Proteomic Profile of Intraluminal Mucus as Potential Diagnostic Biomarkers of Microaspiration in Bronchiolocentric Interstitial Pneumonitis

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Rationale: Bronchiolocentric interstitial pneumonitis with fibrosis (BIPF) is a pathological pattern seen on surgical lung biopsy associated with a wide range of differential diagnosis, including hypersensitivity pneumonitis, connective vascular diseases and chronic microaspiration (CMA). The diagnosis is defined by multidisciplinary discussion (MDD) based on clinical, radiological and pathological findings, despite of the variable degree of diagnostic certainty. Our aims were to confirm BIPF secondary to CMA after MDD. Methods: 3 patients with BIP by surgical lung biopsy (SLB) were diagnosed with CMA after multidisciplinary discussion in 2019. Clinical and tomographic data were collected by electronic medical record. Laser capture microdissection (LCM) was used to isolate intraluminal mucus areas from SLB by Leica LMD6500. Protein extraction and trypsin digestion were carried out and the peptide mixture was analyzed by liquid chromatography coupled with mass spectrometry. The protein identification and quantitation were performed in MaxQuant against 3 databases. Results: Two patients were men and mean age was 67 years old. All patients had documented evidences of gastroesophageal reflux disease and high resolution computer tomography pattern suggested an alternative diagnosis. SLBs showed a bronchiolocentric fibrotic remodeling associated with peribronchiolar lymphocytic infiltrate, peribronchiolar metaplasia and intraluminal basophilic and/or eosinophilic areas. After MDD, the definitive diagnosis was CMA in all cases. Mass spectrometry analysis showed at least 2 unique? peptides of each protein from esophagus, oral mucous, salivary gland, pig, cow and potatoes. The proteins were Type II cytoskeletal 1 keratin of human esophagus, protein-glutamine gamma-glutamyltransferase 2 of human salivary gland, Apolipoprotein A-IV of human oral mucous, trypsin of pig (Sus scrofa), hemoglobin subunit alpha of cow (Bos taurus), Actin-71 of potatoes (Solanum tuberosum). Conclusions: We demonstrate that identification of extra pulmonary proteins within intraluminal mucus is helpful to confirm the final diagnose of BIPF secondary to CMA, improving the diagnostic accuracy of MDD. Proteomic analysis of intraluminal mucus should be considered for the differential diagnosis of BIPF. Further investigation with larger cohorts is warranted.