COPD: Immunopathogenesis and Immunological Markers

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Authors' contributions

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a disease of the lungs characterised by progressive and irreversible airflow limitation associated with chronic inflammation. Despite extensive research, the immunopathogenesis of COPD is still not fully elucidated. In this review, we outline the current understanding of the pathophysiology of COPD with a particular focus on chronic inflammation and the role of inflammatory cells such as neutrophils and macrophages in the disease, describe the exhaled breath condensate, a novel method of detecting inflammatory biomarkers, and suggest novel biomarkers to better characterise the immunopathogenesis of COPD.

Keywords: COPD; biomarkers; exhaled breath condensate; microRNA.

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1. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a disease state characterised by airflow limitation that is progressive, irreversible and associated with an inflammatory response to noxious particles and gases [1]. It is the sixth leading cause of death in the world and is expected to become the third leading cause of mortality in the year 2020 [2].

Currently, COPD consists of three main pathophysiological phenotypes: chronic bronchitis, emphysema and small airway disease [2]. Chronic bronchitis is caused by excess production and secretion of mucus by goblet cells. This culminates in epithelial remodelling and obstruction of small airways which leads to the worsening of airflow obstruction and changes in airway surface tension predisposing to collapse [3]. Emphysema is caused by the degradation of elastin fibres and components of the extracellular matrix due to unregulated proteolysis resulting in irreversible damage to the lung parenchyma [2,4].

Currently, much research is ongoing to find new biomarkers to diagnose COPD and better understand its pathophysiology.

This review explores the current understanding of the pathophysiology of COPD, with reference to the inflammatory cells involved such as neutrophils and macrophages. This review will also describe the exhaled breath condensate, an innovative method of identifying inflammatory markers, and proposes novel biomarkers to better characterise the immunopathogenesis of COPD.

2. PATHOPHYSIOLOGY OF COPD

The pathophysiology of COPD is still not well understood although several theories have been postulated in an attempt to describe it. Currently, 4 main mechanisms are described.

1. Chronic inflammation of the airways due to the influx of inflammatory cells into the lungs in response to cigarette smoke (Fig. 1).
2. Oxidative stress
3. Imbalance between proteolytic and anti-proteolytic activity culminating in lung tissue destruction
4. The apoptosis of lung structural cells has been postulated as a crucial upstream event in the development of COPD [5].

2.1 Chronic Inflammation of the Airways

COPD is mainly caused by exposure to noxious gases (usually cigarette smoke) or particles culminating in inflammation and remodelling in the large and small airways, and the destruction of lung parenchyma [6]. Currently, the inflammation in COPD is thought to consist of two phases: a phase involving the innate immune response, whereby a danger signal such as damage-associated molecular patterns (DAMPs) triggers inflammation, and a subsequent phase involving the acquired immune response [7,8].

2.1.1 Innate immunity stage

Cigarette smoking introduces oxidants into the lungs which then activate pattern recognition receptors expressed in innate immune cells such as alveolar macrophages, dendritic cells and lung epithelial cells. Furthermore, oxidative damage by cigarette smoke has been postulated to cause DAMPs to be released from the injured epithelial cells [8].

Upon activation, these innate immune cells produce various chemotactic factors that recruit inflammatory cells to the lungs. These include CXC-chemokine ligand 8 (CXCL8) (aka IL-8) and CXCL1 (aka GRO-α) which acts via CXC-chemokine receptor 1 (CXCR1) and CXCR2 respectively to recruit neutrophils and monocytes (which subsequently differentiate into lung macrophages), CC-chemokine ligand 2 (CCL2) (aka MCP1), which binds to CC-chemokine receptor 2 (CCR2) to recruit monocytes, and CXCL9, CXCL10 and CXCL11, which binds to CXCR3 to recruit type 1 cytotoxic T (Tc1) cells and Th1 cells [9,10]. Tc1 and Th1 cells then release interferon (IFN)-γ which stimulates further release of CXCR3 ligands, culminating in an inflammatory state that is persistent [11].

In addition, oxidative damage by cigarette smoke causes various chemotactic factors that recruit inflammatory cells to the lungs. These include nuclear factor-kB (NF-kB) and activator protein 1 (AP-1) in airway epithelial cells and macrophages [12,13]. The activated transcription factors result in the transcription of downstream inflammatory cytokines such as tumour necrosis factor α (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8) which then recruit neutrophils
to further amplify the inflammatory process [12]. The disease severity correlates with the magnitude of inflammation as evident by the presence of inflammatory cells [14].

Neutrophils and macrophages release oxidants and proteolytic enzymes such as neutrophil elastase (NE) and matrix metalloproteinase-9 (MMP-9) which breakdown elastin and collagen in lung matrix [8] resulting in tissue damage. They also release cytokines capable of further amplifying the inflammatory response process [15].

The role of neutrophils and macrophages in COPD and the mediators that they produce will be discussed in greater detail in the subsequent sections.

2.1.2 Adaptive immunity stage

In addition to neutrophils and macrophages, a role has been suggested for B cells, lymphoid aggregates and CD8+ T cells in the chronic inflammatory process of COPD. This occurs especially in small airways, and the degree of inflammation correlates positively with the severity of disease [16]. CD8+ T cells and natural killer cells release the proteolytic enzymes perforin and granzyme B which are toxic to lung tissue cells [17,18].

2.1.3 Outcome of chronic inflammation

The recruitment and presence of inflammatory cells such as neutrophils have been shown to culminate in the generation of mucous metaplasia in chronic bronchitis and lung tissue destruction in emphysema. In patients with chronic bronchitis, the excessive mucus production and impaired mucociliary clearance result in airway obstruction [11,19,20].

2.1.4 Persistence of chronic inflammation in COPD

Even after smoking cessation, it is thought that chronic inflammation persists in COPD. The inflammatory process might be sustained by defective antimicrobial responses resulting in microbial colonization or low-grade infections [21,22]. Furthermore, the dysfunctional regulation of tolerogenic immune mechanisms could result in autoimmune reactions which subsequently culminate in chronic inflammation [7,23].

In addition, the chronic inflammation in COPD could be explained by cumulative DNA damage as there is a substantial amount of information that supports the association between DNA damage and chronic inflammation. Inflammatory cells produce reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can cause serious DNA damage such as double-strand breaks, oxidation and nitration [24].

Aoshiba and colleagues (2013) have suggested a two-hit hypothesis explaining how the inflammation in COPD becomes chronic. The first hit occurs from a danger signal such as DAMPs which initiates the inflammation and the second hit is when the inflammation perpetuates due to DNA damage. This hypothesis suggests that the vicious cycle between DNA damage and inflammation causes the inflammation to progressively worsen in COPD patients. In addition, the inflammation in COPD remains even after smoking cessation due to the persistence of DNA damage [24].

2.1.5 Role of neutrophils in COPD

COPD is often thought to be a disease principally caused by neutrophils. Several studies show that neutrophils are found primarily in the lumen of both the small and large airways and also in the bronchial epithelium, glands and airway smooth muscle bundles. This is evident from the sputum, bronchoalveolar lavage (BAL) [16] and bronchial biopsy specimens obtained from COPD patients[25, 26].

Bronchial biopsy specimens from patients with severe COPD show a higher number of subepithelial neutrophils as compared to that of patients with mild COPD, which in turn was higher than in smokers without COPD[27]. Moreover, the number of neutrophils found in the sputum seemed to correlate positively with lung function decline over time [28]. In addition, reduced spontaneous apoptosis of peripheral blood neutrophils was observed in patients with an acute exacerbation of COPD [29].

Neutrophils are known to produce reactive oxygen metabolites, proteases [30], inflammatory cytokines, lipid mediators [31] and antibacterial peptides [32] and are associated with lung tissue destruction in emphysema and mucous cell metaplasia in chronic bronchitis [20] (Fig. 2).

In addition, neutrophils produce proteases/metalloproteases which include NE and MMPs with gelatinase and collagenase activity (MMP-8, MMP-9) and their proteolytic
potential have been investigated by several studies [33]. Metalloproteases are activated from their inactive preforms by proteolysis after exocytosis and are capable of breaking down structural components of the extracellular matrix which include collagens, proteoglycans, fibronectin, gelatin and laminin [34].

Apart from the ability to degrade extracellular matrix, NE can also stimulate mucin production and secretion. The proteolytic cleavage of transforming growth factor α (TGFα), a ligand of epidermal growth factor receptor, by NE induces mucin production. Increased mucus production and defective mucociliary clearance culminates in airway obstruction in patients with COPD [19].

A number of different signals recruit neutrophils to the airways. Elevated levels of neutrophilic chemoattractants such as CXCL8 aka IL-8, leukotriene B4 (LTB4) [35], CXCL1 (aka growth-related oncogene-α, GRO-α) [36] and CXCL5 (epithelial neutrophil activating protein 78, ENA-78) [37] have been found in the airways of patients with COPD. The activation of CXCR2, a high affinity receptor to which several chemokines (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6 and CXCL8) bind, induces chemotaxis of neutrophils [38].

A study conducted by Milara and colleagues (2011) contributed novel insights into the role of neutrophils in COPD. It was demonstrated that subjects who developed severe early onset (age <56 years) COPD had persistently elevated neutrophil count in the peripheral circulation despite years of smoking cessation, compared to age-matched controls without COPD. Furthermore, these neutrophils are highly activated with enhanced chemotaxis, and exhibit increased production of elastase and ROS when stimulated in comparison to controls. Lastly, these activated neutrophils are also more resistant to apoptosis [39]. This may help to explain the disease progression in COPD even after smoking cessation.

Fig. 1. Smoking as the major cause of chronic inflammation in the immune pathogenesis of COPD. Adapted from [9,11,19,20]
Neutrophils activated by cigarette smoke are less deformable as a result of conversion of G-actin into F-actin. Several studies demonstrate that these stiffer neutrophils tend to be sequestered principally in the capillaries of the upper lung regions which are locations typical for smoking-related centrilobular emphysema [40-42]. The prolonged transit times of these activated neutrophils through the lung allows more time for proteases to be released to cause alveolar wall damage [43].

2.1.6 Role of macrophages in COPD

Alveolar macrophages (AMs) can secrete several inflammatory mediators such as reactive oxygen and nitrogen species, lipid mediators, growth factors, cytokines and chemokines (Fig. 3). They have both pro-inflammatory and anti-inflammatory functions in the respiratory tract and may be activated by various stimuli such as cigarette smoke, endotoxin, pro-inflammatory cytokines and immune stimuli. Generally, AMs from COPD patients demonstrate a higher production of inflammatory mediators than that of normal smokers, which in turn is higher than that of non-smokers [9]. AMs are activated by cigarette smoke to release inflammatory mediators, such as TNF-α, IL-8, [47] and leukotriene (LT) B4 [11]. They originate from circulating monocytes which migrate to the lungs in response to chemoattractants such as CXCL1 acting on CXCR2 and CCL2 (aka MCP1) acting on CCR2 [48]. (Table 1) shows the inflammatory mediators produced by macrophages and their role in COPD.

Activated macrophages also play a role in the destruction of lung parenchymal by inducing oxidative stress which is a direct signal for apoptosis of epithelial and endothelial cells [64]. Another signal for apoptosis in COPD is the loss of cell contact with the ECM caused by the degradation of the matrix by proteolytic enzymes [4,65].

![Fig. 2. Role of neutrophils in COPD. Adapted from: [19,30,31,38,44-46]](image-url)
Fig. 3. Role of macrophages in COPD. Adapted from: [9-11,19,44-47,49]

| Inflammatory mediators | Existing literature |
|------------------------|---------------------|
| **Growth factors**     | Human AMs express transforming growth factor-β1 (TGF-β1) and TGF-β3 [50]. In patients with COPD, there is an increased expression of TGF-β1 in airway macrophages [51]. TGF-β1 induces fibrosis and may be responsible for the fibrosis and narrowing of peripheral airways in COPD [45,46]. Furthermore, TGF-β1 activates MMP-9, which then further activates TGF-β1. It is thought that MMP-9 may be able to mediate the proteolysis of TGF-β-binding protein which could account for the physiological release of TGF-β1. This phenomenon could demonstrate a connection between emphysema and small airway fibrosis in COPD [52]. Furthermore, TGF-β1 has been shown to down regulate β2-adrenoceptors [53]. |
| **Proteases**          | Macrophages produce MMP-1 [11], MMP-2, MMP-9, MMP-12, cathepsins K, L and S and NE taken up from neutrophils [49,54]. These proteases damage the alveolar wall attachments which culminates in lung parenchymal destruction, collapsed small airway lumens and reduced alveoli recoil [11]. MMP-9 seems to be the main elastolytic enzyme secreted by alveolar macrophages in COPD patients [55,56]. It is also highly expressed in lungs affected by emphysema, particularly at areas where macrophages gather [57]. MMP-12 (macrophage metalloelastase) is thought to be necessary for the release of activated TNF-α by alveolar macrophages and it plays a vital role in cigarette smoke-induced emphysema in mice [58]. It has been shown that the Th1 produced chemokines IL-10, CXCL10 and MIG/CXCL9 interact with the CXCR3 receptor found in alveolar macrophages to up-regulate MMP-12 production [59]. MMP-12 is the proteinase that is highly involved in mouse models of emphysema [60,61]. However, there are conflicting studies about the role of MMP-12 in human emphysema [62,63]. |
From the aforementioned studies in both human subjects and murine models, it is evident that macrophages play an active role in the destruction of lung parenchyma and the airways. However, the exact pathways and the key mediators have not yet been identified completely [11].

2.2 Oxidative Stress

Oxidative stress is another mechanism involved in the pathogenesis of COPD in which an excessive production of reactive oxygen species overwhelm the antioxidant defence mechanisms [66]. Oxidants are produced by cigarette smoking or are released from inflammatory leukocytes and alveolar epithelial and endothelial cells [67]. Oxidative stress can cause cell dysfunction or apoptosis and lung extracellular matrix damage [5].

As mentioned above, oxidants contribute to the inflammatory process in COPD by activating the transcription factor NF-κB which leads to the transcription of pro-inflammatory genes [12,13]. In addition to its contribution to the inflammatory process, oxidants also react readily with polyunsaturated fatty acids of cell membranes to form lipid peroxidation products such as hydroperoxides [68], endoperoxide and aldehydes such as ethane, pentane, malondialdehyde [69] and 4-hydroxy-2-nonenal which are highly reactive [70]. Lipid peroxidation damages the cell membrane leading to cell destruction [68] and lipid peroxidation products (LPPs) react with DNA to cause adduct formation [71].

2.3 Imbalance between Proteolytic and Anti-proteolytic Activity

2.3.1 α1-antitrypsin (A1AT) deficiency

A1AT deficiency is a known risk factor for COPD. A1AT inhibits NE and therefore protects the lung from NE-induced damage [72]. Anti-proteinases such as α-1-proteinase-inhibitor (α-1-PI) and anti-leukoprotease are inactivated by oxidants [69], leading to a proteinase/anti-proteinase imbalance which culminates in the destruction of lung elastin and connective tissue thereby causing emphysema [73].

2.4 Apoptosis

Apoptosis is suggested as the fourth mechanism to explain the pathogenesis of COPD. The imbalance between apoptosis and replacement of alveolar epithelial and endothelial cells in the lung has been thought to contribute to the lung tissue destruction in response to cigarette smoke, resulting in emphysema [5].

The various mechanisms are strongly interrelated in the pathogenesis of COPD and do not function separately. For instance, oxidative stress contributes to the proteinase and antiproteinase imbalance by inactivating antiproteinases, whereas an accumulation of apoptotic cells results in secondary necrosis and can amplify ongoing lung inflammation [8].

3. NOVEL BIOMARKERS TO CHARACTERISE THE IMMUNOPATHOGENESIS OF COPD

Currently, the immunopathogenesis of COPD is still not fully understood. Increasing evidence suggests that either local or systemic sampling of biological molecules known as biomarkers can aid in better understanding the pathophysiological mechanisms of COPD [74].

The definition of a biomarker includes any cell or tissue, or molecule, or biochemical feature that can be measured in the body or its products, and which could be used to understand the disease process or predict its outcome [75,76]. In addition, an ideal biomarker should be sensitive, specific, presents with a high predictive value, reproducible, and easy and cheap to determine [77]. The inflammatory cells, mediators, products and enzymes mentioned in Figs. 1-3 are examples of biomarkers for COPD.

Currently, there is still a lack of viable and established biomarkers to monitor disease severity, progression, clinical subtypes, or response to treatment with regards to COPD. A substantial number of inflammatory cells, mediators and enzymes are involved in the complicated immunopathogenesis of COPD but their relative importance is still not well understood [75]. The identification of biomarkers for COPD could help develop better methods to classify the different disease phenotypes, facilitate earlier diagnosis and to monitor response to novel therapeutic treatment in early clinical studies [45,78].

In COPD, numerous types of biomarkers have been detected and measured in various sample sites such as exhaled breath condensate,
peripheral blood, urine, induced sputum, bronchial biopsy, and bronchoalveolar lavage fluid (BALF) [75,76]. This review article will focus on the exhaled breath condensate as a technique for sampling biomarkers for COPD.

4. EXHALED BREATH CONDENSATE AS A TOOL FOR SAMPLING BIOMARKERS

Exhaled breath condensate (EBC) is an emerging non-invasive technique that can detect biomarkers in various lung diseases. EBC is produced by the cooling of exhaled breath vapour and it contains water vapour and aerosolised particles which are produced by the airway lining fluid. EBC allows the investigation of the composition of the airway lining fluid which may provide a sample of inflammatory mediators from inflammatory lung conditions [44].

Several studies demonstrate the utility of EBC to detect a broad range of organic and inorganic compounds including small inorganic molecules (H$_2$O$_2$, pH and nitric oxide related biomarkers), lipid mediators (8-isoprostane, leukotrienes and prostaglandins), small proteins (cytokines and chemokines) and nucleic acid derivatives (Table 2). These clinically relevant compounds are either due to chronic inflammation of the respiratory tract or acute oxidative stress or both. However, the majority of these compounds are of minute concentrations which may affect the accuracy of their detection in EBC[79].

The utility of EBC to sample biomarkers has several advantages. It is non-invasive, inexpensive [80], does not affect or aggravate an ongoing pulmonary inflammatory process[81], conveniently performed and highly reproducible [82].

EBC possesses the potential to be utilised for diagnosing COPD, disease phenotyping, evaluating treatment response as well as defining patient’s prognosis [83]. For instance, EBC can be utilised to measure airway inflammation which allows the monitoring of response to anti-inflammatory treatment. It may also permit early interventions for COPD patients before the occurrence of symptom development and lung function decline [84,85].

| Category                      | Biomarker          | Findings in COPD patients | Studies     |
|-------------------------------|--------------------|---------------------------|-------------|
| pH                            | pH                 | Lower                     | [86,87]     |
| Reactive oxygen species       | Hydrogen peroxide  | Increased                 | [88]        |
| Reactive nitrogen species     | Nitric oxide       | Higher                    | [89]        |
|                               | Nitrite (NO$_2^-$) | Elevated                  | [90]        |
|                               | Nitrate            | No significant difference | [89]        |
|                               | Peroxynitrite      | Higher                    | [91]        |
|                               | Nitrosothiols      | Higher                    | [90]        |
| Cytokines                     | TNF-α              | Increased                 | [92]        |
|                               | IL-1β              | Increased in exacerbation  |             |
|                               | IL-6               | IL-6 increased            | [93]        |
|                               | IL-8               | Increased in exacerbation  | [92]        |
|                               | IL-10              | Increased in exacerbation  |             |
|                               | IL-12p70           | Increased in exacerbation  |             |
|                               | IL-17              | No difference             | [94]        |
| Collagenase                   | MMP-9 TIMP-1       | Increased in COPD         | [95]        |
|                               | Neopterin          | No significant difference | [96]        |
|                               | IP-10              | No significant difference | [96]        |
|                               | 8-IP               | Elevated in COPD          | [79]        |
| Arachidonic acid derivatives  | Malondialdehyde    | Increased                 | [97]        |
|                               | PGE2 LTB4          | Increased                 | [98]        |
|                               | Prostaglandin F2-alpha | No significant difference |             |
| Nucleic acids                 | microRNAs         | Lower expression of Let-7a, miR-328, miR-21 in COPD | [99]        |

Table 2. Summary of EBC biomarkers studied in COPD patients
However, the disadvantages of EBC include salivary contamination which may affect EBC measurement [80,81]. Furthermore, the collected condensate is not anatomic-site specific as the precise location where aerosol particles are derived from the lower respiratory tract and the relative contribution of the various sites to the particles is still unknown [82].

(Table 2) summarises the variety of biomarkers studied in EBC of COPD patients. Studies on certain biomarkers such as TGF-β, MMP-8, neutrophil elastase and miR-223 have not been carried out yet and remains a potential area of exploration.

Recently, microRNAs have been an area of interest in identifying novel biomarkers for COPD.

5. MICRORNAS

MicroRNAs (miRNAs) are small noncoding RNAs comprising 20 to 25 nucleotides that are expressed in bodily fluids and tissue. They are emerging as potential biomarkers that are vital in the regulation of inflammation [99]. miRNAs control gene expression by initiating mRNA degradation or inhibiting mRNA translation [100]. miRNA expression profiling can aid in the identification of miRNAs that regulate a range of vital biological processes. In addition, measuring miRNA expression has led to the development of miRNA-based biomarkers for diverse molecular diagnostic applications in cancer, autoimmune and cardiovascular and forensics [101].

However, it is a challenge to develop accurate, unbiased quantification techniques due to the sequence homology, common secondary structures and wide range of abundance of miRNAs. For miRNA detection to be applied in a clinical setting, a high-throughput processing, the flexibility to develop custom assays and large coding libraries for multiplexed analysis are required [102].

At present, miRNA detection can be carried out by a range of methods such as Northern blotting, microarrays, and quantitative RT-PCR (qRT-PCR) etc. Each of these methods has its relative strengths and limitations [103] as shown in (Table 3).

Despite the limitations of miRNA detection and quantification, there is increasing literature suggesting that there is abnormal expression of specific miRNAs in certain lung diseases such as COPD [105].

In a study comparing the miRNA expression profile of bronchial epithelial cells from never-smokers and smokers, 28 miRNAs were found to be differentially expressed. In particular, miR-218 was thought to be important in modulating epithelial gene expression following cigarette smoke exposure [106].

Table 3. Strengths and limitations of miRNA detection and quantification methods. Adapted from: [101-104]

| Method            | Strengths                                  | Limitations                                                                 |
|-------------------|--------------------------------------------|-----------------------------------------------------------------------------|
| Northern blotting | Used to identify novel miRNAs that are previously unidentified | Often fails to detect miRNAs with low abundance                             |
|                   |                                            | A substantial amount of total RNA (hundreds of micrograms) is needed as starting material |
| Microarray approaches | High sensitivity and multiplexing capacity | Low throughput, complexity, and fixed design                                |
|                   |                                            | Less than ideal for use in a clinical setting                               |
| PCR-based strategies | Highly sensitive and specific detection for genome-wide miRNA expression profiling | Low throughput                                                               |
|                   |                                            | Availability of a well-annotated primary sequence for the species of interest |
| Alternative bead-based systems | High sample throughput | Low dynamic range, sensitivity and multiplexing capacities                   |
| Deep sequencing | Powerful tool for small RNA analysis | High cost of implementation                                                 |
|                   |                                            | Need for large amounts of input RNA                                         |
Another study showed that miR-638 was upregulated in emphysema. miR-638 is thought to respond to oxidative stress by culminating in an accelerated lung aging response and dysfunctional ECM repair [107].

5.1 MicroRNA-223

MicroRNA-223 is myeloid-specific and was shown to down leukoprotease are progenitor proliferation and granulocyte differentiation and activation [108].

In a study by Fazi, et al. the authors have identified that miR-223 is an important modulator of human myeloid differentiation that is specifically expressed in myeloid cells. In addition, miR-233 is upregulated during retinoic acid mediated granulocytic differentiation of acute promyelocytic leukemia cells both in vivo and in vitro. Both over expression and knockdown experiments show the relevant role of miR-223 in the differentiation process. For the first case, there was a twofold increase in the cells committed to the granulocyte-specific lineage, whereas decreased miR-223 levels resulted in the opposite effect [108].

Detection of miRNA-223 in human EBC for COPD patients has not been carried out yet and hence remains a potential area for exploration. The following presents current studies done on miR-223 in relation to COPD.

5.1.1 Murine studies

miR-223 has been known to target Mef2c, a transcription factor that promotes myeloid progenitor proliferation. miR-223-deficient granulocytes demonstrate hypermaturity, are more sensitive to activating stimuli and show stronger fungicidal activity. miR-223 mutant mice was observed to develop increased tissue damage and inflammatory lung pathology after endotoxin challenge as a result of neutrophil hyperactivity [108].

Another study showed that environmental cigarette smoke led to the down regulation of miR-223 expression in the lungs of rats.

5.1.2 Human lung tissue samples

There is a conflicting study which showed that miR-223 was increased in expression by nearly threefold in lung tissue samples from COPD patients compared with smokers without airflow limitation [110]. A possible reason could be due to the difference in genetic makeup in humans and mice and thus more studies on miR-233 could be done especially in human subjects.

As neutrophils play an important role in the immunopathogenesis of COPD, and miRNA-223 is essential in neutrophil production and development, the role of miRNA-223 in COPD remains a potential area of interest. This could also pave the way for novel therapeutic strategies for the disease.

6. CONCLUSION

Despite extensive research carried out for many decades, the immunopathogenesis of COPD and the exact mechanisms of the disease are still not fully understood. EBC could be utilised as a non-invasive method to diagnose COPD and aid in better understanding the immunopathogenesis of COPD by the identification of novel biomarkers. More studies could be done on microRNAs in relation with COPD.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vestbo J. COPD: Definition and phenotypes. Clin Chest Med. 2014;35(1):1-6.
2. Noujeim C, P Bou-Khalil. COPD updates: What's new in pathophysiology and management? Expert Rev Respir Med. 2013;7(4):429-437.
3. Kim V, Criner GJ. Chronic bronchitis and chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2013;187(3):228-237.
4. Chapman HA, G-P Shi. Protease Injury in the Development of COPD: Thomas A. Neff Lecture. CHEST Journal. 2000;117(5_suppl_1):295S-299S.
5. Demedts IK, Demoor T, Bracke KR, Joos GF, Brusselle GG. Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. Respir Res. 2006;7(1):53.
6. Nicholas BL. Search for biomarkers in chronic obstructive pulmonary disease: Current status. Curr Opin Pulm Med. 2013;19(2):103-108.
7. Cosio MG, Saetta M, Agusti A. Immunologic Aspects of Chronic Obstructive Pulmonary Disease. New England Journal of Medicine. 2009;360(23):2445-2454.
8. Brusselle GG, Joos GF, Bracke KR. New insights into the immunology of chronic obstructive pulmonary disease. The Lancet. 2011;378(9795):1015-1026.
9. Barnes PJ. Alveolar macrophages as orchestrators of COPD. COPD: Journal of Chronic Obstructive Pulmonary Disease. 2004;1(1):59-70.
10. Chung K. Cytokines in chronic obstructive pulmonary disease. Eur Respir J. 2001;18(suppl):50s-59s.
11. Pappas K, Papaioannou AI, Kostikas K, Tzanakis N. The role of macrophages in obstructive airways disease: Chronic obstructive pulmonary disease and asthma. Cytokine. 2013;64(3):613-625.
12. Drost E, Skwarski K, Sauleda J, Soler N, Roca J, Agusti A, et al. Oxidative stress and airway inflammation in severe exacerbations of COPD. Thorax. 2005;60(4):293-300.
13. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. Nat Rev Immunol. 2008;8(3):183-192.
14. Yao H, Rahman I. Current concepts on the role of inflammation in COPD and lung cancer. Curr Opin Pharmacol. 2009;9(4):375-383.
15. Tetley TD. Inflammatory cells and chronic obstructive pulmonary disease. Curr Drug Targets Inflamm Allergy. 2005;4(6):607-618.
16. Sohal SS, Ward C, Danial W, Wood-Baker R, Walters EH. Recent advances in understanding inflammation and remodeling in the airways in chronic obstructive pulmonary disease. Expert Rev Respir Med. 2013;7(3):275-288.
17. Saetta M, Di Stefano A, Turato G, Facchini FM, Corbino L, Mapp CE, et al. CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1998;157(3):822-826.
18. Urbanowicz RA, Lamb JR, Todd I, Corne JM, Fairclough LC. Enhanced effector function of cytotoxic cells in the induced sputum of COPD patients. Respir Res. 2010;11:76.
19. Fahy JV, Dickey BF. Airway mucus function and dysfunction. New England Journal of Medicine. 2010;363(23):2233-2247.
20. O'Donnell R, Breen D, Wilson S, Djukanovic R. Inflammatory cells in the airways in COPD. Thorax. 2006;61(5):448-454.
21. Hogg JC. Role of latent viral infections in chronic obstructive pulmonary disease and asthma. Am J Respir Crit Care Med. 2001;164(supplement_2):S71-S75.
22. Sethi S, Maloney J, Grove L, Wrona C, Berenson CS. Airway inflammation and bronchial bacterial colonization in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2006;173(9):991.
23. Van Pottelberge GR, Mestdagh P, Bracke KR, Thas O, Van Durme YMTA, Joos GF, et al. MicroRNA expression in induced sputum of smokers and patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2011;183(7):898-906.
24. Aoshiba K, Tsuji T, Yamaguchi K, Itoh M, Nakamura H. The danger signal plus DNA damage two-hit hypothesis for chronic inflammation in COPD. Eur Respir J. 2013;42(6):1689-1695.
25. Saetta M, Turato G, Facchini FM, Corbino L, Lucchini RE, Casoni G, et al. Inflammatory cells in the bronchial glands of smokers with chronic bronchitis. Am J Respir Crit Care Med. 1997;156(5):1633-1639.
26. Riise GC, Ahlstedt S, Larsson S, Enander I, Jones I, Larsson P, et al. Bronchial inflammation in chronic bronchitis assessed by measurement of cell products in bronchial lavage fluid. Thorax. 1995;50(4):360-365.
27. Di Stefano A, Capelli A, Lusuardi M, Balbo P, Vecchio C, Maestrelli P, et al. Severity of airflow limitation is associated with severity of airway inflammation in smokers. Am J Respir Crit Care Med. 1998;158(4):1277-1285.
28. Stănescu D, Sanna A, Veriter C, Kostianev S, Calcagni P, Fabbri L, et al. Airways obstruction, chronic expectoration, and rapid decline of FEV1 in smokers are associated with increased levels of sputum neutrophils. Thorax. 1996;51(3):267-271.
29. Pletz M, Ioanas M, De Roux A, Burkhardt O, Lode H. Reduced spontaneous apoptosis in peripheral blood neutrophils during exacerbation of COPD. Eur Respir J. 2004;23(4):532-537.
30. Segal AW. How neutrophils kill microbes. Annu Rev Immunol. 2005;23:197.

31. Sadik CD, Kim ND, Luster AD. Neutrophils cascading their way to inflammation. Trends Immunol. 2011;32(10):452-460.

32. Hiemstra P, Van Wetering S, Stolk J. Neutrophil serine proteinases and defensins in chronic obstructive pulmonary disease: Effects on pulmonary epithelium. Eur Respir J. 1998;12(5):1200-1208.

33. Hibbs MS, Hasty KA, Seyer JM, Kang AH, Mainardi CL. Biochemical and immunological characterization of the secreted forms of human neutrophil gelatinase. Journal of Biological Chemistry. 1985;260(4):2493-2500.

34. Delclaux C, Delacourt C, D’Ortho M-P, Boyer V, Lafuma C, Harf A. Role of gelatinase B and elastase in human polymorphonuclear neutrophil migration across basement membrane. Am J Respir Cell Mol Biol. 1996;14(3):288-295.

35. Larsson K. Aspects on pathophysiological mechanisms in COPD. Journal of Internal Medicine. 2007;262(3):311-340.

36. Milara J, Juan G, Peiró T, Serrano A, Cortijo J. Neutrophil activation in severe, early-onset COPD patients versus healthy non-smoker subjects in vitro: Effects of antioxidant therapy. Respiration. 2011;83(2):147-158.

37. Drost EM, Selby C, Bridgeman MM, Macnee W. Decreased leukocyte deformability after acute cigarette smoking in humans. Am Rev Respir Dis. 1993;148:1277-1277.
recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1998;158(6):1951-1957.

52. Dallas SL, Rosser JL, Mundy GR, Bonewald LF. Proteolysis of latent transforming growth factor-β (TGF-β) binding protein-1 by osteoclasts: A cellular mechanism for release of TGF-β from bone matrix. Journal of Biological Chemistry. 2002;277(24):21352-21360.

53. Mak JC, Rousell J, Haddad E-B, Barnes PJ. Transforming growth factor-β1 inhibits β2-adrenoceptor gene transcription. Naunyn Schmiedebergs Arch Pharmacol. 2000;362(6):520-525.

54. Russell RE, A Thorley, SV Culpitt, S Dodd, LE Donnelly, C Demattos, et al. Alveolar macrophage-mediated elastolysis: Roles of matrix metalloproteinases, cysteine, and serine proteases. Am J Physiol Lung Cell Mol Physiol. 2002;283(4):L867-L873.

55. Russell RE, SV Culpitt, C DeMatos, L Donnelly, M Smith, J Wiggins, et al. Release and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol. 2002;26(5):602-609.

56. Finlay GA, O'DRISCOLL LR, Russell KJ, D'arcy EM, Masterson JB, Fitzgerald MX, et al. Matrix metalloproteinase expression and production by alveolar macrophages in emphysema. Am J Respir Crit Care Med. 1997;156(1):240-247.

57. Ohnishi K, Takagi M, Kurokawa Y, Satomi S, Konttinen YT. Matrix metalloproteinase-mediated extracellular matrix protein degradation in human pulmonary emphysema. Lab Invest. 1998;78(9):1077-1087.

58. Churg A, Wang RD, Tai H, Wang X, Xie C, Dai J, et al. Macrophage metalloelastase mediates acute cigarette smoke-induced inflammation via tumor necrosis factor-α release. Am J Respir Crit Care Med. 2003;167(8):1083-1089.

59. Grumelli S, Corry DB, Song L-Z, Song L, Green L, Huh J, et al. An immune basis for lung parenchymal destruction in chronic obstructive pulmonary disease and emphysema. PLoS Med. 2004;1(1):e8.

60. Hautamaki RD, Kobayashi DK, Senior RM, Shapiro SD. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. Science. 1997;277(5334):2002-2004.

61. Churg A, Zay K, Shay S, Xie C, Shapiro SD, Hendricks R, et al. Acute cigarette smoke–induced connective tissue breakdown requires both neutrophils and macrophage metalloelastase in mice. Am J Respir Cell Mol Biol. 2002;27(3):368-374.

62. Molet S, Belleguic C, Lena H, Germain N, Bertrand C, Shapiro S, et al. Increase in macrophage elastase (MMP-12) in lungs from patients with chronic obstructive pulmonary disease. Inflammation Research. 2005;54(1):31-36.

63. Imai K, Dalal SS, Chen ES, Downey R, Schulman LL, Ginsburg M, et al. Human collagenase (matrix metalloproteinase-1) expression in the lungs of patients with emphysema. Am J Respir Crit Care Med. 2001;163(3):786-791.

64. MacNee W. Pulmonary and systemic oxidant/antioxidant imbalance in chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2005;2(1):50-60.

65. Giancotti FG, Ruoslahti E. Integrin signaling. Science. 1999;285(5430):1028-1033.

66. Barnes P, Shapiro S, Pauwels R. Chronic obstructive pulmonary disease: Molecular and cellular mechanisms. Eur Respir J. 2003;22(4):672-688.

67. Pandey R, Singh M, Singhal U, Gupta KB, Aggarwal SK. Oxidative/nitrosative stress and the pathobiology of chronic obstructive pulmonary disease. J Clin Diagn Res. 2013;7(3):580-588.

68. Nagorni-Obradović L, Pešut D, Škodrić-Trifunović V, Adžić T. Influence of tobacco smoke on the appearance of oxidative stress in patients with lung cancer and chronic obstructive pulmonary diseases. Vojnosanitetski pregled. 2006;63(10):893-895.

69. Rahman I, MacNee W. Role of oxidants/antioxidants in smoking-induced lung diseases. Free Radic Biol Med. 1996;21(5):669-681.

70. Rahman I, Van Schadewijk AA, Crowther AJ, Hiemstra PS, Stoik J, MacNee W, et al. 4-Hydroxy-2-nonenal, a specific lipid peroxidation product, is elevated in lungs of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2002;166(4):490-495.

71. Marrett LJ. Oxyradicals and DNA damage. Carcinogenesis. 2000;21(3):361-370.
72. Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L. Neutrophils: molecules, functions and pathophysiological aspects. Laboratory investigation. 2000;80(5):617-653.
73. Abboud R, Vimalanathan S. Pathogenesis of COPD. Part I. The role of protease-antiprotease imbalance in emphysema. [State of the Art Series. Chronic obstructive pulmonary disease in high-and low-income countries. Edited by G. Marks and M. Chan-Yeung. Number 3 in the series]. Int J Tuberc Lung Dis. 2008;12(4):361-367.
74. Koutsokera A, Kostikas K, Nicod LP, Fitting JW. Pulmonary biomarkers in COPD exacerbations: A systematic review. Respir Res. 2013;14(1).
75. Tzortzaki EG, Lambiri I, Vlachaki E, Siafakas NM. Biomarkers in COPD. Curr Med Chem. 2007;14(9):1037-1048.
76. Barnes PJ, Chowdhury B, Kharitonov SA, Magnussen H, Postma D, et al. Pulmonary biomarkers in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2012;174(1).
77. Tzouvelekis A, Pneumatikos I, Bouros D. Serum biomarkers in acute respiratory distress syndrome an ailing prognosticator. Respir Res. 2005;6(1):62.
78. Bhattacharya S, Srisuma S, DeMeo DL, Shapiro SD, Bueno R, Silverman EK, et al. Molecular biomarkers for quantitative and discrete COPD phenotypes. Am J Respir Cell Mol Biol. 2009;40(3):359.
79. Kubafi P, Foret F. Exhaled breath condensate: Determination of non-volatile compounds and their potential for clinical diagnosis and monitoring. A review. Anal Chim Acta. 2013;805:1-18.
80. Hunt J. Exhaled breath condensate: An evolving tool for noninvasive evaluation of lung disease. Journal of Allergy and Clinical Immunology. 2002;110(1):28-34.
81. Rosias P. Methodological aspects of exhaled breath condensate collection and analysis. J Breath Res. 2012;6(2):027102.
82. Mutlu GM, Garey KW, Robbins RA, Danziger LH, Rubinstein I. Collection and analysis of exhaled breath condensate in humans. Am J Respir Crit Care Med. 2001;164(5):731-737.
83. O'Reilly P, Bailey W. Clinical use of exhaled biomarkers in COPD. Int J Chron Obstruct Pulmon Dis. 2007;2(4):403.
asthma attacks. J Formos Med Assoc. 2012.

95. Kwiatkowska S, K Noweta, M Zieba, D Nowak, P Bialasiewicz. Enhanced exhalation of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in patients with COPD exacerbation: A prospective study. Respiration. 2012;84(3):231-241.

96. Warwick G, PS Thomas, DH Yates. Non-invasive biomarkers in exacerbations of obstructive lung disease. Respirology. 2013;18(5):874-884.

97. Corradi M, Rubinstein I, Andreoli R, Manini P, Caglieri A, Poli D, et al. Aldehydes in exhaled breath condensate of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2003;167(10):1380-1386.

98. Montuschi P, Khartonov S, Ciabattoni G, Barnes P. Exhaled leukotrienes and prostaglandins in COPD. Thorax. 2003;58(7):585-588.

99. Pinkerton M, Chinchilli V, Banta E, Craig T, August A, Bascom R, et al. Differential expression of microRNAs in exhaled breath condensates of patients with asthma, patients with chronic obstructive pulmonary disease, and healthy adults. The Journal of allergy and clinical immunology. 2013;132(1):217-219.e2.

100. Bartel DP. MicroRNAs: Target Recognition and Regulatory Functions. Cell. 2009;136(2):215-233.

101. Pritchard CC, Cheng HH, Tewari M. MicroRNA profiling: Approaches and considerations. Nat Rev Genet. 2012;13(5):358-369.

102. Chapin SC, Appleyard DC, Pregibon DC, Doyle PS. Rapid microRNA profiling on encoded gel microparticles. Angew Chem Weinheim Bergstr Ger. 2011;123(10):2337-2341.

103. Dong H, Lei J, Ding L, Wen Y, Ju H, Zhang X. MicroRNA: function, detection, and bioanalysis. Chem Rev. 2013;113(8):6207-6233.

104. Yin JQ, Zhao RC, Morris KV. Profiling microRNA expression with microarrays. Trends Biotechnol. 2008;26(2):70-76.

105. Pagdin T, Lavender P. MicroRNAs in lung diseases. Thorax. 2012;67(2):183-184.

106. Schembri F, Sridhar S, Perdomo C, Gustafson AM, Zhang X, Ergun A, et al. MicroRNAs as modulators of smoking-induced gene expression changes in human airway epithelium. Proceedings of the National Academy of Sciences. 2009;106(7):2319-2324.

107. Christenson SA, Brandsma CA, Campbell JD, Knight DA, Pechkovsky DV, Hogg JC, et al. MiR-638 regulates gene expression networks associated with emphysematous lung destruction. Genome Med. 2013;114.

108. Johnnidis JB, Harris MH, Wheeler RT, Stehling-Sun S, Lam MH, Kirak O, et al. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. Nature. 2008;451(7182):1125-1129.

109. Fazi F, Rosa A, Fatica A, Gelmetti V, De Marchis ML, Nervi C, et al. A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBPa regulates human granulopoiesis. Cell. 2005;123(5):819-831.

110. Ezze ME, Crawford M, Cho J-H, Orellana R, Zhang S, Gelinas R, et al. Gene expression networks in COPD: microRNA and mRNA regulation. Thorax. 2012;67(2):122-131.