

# The evolution of sputum cytometry to assess bronchitis

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Airway inflammation is not routinely assessed in the management of airway diseases such as asthma, chronic obstructive pulmonary disease or chronic cough. This is an important limitation because the pathogenesis of airway disease includes three nonoverlapping components: airway inflammation (ie, bronchitis); airflow obstruction; and hyper-responsiveness (1). Bronchitis can be measured noninvasively by sputum cell counts, the fraction of exhaled nitric oxide (FENO) and exhaled breath measurements (2). Sputum cell count quantification has been shown to have important clinical use in reducing eosinophilic asthma exacerbations (3,4), guiding treatment and selecting patients for treatment with biologics (5). Although exhaled nitric oxide can predict steroid responsiveness in patients with mild airway diseases (6), it may have less clinical applicability, particularly in patients with severe airway diseases because it may not be directly related to the degree of sputum eosinophilia (7) and, thus, may not be able to monitor disease and titrate treatment (8). Sputum cell counts can help advance our understanding of different endophenotypes and their pathobiological pathways in conditions such as asthma and chronic obstructive lung disease (9,10). Compared with FENO, sputum cell counts provide information on the type (eosinophilic versus neutrophilic) and degree of airway inflammation. This will increase our understanding of various endophenotypes – especially the severe and treatment-refractory types – to help physicians predict response to novel therapies and, importantly, to allocate the appropriate treatment to the correct patient.

The technology for examining sputum has evolved in the past 20 years. In the late 1980s to early 1990s, Gibson et al (11) and Pin et al (12) demonstrated a method for obtaining sputum differential cell counts from sputum smears (self-expectorated or induced) that was reproducible and valid. However, the problem with using sputum smears was that cellular definition was poor and this made cell counting very laborious. Subsequently, this problem was overcome when sputum, separated from saliva, was treated with dithiothreitol to disperse mucus and cytopins were used to facilitate cell dispersion (13). The treated sputum is then filtered through a nylon mesh over a funnel, and the final sputum specimen is examined using an inverted microscope that selects parts that are uncontaminated by squamous cells. The cell counts obtained using this method show excellent inter- and intraobserver repeatability and are responsive to changes in and across disease states (14). To simplify and standardize this process, a novel processing device (Accufilter, Cellometrics Inc, Canada) has been developed (15). The simplified technology can reduce the workload in centres with large sample loads.

Establishing normal values is a prerequisite for the introduction of a biomarker into clinical practice. Three previous studies have attempted to describe the reference ranges of sputum cell counts in normal individuals (16-18). In the current issue of the *Journal*, Davidson et al (19) (pages 424-428) show that the current reference values based on the original study by Belda et al (16) are equally applicable in western Canada and at elevated altitude. Appropriate statistical methods were used, although the sample size was not justified and the lower sample size compared with previous studies may

have contributed to the slightly higher variability in cell counts observed in this study. A 'normal' reference range may vary according to demographic factors, comorbidities and exposure to environmental influences such as pollution and smoking. The study by Belda et al (16) reported a higher total and absolute neutrophil cell count compared with the other studies. It was conducted in Hamilton (Ontario) and may have been skewed by the ambient pollution of an industrialized city and by prevailing climatic conditions that may affect the neutrophil and macrophage counts (20). They also reported increased sputum eosinophilia with atopy and female sex. The study by Thomas et al (18) found that the sputum neutrophil differential counts increased significantly with age. Yet, it is reassuring that the central measures and variances reported from these centres from three different continents are remarkably similar.

Currently, there are several limitations in the routine sputum processing methods in practice: first, sputum specimens must be examined within 2 h; second, as described above, it requires multiple steps; and third, it requires a certified technologist trained in the examination of cell counts for it to be standardized, reliable and real time. Refrigeration at 4°C can preserve cell morphology and accuracy of cell counts for up to 9 h (21). Sputum fixation or preservation of sputum may extend the time between collection and processing by allowing sputum examination to be delayed for up to 72 h (22,23), thus facilitating transport to a dedicated laboratory for subsequent examination. Sputum can be preserved in 10% buffered formalin or 2% paraformaldehyde-dithiothreitol fixative. The preservatives may increase cellular counts by rapidly inactivating cellular enzymes and by stabilizing cell membranes, thus resulting in increased cell recovery. However, the effect of preservation on total expectorate compared with saliva-free expectorate may be different and this may affect the interobserver repeatability of cell differentials (24). Thus, it is important to note that the normal reference range using the preservation methods described in the article by Davidson et al (19) may be slightly different from the methods that examine freshly expectorated sputum; however, the differences may not be clinically relevant. This method may help to promote widespread use of sputum analysis in clinical practice and research, and remove one barrier from its implementation in the routine care of patients with airway diseases. In the future, development of a point-of-care biomarker for bronchitis would further enhance its widespread adoption and clinical application (25).

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