Supplementary Material

Exhaled breath metabolomics as non–invasive diagnostic tool for ARDS.

Lieuwe DJ Bos, MSc [1,2], Hans Weda, PhD [4], Yuanyue Wang [4], Hugo H Knobel, Ir [4], Tamara ME Nijsen, PhD [4], Teunis J Vink, PhD [4], Aeilko H. Zwinderman PhD [3], Peter J Sterk, MD PhD [2], Marcus J Schultz, MD PhD [1]

Academic Medical Center – University of Amsterdam, The Netherlands:
[1] Intensive Care, [2] Department of Respiratory Medicine, [3] Clinical Epidemiology and Biostatistics
[4] **Philips Research, Eindhoven, The Netherlands**

Supplement Materials and Methods
Supplement Tables
Supplement Figures

Supplement Tables

Table E1: Adherence to STARD guidelines.

Section and Topic	Item		On page
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy(recommend MeSH heading 'sensitivity and specificity').	2
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	4
METHODS			
Participants	3	Describe the study population: The inclusion and exclusion criteria, setting and locations where the data were collected.	5
	4	Describe participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the (evaluated) index tests or the (golden) reference standard?	5
	5	Describe participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in items 3 and 4? If not, specify how participants were further selected.	5
	6	Describe data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	5–6
Test methods	7	Describe the reference standard and its rationale.	4
	8	Describe technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	*Online supplement*
	9	Describe definition of and rationale for the units, cut–offs and/or categories of the results of the index tests and the reference standard.	N.A.
	10	Describe the number, training and expertise of the persons executing and reading the index tests and the reference standard.	4 and *Online supplement*
	11	Describe whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	*Online supplement*
Statistical methods	12	Describe methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (*e.g.* 95% confidence intervals).	5–6 and *Online supplement*
	13	Describe methods for calculating test reproducibility, if done.	*Online supplement*
RESULTS			
Participants	14	Report when study was done, including beginning and ending dates of recruitment.	4
	15	Report clinical and demographic characteristics of the study population (*e.g.* age, sex, spectrum of presenting symptoms, co morbidity, current treatments, recruitment centers).	6 & Table 1, E3 and E4
	16	Report the number of participants satisfying the criteria for inclusion that did or did not undergo the index tests and/or the reference standard; describe why participants failed to receive either test (a flow diagram is strongly recommended).	6 & Figure 1
Test results	17	Report time interval from the index tests to the reference standard, and any treatment administered between.	*Online supplement*
	18	Report distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	6 & Table 1
	19	Report a cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	6–8
	20	Report any adverse events from performing the index tests or the reference standard.	N.A.
Estimates	21	Report estimates of diagnostic accuracy and measures of statistical uncertainty (*e.g.* 95% confidence intervals).	6–8
	22	Report how indeterminate results, missing responses and outliers of the index tests were handled.	N.A.
	23	Report estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	8
	24	Report estimates of test reproducibility, if done.	7
DISCUSSION	25	Discuss the clinical applicability of the study findings.	9–13

Table E2: Criteria for community– and hospital acquired pneumonia.

Community acquired pneumonia	*Symptoms of pneumonia started at home or in first 48h of hospital admission*	
Possible	Uncertainty about infiltrates on CXR	
	Low clinical suspicion, one or more of the following	Cough
		Purulent sputum
		Fever or hypothermia
		Leucocytosis
		Increased CRP (>30mg/L)
		Hypoxia (pO2<60mmHg)
Probable	Evident infiltrates on CXR	
	High clinical suspicion, one or more of the following	Crepitations during auscultation
		Positive pneumococcal or legionella urine test
Definite	Evident infiltrates on CXR	
	High clinical suspicion	
	Causative organism detected, one or more of the following	Positive blood culture
		High growth in tracheal aspirate
		Isolation of virus
		Positive serology
		Histopathology
Hospital acquired pneumonia	*Symptoms of pneumonia started after 48h of hospital admission*	
Possible	Uncertainty about infiltrates on CXR	
	Low clinical suspicion, one or more of the following	Cough
		Purulent sputum
		Fever or hypothermia
		Leucocytosis
		Increased CRP (>30mg/L)
		Hypoxia (pO2<60mmHg)
Probable	Evident infiltrates on CXR	
	High clinical suspicion, one or more of the following	Crepitations during auscultation
		PaO2/FiO2 ratio < 300
		Mechanical ventilation
	Causative organism detected, one or more of the following	Detection of pathogen in respiratory secretion
		Quantitative culture of BAL/PSB but below threshold for definite
Definite	Evident infiltrates on CXR	
	High clinical suspicion with one or more of the following	Crepitations during auscultation
		PaO2/FiO2 ratio < 300
		Mechanical ventilation
	Causative organism detected, one or more of the following following	Positive blood culture with respiratory pathogen
		Quantitative culture of BAL/PSB but above threshold (10^3 for PSB and 10^4 for BAL)
		Isolation of virus
		Positive serology
		Histopathology

Table E3: Logistic regression model

VOC	Fragment	Regression coefficient
Octane	m/z 84	-2.03
Octane	m/z 85	7.02
Octane	m/z 114	3.65
Acetaldehyde	m/z 44	0.49
3-Methylheptane	m/z 84	0.67

Table E4: Patient characteristics in training cohort.

		Control (25)	ARDS (23)	P–value
Age	*(years)*	60 (51–74)	66 (43–75)	0.79
Male	*(yes)*	14 (56)	15 (65)	0.91
APACHE II		24 (17–26)	24 (20–29)	0.23
SAPS II		48 (39–58)	46 (39–63)	0.65
Admission type	Medical	14 (56)	16 (70)	0.72
	Elective surgery	4 (16)	6 (26)	
	Emergency surgery	2 (8)	1 (4)	
Comorbidities	Asthma	0 (0)	0 (0)	-
	COPD	3 (12)	2 (9)	0.60
	Other respiratory	0 (0)	2 (9)	0.16
	Malignancy	2 (8)	3 (13)	0.67
	DM	1 (4)	7 (30)	0.02
Pmax	*(cmH_2O)*	17 (14–18)	27 (23–31)	<0.001
PEEP	*(cmH_2O)*	5 (5–5)	10 (7–11)	<0.001
Tidal volume	*(ml)*	464 (373–518)	447 (383–500)	0.85
Minute volume	*(l/min)*	8.7 (7.1–10.4)	12.5 (9.8–14.2)	<0.001
PaCO2	*(kPa)*	5.1 (4.6–5.7)	5.5 (4.6–6.1)	0.37
PaO_2/FiO_2	*(mmHg/%)*	341 (272–438)	219 (147–286)	<0.001
LIPS		3 (2–4)	5 (5–5)	<0.001
ICU Mortality		2 (8)	8 (35)	0.06

Differences between groups are tested using Mann-Whitney U or Chi–square test (with Yates' correction if necessary) and P–values are reported.

Table E5: Patient characteristics in temporal validation cohort.

		Control (27)	ARDS (19)	P–value
Age	*(years)*	60 (37–67)	68 (51–72)	0.19
Male	*(yes)*	16 (59)	16 (84)	0.27
APACHE II		17 (13–23)	22 (17–24)	0.14
SAPS II		41 (32–49)	46 (40–56)	0.12
Admission type	Medical	20 (74)	12 (63)	0.53
	Elective surgery	2 (7)	6 (32)	
	Emergency surgery	1 (4)	1 (5)	
Comorbidities	Asthma	0 (0)	1 (5)	0.26
	COPD	1 (4)	0 (0)	0.36
	Other respiratory	0 (0)	0 (0)	-
	Malignancy	1 (4)	4 (21)	0.10
	DM	5 (19)	2 (11)	0.33
Pmax	*(cmH_2O)*	15 (14–20)	20 (17–24)	0.05
PEEP	*(cmH_2O)*	5 (5–5)	8 (5–10)	0.002
Tidal volume	*(ml)*	451 (429–517)	549 (479–620)	0.06
Minute volume	*(l/min)*	9.7 (7.9–11)	9.4 (7.6–10.8)	0.93
PaCO2	*(kPa)*	4.9 (4.4–5.4)	5.3 (4.8–5.9)	0.04
PaO_2/FiO_2	*(mmHg/%)*	375 (307–480)	241 (204–290)	<0.001
LIPS		2 (1–3)	4 (4–5)	<0.001
ICU Mortality		3 (11)	4 (21)	0.39

Differences between groups are tested using Mann-Whitney U or Chi–square test (with Yates' correction if necessary) and P–values are reported.

Supplement Figures

Figure E1: Patient classification

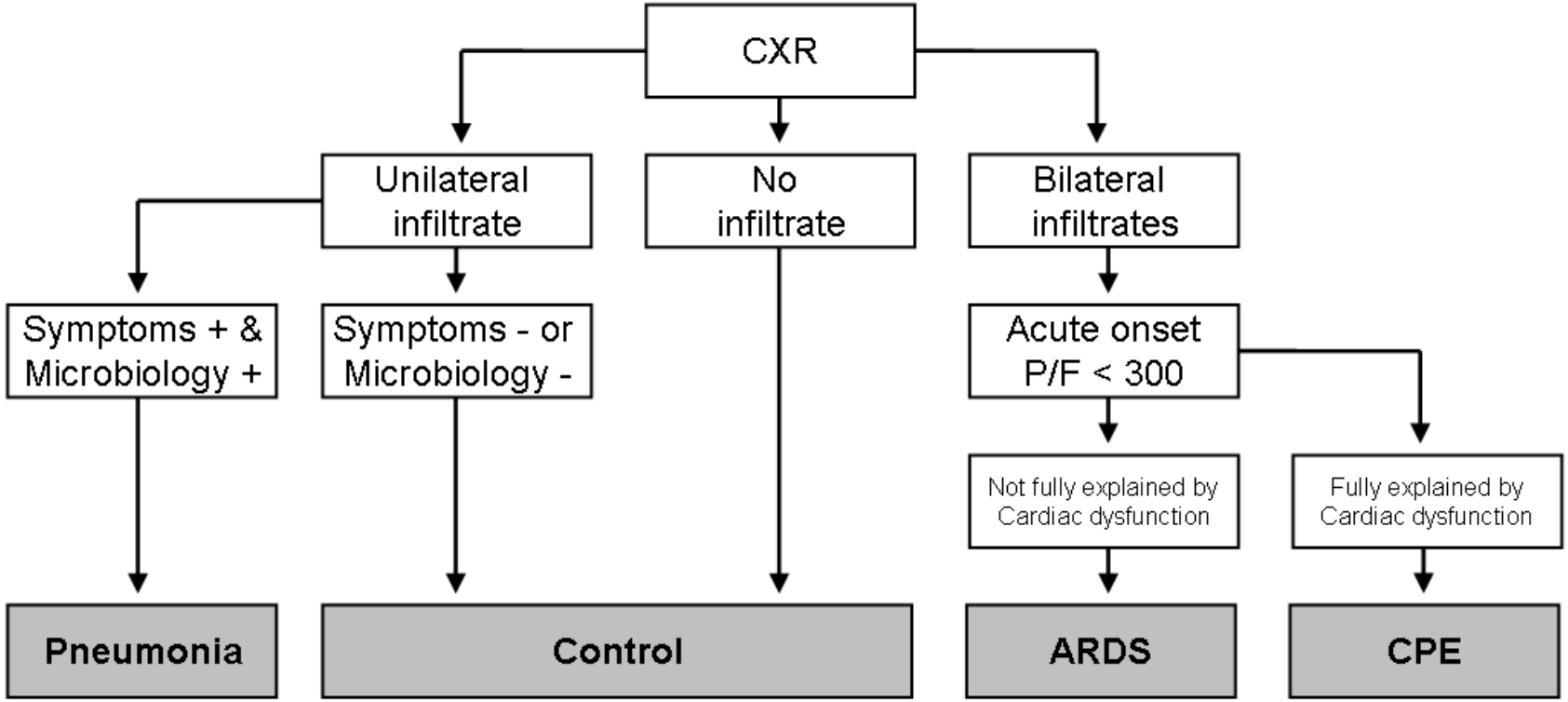


Figure E2: Relative abundances of selected fragments between ARDS patients and controls in the *training* cohort.

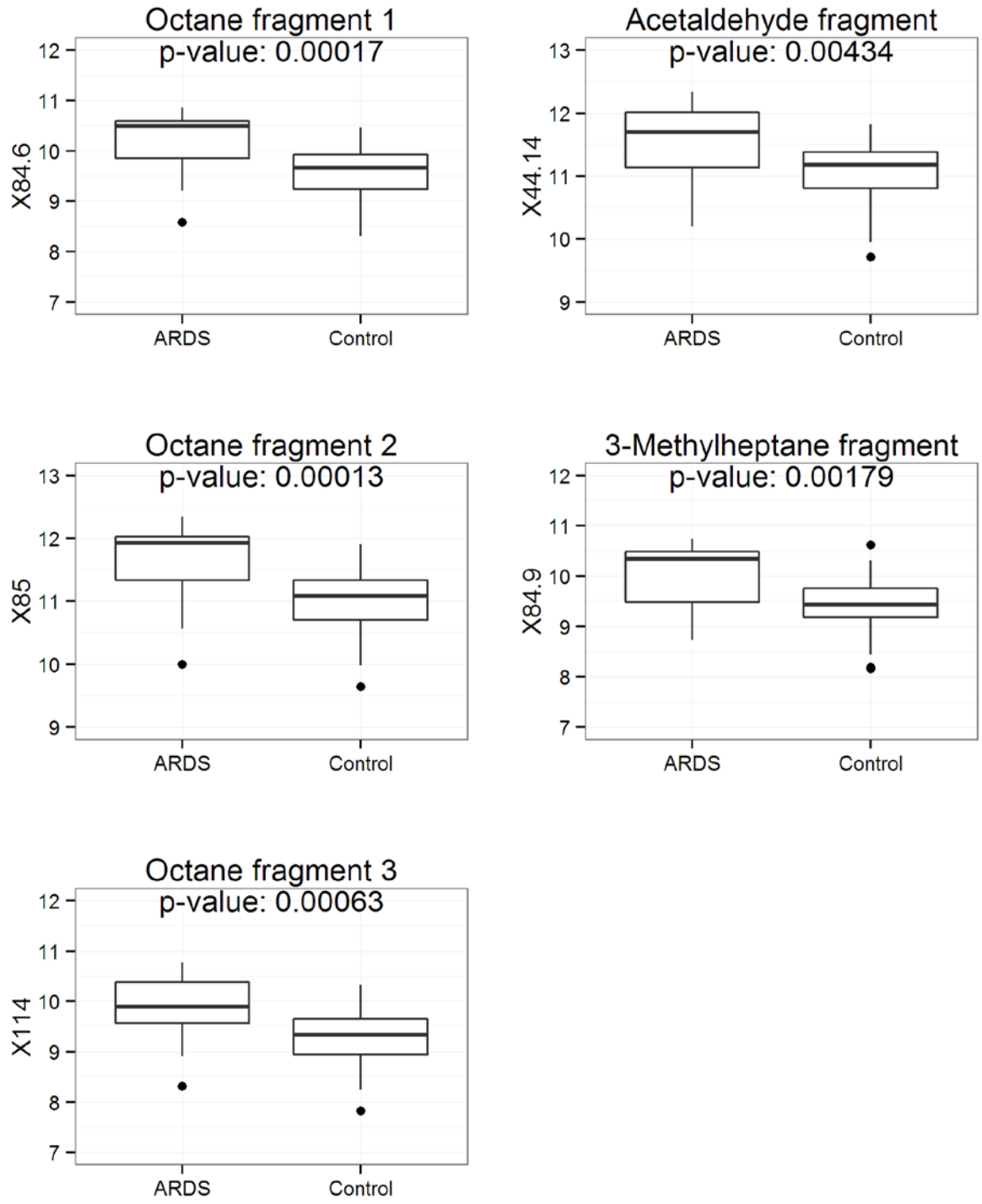
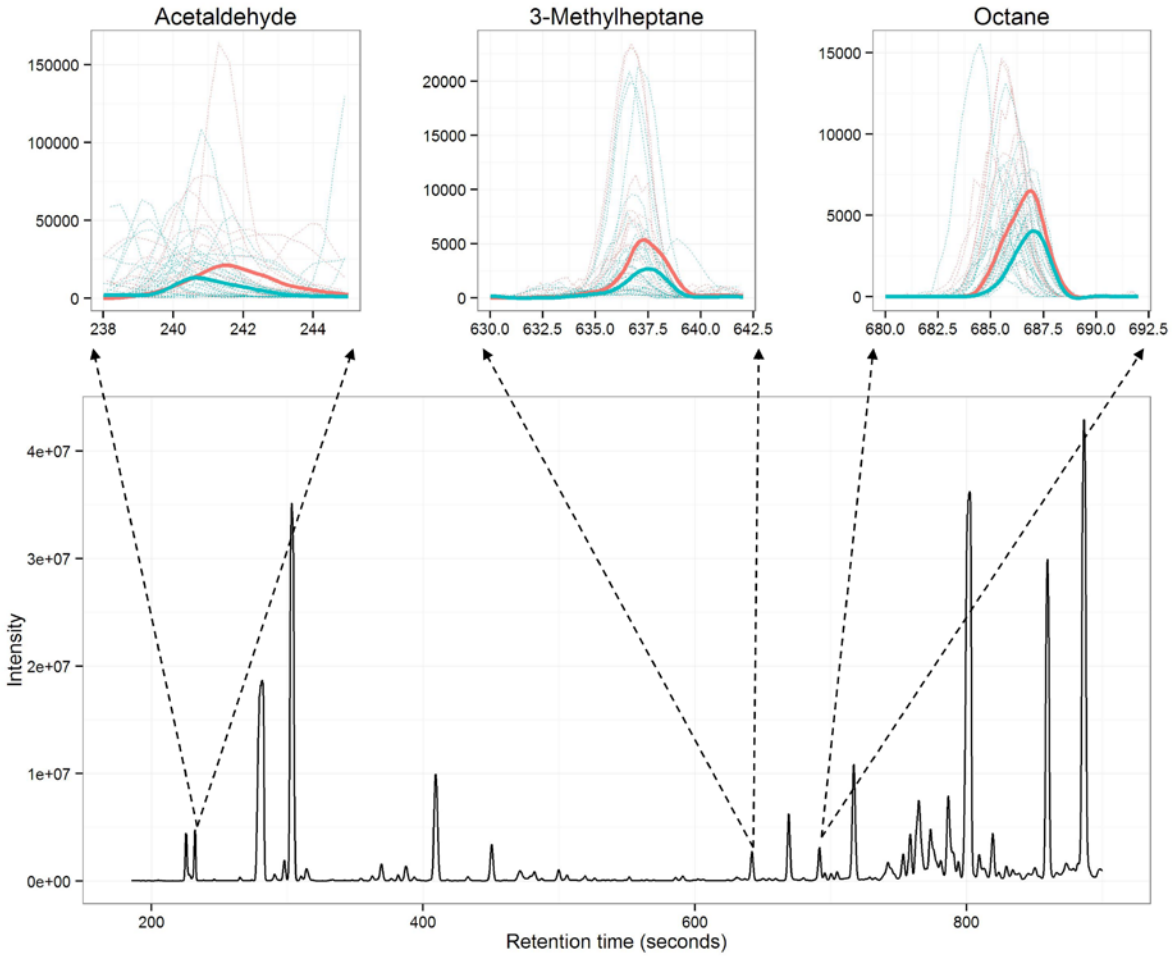


Figure E3: Typical chromatogram with selected volatile organic compounds.



The bottom panel shows a typical chromatogram from one patient. Every peak represents a volatile metabolite and the area under the peak is a linearly associated with the relative concentration. The top panel show the individual chromatograms of the three VOCs that were used for ARDS classification. The solid red line shows the median ARDS chromatogram and the solid blue line shows the median control chromatogram.

Figure E4: Relative abundances of selected fragments between ARDS patients and controls in the *temporal external validation* cohort.

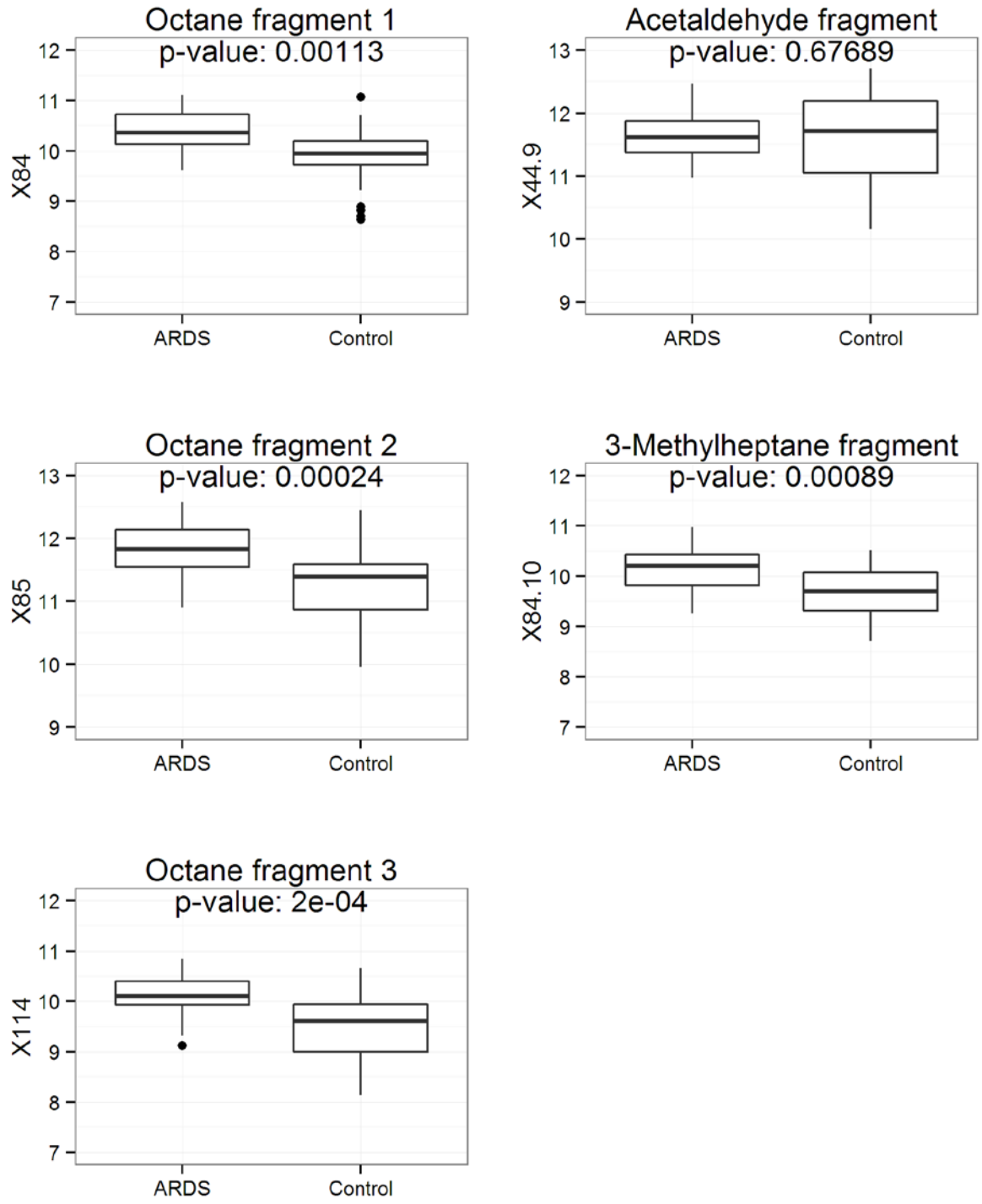


Figure E5: Calibration of the classification algorithm in the training and temporal validation cohort.

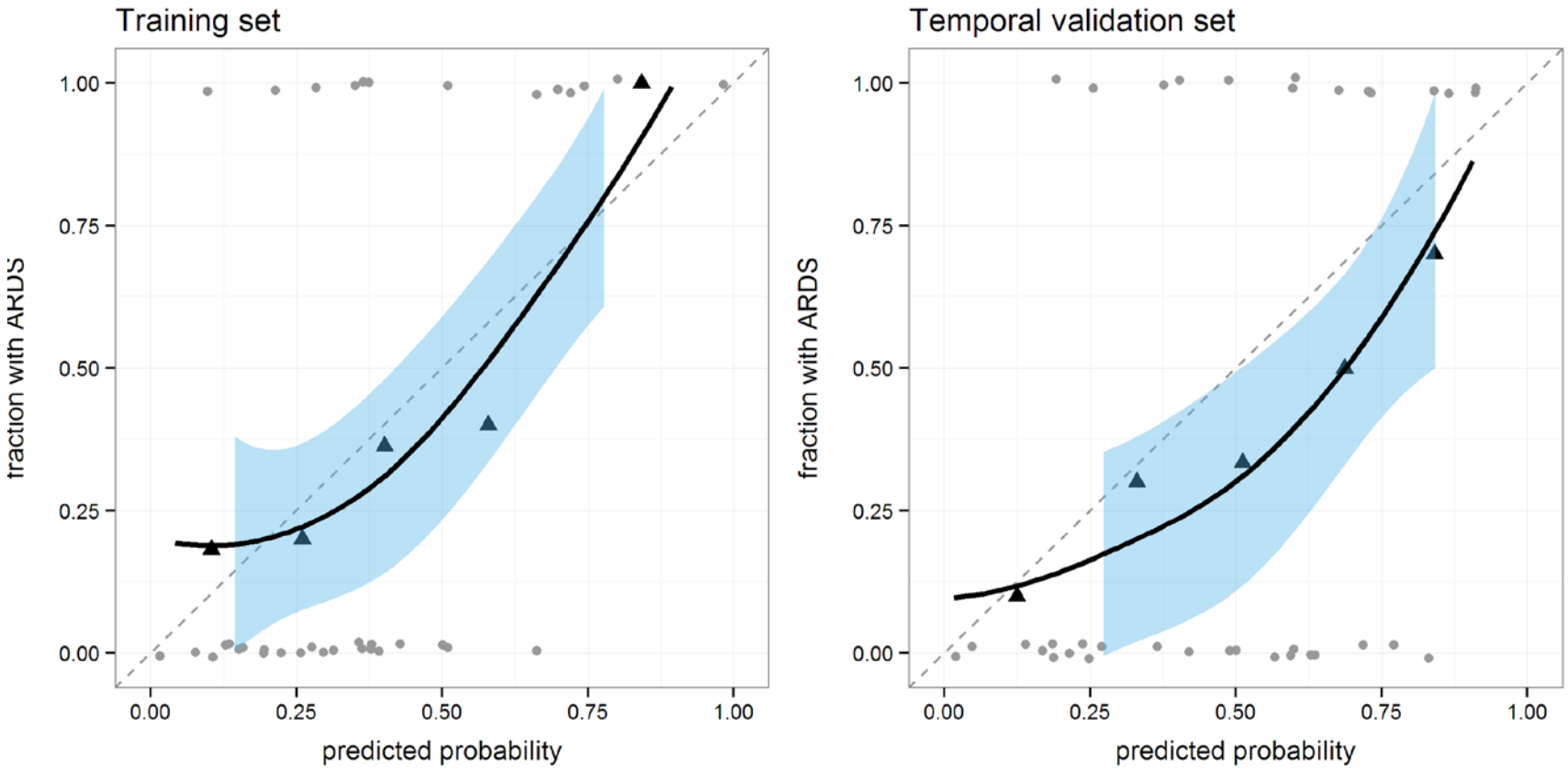


Figure E6: Result of exhaled breath analysis per subgroup

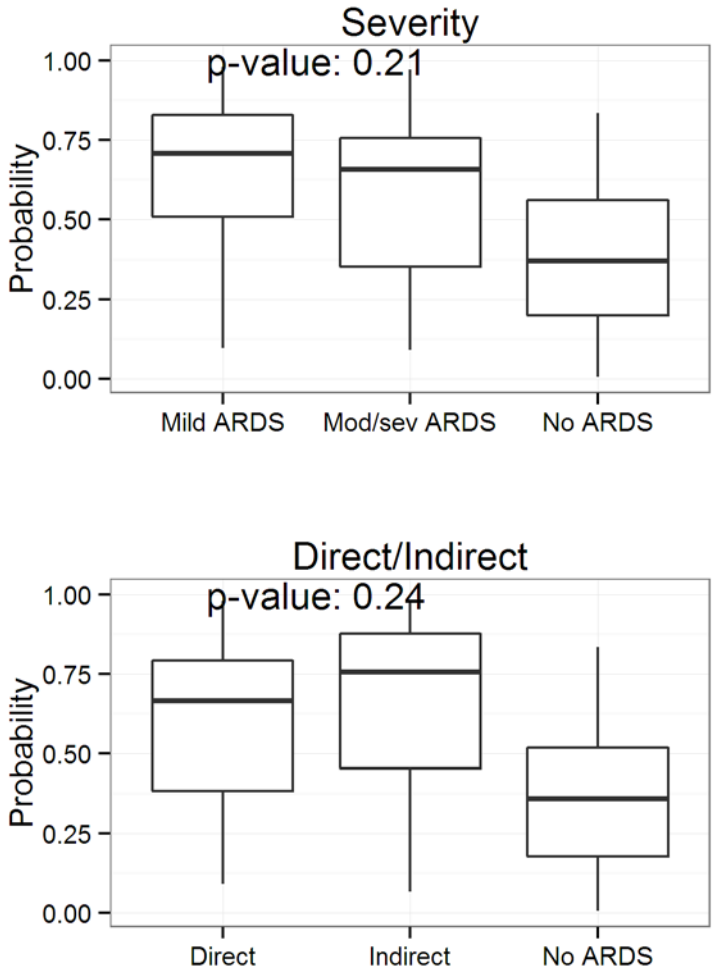


Figure E7: Relative abundance of isoprene in patients with and without ARDS.