Letter to the Editor

Discriminating sputum-eosinophilic asthma: Accuracy of cutoffs in blood eosinophil measurements versus a composite index, ELEN

To the Editor:

"Tailored treatment using sputum eosinophil count results in fewer asthma attacks than traditional management in adults with severe asthma" was a primary conclusion of a systematic review and meta-analysis study of eosinophilic markers.[1] Although cutoffs in eosinophil percentage in induced sputum are considered the criterion standard for discriminating eosinophilic and noneosinophilic asthma phenotypes, it is deemed impractical for routine clinical use.[2] Because a complete blood cell count with differential can be performed at a lower cost and greater accessibility than induced sputum, eosinophil counts in peripheral blood have potential clinical utility in identifying asthma patients with eosinophilia.[3] A recent study reported that blood eosinophils do not accurately predict sputum eosinophil percentages in subjects with asthma.[4] However, 2 cutoffs in blood eosinophils were used to select patients in successful clinical trials of anti-IL5 biologics: 300 cells/uL (mepolizumab, NCT01000506) and 400 cells/uL (reslizumab, NCT01287039). Another successful phase 3 trial of mepolizumab (NCT01691521) used the inclusion criterion of blood eosinophils being more than or equal to 150 cells/uL at screening or, alternatively, more than or equal to 300 cells/uL at some time during the previous year.[5] In yet another recent study, Zhang et al[2] recommended other alternate cutoffs: 0.26 eosinophil count ($\times 10^9$/L), 2.70 eosinophil (%), 0.10 eosinophil/lymphocyte ratio (ELR), 0.05 eosinophil/neutrophil ratio (ENR), and 0.26 eosinophil/monocyte ratio; they reported that blood eosinophil percentage was the best predictor for eosinophilic asthma and that eosinophil count and eosinophil ratios were also efficient. Some have questioned the utility of a single eosinophil count after observing large variability in blood eosinophil counts.[6] Others note that "using different cutoffs for defining eosinophilia would have significant impact on the interpretation of the accuracy of the biomarkers and the clinical efficacy of the drug in question."[7] Clearly, this topic continues to generate considerable clinical research interest.

We were particularly intrigued by cutoffs in ELR and ENR in the study by Zhang et al because we previously reported the utility of an easy-to-use peripheral blood–based algorithm (the ELEN Index).[8] The ELEN index combines ELR and ENR (hence the name ELEN) without requiring a fixed cutoff to predict sputum-eosinophilic asthma phenotype. The algorithm uses weighted ELR and ENR to derive probability-based discrimination scores of binary group association and uses a decision rule to assign each case to either the sputum-eosinophilic group or the sputum noneosinophilic group in 3 steps: (1) calculate score 1 = −9.5243 + [70.0975 × eosinophil/lymphocyte] − [3.7790 × natural log (eosinophil/neutrophil)], (2) calculate score 2 = −14.5853 + [101.2198 × eosinophil/lymphocyte] − [3.9567 × natural log (eosinophil/neutrophil)], and (3) use decision rule: if score 1 > score 2, assign case to the sputum noneosinophilic group; otherwise, assign case to the sputum-eosinophilic group. The ELEN Index was used prospectively to stratify patients with asthma in a successful randomized phase 2b study of benralizumab, an anti-IL5rα mAb (NCT01238861). The study yielded statistically significant results at prespecified alpha and met the protocol-specified primary end point of asthma exacerbation reduction in a dose-dependent fashion in the eosinophilic group but not in the noneosinophilic group. Groups were stratified before randomization using the ELEN Index and/or fraction of exhaled nitric oxide (FENO) of 50 ppb or more (7% of the subjects were enrolled by FENO only).[8] Two key secondary end points related to improvements in lung function and asthma control were also met. In a prespecified post hoc analysis in the study, blood eosinophil cutoff level of 300 cells/μL or more also yielded statistically significant results, but the post hoc strata were formed after randomization and had a higher exacerbation rate in the placebo arm.

In this article, we compare diagnostic test (Dx) accuracy of the ELEN Index against the above-cited cutoffs in peripheral blood. Our analysis data set (n = 154) was pooled across 3 studies of adults with mild to severe asthma (described in Castro et al[8]). We report here Dx comparisons for 2 commonly used sputum eosinophil cutoffs: 3% or more[4,9] and 2% or more.[4,8,9] The 2% cutoff was also recommended by an expert panel.[10] Comparisons include test sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and overall classification accuracy. Difference in the prevalence of the eosinophilic phenotype will affect predictive values (PPV and NPV) but not a test's sensitivity, specificity, and accuracy, which can be directly compared.

Results are presented in Table I and in Table E1 in this article's Online Repository at www.jacionline.org. The 3% or more sputum eosinophil cutoff resulted in 5 fewer true-positive subjects than did the 2% or more cutoff (56 vs 61 true-positive subjects in our sample of 154 subjects with asthma). Classification agreement between the 2 cutoffs was 96.8%. Sputum eosinophil cutoff at 2% versus 3% resulted in small differences in Dx accuracy, but overall results were consistent. The ELEN Index had the highest accuracy at both 3% or more and 2% or more cutoffs in sputum eosinophils (79.9% and 79.2%, respectively). The algorithm, designed to maximize overall diagnostic accuracy (by balancing the PPV and the NPV) without purposefully biasing toward either sensitivity or specificity, is more specific than sensitive (Table I). Its sensitivity may be increased without significantly lowering the accuracy by combining with other highly specific diagnostics; for example, Castro et al used a FENO value of 50 ppb or more.[8] Because Zhang et al reported high accuracy of multiple cutoffs in the complete blood cell count, we were particularly interested in testing those cutoffs in our data and comparing against the performance of the ELEN Index. Surprisingly, the most accurate test in Zhang et al, a 2.70% eosinophil percentage cutoff (area under the curve = 90.7%), was the least accurate in our data (62% and 66% accuracy for ≥3% and ≥2% eosinophil cutoffs in induced sputum, respectively). Their optimal ENR cutoff of 0.05 or more (area under the curve = 89.1%) was also among the least accurate in our analysis (62.3% and 65.6% for sputum eosinophil ≥3% and ≥2%, respectively). Their optimal cutoff in eosinophil count at 0.26 × 10^9/L or more and 0.3 × 10^9/L or more used by Wenzel et al[11] yielded 72% to 74% overall accuracy in our data, which was lower than the ELEN Index accuracy at 79% to 80%. Statistics on accuracy comparisons of the ELEN Index with the other tests are provided in Table E1. Part A compares accuracy, sensitivity, and specificity (measures not affected by

TABLE I. Comparison of diagnostic accuracy in discriminating sputum eosinophilic and noneosinophilic asthma patients using different peripheral blood tests

Test	True negative	False positive	False negative	True positive	Total (n)	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	Accuracy (%)
Eosinophil <3% vs ≥3% in induced sputum (prevalence of ≥3% = 36.4%)										
≥0.26 eosinophils ($\times 10^9$/L)*	79	19	20	36	154	64.3	80.6	79.8	65.5	74.7
≥2.70 eosinophils (%)*	50	48	10	46	154	82.1	51.0	83.3	48.9	62.3
≥0.10 ELR*	65	33	10	46	154	82.1	66.3	86.7	58.2	72.1
≥0.05 ENR*	52	46	12	44	154	78.6	53.1	81.3	48.9	62.3
≥0.30 eosinophils ($\times 10^9$/L)†	80	18	24	32	154	57.1	81.6	76.9	64.0	72.7
≥0.40 eosinophils ($\times 10^9$/L)‡	90	8	36	20	154	35.7	91.8	71.4	71.4	71.4
ELEN Index§	85	13	18	38	154	67.9	86.7	82.5	74.5	79.9
Eosinophil <2% vs ≥2% in induced sputum (prevalence of ≥2% = 39.6%)										
≥0.26 eosinophils ($\times 10^9$/L)*	76	17	23	38	154	62.3	81.7	76.8	69.1	74.0
≥2.70 eosinophils (%)*	50	43	10	51	154	83.6	53.8	83.3	54.3	65.6
≥0.10 ELR*	64	29	11	50	154	82.0	68.8	85.3	63.3	74.0
≥0.05 ENR*	52	41	12	49	154	80.3	55.9	81.3	54.4	65.6
≥0.30 eosinophils ($\times 10^9$/L)†	77	16	27	34	154	55.7	82.8	74.0	68.0	72.1
≥0.40 eosinophils ($\times 10^9$/L)‡	86	7	40	21	154	34.4	92.5	68.3	75.0	69.5
ELEN Index§	82	11	21	40	154	65.6	88.2	79.6	78.4	79.2

*Zhang et al.[2]
†Wenzel et al[11] and used in mepolizumab clinical trial NCT01000506.
‡Used in reslizumab clinical trial NCT01287039.
§Castro et al.[8]

prevalence) in which many comparisons are statistically significant. Part B compares the PPV and the NPV (affected by prevalence) and shows higher prediction accuracy of the ELEN Index. Many comparisons in Part B are not statistically significant, likely due to smaller sample sizes in variable marginal totals.

We used the classification and regression tree to investigate optimal binary cutoffs in our data. Optimal cutoffs differed in the 3 studies, indicating lack of stability (and, therefore, reliability) of binary cutoffs in general. In pooled data, our optimal cutoffs (vs Zhang et al) were 0.25 (vs 0.26) eosinophil count ($\times 10^9$/L), 4.00 (vs 2.70) eosinophil (%), 0.15 (vs 0.10) ELR, and 0.16 (vs 0.05) ENR. Using fixed cutoff in cells assumes that measurements will be unaffected by potential variations in time, laboratory equipment, reagents, and so forth. Such an assumption ignores biases, for example, sample degradation (some blood samples may be analyzed after 6 hours while others after 24 hours postextraction), or variability in calibration and performance of different hematology analyzers. Additional biases could result from differences in therapies such as from using oral corticosteroids. Using ratios of blood cell types contributes toward measurement standardization, which should increase accuracy by reducing some systematic biases. The ELEN Index, which integrates 2 ratios of 3 cell types (ELR and ENR), was standardized and optimized mathematically. Thus, the algorithm is expected to be more accurate than tests with nonstandardized fixed cutoffs, while retaining generalizability and practicality (the discriminant scores can be hand-calculated or programmed into a computer). Besides yielding the highest accuracy (~5% to 15% higher), the ELEN Index was the only test that yielded both the NPV and the PPV (objective measures of clinical utility) of 75% or higher for both 2% and 3% eosinophil cutoffs in induced sputum.

We conclude that the diagnostic performance results in this analysis show potential utility of the ELEN Index in predicting elevated sputum eosinophils in those with asthma. At this stage, the algorithm should be considered a research tool despite its successful implementation in a large, multicenter, prospective phase 2b randomized clinical trial of subjects with moderate to severe asthma; readers are referred to Castro et al[8] (and their article's Online Repository at www.thelancet.com) for details on the algorithm's performance on clinical outcomes. Because the ELEN Index was developed and validated using matching sputum and blood data from screening visits at study enrollment, its present utility lies in initial diagnostic assessment of patients with asthma similar to those studied. Its potential utility for follow-up patient visits needs further verification with matching sputum and blood data collected at such visits. The ELEN Index has potential to be relevant for clinical practice if additional independent verifications validate its accuracy in a broader asthma population. We encourage interested researchers to test the algorithm and report findings.

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Disclosure of potential conflict of interest: D. B. Khatry and J. M. Parker are employed by MedImmune/AstraZeneca, own AstraZeneca stock, and report a patent (Pub no. US 2012/0328606 A1, Pub date: December 27, 2012) pending to MedImmune. D. L. Gossage was employed by MedImmune/AstraZeneca and owned AstraZeneca stock during his employment within 36 months of this publication; is currently an employee of Gilead Sciences, Inc, and owns Gilead stock; and reports a patent (Pub no. US 2012/0328606 A1, Pub date: December 27, 2012) pending to MedImmune. G. P. Geba and N. A. Molfino report a patent (Pub no. US 2012/0328606 A1, Pub date: December 27, 2012) pending to MedImmune. N. N. Jarjour has received study materials from GlaxoSmithKline. W. W. Busse has consultant arrangements with Novartis, GlaxoSmithKline, Genentech, and Roche; is on data monitoring boards for Boston Scientific and Circassia; and is on a study oversight committee for ICON.

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TABLE E1. Statistical comparison of accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of ELEN with other diagnostic tests that use fixed binary cutoffs in peripheral blood measurements

Part A

Diagnostics measure	Reference data	Contrast test (vs the ELEN Index)	Both tests (−ve)	ELEN (+ve); contrast (−ve)	ELEN (−ve); contrast (+ve)	Both tests (+ve)	Total	*P* value (exact McNemar test)	Odds ratio	95% CI (low)	95% CI (high)
Accuracy	All data	≥0.26 eosinophils ($\times 10^9$/L)*	92	7	11	44	154	.4807	1.57	0.56	4.78
		≥2.70 eosinophils (%)*	60	0	43	51	154	**<.00001**	∞	11.16	∞
		≥0.10 ELR*	75	0	28	51	154	**<.00001**	∞	7.10	∞
		≥0.05 ENR*	62	2	41	49	154	**<.00001**	20.50	5.32	174.96
		≥0.30 eosinophils ($\times 10^9$/L)†	94	9	10	41	154	1.00000	1.11	0.41	3.09
		≥0.40 eosinophils ($\times 10^9$/L)‡	101	2	25	26	154	**<.00001**	12.50	3.12	108.89
Sensitivity	Sputum eosinophil cutoff ≥3%	≥0.26 eosinophils ($\times 10^9$/L)*	15	5	3	33	56	.7266	0.60	0.09	3.08
		≥2.70 eosinophils (%)*	10	0	8	38	56	**.0078**	∞	1.71	∞
		≥0.10 ELR*	10	0	8	38	56	**.0078**	∞	1.71	∞
		≥0.05 ENR*	10	2	8	36	56	.1094	4.00	0.80	38.67
		≥0.30 eosinophils ($\times 10^9$/L)†	16	2	8	30	56	.1094	4.00	0.80	38.67
		≥0.40 Eosinophils ($\times 10^9$/L)‡	17	1	19	19	56	**<.00001**	19.00	3.02	789.46
Specificity	Sputum eosinophil cutoff <3%	≥0.26 eosinophils ($\times 10^9$/L)*	77	2	8	11	98	.1094	4.00	0.80	38.67
		≥2.70 eosinophils (%)*	50	0	35	13	98	**<.00001**	∞	9.00	∞
		≥0.10 ELR*	65	0	20	13	98	**<.00001**	∞	4.94	∞
		≥0.05 ENR*	52	0	33	13	98	**<.00001**	∞	8.46	∞
		≥0.30 eosinophils ($\times 10^9$/L)†	78	2	7	11	98	.1797	3.50	0.67	34.53
		≥0.40 eosinophils ($\times 10^9$/L)‡	84	1	6	7	98	.125	6.00	0.73	275.99
Sensitivity	Sputum eosinophil cutoff ≥2%	≥0.26 eosinophils ($\times 10^9$/L)*	18	5	3	35	61	.7266	0.60	0.09	3.08
		≥2.70 eosinophils (%)*	10	0	11	40	61	**.001**	∞	2.51	∞
		≥0.10 ELR*	11	0	10	40	61	**.002**	∞	2.24	∞
		≥0.05 ENR*	10	2	11	38	61	**.0225**	5.50	1.20	51.07
		≥0.30 eosinophils ($\times 10^9$/L)†	19	2	8	32	61	.1094	4.00	0.80	38.67
		≥0.40 eosinophils ($\times 10^9$/L)‡	20	1	20	20	61	**<.00001**	20.00	3.20	828.96
Specificity	Sputum eosinophil cutoff <2%	≥0.26 eosinophils ($\times 10^9$/L)*	74	2	8	9	93	.1094	4.00	0.80	38.67
		≥2.70 eosinophils (%)*	50	0	32	11	93	**<.00001**	∞	8.81	∞
		≥0.10 ELR*	64	0	18	11	93	**<.00001**	∞	4.40	∞
		≥0.05 ENR*	52	0	30	11	93	**<.00001**	∞	7.64	∞
		≥0.30 eosinophils ($\times 10^9$/L)†	75	2	7	9	93	.1797	3.50	0.67	34.53
		≥0.40 eosinophils ($\times 10^9$/L)‡	81	1	5	6	93	.2188	5.00	0.56	236.49

Part B

Diagnostics measure	Reference data	Contrast test (vs the ELEN Index)	True (ELEN)	True (contrast)	False (ELEN)	False (contrast)	Total	*P* value (2-sided Fisher exact test)	Odds ratio	95% CI (low)	95% CI (high)	ELEN's prediction
PPV	Sputum eosinophil cutoff ≥3%	≥0.26 eosinophils ($\times 10^9$/L)*	38	36	13	19	106	.3978	1.54	0.63	3.73	Higher
		≥2.70 eosinophils (%)*	38	46	13	48	145	**.0030**	3.03	1.37	7.03	Higher
		≥0.10 ELR*	38	46	13	33	130	.0634	2.09	0.94	4.57	Higher
		≥0.05 ENR*	38	44	13	46	141	**.0043**	3.03	1.43	6.61	Higher
		≥0.30 eosinophils ($\times 10^9$/L)†	38	32	13	18	101	.2860	1.64	0.67	4.09	Higher

(Continued)

TABLE E1. *(Continued)*

Part B

Diagnostics measure	Reference data	Contrast test (vs the ELEN Index)	True (ELEN)	True (contrast)	False (ELEN)	False (contrast)	Total	*P* value (2-sided Fisher exact test)	Odds ratio	95% CI (low)	95% CI (high)	ELEN's prediction
		≥0.40 eosinophils ($\times 10^9$/L)‡	38	20	13	8	79	.7945	1.17	0.39	3.47	Higher
NPV	Sputum eosinophil cutoff <3%	≥0.26 eosinophils ($\times 10^9$/L)*	85	79	18	20	202	.7193	1.19	0.58	2.52	Higher
		≥2.70 eosinophils (%)*	85	50	18	10	163	1.0000	0.94	0.40	2.30	Lower
		≥0.10 ELR*	85	65	18	10	178	.5344	0.73	0.31	1.73	Lower
		≥0.05 ENR*	85	52	18	12	167	.8385	1.09	0.47	2.50	Higher
		≥0.30 eosinophils ($\times 10^9$/L)†	85	80	18	24	207	.3880	1.41	0.69	2.87	Higher
		≥0.40 eosinophils ($\times 10^9$/L)‡	85	90	18	36	229	.0604	1.88	0.98	3.61	Higher
PPV	Sputum eosinophil cutoff ≥2%	≥0.26 eosinophils ($\times 10^9$/L)*	40	38	11	17	106	.3782	1.62	0.67	3.96	Higher
		≥2.70 eosinophils (%)*	40	51	11	43	145	**.0042**	3.04	1.39	7.09	Higher
		≥0.10 ELR*	40	50	11	29	130	.0813	2.10	0.89	5.12	Higher
		≥0.05 ENR*	40	49	11	41	141	**.0062**	3.02	1.36	7.09	Higher
		≥0.30 eosinophils ($\times 10^9$/L)†	40	34	11	16	101	.2672	1.70	0.68	4.19	Higher
		≥0.40 eosinophils ($\times 10^9$/L)‡	40	21	11	7	79	.7827	1.21	0.39	3.77	Higher
NPV	Sputum eosinophil cutoff <2%	≥0.26 eosinophils ($\times 10^9$/L)*	82	76	21	23	202	.7334	1.18	0.58	2.38	Higher
		≥2.70 eosinophils (%)*	82	50	21	10	163	.6801	0.78	0.34	1.81	Lower
		≥0.10 ELR*	82	64	21	11	178	.4296	0.67	0.28	1.53	Lower
		≥0.05 ENR*	82	52	21	12	167	.8440	0.90	0.40	2.02	Higher
		≥0.30 eosinophils ($\times 10^9$/L)†	82	77	21	27	207	.4108	1.37	0.71	2.73	Higher
		≥0.40 eosinophils ($\times 10^9$/L)‡	82	86	21	40	229	.0709	1.81	0.95	3.38	Higher

$P < .05$ rejects the null hypothesis that the 2 contrasted tests are not independent. *P* values in bold are statistically significant.

*Zhang et al.[2]

†Wenzel et al[11] and used in mepolizumab clinical trial NCT01000506.

‡Used in reslizumab clinical trial NCT01287039.