

Assessment of a rapid liquid based cytology method for measuring sputum cell counts

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Abstract

Differential sputum cell counting is not widely available despite proven clinical utility in the management of asthma. We compared eosinophil counts obtained using liquid based cytology (LBC), a routine histopathological processing method, and the current standard method. Eosinophil counts obtained using LBC were a strong predictor of sputum eosinophilia ($\geq 3\%$) determined by the standard method suggesting LBC could be used in the management of asthma.

Background

Differential cell counts (DCC) of induced sputum samples have been shown to be useful in the management of patients with moderate-to-severe asthma(1) and in the diagnosis of eosinophilic bronchitis(2). Unfortunately, sputum processing is time consuming(3) taking approximately 4 hours and a meta-analysis concluded that its routine use could not be justified due to the technical expertise required(4). We aimed to establish if a simplified method using routine liquid based cytology (LBC), used in histopathology laboratories throughout the NHS, could provide similar results.

Method

Subjects with asthma or chronic cough presenting to an outpatient respiratory clinic who were willing to take part in the study were consented (REC 08/H0407/2) and spirometry performed. Sputum samples were induced using hypertonic saline as previously described(3), following which sputum plugs were isolated, weighed and divided into two equal portions.

The first sample was processed as described previously(3) and the resultant slide counted by a trained research scientist. Cell counts were later reviewed by a second scientist blinded to the results and any discrepancies in counts were resolved by reassessment by both scientists.

For the LBC method, sputum samples were manually mixed in 10ml CytoRich Red (CRR) solution with a pipette. The mixture was shaken on an Ika-Vibrax-VXR shaker at 1000rpm for 30 mins to homogenise the sample and then centrifuged at 780g for 5 minutes. The resultant pellets were re-suspended in 1.5ml CRR and 0.75ml aliquots were deposited into settling chambers positioned on coated slides for a minimum of 10 minutes. Any excess fluid was extracted from the chambers using a pipette. The chambers were then removed and the slides fixed and stained using the Papanicolaou method. Eosinophils in 100 high power fields (x400) were counted and averaged for each slide by a single consultant histopathologist (IS) blinded to the DCC results. Each slide was graded on an ordinal scale of 0-4 based on the approximate number of eosinophils per high power field (eos/hpf) where: 0=0 eos/hpf, 1=1-2 eos/hpf, 2=3-4 eos/hpf, 3=5-10 eos/hpf and 4= >10 eos/hpf.

Data were entered into GraphPad Prism. Empirical receiver operator characteristics (ROC) curves and sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) for each LBC 'grade' to identify a sputum eosinophil count of $\geq 3\%$ (1) were generated. This analysis was repeated with exclusion of samples with $\leq 50\%$ viability and/or $\geq 20\%$ squamous cell contamination for comparative purposes with previous studies investigating the inter-observer agreement of DCCs(5).

Results

Demographic and lung function data are available in the online supplement. Of 55 patients, 6 produced samples of insufficient volume to process by either technique, and 4 samples were uncountable by both methods, leaving 45 pairs of slides available for counting. Four slides were identified by DCC with $\leq 50\%$ viability and 2 slides had $\geq 20\%$ squamous contamination leaving 39 pairs of slides for counting in the 'exclusion set'. The empirical ROC curve to assess the utility of the LBC based method to identify a sputum eosinophil count of $\geq 3\%$ had an area under the curve (ROC AUC) of 0.90 ($p < 0.001$) (Fig

1) which increased to 0.95 ($p < 0.001$) upon excluding slides with low viability or significant squamous contamination. The cut-point with the highest combined set of values for sensitivity, specificity, PPV and NPV for identifying a sputum eosinophil count of $\geq 3\%$ was >10 eos/hpf, as shown in table 1. Processing of sputum took approximately 1 hour with LBC compared with approximately 4 hours using the current cytopsin and DCC technique. Examples of slides produced by both methods are shown in the online supplement.

LBC grade (eos/hpf)	Sensitivity%	95% CI (%)	Specificity%	95% CI (%)	PPV%	NPV%
1 (1-2)	100	78.2 - 100	20	7.7 - 38.6	37.5	100
2 (3-4)	93.3	68.1 - 99.8	56.7	37.4 - 74.5	51.9	95.4
3 (5-10)	93.3	68.1 - 99.8	70	50.6 - 85.3	60.9	95.5
4 (>10)	66.7 (83.3*)	38.4 - 88.2	96.7	82.8 - 99.9	90.9	85.3 (93.1*)

Table 1: Sensitivity and specificity values for each LBC grade to identify a DCC sputum eosinophil count of $\geq 3\%$ for all slides. *Values for sensitivity and NPV after excluding slides with low viability/squamous contamination ($n=6$)

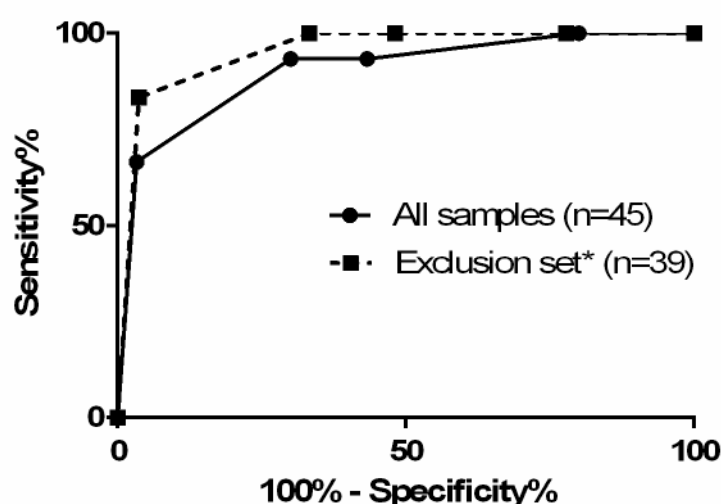


Figure 1: Empirical ROC curve showing the sensitivity (%) and 100-specificity (%) of LBC derived eosinophil count (eos/hpf) to identify a sputum differential eosinophil count of $\geq 3\%$ for all samples and after exclusion of samples with $\leq 50\%$ viability and/or $\geq 20\%$ squamous contamination*

Discussion

Our results indicate that a cut-off point of >10 eos/hpf from the LBC technique has a high PPV and NPV to identify patients with a sputum differential eosinophil count of \geq or $< 3\%$. This is important in asthma management because this value is associated with increased risk of exacerbations and can be modified with additional oral or inhaled corticosteroids(1).

Sputum differential cell counts have demonstrated utility in guiding treatment decisions in patients with more severe asthma(1) but the DCC technique has not been widely adopted into clinical practice because of the time and technical expertise required to perform it. Any novel method of assessing sputum eosinophilia must therefore be compared with DCC. However, differences in results do not necessarily mean the DCC

method is superior to the LBC approach. Firstly, it cannot be excluded that differences in eosinophil counts produced by the two methods were due to heterogenous distribution of the cellular portion of the sample, resulting in a higher eosinophil concentration in one part of the sample than the other. Secondly, the DCC technique is not subject to the formal quality control procedures in place for laboratory investigations performed in the NHS. It remains possible that LBC with an eosinophil count performed by a consultant histopathologist is more accurate than research scientists performing DCC. LBC also has some practical advantages: 1) samples do not have to be processed on the day of collection as for DCC(6) because CRR acts as a mucolytic and a fixative and 2) the LBC technique is less time intensive making it more suitable for use in NHS histopathology departments.

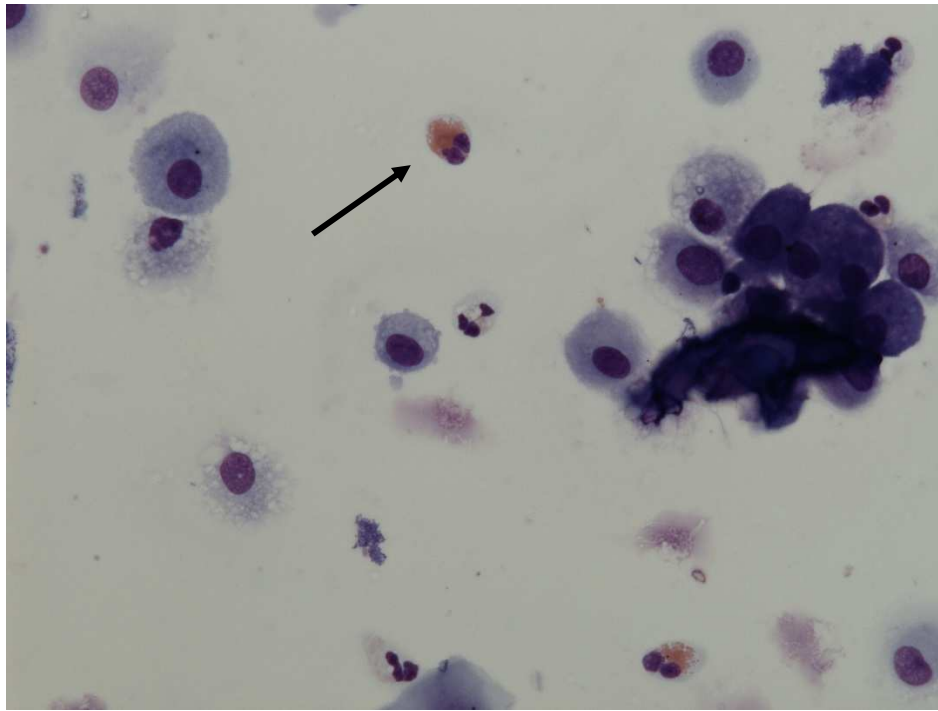
The next step is to demonstrate that these results can be reproduced at other centres and that treatment decisions based on LBC are beneficial in terms of asthma outcomes as has been shown for DCC's(1). These studies should include blood eosinophil counts which are more easily obtained and have been demonstrated to be a good predictor of sputum eosinophilia in subjects undergoing COPD exacerbations(7) and a moderate predictor of sputum eosinophilia in asthma(8). The reliability of LBC eosinophil counts from mucopurulent sputum samples from subjects with COPD also needs to be assessed.

References:

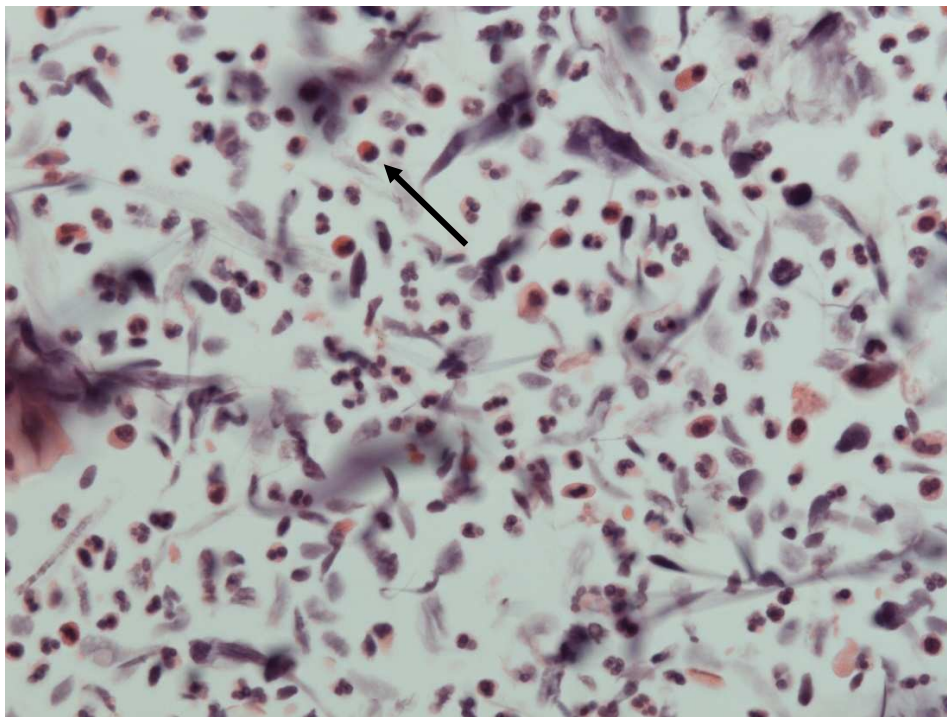
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	Subjects with asthma	Subjects with chronic cough
Total number recruited to study	43	12
Total number who produced adequate samples for processing	41	8
Total number who produced 'countable' samples	38	7
Mean age (range) ^a	55 (26-76)	59 (41-79)
Sex: male (%)	27 (62.8)	5 (41.7)
Ethnic group:		
White Or White British (%)	43 (100)	12 (100)
Mean FEV ₁ % predicted (SD)	63 (15)	73 (12)
Mean FEV ₁ /FVC % (SD)	67 (23)	107 (25)
Median blood eosinophil count x10 ⁹ /L (IQR)	0.75 (3.25)	1.9 (2.8)

Table S1: Demographics and clinical measures of study subjects with asthma and chronic cough



A



B

Figure S1: Cytological analysis of sputum samples from the study. Eosinophils are indicated with arrows. (A) A cytocentrifuge slide prepared by the standard (DCC) technique (Giemsa stain, original magnification x400) (B) A slide prepared by the LBC technique (Papanicolaou stain, original magnification x400)