014) 41–51

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Influences of nasal lavage collection-, processing- and storage methods on inflammatory markers — Evaluation of a method for non-invasive sampling of epithelial lining fluid in cystic fibrosis and other respiratory diseases

Julia Hentschel [a,*,1], Ulrike Müller [a,1], Franziska Doht [a], Nele Fischer [a], Klas Böer [b], Jürgen Sonnemann [c], Christina Hipler [d], Kerstin Hünniger [e,f], Oliver Kurzai [e,f], Udo R. Markert [g], Jochen G. Mainz [a]

[a] CF-Center, Pediatrics, Jena University Hospital, Germany
[b] Institute for Clinical Chemistry and Laboratory Diagnostics, Jena University Hospital, Germany
[c] Pediatric Haematology and Oncology, Pediatrics, Jena University Hospital, Germany
[d] Department of Dermatology, Jena University Hospital, Germany
[e] Septomics Research Center, Friedrich Schiller University, Germany
[f] Leibniz Institute for Natural Product Research and Infection Biology — Hans Knoell Institute, Jena, Germany
[g] Department of Obstetrics, Placenta Laboratory, Jena University Hospital, Germany

ARTICLE INFO

Article history:
Received 30 September 2013
Received in revised form 13 December 2013
Accepted 16 December 2013
Available online 23 December 2013

Keywords:
Nasal lavage
Sampling technique
Centrifugation
Cytology
Cytokines
Cystic fibrosis

ABSTRACT

Background: Non-invasive sampling of airway epithelial-lining-fluid by nasal lavage (NL) is an emerging method to monitor allergy, infection and inflammation in patients with respiratory diseases. However, the influences of collection-, processing- and storage-methods have not been sufficiently evaluated and standardized.
Methods: Influences of repeated NL, centrifugation setups, repeated freezing and thawing, and protease inhibitors on mediator concentration were evaluated in healthy controls and CF patients, which serve as a model for chronic bacterial infection and inflammation.
Polymorphonuclear leukocyte elastase (NE)/myeloperoxidase (MPO)/interleukin (IL)-1/IL-6/IL-8 and tumour necrosis factor alpha (TNF) concentrations were measured using ELISA and Multiplex Bead-Arrays.
Results: NL-repetition within 0.5–4 h markedly decreased NE, IL-8 and MPO-concentrations for up to 70%. NL centrifugation up to 250 $\times g$ for cellular differentiation did not significantly influence mediator concentration in native and processed NL fluid. NL freezing and thawing markedly decreased IL-8 and MPO concentrations by up to 50% while NE remained stable. In contrast to preceding reports, storing at −70 °C for ≥5 years led to significantly reduced mediator concentrations in NL compared to contemporary analyses, being most pronounced for IL-1β, IL-6 and TNFa. Storing of samples in the presence of protease inhibitors led to an increase in marker concentration for IL-8 (+27%) and MPO (+15%) even after one year of storage.
Conclusions: NL is an easy and robust technique for inflammation monitoring of the upper airways. For the first time we have shown that diagnostic NL should be performed only once daily to get comparable results. Whereas NL-fluid can be stored unprocessed at −70 °C for

* Corresponding author at: Cystic Fibrosis Center, Department of Pediatrics, Jena University Hospital, Kochstrasse 2, D 07740 Jena, Germany. Tel.: +49 3641 938 399; fax: +49 3641 938 314.
E-mail address: Julia.Hentschel@med.uni-jena.de (J. Hentschel).
[1] Both authors contributed equally to this publication.

0022-1759/$ – see front matter
http://dx.doi.org/10.1016/j.jim.2013.12.003

cytokine analysis over 1–2 years with protease inhibitors supporting stability, ≥5 years storage as well as repeated freezing and thawing should be avoided.

1. Introduction

The upper airways (UAW) have an important climate and filter function in the respiratory system as about 12,000 L of airflow pass the adult nose every day, being hydrated and with up to 95% of particles being filtered out (Beule, 2010). Dysfunction of the UAW results in bronchial inhalation of cold, dry and non-filtered air which negatively affects lower airway (LAW) function and increases bronchial hyper-sensitivity as described in asthma (Leynaert et al., 2000; Bresciani et al., 2001; Bergeron and Hamid, 2005). Consequently, in patients with allergic rhinitis stimulated by allergens, enhanced inflammatory mediators in sputum samples were detected (Semik-Orzech et al., 2009). Moreover a descend of pathogens from the upper to the lower airways by postnasal drip and their aspiration was shown (Ostertag et al., 2003).

In Cystic Fibrosis (CF), an autosomal recessive disorder, known as the causative Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) alteration, is present in the upper and lower respiratory system. This leads to viscous secretions (Rowe et al., 2005) facilitating airway-colonisation with opportunistic bacteria like *Pseudomonas* (*P.*) *aeruginosa* which are found in CF upper airways (UAW) as the site of first and persistent colonisation (Hansen et al., 2012; Mainz et al., 2011, 2012). This is important as *P. aeruginosa* can be eradicated in new colonisation before changing from planktonic to sessile mucoid phenotype. Additionally, mucoid *P. aeruginosa* is associated with enhanced infection and inflammation (Kerem et al., 1990; Henry et al., 1992). Almost 100% of CF patients reveal UAW abnormalities in Computer Tomography scans (Eggesbo et al., 2001); chronic rhinosinusitis (CRS) and nasal polyps were found in 25 to 40% of CF patients above the age of 5 (Fokkens et al., 2012) and the resulting impairment of quality of life is substantial for many patients.

Otherwise, pathogen colonisation of the paranasal sinuses in mechanically ventilated patients appears to be a risk for ventilator associated pneumonia (VAP), as shown by Holzapfel et al. (Odds-ratio 3.8) (Holzapfel et al., 1999).

Lower airway (LAW) sampling of epithelial lining fluid (ELF) for microbiological and inflammatory analyses by bronchoalveolar lavage (BAL) is limited by its invasiveness. In contrast, nasal lavage (NL) is an easy, non-invasive, sensitive and cheap method to monitor ELF for pathogen colonisation, changes in cytological distribution, and soluble inflammatory markers (Mainz et al., 2009; Beiersdorf et al., 2013). In this regard, NL allows differentiation of characteristic diseases of the nose, the sinuses, and the overall airway system in certain limits (Biewenga et al., 1991). Additionally, and most interestingly, assessment of mediators in ELF sampled by NL may provide a meaningful outcome parameter for the evaluation of systemic therapeutic effects, e.g. in antibiotic treatment (Hentschel et al., submitted for publication). Moreover, especially in CF patients, early detection of critical pathogens like *P. aeruginosa* in the upper airways by NL has the highest impact on eradication concepts. This could be done conservatively, e.g. by sinonasal inhalation of antibiotics via Pari Sinus™ (Mainz et al., 2011, 2012) or by sinus surgery followed by antibiotic lavages (Aanaes et al., 2013).

Secretions sampled by NL derive from the nose and, to some limited extent, also from the paranasal sinuses. They are mixtures of plasma exudation, excretions from goblet cells, and seromucous glands as well as cellular components (Watelet et al., 2004), which comprise immunocompetent cells and expelled epithelial cells. The suggestion that transudation is occurring derives from the predominance of albumin as protein (Maremmani et al., 1996) in the sinonasal ELF which contains about 95% water, 3% organic, and 2% mineral elements. Defensively active substances like secretory IgA, kallikrein, lysozyme, and lactoferrin occur in NL, as well as antioxidants, antibacterial - and inflammatory substances (Kirkeby et al., 2000).

In addition to its non-invasiveness, sampling of NL-fluid has the advantage of facilitated freezing, storing and time-delayed analysis which is essential for longitudinal and multicentric studies. Nevertheless, a review of literature also reveals many diverging protocols for processing (Bergoin et al., 2002; Nikasinovic-Fournier et al., 2002; Howarth et al., 2005; Pitrez et al., 2005).Establishment of NL as a scientific tool in CF centres and laboratories requires easy and quick handling and processing procedures. Furthermore, the comparability of publications in this field is only possible using standardised processing procedures and calculated dilution factors.

Therefore, the aims of the present study were to analyse the effects of sampling and processing methods including repeated NL-collection, centrifugation, addition of protease inhibitors, storage time, and freezing and thawing on cells and cytokine concentrations.

2. Material and methods

2.1. Study cohort

For the majority of experiments, nasal lavages from the Jena University Hospital CF centres' patient cohort were analysed. In order to assess the marker concentration degradation over time, we compared material from a recent CF-cohort used within the study of Hentschel et al. (submitted for publication) with material from a CF-cohort stored for five years as reported by Beiersdorf et al. (2013). The patients in both cohorts were comparable regarding demographics and microbiological characteristics. For analysis of the influence of short term storage with or without protease inhibitors (PI), we used material collected from 10 CF patients of our outpatient clinic during routine sampling. Samples were stored with and without PI for one year and cytokines were quantified directly after collection and after one year of storage. For freeze-and-thaw-experiments and the investigation of repetition effects material from healthy controls of our centre staff was used.

2.2. NL collection and processing

NL-fluid was sampled as described previously (Mainz et al., 2009; Beiersdorf et al., 2013). Before lavaging (sino)nasal