ABSTRACT

Asthma-COPD overlap (ACO) refers to a complex heterogeneous disease consisting of poorly characterised patients reporting with disease presentations of both asthma and COPD. This overlap disease has no clear diagnostic or therapeutic guidelines, thereby making both diagnosis and treatment challenging for the clinicians. ACO patients exhibit a higher burden in terms of both mortality and morbidity in comparison to patients with only asthma or COPD. The pathophysiology of the disease, its characteristic features, and existence as a unique disease entity remains unclear. The present study aims to determine whether ACO has a distinct metabolic and immunological mediator profile in comparison to asthma and COPD. It also aims to establish whether a combination of the identified set of markers obtained using various tools is able to adequately distinguish ACO from both asthma and COPD.

In the first part of this study, serum is used to determine the global metabolomic fingerprint of ACO with respect to asthma and COPD. A combination of nuclear magnetic resonance (NMR) based metabolomics and gas chromatography mass spectrometry (GC-MS) based metabolomics is used for this purpose. Two different groups of patients – a discovery cohort and validation cohort were recruited. A total of 12 metabolites [lipids, isoleucine, N-acetylglycoproteins (NAG), valine, glutamate, citric acid, glucose, L-leucine, lysine, asparagine, phenylalanine and histidine] were dysregulated in ACO using NMR metabolomics. Using GC-MS based metabolomics in the same set of samples, 11 metabolites [serine, threonine, ethanolamine, glucose, cholesterol, 2-palmitoylglycerol, stearic acid, lactic acid, linoleic acid, D-mannose and succinic acid] were found to be significantly altered in ACO as compared with asthma and COPD. These trends observed in the discovery cohort were further validated in a fresh cohort of patients.

In the second part of the study, multiplexed analysis of 25 immunological markers (IFN-γ (interferon gamma), TNF-α (tumor necrosis factor alpha), IL-12p70 (interleukin 12p70), IL-2, IL-4, IL-5, IL-13, IL-10, IL-1α, IL-1β, TGF-β (transforming growth factor), IL-6, IL-17E, IL-21, IL-23, eotaxin, GM-CSF (granulocyte macrophage-colony stimulating factor), IFN-α (interferon alpha), IL-18, NGAL (neutrophil gelatinase-associated lipocalin), periostin, TSLP (thymic stromal lymphopoietin), MCP-1

(monocyte chemoattractant protein- 1), YKL-40 (chitinase 3 like 1) and IL-8 was performed in serum of the subjects recruited in the discovery cohort. Thirteen immunological mediators including TNFα, IL-1β, IL-17E, GM-CSF, IL-18, NGAL, IL-5, IL-10, MCP-1, YKL-40, IFN-γ, IL-6 and TGF-β showed distinct expression patterns in ACO.

A quick non-invasive way to access information about the lower respiratory tract with minimal technical skills and without any patient discomfort is sampling of exhaled breath condensate (EBC). EBC consists of water vapor containing volatile and non-volatile substances from the central airways. EBC was collected from all patients recruited in the discovery and validation cohorts earlier. NMR based metabolomic profiling was conducted in these samples. A total of 8 metabolites (lactate, acetone, methanol, formate, valine, fatty acids, propionate, and isopropanol) were found to be dysregulated in ACO in comparison to both asthma and COPD. These trends were yet again validated in the fresh validation cohort.

The findings of the present thesis suggest that ACO is associated with an enhanced energy and metabolic burden as compared to asthma and COPD. Also, the energy metabolites, cholesterol and fatty acids correlate significantly with the immunological mediators, suggesting existence of a possible link between the inflammatory status of these patients and impaired metabolism.

Finally, 46 variables including dysregulated metabolites, immunological mediators and clinical parameters identified in ACO cases have been correlated, receiver operator curve (ROC) models generated and feature extraction algorithms run to ascertain how well ACO can be discriminated from both asthma and COPD with good sensitivity and specificity. It is anticipated that the findings of the present thesis will stimulate researchers to further explore ACO and unravel the pathophysiological complexities associated with the disease. The present findings could be possibly extended to better define the ACO diagnostic criteria, improve management and tailor therapies exclusively for the disease.

Keywords: Asthma COPD overlap (ACO), metabolomics, immunological mediators, serum, exhaled breath condensate (EBC)