Chapter 6
Salivary Biomarkers in Respiratory Diseases

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6.1 Introduction

Respiratory diseases englobe diseases that affect any part of the respiratory system impeding adequate gas exchange by narrowing or blocking the airways. Respiratory diseases are among the most frequently occurring diseases in humans and animals (WHO 2018). Due to their high variability in terms of origin (e.g., inflammatory, infectious or neoplastic), intensity (acute, chronic), severity (self-limiting to life-threatening), and anatomical localization (trachea, bronchi, bronchioles, alveoli, pleurae, pleural cavity, and the nerves and muscles of respiration) the diagnostics of respiratory tract disorders is complex (Table 6.1).

In the present chapter, the existing information about the possible utility of salivary biomarkers for diagnosis and monitoring of the most commonly studied respiratory diseases are reviewed. Furthermore, since sputum (a coughed-up mucus from the lower airways, trachea and bronchi) is a sample already used in clinics and has a close relationship with saliva, data related to sputum analysis was also included. Nevertheless, animal salivary biomarkers are not described in this chapter as they were mainly studied in respiratory diseases of infectious origin and, though, are reviewed in Chap. 12.

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Table 6.1 Respiratory disease diagnosis

Steps to follow
(a) Complaints of the patient and medical history; (b) Physical examination findings (inspection, auscultation and percussion); (c) Radiological tests (X-ray, computer tomography, scintigraphy, angiography, ultrasound and magnetic resonance test); (d) Functional tests (spirometry, blood gas analysis, lung diffusion test, lung capacity measurement); (e) Salivary sputum tests (cytological and microbiological tests, biomarkers). (f) Bronchoscopy (including tests of bronchoalveolar lavage); (g) Biopsies (including tests of pleural fluid);

6.2 Bronchial Asthma

The heterogeneity of asthma phenotypes represents a challenge for adequate assessment and treatment of the disease (Wenzel 2012). The molecular analysis of highly characterized cohorts of asthma patients was suggested to help to identify biomarkers of asthma subtypes, which may lead to more efficient and personalized therapies (Wenzel 2012; Wagener et al. 2013). So far, asthma has been mainly divided into two subtypes based on airway and systemic eosinophilia and response to glucocorticoids: T-helper cell type 2 (Th2)-high asthma (presence of eosinophilia, respond to glucocorticoids) and Th2-low asthma (without airway or systemic eosinophilia; do not respond to glucocorticoids) (Fahy 2015).

Sputum Patients with poorly controlled asthma present higher sputum eosinophils, leukotriene E4 (LTE4), eosinophil cationic protein (ECP) and regulated upon activation normal T-cell expressed and secreted (RANTES) levels, and interleukin (IL) 13, but not IL-8, compared to patients with controlled asthma (Romagnoli et al. 2002; Truyen et al. 2006). However, sputum eosinophilia and sputum inflammatory biomarkers were associated with poorly controlled asthma rather than with the severity (Romagnoli et al. 2002). On the other hand, sputum IL-6 was suggested as a biomarker for asthma monitoring since strongly correlated with forced expiratory volume in 1 s (FEV_1) and FEV_1/FVC (forced vital capacity) (Neveu et al. 2010; Poynter and Irvin 2016). Both clinical and functional responsiveness of patients with asthma to treatment with steroids was related to sputum eosinophilia indicating its utility for disease monitoring (Romagnoli et al. 2002).

Gene expression analysis of induced sputum revealed the tryptase *TPSAB1*, *CPA3* (a mast cell granule marker) *and CLC* (an eosinophilic granule protein) genes to be mostly upregulated in patients with asthma *versus* control subjects (Foresi et al. 1997; Saha et al. 2008; Wang et al. 2016; Reber and Fahy 2016). According to tryptase *TPSAB1* and *CPA3* gene expression in sputum, patients with asthma could be grouped into 3 groups: (1) low tryptase *TPSAB1* and *CPA3* expression; (2) detectable tryptase *TPSAB1* (MC_T); (3) detectable both tryptase and *CPA3* genes ($MC_{T/CPA3}$). The $MC_{T/CPA3}$ group was characterized by elevated exhaled NO, sputum eosinophilia, bronchial sensitivity and reactivity compared with the MC_T group and responded

better to the therapy with corticosteroids (Dougherty et al. 2010). Furthermore, the close relation between mRNA of *CLC* and *CPA3* in induced sputum and response to glucocorticoid treatment was observed reinforcing their clinical utility (Saha et al. 2008). Other gene expression profiles have been also identified in induced sputum including IL-4, IL-5 and IL-13 being useful in phenotyping asthma as Th2-low and Th2-high (Naseer et al. 1997). Overall, although technically more complex than blood analysis, sputum gene expression signatures were reported to have a great potential in detecting underlying mechanisms and guiding personalized treatment and management strategies in patients suffering from asthma (Wang et al. 2016).

Saliva Patients with asthma were shown to present alterations in salivary stress markers. Just for instance, salivary alpha-amylase (sAA) and cortisol were lower in patients with asthma than in saliva of healthy controls (Tan and Chew 2009; Bakkeheim et al. 2010). Furthermore, lower morning salivary cortisol levels were observed in patients with uncontrolled asthma as compared to patients with controlled asthma (Shin et al. 2014). For this, the authors recommended measuring the morning salivary cortisol levels to assess asthma control status. However, cautions should be taken since a big overlap in salivary cortisol levels between groups existed (Shin et al. 2014).

Inflammatory biomarkers, such as C-reactive protein (CRP), haptoglobin (Hp), leukotriene and Eosinophil cationic protein (ECP), were increased in patients with asthma (Schmekel et al. 2001; Gaber et al. 2008; Rao et al. 2011). Interestingly, salivary leukotriene contributed to the differentiation of aspirin-intolerant asthmatic patients from tolerant counterparts (Gaber et al. 2008). ECP, a protein located in the eosinophil primary matrix and released during degranulation of eosinophils, was associated with asthma activity (Schmekel et al. 2001).

6.3 Bronchiectasis

Bronchiectasis is a chronic destructive disease of respiratory tract characterized by persistent bacterial colonization, purulent inflammation and chronic irreversible dilatation of bronchi.

Sputum Sputum neutrophil elastase (NE), a 29-kDa serine protease released from the azurophilic granules of neutrophils, is considered one of the best biomarkers of bronchiectasis being related to disease activity, severity (including clinical data, antibiotic responsiveness, quality of life), and inflammatory biomarkers (leukocytes, IL-1b, and TNF-a). Patognostic features of NE include degradation of elastin present in the airways and impairment of the immune response (Gramegna et al. 2017; Polverino et al. 2017). Some authors suggest sputum NE be able to predict future exacerbations in bronchiectasis patients (Chalmers et al. 2017; Oscullo Yepez et al. 2019), although their levels did not increase in all cases (Sibila et al. 2019). Therefore, NE could not be an ideal marker for diagnosing bronchiectasis exacerbations. Furthermore, it should be noted that sputum NE is not a pathognomonic bio-

Table 6.2 Behavior antimicrobial peptides in the sputum of patients with bronchiectasis according to Sibila et al. (2019)

Antimicrobial peptide and its behavior	Associated with	Clinical utility
At diagnosis		
↑ LL-37 ↓ SLPI	Frequent exacerbator phenotype of bronchiectasis Bronchiectasis severity index Lower FEV_1 (forced expiratory volume in 1 s) *Pseudomonas aeruginosa* infection	Could predict future risk of exacerbations in bronchiectasis
At follow-up		
↑ LL-37 ↓ SLPI	Shorter time to the next exacerbation	Prediction of future risk exacerbations in bronchiectasis and frequency
↑ LL-37	Exacerbation frequency over the next 12 months	

LL-37 cathelicidin, *SLPI* secretory leukocyte protease inhibitor

marker of bronchiectasis and can also increase in other respiratory diseases that present inflammatory status such as COPD or cystic fibrosis (Gramegna et al. 2017; Polverino et al. 2017). Nevertheless, scientific evidences suggest NE be a key molecule in the pathophysiology of bronchiectasis in most patients, and, therefore, its use in clinical practice would greatly contribute to the accurate disease management, although point-of-care devices facilitating its determination in situ are needed (Oscullo Yepez et al. 2019).

Altered sputum antimicrobial peptides (AMP) was reported in patients with bronchiectasis (Table 6.2) (Sibila et al. 2019).

6.4 Chronic Obstructive Pulmonary Disease (COPD)

COPD is characterized by airway inflammation, chronic airflow limitation, progressive tissue destruction, extra-pulmonary manifestations and systemic inflammation (Agustí et al. 2012; Thomsen et al. 2013).

Sputum The inflammatory response in COPD is dominated by neutrophils and chemokines/cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-8 (IL-8), which are of importance for neutrophils recruitment (Keatings et al. 1996; Lipińska-Ojrzanowska et al. 2017). In consequence, sputum from patients with COPD contains increased levels of eosinophils, IL-5, IL-8, and TNF-α being related to the severity of COPD and its exacerbation (Papi et al. 2006). Furthermore, IL-8 and IL-6 were able to differentiate patients with COPD with and without coexisting bronchiectasis (Patel et al. 2004).

Sputum mucin concentration, microstructure and biochemical properties of main airway mucins, MUC5AC and MUC5B, were also suggested to contribute to clinical management and follow-up of COPD (Kesimer et al. 2017; Garudadri and Woodruff 2018; Chisholm et al. 2019). Sputum mucin concentrations were higher in patients with two or more respiratory exacerbations per year than in those with zero exacerbations (Kesimer et al. 2017; Garudadri and Woodruff 2018). Furthermore, both MUC5AC and MUC5B sputum concentrations increase with increasing airflow obstruction in COPD. In addition, since MUC5AC increases to a greater degree than MUC5B, ratio MUC5AC:MUC5B was related to the severity of the obstruction (Kesimer et al. 2017). Nevertheless, smoking (current or former) was also related to increment concentrations of mucins, MUC5B and MUC5AC, in sputum, thus, smoking should be taken into account when interpreting results (Kesimer et al. 2017). Recently, Chisholm et al. (2019) reported that microscopic mucus structure could serve as a risk factor for COPD progression and severity, although further studies are needed to collaborate these findings.

Saliva Salivary CRP, procalcitonin (PCT) and neutrophil elastase (NE) increase during COPD exacerbations (Ilumets et al. 2008; Özçaka et al. 2011; Patel et al. 2015). Furthermore, CRP and PCT concentrations in saliva were correlated with their concentrations in serum, while salivary NE did not correlate significantly with its levels in serum, but did reflect sputum NE levels (Patel et al. 2015). Therefore, salivary CRP, PCT and NE could have clinical value for COPD management.

6.5 Pneumonia

Pneumonia is an inflammation of the lung tissue affecting alveoli. Due to infection microorganisms, edemic liquid and inflammatory cells accumulate in alveoli and cause damage to normal gas exchange. Death rate from pneumonia takes 5-6th places in industrial countries (WHO 2018) being the greatest risk in the neonatal period (Nissen 2007). Thus, accurate early diagnosis is of high importance to enable efficient treatment and improve the prognosis.

Sputum Alpkvist et al. (2018) reported sputum *lytA* load being associated with respiratory failure and severity of disease. While, although sputum HMGB1 (High Mobility Group Box 1) levels were not related to severity (pneumonia severity index or presence of sepsis), its high levels were associated with pneumococcal etiology, indicating a potential role for HMGB1 in bacterial dissemination (Alpkvist et al. 2018).

Saliva A number of studies reported significant increments of salivary concentrations of CRP in neonates with late-onset pneumonia in comparison with healthy controls (Xiao et al. 2015; Omran et al. 2018). Therefore, salivary CRP was indicated to be one of the best single tests for the early non-invasive detection of pneumonia in children (Hansen et al. 2000; Omran et al. 2018)

6.6 Tuberculosis (TB)

TB is an infectious disease usually affecting the lungs but also may pleura and lymph nodes among others (Ketata et al. 2015). In 2017, globally 10.0 million people developed TB disease and 1.3 million people died from this disease (WHO 2019). Furthermore, a number of patients present drug-resistant TB constituting so denominated a public health crisis (WHO 2019). For these reasons, TB is considered to be one of the most important and dangerous diseases worldwide (Jacobs et al. 2016).

Sputum Sputum mRNA, especially isocitrate lyase (*icl)* mRNA, quantified by RT-PCR was reported to be a promising marker of *M. tuberculosis* viability and, therefore, useful to monitor the response to short and long-term TB treatments. Furthermore, *icl* mRNA was stated to potentially replace the quantitative culture (CFU counts) (Li et al. 2010). On the other hand, the utility of a commercially available real-time PCR assay, Xpert MTB/RIF, to detect Mycobacterium-tuberculosis-specific DNA sequences in sputum samples was evaluated (Friedrich et al. 2013). And, although high sensitivity was observed (97·0%, 95% CI 95·8-97·9), specificity was poor (48·6%, 45·0-52·2). Therefore, the authors concluded that the method was not clinically useful for monitoring tuberculosis treatment and should not replace standard analyses (culture, smear microscopy) (Friedrich et al. 2013). Nevertheless, in any case, longitudinal studies are needed in order to confirm the reliability of the sputum as a sample for TB detection and monitoring.

Saliva Salivary pro-inflammatory proteins including ILs (5, 6, 15), TNFα and CRP were showed to present potential in the diagnosis of TB disease (Phalane et al. 2013; Jacobs et al. 2016). However, only CRP and Hp were shown to present clinical utility independently presenting AUC >0.8 when data of patients with TB and other respiratory diseases (ORD) were studied by ROC curve analysis (Jacobs et al. 2016). Nevertheless, ideally, the use of a combination of salivary biomarkers was proposed in order to increase the specificity and sensitivity of the diagnosis of TB disease. The proposed panel would ideally include CRP, ferritin, serum amyloid P (SAP), monocyte chemotactic protein (MCP)-1, alpha2 macroglobulin (A2M), fibrinogen and haptoglobin (Jacobs et al. 2016). Finally, a number of salivary biomarkers, namely IL-15, granzyme A, MCP-1, IL-1β, IL-9, MIP-1β, IL-10, SAA and ferritin, significantly changed in a course of treatment suggesting their potential as biomarkers of TB disease monitoring (Jacobs et al. 2016). Further large scale studies are now required in order to confirm these promising findings and to deepen the subject since the use of saliva could greatly improve the screening of the potential patents permitting early diagnosis and initiation of treatment and follow-up.

6.7 Lung Cancer

Lung cancer was the most frequently diagnosed cancer type (11.6%) and a reason for death (18.4%) in 2018 as compared to other types of cancers (International Agency for Research on Cancer and WHO). Both sputum and saliva were studied as a possible source of lung cancer biomarkers such as different proteins, RNA and DNA (I and Cho 2015). Furthermore, since saliva can be noninvasively collected and contains a large array of biomarkers, it was suggested to be a good biofluid for early detection of lung cancer (I and Cho 2015). For more information, related to salivary biomarkers in cancer, see Chap. 13.

6.8 Conclusion

Overall, saliva presents the potential for respiratory diseases diagnosis and treatment monitoring. However, further studies are now required in order to confirm these evidences and increase the knowledge related to a broader range of diseases, therapies, biomarkers among others, what would permit the use of this non-invasive biofluid in clinics.

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