Biomarkers in bronchiectasis

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Bronchiectasis is a heterogeneous disease with multiple causes and a highly variable clinical presentation. Biomarkers and the identification of endotypes hold promise in guiding clinical care and therapy. This review examines the evidence for biomarkers. https://bit.ly/3vqohYG

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Abstract

Bronchiectasis is a heterogeneous disease with multiple aetiologies and diverse clinical features. There is a general consensus that optimal treatment requires precision medicine approaches focused on specific treatable disease characteristics, known as treatable traits. Identifying subtypes of conditions with distinct underlying biology (endotypes) depends on the identification of biomarkers that are associated with disease features, prognosis or treatment response and which can be applied in clinical practice. Bronchiectasis is a disease characterised by inflammation, infection, structural lung damage and impaired mucociliary clearance. Increasingly there are available methods to measure each of these components of the disease, revealing heterogeneous inflammatory profiles, microbiota, radiology and mucus and epithelial biology in patients with bronchiectasis. Using emerging biomarkers and omics technologies to guide treatment in bronchiectasis is a promising field of research. Here we review the most recent data on biomarkers in bronchiectasis.

Introduction

Bronchiectasis is an increasingly common, and uniquely heterogeneous disease diagnosed when permanent bronchial dilatation is seen on chest high-resolution computed tomography (HRCT) [1–4]. This bronchial dilatation is the outcome of a "vicious vortex" of airway inflammation, recurrent infection and epithelial dysfunction where these factors become mutually reinforcing and lead to irreversible structural airway damage [5, 6]. Bronchiectasis has a major impact on patients' quality of life due to symptoms of daily cough, sputum production and recurrent respiratory infection which ultimately result in long-term lung function decline and increased mortality [7, 8]. It is associated with substantial healthcare costs, driven primarily by hospitalisation and medication costs among patients with severe disease [9].

Disease heterogeneity in bronchiectasis

Multiple documented underlying aetiologies and susceptibility factors lead to bronchiectasis. Predominant aetiologies, clinical features and comorbidities vary substantially between individual patients and communities worldwide [10–12]. This poses a treatment challenge, as different aetiologies and clinical phenotypes may require vastly different treatment approaches [13]. Drug trials in bronchiectasis have often failed to reach their end-points, leading to a lack of evidence-based treatments [14]. There are currently no licensed bronchiectasis-specific therapies and treatment is primarily airway clearance and short- and long-term antibiotics [2].

In recognition of the challenge posed by disease heterogeneity, the bronchiectasis paradigm has shifted in recent years towards a "treatable traits" approach, seeking to group patients by clinical phenotype to target effective treatments and guide recruitment to clinical trials [14–16].

One approach to phenotyping is by aetiology. This is important in clinical practice because some underlying conditions require specific treatment, such as immunodeficiency, allergic bronchopulmonary aspergillosis, nontuberculous mycobacterial infection, connective tissue disease or cystic fibrosis [1–3].

However, disease severity and prognosis can vary within aetiologies, and a recent European Multicentre Bronchiectasis Audit and Research Collaboration (EMBARC) analysis of 16 963 patients across Europe found 59% of patients had idiopathic or post-infective disease which has no specific treatment [12, 13].

Several studies have sought to identify bronchiectasis phenotypes based on clinical features. Across three separate cluster analyses by Guan *et al.* [17], Martínez-García *et al.* [18] and Aliberti *et al.* [19], *Pseudomonas aeruginosa* infection was persistently linked to poorer outcomes. Chalmers *et al.* [20] identified a "frequent exacerbator" phenotype associated with higher risk of future exacerbations, hospitalisations and mortality. Phenotypic features have been combined to create bronchiectasis severity scores, including the Bronchiectasis Severity Index (BSI) and the FACED (forced expiratory volume in 1 s (FEV_1), age, chronic colonisation by *P. aeruginosa*, radiological extension and dyspnoea) and E-FACED (FACED plus exacerbations) scores, which accurately predict exacerbations, hospitalisation and mortality [21–23].

Why do we need biomarkers in bronchiectasis?

Clinical phenotypes are a useful means of stratifying patients based on risk of adverse outcomes, but they provide little information about underlying pathophysiology, and in most cases do not predict treatment response. A good example of this is the frequently exacerbating patients. This is clearly a phenotype, in that many patients experience persistent exacerbations and this is linked to poorer outcomes, but the mechanisms leading to exacerbations are diverse, so treatments to prevent exacerbations must be personalised.

To identify targets for new bronchiectasis treatments, identify which patients will benefit from these treatments, and accurately measure treatment response, the biological processes driving the disease must be understood and patients grouped by predominant biological mechanism or "endotype".

This has become particularly clear as the central role of inflammation in the pathophysiology of bronchiectasis is recognised [6]. Research advances including multiomics technologies allow complex immune profiling of blood, sputum and bronchoalveolar lavage (BAL) samples from bronchiectasis patients to investigate disease heterogeneity on a molecular level. Distinct inflammatory endotypes have been identified associated with clinical outcomes [5, 24–26]. New therapies targeting inflammatory pathways have shown promising initial results [27].

Identifying biomarkers indicative of inflammatory and molecular endotypes holds potential to guide individualised therapies for bronchiectasis. This review discusses biomarkers relating to each aspect of the bronchiectasis vicious vortex: inflammation, infection, epithelial dysfunction and impaired mucociliary clearance; the outcome of structural airway damage; and how these may be applied to aid precision medicine (figure 1).

Biomarkers of inflammation

Airway biomarkers

Neutrophilic inflammation

Studies have consistently identified neutrophilic inflammation as a driver of disease severity in bronchiectasis. High levels of neutrophils and neutrophil-associated proteins are observed in bronchiectasis sputum and BAL (figure 2) [28, 29]. Neutrophils are recruited to the airways by inflammatory cytokines in response to infection and, under normal physiological conditions, contribute to host defence primarily through phagocytosis of pathogens [30, 31]. Other neutrophil defence mechanisms include degranulation and release of neutrophil extracellular traps (NETs) which are web-like structures, composed of decondensed chromatin, DNA, histones and granule contents extruded into the extracellular space to disarm and trap pathogens [32, 33].

The most clinically accessible and inexpensive neutrophil biomarker in practice is sputum colour. The green colour of purulent sputum is due to haem pigment present in myeloperoxidase (MPO) released into the airways during degranulation and NETosis [34–36]. Sputum purulence is associated with bacterial infection, radiological severity and quality of life in bronchiectasis [36, 37]. In the AIR-BX studies, patients with more cough and purulent sputum were more likely to experience symptomatic improvement in response to inhaled aztreonam [38]. Therefore, sputum colour alone, assessed using tools such as the Murray chart, is a biomarker of neutrophilic airways inflammation with potential to guide therapy [37]. However, it remains a proxy measure. Markers of specific elements of the inflammatory cascade hold potential to further characterise neutrophilic endotypes and aid drug development.

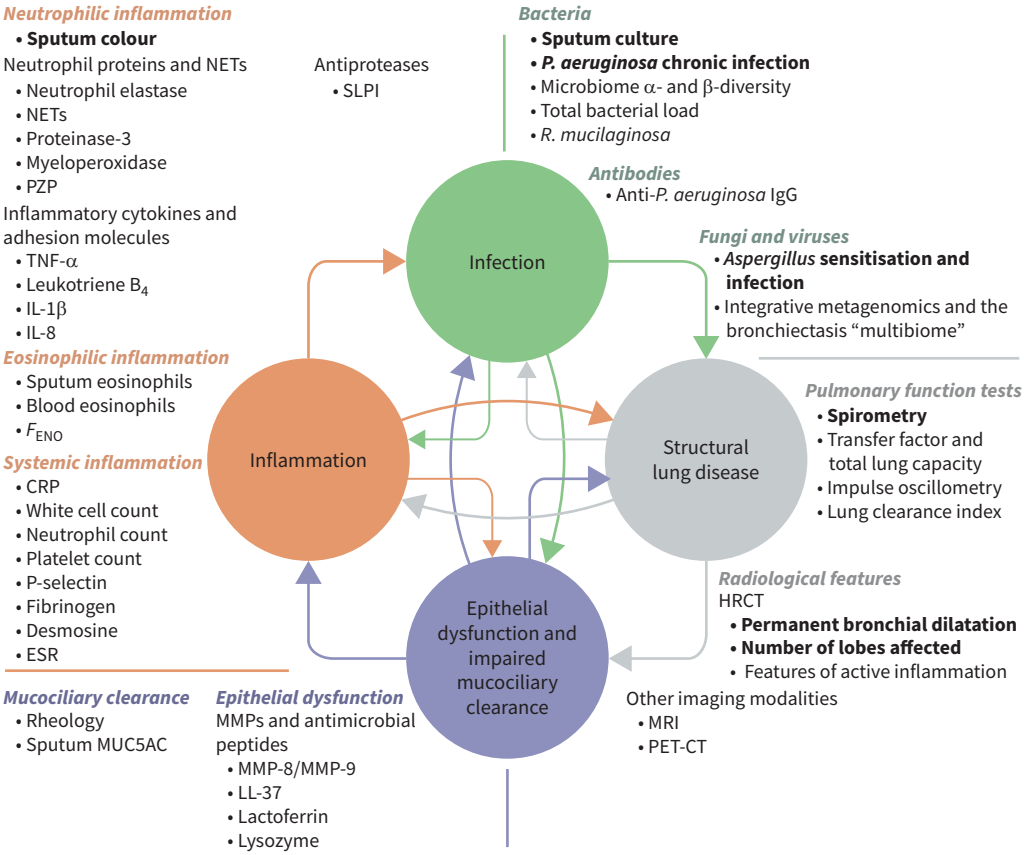

FIGURE 1 Established and exploratory biomarkers related to each aspect of the bronchiectasis vicious vortex: inflammation, infection, epithelial dysfunction and impaired mucociliary clearance, and structural lung damage. Established biomarkers are shown in bold. NETs: neutrophil extracellular traps; PZP: pregnancy zone protein; SLPI: secretory leukocyte protease inhibitor; TNF: tumour necrosis factor; IL: interleukin; F_{ENO}: exhaled nitric oxide fraction; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; MMP: matrix metalloproteinase; *P. aeruginosa*: *Pseudomonas aeruginosa*; *R. mucilaginosa*: *Rothia mucilaginosa*; HRCT: high-resolution computed tomography; MRI: magnetic resonance imaging; PET: positron emission tomography; CT: computed tomography. Reproduced and modified from [5] with permission. Figure created with biorender.com.

Neutrophil proteases and NETs

NETs and NET-associated proteins are prospective biomarkers of a bronchiectasis endotype defined by excessive, dysregulated neutrophilic inflammation which may respond to antineutrophil therapies. A multicohort observational study found sputum NET concentration, measured using complexes between DNA/histones and neutrophil elastase, was associated with multiple bronchiectasis severity markers including BSI, exacerbations and mortality (table 1). Proteomic analysis also showed that NET-associated proteins including MPO, neutrophil elastase, resistin and azurocidin-1 identified a group of patients with severe disease. Sputum NET concentration has been linked to microbial dysbiosis, and both intravenous antibiotic therapy and macrolide therapy successfully reduced sputum NETs [29].

Of the NET-associated proteins, neutrophil elastase is furthest advanced in the process of becoming a validated biomarker. Neutrophil elastase is a neutrophil serine protease (NSP) stored in the primary (azurophilic) granules. It is released through both degranulation and NETosis and degrades extracellular matrix proteins and virulence factors of various Gram-negative bacteria. However, it also cleaves proteins on host cells, leading to epithelial damage and airway remodelling as well as inducing mucus hypersecretion and hyperviscosity [75–77]. Sputum neutrophil elastase activity correlates with exacerbations, lung function decline and bacterial infection in bronchiectasis [39–42]. A point-of-care test has been developed (NEATstik) which scores sputum neutrophil elastase concentration on a 10-point scale and is able to identify patients at higher risk of exacerbation [78].

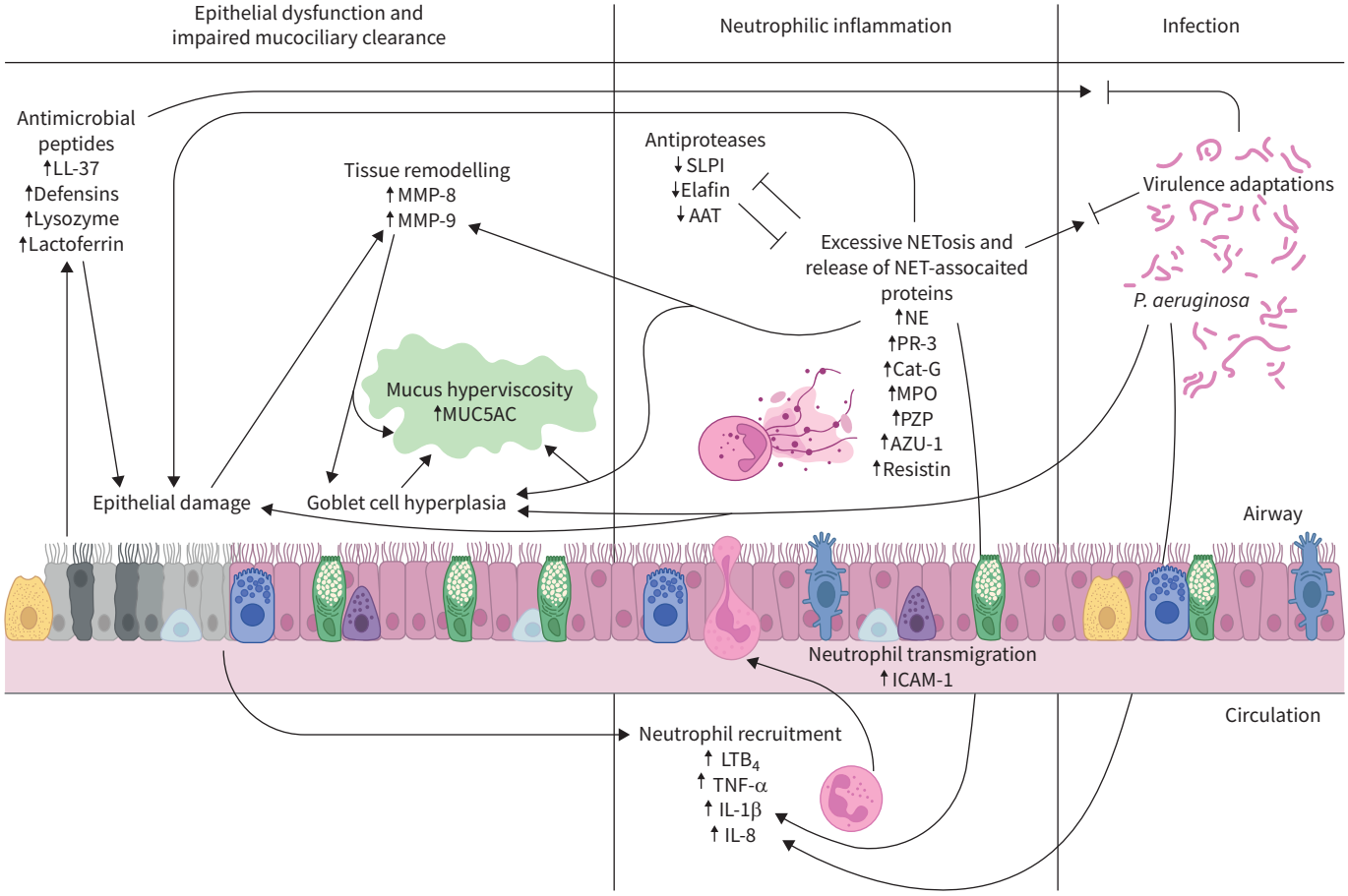

FIGURE 2 Known interactions between biomarkers of neutrophilic inflammation, infection, epithelial dysfunction and mucociliary clearance in bronchiectasis. MMP: matrix metalloproteinase; SLPI: secretory leukocyte protease inhibitor; AAT: alpha-1 antitrypsin; NETs: neutrophil extracellular traps; NE: neutrophil elastase; PR: proteinase; MPO: myeloperoxidase; PZP: pregnancy zone protein; AZU: azurocidin; ICAM: intracellular adhesion molecule; LTB_4: leukotriene B_4; TNF: tumour necrosis factor; IL: interleukin. Figure created with biorender.com.

Neutrophil elastase is a particularly clinically relevant biomarker because novel anti-inflammatory therapies targeting NSPs are currently in development. Preliminary trials of direct neutrophil elastase inhibition have shown mixed results [79, 80]. However, dipeptidyl peptidase (DPP)-1 inhibitors are a promising new therapy in development. DPP-1 cleaves and activates NSPs including neutrophil elastase during neutrophil maturation in the bone marrow [81, 82]. In the WILLOW phase 2 trial (clinicaltrials.gov identifier NCT03218917) of the DPP-1 inhibitor brensocatib (10 mg and 25 mg) *versus* placebo, brensocatib prolonged time to first exacerbation and reduced neutrophil elastase activity in patients with bronchiectasis, and a large phase 3 trial is now underway (clinicaltrials.gov identifier NCT04594369) [27].

Brensocatib has also been shown to reduce the NSPs proteinase (PR)-3 and cathepsin-G (Cat-G) which are also activated by DPP-1 and have similar proteolytic activity to neutrophil elastase [83, 84]. Both have been linked to disease severity in COPD [85, 86]. Preliminary data suggest that sputum PR-3 is raised in bronchiectasis exacerbations [43]. Further clinical associations with these proteases in bronchiectasis, and their role in response to anti-inflammatory therapies, are yet to be uncovered.

MPO is another NET-associated enzyme responsible for reactive oxygen species generation. As well as its role in sputum purulence it is independently associated with radiological severity and exacerbation risk in bronchiectasis [19, 44]. In a study of 28 bronchiectasis patients randomised to inhaled gentamicin *versus* control, sputum MPO fell significantly in response to treatment in association with reduced sputum volume and improved peak expiratory flow and 6-min walk distance [45].

Additionally, Finch *et al.* [47] demonstrated that pregnancy-zone protein (PZP), a serum protein with known antiprotease and T-cell immunosuppressive effects, was present in the cytoplasm of neutrophils and

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TABLE 1 Inflammatory, epithelial dysfunction and mucociliary clearance biomarkers in bronchiectasis and clinical associations

	Clinical associations								
	Radiological severity	Lung function	Exacerbations	Bacterial colonisation	Severity scores	Acute infection	Antibiotic response	Mortality	References
Neutrophilic inflammation									
Neutrophil proteins									
NE	●	●	●	●	●	●	●		[39–42]
NETs			●	●	●		●	●	[29]
PR-3						●			[43]
MPO	●			●	●		●		[19, 44–46]
PZP			●	●	●				[47]
Protease/antiprotease imbalance									
SLPI		●	●	●	●				[26, 48]
Inflammatory cytokines and adhesion molecules									
LTB_4	●	●	●						[49]
TNF-α	●			●		●	●		[50, 51]
IL-1β	●			●		●	●		[19, 44, 52]
IL-8	●			●		●	●		[44, 50, 52]
Eosinophilic inflammation									
Blood eosinophils			●		●			●	[53, 54]
F_{ENO}		●			●				[55]
Epithelial dysfunction and mucociliary clearance									
Mucins									
MUC5AC				●	●		●		[56–58]
MMPs									
MMP-9	●	●	●		●				[26, 59–61]
MMP-8	●	●			●				[59, 60, 62]
Antimicrobial peptides									
LL-37		●	●	●	●				[26, 48]
Lactoferrin				●					[26, 48]
Lysozyme				●					[26, 48]
Systemic inflammatory markers									
CRP	●		●	●	●	●	●	●	[50, 63–66]
White cell count	●	●				●	●		[50, 51, 63, 67, 68]
Neutrophil count	●	●				●	●		[46, 51, 68]
Neutrophil/lymphocyte ratio				●		●			[63, 69]
Platelet count			●		●	●		●	[70]
p-Selectin					●				[71]
Fibrinogen			●	●	●	●			[72, 73]
ESR			●				●		[67, 68]
Desmosine								●	[74]

NE: neutrophil elastase; NETs: neutrophil extracellular traps; PR: proteinase; MPO: myeloperoxidase; PZP: pregnancy zone protein; SLPI: secretory leukocyte protease inhibitor; LTB_4: leukotriene B_4; TNF: tumour necrosis factor; IL: interleukin; F_{ENO}: exhaled nitric oxide fraction; MMP: matrix metalloproteinase; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate. Reproduced and modified from Amaro *et al.* [25] with permission.

released in NETs. Sputum PZP was linked with high BSI and exacerbation risk, as well as higher airway bacterial load, and was reduced in response to antibiotics. This study determined that PZP is a biomarker of an endotype characterised by NET formation and high bacterial load and is a possible mechanism through which neutrophils alter the adaptive immune response to bacteria by modifying T-cell function.

Protease/antiprotease imbalance

In the normal immune response, the actions of NSPs are tightly regulated by antiproteases. Three key antiproteases that regulate neutrophilic inflammation in the lung are secretory leukocyte protease inhibitor (SLPI), elafin and alpha-1 antitrypsin (AAT). Protease/antiprotease imbalance leading to unopposed NSP activity is probably a contributor to excessive neutrophil-mediated epithelial damage in bronchiectasis [87].

SLPI is an inhibitor of neutrophil elastase and Cat-G, and elafin inhibits both neutrophil elastase and PR-3 [88]. Both are cleaved and degraded by neutrophil elastase, diminishing their ability to mitigate NSP-mediated epithelial damage [89–91]. Deficiency of both these antiproteases is linked to disease severity in COPD and cystic fibrosis [92, 93]. In bronchiectasis, SLPI deficiency is linked to higher BSI, poorer pulmonary function and *P. aeruginosa* infection (table 1) [26, 48]. In sputum proteomic analysis, SLPI was upregulated in response to antibiotic therapy [29]. SLPI is therefore a potential future tool to identify bronchiectasis endotypes defined by protease/antiprotease imbalance to target anti-inflammatory therapies [87].

Inflammatory cytokines

In addition to neutrophils themselves, multiple inflammatory cytokines contribute to neutrophilic inflammatory pathways in the lung (figure 2). Neutrophils are recruited to the airways by cytokines including leukotriene B_4 (LTB_4), interleukin (IL)-8, IL-1β and tumour necrosis factor (TNF)-α released by macrophages, endothelial/epithelial cells and other cell types primarily in response to bacteria, but also in the absence of active infection in bronchiectasis [31, 67, 94].

In a small study by Bedi *et al.* [49], the powerful chemoattractant LTB_4 was significantly increased in patients with moderate to severe compared with mild bronchiectasis and healthy controls and correlated with lower FEV_1 % predicted and higher number of antibiotic courses for exacerbations. Other studies have shown that sputum IL-8, IL-1β and TNF-α levels correspond to radiological severity and sputum bacterial load and decrease following intravenous and nebulised antibiotic treatment (table 1) [44, 50, 52].

Unanswered questions for markers of neutrophilic inflammation

The described studies have established a clear link between neutrophilic inflammation and bronchiectasis severity and identified multiple candidate biomarkers for development of targeted antineutrophil therapies. Ongoing research must clarify the relationships between these biomarkers and whether all are equally representative of neutrophilic inflammation or whether multiple neutrophilic endotypes exist defined by different neutrophil proteins or processes.

There is evidence that neutrophils in bronchiectasis have altered function. Notably, Bedi *et al.* [46] reported impaired phagocytosis, delayed apoptosis and prolonged lifespan in neutrophils from bronchiectasis patients compared with controls [46, 95, 96].

A possible mechanism for altered neutrophil function is neutrophil heterogeneity. Discrete neutrophil subsets, identifiable by cell surface markers, are now recognised. Well-characterised neutrophil subsets include the $CD63^+$, $CD177^+$, $CXCR4^{hi}CD62L^{low}$ subset, and $CD64^+$ subsets. CD63 is a tetraspanin involved in retaining neutrophil elastase in neutrophil granules and surface CD63 expression correlates with extracellular neutrophil elastase release [97, 98]. The $CD177^+$ neutrophil subset expresses PR-3 on the cell surface, which is proteolytically active and more resistant to inhibition than soluble PR-3 [99, 100]. The $CXCR4^{hi}CD62L^{low}$ subset is indicative of "aged" neutrophils marked for clearance to the bone marrow [100] and Zhang *et al.* [101] demonstrated that aged neutrophils display increased NETosis. CD64 is a complement receptor responsible for phagocytosis of opsonised pathogens. Additionally, the $CD63^+$ and $CXCR4^{hi}CD62L^{low}$ subsets both have increased expression of Cd11b, an integrin molecule involved in neutrophil migration and attachment [100].

Changes in neutrophil subsets have been reported in several inflammatory diseases including asthma, COPD and coronavirus disease 2019, but their role in neutrophilic inflammation and clinical outcomes in bronchiectasis has not yet been studied [79, 102–105]. Bedi *et al.* [46] found increased neutrophil cell-surface CD11b and decreased CD62L expression in severe bronchiectasis. However, other studies have found no difference in neutrophil phagocytosis or CD11b expression in bronchiectasis suggesting further study is needed [95, 96, 106].

Eosinophilic inflammation

Neutrophilic inflammation has been considered the predominant inflammatory endotype in bronchiectasis. However, it has been recognised recently that eosinophilic inflammation also plays a role, at least in a subset of bronchiectasis patients.

Eosinophils are markers of type 2 T-helper (Th2) inflammation which plays a well-established role in atopic and allergic lung diseases [107]. Sputum eosinophilia is the gold standard for identifying asthma and COPD endotypes with improved response to inhaled corticosteroids [108]. In bronchiectasis patients without asthma, sputum eosinophilia has been linked to greater bronchodilator reversibility and to other sputum markers of Th2 response including IL-13 [109, 110].

In a European multicohort study of 1007 patients with bronchiectasis, SHOEMARK *et al.* [53] found that 22.6% of patients with bronchiectasis had raised sputum eosinophils >3%. Importantly, they showed that a blood eosinophil count >300 cells·μL^{-1} correlated with sputum eosinophilia, making it a credible biomarker of airway Th2 inflammation. Low blood eosinophil counts were linked to disease severity and mortality and, following antibiotic treatment for *P. aeruginosa*, eosinophilic patients had shorter time to first exacerbation compared to non-eosinophilic patients.

Exhaled nitric oxide fraction (F_{ENO}) is an additional marker of airway Th2 inflammation already routinely used as a point-of-care test in asthma [111]. In an observational study of 249 bronchiectasis patients, ORIANO *et al.* [55] identified that a Th2-high endotype defined by increased F_{ENO} ⩾25 dpp and blood eosinophils >300 cells·μL^{-1} was present in 31% of bronchiectasis patients without asthma. In this study, Th2 inflammation was associated with higher BSI score, lower FEV_1 % predicted and increased dyspnoea.

Existence of an eosinophilic phenotype identifiable by easily obtainable blood and F_{ENO} tests has important implications for treatment. Treatments used for Th2-mediated lung disease, including corticosteroids and biological agents, are not currently recommended in bronchiectasis [1, 2]. *Post hoc* analysis of a randomised control trial of inhaled fluticasone in bronchiectasis found that patients with blood eosinophils >3% had a greater improvement in quality of life compared with noneosinophilic patients [54]. A case series of 12 patients showed improvement in lung function, chronic symptoms and exacerbations following treatment with the monoclonal antibodies mepolizumab and benralizumab in bronchiectasis patients with blood eosinophils >300 cells·μL^{-1} [112]. Large-scale prospective studies are required to confirm these reports. Further investigation of the interaction between Th1 and Th2 inflammatory endotypes and their respective biomarkers is also needed to guide therapies targeting Th2 inflammation.

Systemic inflammatory markers

A number of studies have examined "routine" blood tests and their relationship to disease characteristics (table 1). Blood white cell and neutrophil counts were shown to rise in acute bronchiectasis exacerbations, fall in response to therapy, and correlate with radiological severity and poorer pulmonary function [17, 63, 68, 69]. Additionally, a study of 802 patients from the Spanish Registry of Bronchiectasis (RIBRON) found that chronically raised C-reactive protein (CRP) was associated with increased risk of severe exacerbations [113]. Raised CRP has also been found to predict bacterial infection, decrease in response to antibiotic therapy and correlate with radiological disease severity [64, 65, 114]. However, these markers are induced by multiple inflammatory processes and lack specificity to identify distinct bronchiectasis endotypes that will respond to targeted anti-inflammatory therapies.

Fibrinogen, a key component of the coagulation cascade as well as acute phase reactant, has been suggested as a biomarker of a pro-inflammatory phenotype in COPD [115, 116]. In bronchiectasis, LEE *et al.* [72] found that fibrinogen was associated with increased BSI and FACED scores, as well as increased exacerbation risk. Principal component analysis of 31 proteins from 90 bronchiectasis patients conducted by SALEH *et al.* [73] also distinguished fibrinogen as a marker of disease severity, driven primarily by poorer lung function and *P. aeruginosa* colonisation. Similarly, erythrocyte sedimentation rate, which is dependent primarily on the concentration of circulating fibrinogen, has been shown to mark exacerbations and treatment response in bronchiectasis [67, 68].

Aα-Val360, produced specifically when fibrinogen is cleaved by neutrophil elastase, has been measured in the plasma of COPD patients and found to correlate with multiple severity markers and rise during exacerbations, although it has not yet been studied in bronchiectasis [117, 118]. Another degradation product, desmosine, which is released when elastin (a key component of the extracellular matrix in blood vessels and the lung) is degraded by neutrophil elastase, was found to correlate with both sputum

neutrophil elastase and severe exacerbations in bronchiectasis [39, 119]. In cardiovascular disease, plasma desmosine is associated with greater atherosclerotic burden and poorer outcomes after myocardial infarction [120, 121]. In bronchiectasis, HUANG and co-workers [74, 120, 121] demonstrated a link between serum desmosine and increased cardiovascular mortality, including when other risk factors such as age and comorbidities were adjusted for.

Systemic inflammation is also associated with platelet aggregation at sites of infection [122]. In a study by ALIBERTI *et al.* [70] of 1771 bronchiectasis patients, those with thrombocytosis experienced more severe disease, poorer quality of life, higher exacerbation risk severity and higher 3- and 5-year mortality. MÉNDEZ *et al.* [71] found that soluble p-selectin, a marker of platelet activation also involved in leukocyte recruitment, was increased in severe bronchiectasis, further supporting the association between platelet aggregation and activation and bronchiectasis severity.

Levels of intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 are also upregulated through inflammatory pathways in bronchiectasis [67, 123]. In particular, circulating ICAM-1, which is released by endothelial cells and immune cells and regulates neutrophil transmigration into the airways, is associated with radiological severity and sputum bacterial load and decreases following treatment with intravenous and nebulised antibiotics [44, 50, 52, 124]. Both ICAM-1 and VCAM-1 are linked to vascular inflammation and cardiovascular disorders [125].

Bronchiectasis is associated with increased cardiovascular risk. ∼30% of patients with bronchiectasis have a cardiovascular comorbidity and this risk increases with disease severity [12, 126, 127]. These markers provide insight into possible mechanisms behind the relationship between neutrophilic lung inflammation, systemic inflammation, exacerbations and cardiovascular disease and may in future aid risk stratification and targeted interventions to address cardiovascular risk in bronchiectasis [128].

Infection biomarkers

Sputum culture is an easily obtainable "biomarker" to identify bacteria causing acute exacerbations and chronic symptoms [129]. *Haemophilus influenzae* and *P. aeruginosa* are the bacterial species most frequently observed in bronchiectasis [130]. Both are associated with disease severity, but *P. aeruginosa* is currently the most well-established as prognostic marker. *P. aeruginosa* is capable of biofilm formation which conveys resistance to antimicrobials, and other virulence adaptations which disarm neutrophil defence mechanisms including NETs (figure 2) [131–134]. *P. aeruginosa* infection can be chronic or intermittent and bronchiectasis patients with chronic infection display poorer lung function, higher number of exacerbations and increased mortality [17–19, 135–137].

Identifying patients with chronic infection is therefore important to guide appropriate suppressive antibiotic treatment. In addition to sputum culture, serum anti-*P. aeruginosa* IgG measured using ELISA was found to accurately detect chronic *P. aeruginosa* infection in a study of 408 bronchiectasis patients [138]. This raises the possibility of future point-of-care testing to enable immediate treatment decisions in bronchiectasis clinic.

Interestingly, patients with chronic *P. aeruginosa* infection show therapeutic response to macrolide therapy despite a lack of susceptibility to this antibiotic [139]. There is evidence that the broader immunomodulatory effects of macrolides, including direct action against NETs, are responsible for this effect [29], but it is also becoming clear that the relevance of bacteria in bronchiectasis extends beyond dominant pathogens identified by culture. DICKER *et al.* [137] used 16s rNA sequencing to profile the microbiome and found proteobacteria, particularly *P. aeruginosa*, predominated in severe disease. However, they also demonstrated that the composition of the microbiome remained relatively stable even during exacerbations and that α-diversity (a measure of the richness and evenness of different bacterial populations within a sample) was associated with increased exacerbation risk, poorer lung function and more severe disease. It has also been recognised that some bacteria, such as *Rothia mucilaginosa* exert anti-inflammatory properties and that such commensal organisms are depleted in severe disease [140]. This suggests that interplay between bacteria may be equally or more significant than individual pathogens alone.

Total bacterial load is also a potentially useful and accessible biomarker. Patients with higher overall bacterial load are at higher risk of exacerbation and this correlates with increased inflammation [44]. A retrospective analysis of the AIR-BX studies showed that patients with high overall sputum bacterial load experienced significantly improved quality of life when treated with inhaled aztreonam, whereas there was no improvement in those with low airway bacterial load, suggesting this is a biomarker of risk and treatment response [141].

The roles of the mycobiome and virome in the pathophysiology of bronchiectasis are also being increasingly studied. *Aspergillus* sensitisation and infection are common among bronchiectasis patients and distinct mycobiome profiles have been linked to clinical outcomes [142–144]. Similarly, viruses are an important but under-reported cause of exacerbations [145, 146]. Integrated analysis of the interaction between bacterial, fungal and viral communities in the bronchiectasis "multibiome" has been proposed as the future of stratifying bronchiectasis patients and holds exciting potential as a biomarker strategy for endotype-guided therapies [147, 148].

It is increasingly clear that molecular diagnostics such as PCR and sequencing provide additional information beyond that available from culture. These methods have previously been prohibitively time consuming and costly but rapid molecular diagnostics are increasingly available in clinical practice.

Epithelial dysfunction and mucociliary clearance biomarkers

Mucociliary clearance

Viscous sputum is a defining clinical feature of bronchiectasis. Respiratory physiotherapy and mucolytic drugs to aid mucus clearance are core aspects of bronchiectasis management.

It is possible to directly measure sputum parameters using rheology, which has demonstrated higher sputum elasticity and viscosity in bronchiectasis patients than healthy controls [149, 150]. Rheological parameters are associated with pulmonary function and exacerbations in cystic fibrosis [151]. A small study by Ramos *et al.* [152] of bronchiectasis physiotherapy techniques used rheology to demonstrate increased removal of viscoelastic mucus using postural drainage and percussion or huffing, compared with coughing alone.

Viscosity of airway mucus can also be determined by measuring the mucins MUC5B and MUC5AC. Bronchiectasis patients display increased airway concentration of these mucins and an increase in the ratio of MUC5AC to MUC5B, resulting in failure of mucociliary transport and mucus impaction of the airways (figure 2) [150]. Contributing mechanisms include *P. aeruginosa* secretion of the exotoxin pyocyanin which increases production of both MUC5B and MUC5AC and neutrophil elastase also upregulates MUC5AC [76, 153, 154]. Increased MUC5AC levels correlate with rheological parameters and with increased BSI score and bacterial colonisation, while use of prophylactic macrolide therapy reduces sputum MUC5AC concentrations with a resultant symptomatic improvement (table 1) [56–58].

Matrix metalloproteinases

Matrix metalloproteinases (MMPs), produced by a variety of cell types in response to tissue damage, degrade collagen and are responsible for lung tissue remodelling. Overexpression of MMPs in chronic inflammation can lead to excessive tissue destruction [155, 156]. Taylor *et al.* [59] studied nine MMPs in sputum from bronchiectasis patients and discovered five were significantly altered in patients with bronchiectasis compared to healthy controls. In particular, MMP-2, which is produced by lymphocytes, epithelial cells and fibroblasts, was increased in patients with less severe airway remodelling. Conversely, MMP-9, which is released along with MMP-8 from neutrophil-specific granules, was raised in patients with extensive structural airway damage. MMP-9 expression is increased by *P. aeruginosa*, and there is evidence that MMP-9 induces goblet cell hyperplasia and upregulates MUC5AC production with implications for mucociliary clearance (figure 2) [157, 158]. Subsequent studies have linked high airway levels of MMP-8 and MMP-9 to more extensive structural disease, impaired lung function, *P. aeruginosa* infection and increased BSI (table 1) [60–62, 159]. MMP-9 is directly activated by neutrophil elastase, suggesting that therapies targeting NSPs may effectively reduce MMP-9 [77].

Antimicrobial peptides

Antimicrobial peptides (AMPs) form part of the innate immune response. Key AMPs include LL-37, defensins, lysozyme and lactoferrin. In the lung, these peptides are produced primarily by respiratory epithelial cells, but also by macrophages and neutrophils, and are present in airway surface liquid. They demonstrate direct antimicrobial activity, but also induce expression of pro-and anti-inflammatory cytokines and contribute to epithelial cell repair [160]. However, in the dysregulated inflammatory environment of the bronchiectasis lung, AMPs may contribute to perpetuating chronic inflammation (figure 2). Higher levels of AMPs are linked to chronic *P. aeruginosa* infection in bronchiectasis, while LL-37 is also associated with poorer lung function and higher BSI score [48]. A recent cluster analysis by Perea *et al.* [26] was able to group patients according to AMP levels, with the highest levels identifying patients with the most severe disease (table 1).

Structural lung damage biomarkers

Radiology

HRCT evidence of permanent bronchial dilatation demonstrates irreversible structural airways damage and is the current gold-standard diagnostic criteria for bronchiectasis [1, 2]. Radiological criteria for diagnosis are an increased bronchoarterial ratio, bronchus visible within 1 cm of the pleural surface and lack of normal airway tapering [161].

Radiological characteristics are heterogenous and can give important clues to aetiology [162]. For example, lower lobe bronchiectasis is more common in infection or aspiration; middle lobe bronchiectasis is associated with nontuberculous mycobacteria or primary ciliary dyskinesia; and central bronchiectasis is suggestive of allergic bronchopulmonary aspergillosis (figure 3) [163]. However, radiological diagnosis is far from standardised due to differences in scan protocols between centres and scan appearances at different levels of inspiration, lack of age- and sex-related reference values, and dependence on the size of the adjacent pulmonary artery, which is altered in several diseases [164–166]. Radiological bronchiectasis has been documented in up to 20% of asymptomatic adults aged >65 years, while patients with persistent bacterial bronchitis at risk of progression to bronchiectasis may have significant symptom burden, but miss early preventative treatment as they do not fulfil radiological diagnostic criteria [167–169].

HRCT is also used to assess disease severity. The classification system developed by Lynne McArthur Reid in 1950 into cylindrical, varicose and saccular subtypes has been expanded into radiological severity scores. The Bhalla score includes factors such as bronchial wall thickening and mucus plugging, which are suggestive of active inflammation [170]. However, due to its complexity, it is ill-suited for use in clinical practice. The Reiff score, which focuses on extent of bronchial dilatation and number of lobes involved, or the Bronchiectasis Radiologically Indexed CT Score, which incorporates bronchial dilatation and number of bronchopulmonary segments with emphysema, are more commonly used [171, 172]. Extent of bronchial dilatation is associated with poorer baseline FEV_1 % predicted, increased hospital admissions, sputum production and *P. aeruginosa* infection [173–175]. However, evidence is mixed or lacking regarding its association with exacerbations, quality of life and mortality risk [7, 176–179].

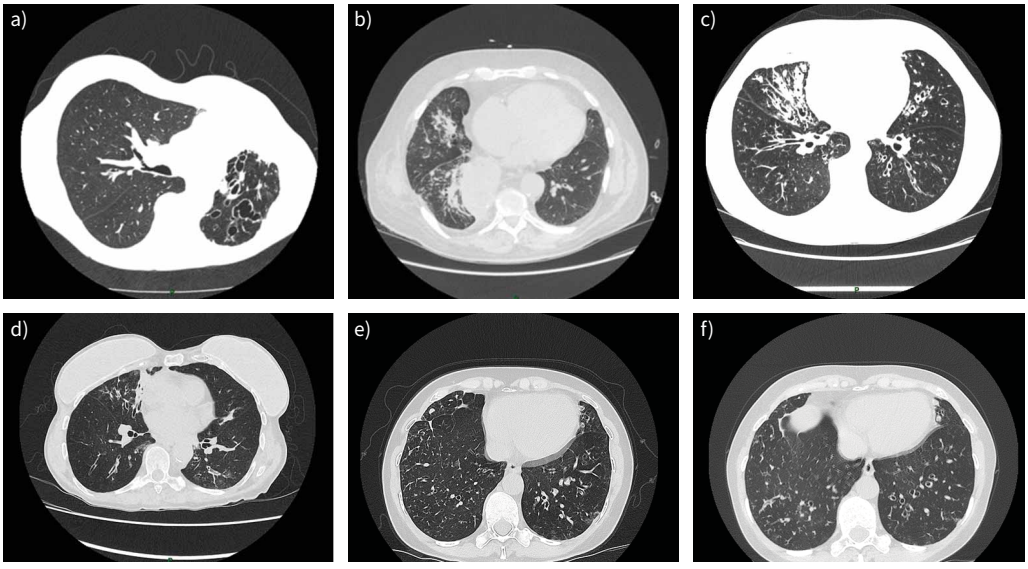

FIGURE 3 High-resolution computed tomography findings associated with different bronchiectasis aetiologies, and with treatment response. a) Localised bronchiectasis affecting the lingula and left lower lobe (with previous left lower lobectomy) due to severe childhood infection; b) bilateral lower-lobe bronchiectasis with mucus plugging, worse on the right with associated inflammatory nodules, in a patient with chronic aspiration due to previous oesophagectomy and gastric pull-up; c) middle-lobe varicose bronchiectasis with bronchial wall thickening and extensive mucus plugging due to primary ciliary dyskinesia; d) middle-lobe bronchiectasis with tree-in-bud nodularity due to *Mycobacterium avium* pulmonary disease; e) idiopathic bilateral lower lobe bronchiectasis with mucus plugging most marked in the left lower lobe due to *Pseudomonas aeruginosa* infection; f) improvement in appearance with reduced mucus plugging following nebulised colomycin to treat *P. aeruginosa*.

TABLE 2 Unanswered questions for biomarkers of inflammation, infection, epithelial dysfunction and mucociliary clearance, and structural lung disease in bronchiectasis

Inflammation	Are biomarkers of neutrophilic inflammation able to reliably identify patients who will respond to antineutrophil/anti-inflammatory therapies?
	Do changes in markers of neutrophilic inflammation over time correspond with changes in clinical outcomes, allowing them to be used as markers of disease activity and treatment response?
	Is peripheral blood neutrophil function truly altered in bronchiectasis and what are the underlying mechanisms?
	What is the potential of markers of neutrophilic inflammation beyond the NSP (*e.g.* other NET-associated proteins, inflammatory cytokines, antiproteases) as therapeutic targets in bronchiectasis?
	What is the interaction between neutrophilic inflammation and eosinophilic inflammation in bronchiectasis and how does this impact treatment response with both antineutrophil and anti-eosinophil/anti-Th2 therapies?
	What is the role of systemic inflammation in bronchiectasis in terms of pulmonary and nonpulmonary outcomes (such as cardiovascular disease)?
	How can inflammatory biomarkers be best incorporated into clinical practice?
Infection	Is it possible to identify microbiome profiles associated with enhanced response to inhaled or systemic antibiotic treatments?
	How can rapid molecular diagnostics be used in clinical practice, *e.g.* to more reliably detect infection, to measure the impact of antibiotic or other therapies on the respiratory microbiome?
	Is there a therapeutic role for "good bacteria" such as *Rothia mucilaginosa* in bronchiectasis?
	How do interaction networks in the respiratory "multibiome" impact on clinical outcomes and how can this be used to guide treatments?
	Can antibody therapies for *e.g. Pseudomonas aeruginosa* be used as an alternative to broad-spectrum antibiotics and what would be the effects of the microbiome?
Epithelial dysfunction and mucociliary clearance	Can rheology and/or mucins be used in practice to identify patients with the highest sputum burden and guide physiotherapy or mucoactive treatments?
	How can we effectively, and noninvasively, measure epithelial function and mucociliary function in practice?
	Which epithelial-derived mediators/biomarkers (such as antimicrobial peptides) can be used as therapeutic targets or biomarkers of therapeutic response?
Structural lung disease and imaging	What is the relationship between radiological features and clinical outcomes in bronchiectasis beyond radiological extent alone, *e.g.* can dynamic features of active inflammation such as mucus plugging be used to measure risk of exacerbation and clinical response to treatment?
	Is there a role for artificial intelligence in evaluating radiological subtypes or future risk from imaging data?
	Is there a role for imaging modalities beyond CT, such as MRI or PET-CT in assessing disease extent and inflammation?
	Can novel developments in pulmonary function testing such as impulse oscillometry or lung clearance index be used to identify early disease in adults with bronchiectasis?

NSP: neutrophil serine protease; NET: neutrophil extracellular trap; Th2: type-2 T-helper; CT: computed tomography; MRI: magnetic resonance imaging; PET: positron emission tomography.

There is a need for more detailed studies of the relationship between radiological features and clinical outcomes in bronchiectasis. Mucus plugging and bronchial wall thickening are features of inflammation which may change with therapy, while extent of bronchial dilatation or emphysema may be more suggestive of the extent of lung damage. Therefore, HRCT has the potential to provide markers of both disease activity and severity. Future directions include development of automated systems to improve standardisation of scan reporting, and the use of MRI to further characterise features of active inflammation and ventilatory impact in bronchiectasis [180–182].

Pulmonary function tests

The outcome of structural lung damage is reduced pulmonary function. Bronchiectasis is categorised as an obstructive airways disease and frequently coexists with other obstructive airways diseases such as asthma and COPD. Accordingly, spirometry is recommended to monitor disease progression [1, 3].

Low FEV_1 is associated with greater radiological extent of disease, increased exacerbations and *P. aeruginosa* infection [183, 184]. Spirometry can be useful in guiding treatment, as up to a third of bronchiectasis patients have evidence of reversible airway obstruction associated with sustained improvement in FEV_1 following long-term bronchodilator therapy [51, 185]. However, in bronchiectasis drug trials FEV_1 has rarely shown a response to treatment, and, in a recent trial of inhaled tiotropium,

FEV_1 improved, but exacerbations or symptom scores did not, suggesting that increased FEV_1 may not reflect meaningful improvement in quality of life [14, 186].

While we think of bronchiectasis as an obstructive disease, recent studies have highlighted the heterogeneity of lung function abnormalities observed. The EMBARC multicentre European study found that, while airflow obstruction affected 34.9% of patients, spirometry was normal in 31.2% and a further 23.9% displayed preserved ratio impaired spirometry [12]. Other work has found parameters suggesting that hyperinflation, such as increased lung capacity and residual volume, as well as reduced diffusion capacity of the lung for carbon monoxide, are common and associated with increased dyspnoea and reduced long-term survival [66, 187].

One explanation for the prevalence of normal spirometry is that spirometry primarily reflects airway flow in the large airways, whereas bronchiectasis often begins as an inflammatory process of the small airways [188]. New developments in pulmonary function testing may be able to identify early lung disease. Impulse oscillometry, which provides detailed evaluation of small airways resistance and reactivity, correlates with bronchiectasis symptoms and severity scores [188–190]. Lung clearance index, measured by multiple-breath washout of an inert tracer gas, measures uneven ventilation. It has shown value as an early indicator of lung disease in children with cystic fibrosis with normal spirometry and in adults with bronchiectasis it correlates with spirometry and symptom scores [191, 192].

Conclusions: developing the ideal bronchiectasis biomarker

This is an exciting time for bronchiectasis research, as growing understanding of inflammatory pathways is rapidly identifying inflammatory endotypes. Meanwhile, use of multi-omics has overcome previous dependence on known targets and opened up a wealth of possibilities for identification of proteins defining these endotypes [193].

There remain many unanswered questions about biomarkers in bronchiectasis (table 2). Markers currently used for diagnosis and monitoring of bronchiectasis such as radiology, pulmonary function tests and sputum culture predominantly reflect the structural damage and chronic infection components of the vicious vortex which occur once the disease has caused irreversible damage. Future biomarkers that identify inflammation at an early stage could allow treatment to avert these outcomes and may be considered markers of disease activity. Biomarkers demonstrating reduction in inflammation in response to treatment could aid drug development and guide prescribing to achieve maximum efficacy while preventing unnecessary use of ineffective treatments.

Future biomarkers must be sufficiently sensitive and specific to reliably differentiate inflammatory endotypes which may overlap. Given our increasing knowledge of the geographical diversity of bronchiectasis, future biomarkers must reproducibly identify endotypes in different populations where patient characteristics may vary [12, 174]. A biomarker should ideally also be easily measurable, noninvasive, time-efficient and affordable to allow widespread use both in busy respiratory clinics and by research teams trialling new treatments.

Therefore, development and validation of future biomarkers is likely to involve initial identification of candidate markers through multi-omics, targeted assays to confirm clinical relevance, development of user-friendly tools for measurement and validation across multiple cohorts including international trials.

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