

Asthma Control and Exhaled Biomarkers

A real-life study

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Background

- Asthma is a chronic inflammatory airways disorder
- Control is the major target of current asthma treatment guidelines (GINA, NAEPP) and is evaluated using:
 - Daytime symptoms
 - Limitations of activities
 - Nocturnal symptoms - awakening
 - Need for reliever/rescue treatment
 - Lung function (PEF or FEV₁)
 - Exacerbations
- No **biomarker of airways inflammation** has been included in current asthma guidelines

NAEPP 2007: Asthma Control

Components of Control		Classification of Asthma Control (Youths ≥12 years of age and adults)		
		Well-Controlled	Not Well-Controlled	Very Poorly Controlled
Impairment	Symptoms	≤2 days/week	>2 days/week	Throughout the day
	Nighttime awakening	≤2x/month	1–3x/week	≥4x/week
	Interference with normal activity	None	Some limitation	Extremely limited
	Short-acting beta ₂ -agonist use for symptom control (not prevention of EIB)	≤2 days/week	>2 days/week	Several times per day
	FEV ₁ or peak flow	>80% predicted/ personal best	60–80% predicted/ personal best	<60% predicted/ personal best
	Validated Questionnaires ATAQ ACQ ACT	0 ≤0.75* ≥20	1–2 ≥1.5 16–19	3–4 N/A ≤15
Risk	Exacerbations	0–1/year	≥2/year (see note)	
		Consider severity and interval since last exacerbation		
	Progressive loss of lung function	Evaluation requires long-term followup care		
	Treatment-related adverse effects	Medication side effects can vary in intensity from none to very troublesome and worrisome. The level of intensity does not correlate to specific levels of control but should be considered in the overall assessment of risk.		

Aims of the Study

- **Primary aim:**
 - to evaluate the levels of two non-invasive, easy-to-perform on-site, exhaled biomarkers (FeNO and EBC pH) in patients with asthma, according to the level of control
- **Secondary aims:**
 - To evaluate the performance of FeNO and EBC pH for the identification of partly or uncontrolled asthma according to the GINA guidelines
 - To evaluate the performance of the combination of the two biomarkers with questionnaires evaluating asthma control (ACQ, ACT)

Materials and Methods

- **Asthma Control**

- was evaluated on consecutive patients with previously diagnosed asthma in asthma clinics of two tertiary hospitals by two asthma specialists (K.K. and S.L.) blinded to FeNO and EBC measurements, according to the GINA guidelines

- **Questionnaires**

- Asthma Control Test (**ACT**, QualityMetric)
- Asthma Control Questionnaire (**ACQ**, Juniper)

- **Measurements**

- **FeNO** (NiOX MiNO, Aerocrine, Sweden), ppb in a 50 mL/sec flow
- **EBC pH** (Ecoscreen, Viasys + pH-meter after 10 min Argon deaeration)
- **Spirometry** (Masterscreen, Viasys): FEV₁, FVC, FEV₁/FVC, FEF_{25-75%}

ROC Curve Analysis

	Disease				
Test	Present	n	Absent	n	Total
Positive	True Positive	<i>a</i>	False Positive	<i>c</i>	<i>a + c</i>
Negative	False Negative	<i>b</i>	True Negative	<i>d</i>	<i>b + d</i>
Total		<i>a + b</i>		<i>c + d</i>	

Sensitivity = $a / (a+b)$:

probability that a test result will be positive when the disease is present (true positive rate)

Specificity = $d / (c+d)$:

probability that a test result will be negative when the disease is not present (true negative rate)

Positive predictive value = $a / (a+c)$:

probability that the disease is present when the test is positive

Negative predictive value = $d / (b+d)$:

probability that the disease is not present when the test is negative

Positive likelihood ratio = True positive rate / False positive rate = Sensitivity / (1-Specificity) :

ratio between the probability of a positive test result given the *presence* of the disease and the probability of a positive test result given the *absence* of the disease

Negative likelihood ratio = False negative rate / True negative rate = (1-Sensitivity) / Specificity :

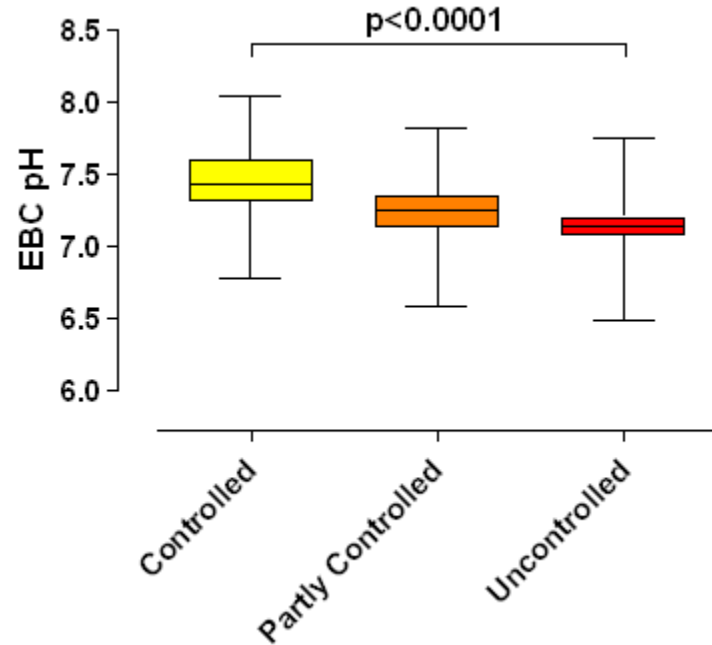
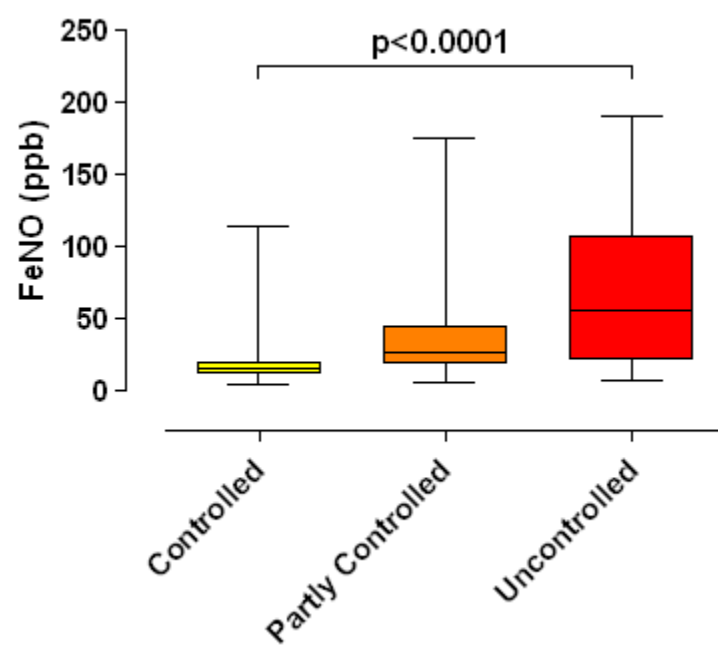
ratio between the probability of a negative test result given the *presence* of the disease and the probability of a negative test result given the *absence* of the disease

Patients' Demographics

	All (n=274)	Well Controlled (n=99)	Partly Controlled (n=115)	Uncontrolled (n=60)
Age (years)	50±17	51±18	51±17	46±15
Gender (F/M)	166/109	64/35	68/47	36/24
BMI (kg/m ²)	27.9±4.9	28.5±4.7	27.5±5.1	28.0±5.0
Smokers	83 (30.3%)	31 (31.3%)	33 (28.7%)	19 (31.7%)
FEV ₁ (% pred.)	85±19	94±17	83±16	74±21
ICS-treated	194 (70.8%)	67 (67.7%)	91 (79.1%)	36 (60.0%)
ACQ	1.43 (0.71 - 2.71)	0.57 (0.29 - 0.86)	1.86 (1.14 - 2.71)	3.43 (2.57 - 4.00)
ACT	19 (16 - 23)	23 (22 - 24)	18 (17 - 19)	14 (11 - 17)

Data are presented as mean±SD when normally distributed, as median (Interquartile ranges) when skewed and as absolute number (%) in the rest of the cases

ALL: FeNO, EBC pH and Control



Comparisons performed with Kruskal-Wallis test with Dunn's post-hoc analysis
 $p < 0.05$ for comparisons between any pairs of groups

FeNO and pH in Subgroups

		All (n=274)	Well Controlled (n=99)	Partly Controlled (n=115)	Uncontrolled (n=60)
All (n=274)	FeNO	22 (16 - 41)	16 (13 - 20)	27 (19 - 44) *	59 (23 - 111) *,#
	EBC pH	7.29 (7.14 - 7.43)	7.44 (7.34 - 7.57)	7.25 (7.12 - 7.36) *	7.14 (7.05 - 7.21) *,#
Group 1 (n=48) ICS-untreated Non-Smokers	FeNO	30 (18-111)	16 (14-21)	40 (27-105)*	116 (63-145)*
	EBC pH	7.25 (7.16-7.40)	7.43 (7.27-7.45)	7.25 (7.19-7.34)*	7.11 (7.08-7.22)*,#
Group 2 (n=32) ICS-untreated Smokers	FeNO	19 (14-22)	16 (12-19)	21 (15-38)	22 (21-108)*
	EBC pH	7.32 (7.14-7.47)	7.47 (7.40-7.58)	7.19 (7.12-7.33)*	7.15 (7.03-7.21)*
Group 3 (n=144) ICS-treated Non-Smokers	FeNO	23 (16-44)	16 (12-20)	28 (20-44)*	61 (35-78)*,#
	EBC pH	7.31 (7.15-7.45)	7.48 (7.34-7.59)	7.29 (7.12-7.36)*	7.15 (7.08-7.21)*,#
Group 4 (n=50) ICS-treated Smokers	FeNO	19 (14-25)	17 (14-22)	19 (13-25)	23 (17-74)
	EBC pH	7.24 (7.10-7.41)	7.38 (7.26-7.53)	7.21 (7.06-7.41)	7.11 (6.96-7.54)

Data are presented as median (Interquartile ranges)

* p<0.05 compared to controlled asthma

p<0.05 compared to partly controlled asthma

FeNO: Partly or Uncontrolled vs. Controlled

	Cut-off point	Sensitivity (95%CI)	Specificity (95%CI)	+LR	-LR	PPV	NPV	AUC (95%CI)
All	>24 ppb	0.61 (0.54 – 0.68)	0.87 (0.79 – 0.93)	4.66	0.45	0.89	0.56	0.790 (0.737 – 0.837)
Group 1	>22 ppb	0.87 (0.71 – 0.96)	0.81 (0.54 – 0.96)	4.67	0.15	0.90	0.76	0.899 (0.778 – 0.967)
Group 2	>19 ppb	0.56 (0.30 – 0.80)	0.75 (0.48 – 0.93)	2.25	0.58	0.69	0.63	0.680 (0.492 – 0.833)
Group 3	>27 ppb	0.64 (0.53 – 0.74)	0.94 (0.84 – 0.98)	11.12	0.38	0.95	0.60	0.844 (0.775 – 0.899)
Group 4	>23 ppb	0.45 (0.29 – 0.63)	0.87 (0.60 – 0.98)	3.43	0.63	0.89	0.41	0.597 (0.449 – 0.733)

Group 1: ICS-untreated, non-smokers

Group 2: ICS-untreated, smokers

Group 3: ICS-treated, non-smokers

Group 4: ICS-treated, smokers

CI: confidence intervals, +LR: positive likelihood ratio, -LR: negative likelihood ratio, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the ROC curve

EBC pH: Partly or Uncontrolled vs. Controlled

	Cut-off point	Sensitivity (95%CI)	Specificity (95%CI)	+LR	-LR	PPV	NPV	AUC (95%CI)
All	≤7.37	0.85 (0.79 – 0.90)	0.66 (0.55 – 0.75)	2.48	0.23	0.81	0.71	0.791 (0.738 – 0.837)
Group 1	≤7.36	0.91 (0.75 – 0.98)	0.63 (0.36 – 0.85)	2.42	0.15	0.83	0.77	0.734 (0.587 – 0.851)
Group 2	≤7.21	0.69 (0.41 – 0.89)	0.88 (0.62 – 0.98)	5.50	0.36	0.85	0.74	0.795 (0.616 – 0.916)
Group 3	≤7.39	0.92 (0.85 – 0.97)	0.65 (0.51 – 0.78)	2.67	0.12	0.82	0.83	0.836 (0.765 – 0.892)
Group 4	≤7.25	0.66 (0.48 – 0.81)	0.80 (0.52 – 0.95)	3.29	0.43	0.89	0.50	0.682 (0.535 – 0.806)

Group 1: ICS-untreated, non-smokers

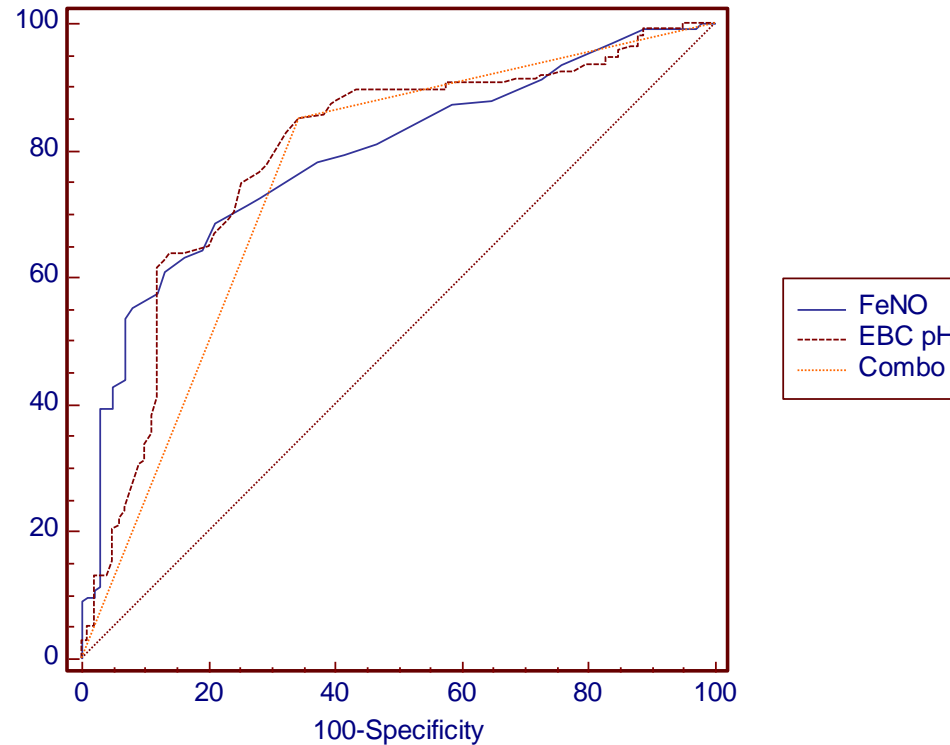
Group 2: ICS-untreated, smokers

Group 3: ICS-treated, non-smokers

Group 4: ICS-treated, smokers

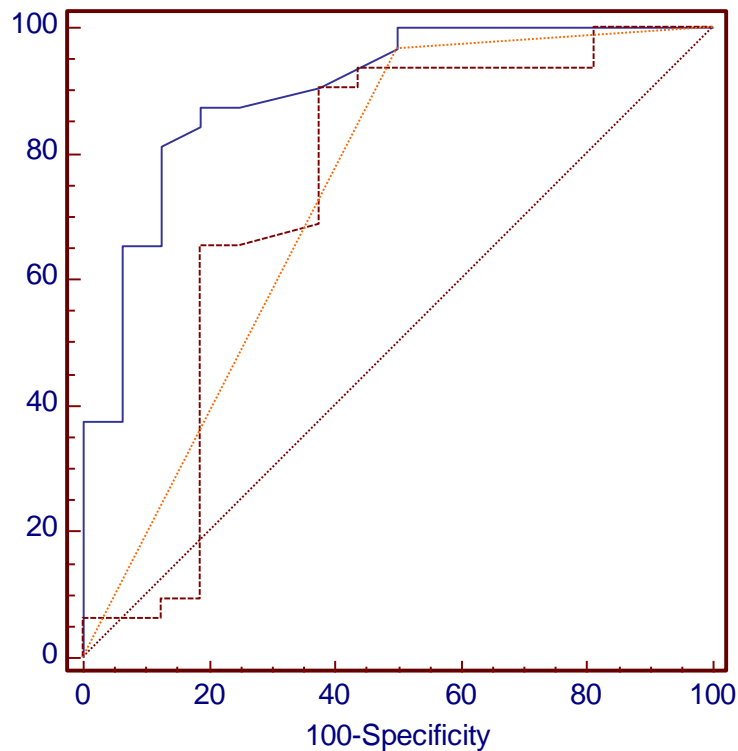
CI: confidence intervals, +LR: positive likelihood ratio, -LR: negative likelihood ratio, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the ROC curve

ALL: Comparison of ROC Curves



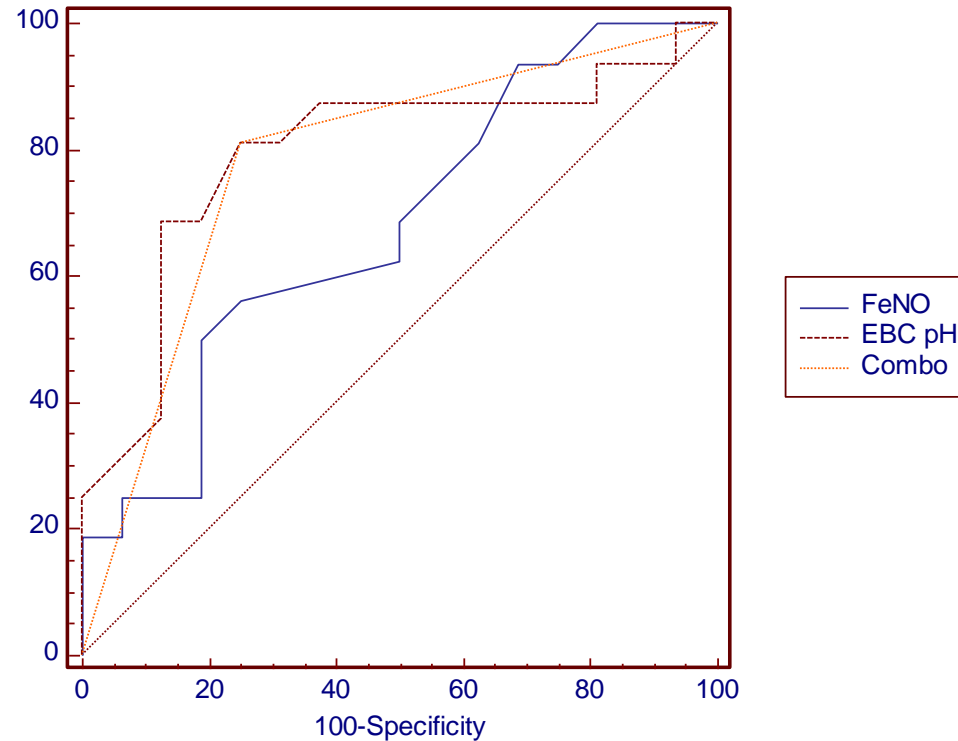
	Cut-off point	Sensitivity (95%CI)	Specificity (95%CI)	+LR	-LR	PPV	NPV	AUC (95%CI)
NO	>24 ppb	0.61 (0.54 – 0.68)	0.87 (0.79 – 0.93)	4.66	0.45	0.89	0.56	0.790 (0.737 – 0.837)
pH	≤7.37	0.85 (0.79 – 0.90)	0.66 (0.55 – 0.75)	2.48	0.23	0.81	0.71	0.791 (0.738 – 0.837)
NO >24 ppb or pH ≤7.37		0.93 (0.88-0.96)	0.66 (0.55-0.75)	2.48	0.23	0.81	0.71	0.754 (0.699 – 0.804)

Group 1: ICS-untreated – non-smokers



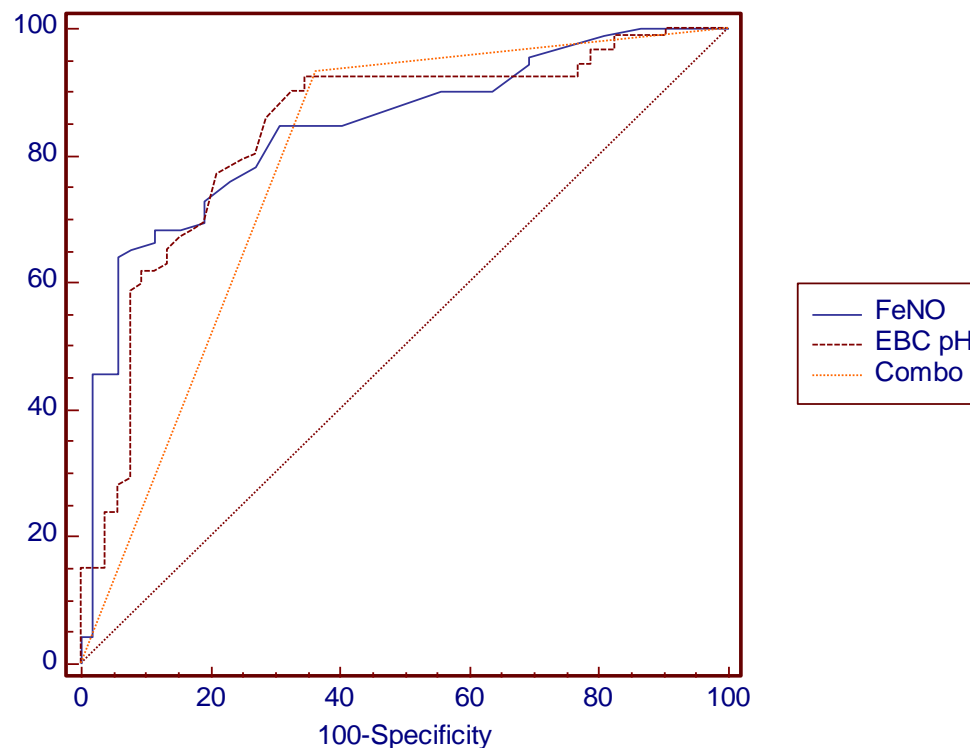
	Cut-off point	Sensitivity (95%CI)	Specificity (95%CI)	+LR	-LR	PPV	NPV	AUC (95%CI)
NO	>22 ppb	0.87 (0.71 – 0.96)	0.81 (0.54 – 0.96)	4.67	0.15	0.90	0.76	0.899 (0.778 – 0.967)
pH	≤7.36	0.91 (0.75 – 0.98)	0.63 (0.36 – 0.85)	2.42	0.15	0.83	0.77	0.734 (0.587 – 0.851)
NO >22 ppb or pH ≤7.36		0.97 (0.84-0.99)	0.50 (0.25-0.75)	1.94	0.06	0.79	0.89	0.734 (0.587 – 0.851)

Group 2: ICS-untreated – smokers



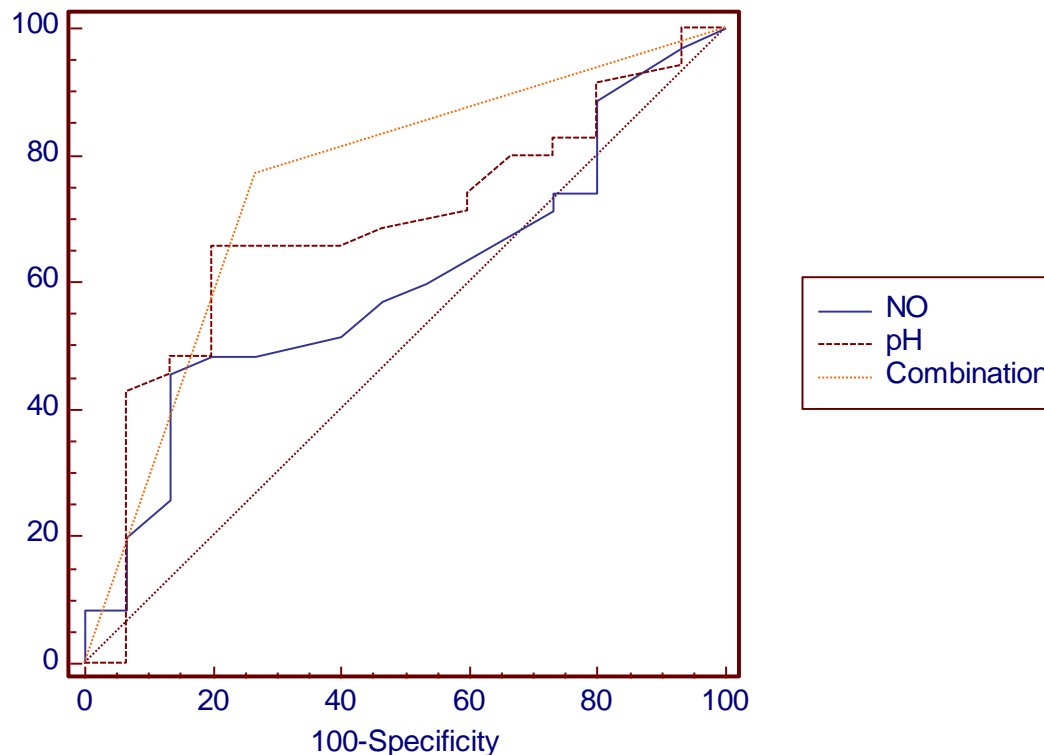
	Cut-off point	Sensitivity (95%CI)	Specificity (95%CI)	+LR	-LR	PPV	NPV	AUC (95%CI)
NO	>19 ppb	0.56 (0.30 – 0.80)	0.75 (0.48 – 0.93)	2.25	0.58	0.69	0.63	0.680 (0.492 – 0.833)
pH	≤7.21	0.69 (0.41 – 0.89)	0.88 (0.62 – 0.98)	5.50	0.36	0.85	0.74	0.795 (0.616 – 0.916)
NO >19 ppb or pH ≤7.21		0.81 (0.54-0.96)	0.75 (0.48-0.93)	3.25	0.25	0.76	0.80	0.781 (0.600 – 0.907)

Group 3: ICS-treated – non-smokers



	Cut-off point	Sensitivity (95%CI)	Specificity (95%CI)	+LR	-LR	PPV	NPV	AUC (95%CI)
NO	>27 ppb	0.64 (0.53 – 0.74)	0.94 (0.84 – 0.98)	11.12	0.38	0.95	0.60	0.842 (0.772 – 0.897)
pH	≤7.39	0.92 (0.85 – 0.97)	0.65 (0.51 – 0.78)	2.67	0.12	0.82	0.83	0.836 (0.765 – 0.892)
NO >27 ppb or pH ≤7.39		0.93 (0.86-0.98)	0.63 (0.49-0.76)	2.56	0.10	0.82	0.85	0.785 (0.709 – 0.849)

Group 4: ICS-treated – smokers



	Cut-off point	Sensitivity (95%CI)	Specificity (95%CI)	+LR	-LR	PPV	NPV	AUC (95%CI)
NO	>23 ppb	0.45 (0.29 – 0.63)	0.87 (0.60 – 0.98)	3.43	0.63	0.89	0.41	0.597 (0.449 – 0.733)
pH	≤7.25	0.66 (0.48 – 0.81)	0.80 (0.52 – 0.95)	3.29	0.43	0.89	0.50	0.682 (0.535 – 0.806)
NO >23 ppb or pH ≤7.25		0.77 (0.60-0.89)	0.73 (0.50-0.92)	2.89	0.31	0.87	0.58	0.752 (0.610 – 0.863)

FeNO for Not-Well Controlled Asthma

	All		Group 1		Group 2		Group 3		Group 4	
	PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV
>10	0.66	0.92	0.68	1.00	0.56	0.80	0.67	1.00	0.72	0.43
>15	0.73	0.65	0.80	1.00	0.57	0.67	0.74	0.72	0.69	0.29
>20	0.83	0.59	0.87	0.75	0.73	0.62	0.85	0.65	0.81	0.38
>25	0.89	0.54	0.90	0.72	0.80	0.56	0.91	0.60	0.82	0.33
>30	0.92	0.50	0.92	0.58	0.89	0.54	0.94	0.56	0.83	0.32
>35	0.94	0.48	0.95	0.56	1.00	0.52	0.94	0.51	1.00	0.32
>40	0.96	0.47	0.95	0.56	1.00	0.52	0.97	0.49	1.00	0.32

Group 1: ICS-untreated, non-smokers

Group 2: ICS-untreated, smokers

Group 3: ICS-treated, non-smokers

Group 4: ICS-treated, smokers

EBC pH for Not-Well Controlled Asthma

	All		Group 1		Group 2		Group 3		Group 4	
	PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV
≤7.10	0.86	0.41	0.62	0.32	0.83	0.56	0.89	0.41	0.92	0.38
≤7.15	0.85	0.44	0.75	0.36	0.79	0.61	0.89	0.44	0.89	0.41
≤7.20	0.87	0.48	0.81	0.40	0.84	0.74	0.91	0.48	0.86	0.41
≤7.25	0.89	0.57	0.78	0.50	0.76	0.80	0.92	0.56	0.89	0.50
≤7.30	0.84	0.58	0.80	0.56	0.70	0.83	0.89	0.60	0.79	0.43
≤7.35	0.82	0.64	0.82	0.71	0.72	0.79	0.84	0.68	0.77	0.43
≤7.40	0.79	0.74	0.80	0.75	0.70	0.83	0.81	0.82	0.74	0.40
≤7.45	0.73	0.70	0.70	0.60	0.61	0.78	0.78	0.80	0.72	0.40
≤7.50	0.71	0.67	0.68	1.00	0.56	0.72	0.75	0.77	0.71	0.33

Group 1: ICS-untreated, non-smokers

Group 2: ICS-untreated, smokers

Group 3: ICS-treated, non-smokers

Group 4: ICS-treated, smokers

Conclusions

- FeNO and EBC pH may have a role in the identification of patients with not-well-controlled asthma in an unselected asthma clinic population
- FeNO is indicative of not-well controlled asthma in non-smoking asthmatics (**PPV=0.90, AUC=0.899, cut-off >22 ppb** for untreated; and **PPV=0.95, AUC=0.844, cut-off >27 ppb** for treated asthmatics, respectively)
- EBC pH values ≤ 7.20 are indicative on not-well controlled asthma in smoking asthmatics (**PPV >0.85**)
- A combination of exhaled NO >23 ppb *or* pH ≤ 7.20 is indicative of not-well controlled asthma in ICS treated smokers (**PPV=0.87**)

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