CDI-00216-2019.R1

Original Article

Urinary volatile organic compound markers and colorectal anastomotic leakage

Victor D. Plat,[1] Boukje T. Bootsma,[1] Matthew Neal,[2] Karin Nielsen,[3] Dirk J.A. Sonneveld,[3] Janneke J.C. Tersteeg,[4] Rogier M.P.H. Crolla,[4] Dieuwertje A. van Dam,[5] Huib A. Cense,[5] Hein B.A.C. Stockmann,[6] James A. Covington,[7] Tim G.J. de Meij,[8] Jurriaan B. Tuynman[1], Nanne K.H. de Boer,[9] and Freek Daams[1]

[1] Department of Gastrointestinal surgery, Amsterdam UMC, VU University Medical Center, Amsterdam, The Netherlands

[2] Department of Statistics, University of Warwick, Coventry, United Kingdom

[3] Department of Gastrointestinal surgery, Dijklander Ziekenhuis, Hoorn, The Netherlands

[4] Department of Gastrointestinal surgery, Amphia Ziekenhuis, Breda, The Netherlands

[5] Department of Gastrointestinal surgery, Rode Kruis Ziekenhuis, Beverwijk, The Netherlands

[6] Department of Gastrointestinal surgery, Spaarne Gasthuis, Haarlem, The Netherlands

[7] School of Engineering, University of Warwick, Coventry, United Kingdom

[8] Department of Paediatric Gastroenterology and Hepatology, Amsterdam UMC, VU University Medical Center, Amsterdam, The Netherlands

[9] Department of Gastroenterology and Hepatology, Amsterdam UMC, VU University Medical Center, Amsterdam, The Netherlands

Word count text: 2991

Financial disclosure: None

Conflict of interest: None

Category: Colorectal neoplasia

Abbreviations

AL: Anastomotic Leakage

ASA: American Society of Anaesthesiologists

AUROC: Area Under the Receiver Operator Characteristics

CI: Confidence Interval

CRP: C-Reactive Protein

FAIMS: Field Asymmetric Ion Mobility Spectrometry

GC-MS: Gas Chromatography combined with Mass Spectrometry

ROC: Receiver Operator Characteristics

ICU: Intensive Care Unit

IQR: Interquartile Range

VOC: Volatile Organic Compound

Author contribution

VD Plat, BSc, participated in the design of the study, in the collection and interpretation of data, wrote and submitted the manuscript. Gave final approval of the version to be published.

BT Bootsma, BSc, was involved in the collection and interpretation of data, revised the manuscript critically and gave final approval of the version to be published.

M Neal, PhD, was involved in the interpretation of data, revised the manuscript critically and gave final approval of the version to be published.

K Nielsen, MD PhD, was involved in the collection of data, revised the manuscript critically and gave final approval of the version to be published.

DJA Sonneveld, MD PhD, was involved in the collection of data, revised the manuscript critically and gave final approval of the version to be published.

J Tersteeg, MD, was involved in the collection of data, revised the manuscript critically and gave final approval of the version to be published.

RMPH Crolla, MD PhD, was involved in the collection of data, revised the manuscript critically and gave final approval of the version to be published.

DA van Dam, MD, was involved in the collection of data, revised the manuscript critically and gave final approval of the version to be published.

HA Cense, MD PhD, was involved in the collection of data, revised the manuscript critically and gave final approval of the version to be published.

HBAC Stockmann, MD PhD, was involved in the collection of data, revised the manuscript critically and gave final approval of the version to be published.

JA Covington, PhD, was involved in the interpretation of data, revised the manuscript critically and gave final approval of the version to be published.

TGJ de Meij, MD PhD, participated in the design of the study, revised the manuscript critically and gave final approval of the version to be published.

J Tuynman, MD PhD, was involved in the collection of data, revised the manuscript critically and gave final approval of the version to be published.

NKH de Boer, MD PhD, participated in the design of the study, revised the manuscript critically and gave final approval of the version to be published.

F Daams, MD PhD, participated in the design of the study, was involved in the interpretation of data, revised the manuscript critically and gave final approval of the version to be published.

Address correspondence to: Mr. Victor Dirk Plat, BSc, Department of Gastrointestinal surgery, Amsterdam UMC, VU University Medical Center, De Boelelaan 1117, ZH 7F020, 1081 HV Amsterdam, The Netherlands. Email: V.plat@vumc.nl

Abstract

Aim: Inflammatory markers such as serum C-reactive protein (CRP) are used as routine markers to detect anastomotic leakage following colorectal surgery. However, CRP is characterized by a relatively low predictive value, emphasizing the need for the development of novel diagnostic approaches. Volatile organic compounds (VOCs) are gaseous metabolic products deriving from all conceivable bodily excrements and reflect (alterations in) the patient's physical status. Therefore, VOCs are increasingly considered as potential non-invasive diagnostic biomarkers. The aim of this study was to assess the diagnostic accuracy of urinary VOCs for colorectal anastomotic leakage.

Methods: In this explorative multicentre study, urinary VOC profiles of 22 patients with confirmed anastomotic leakage and 27 uneventful control patients following colorectal surgery were analysed by field asymmetric ion mobility spectrometry (FAIMS).

Results: Urinary VOCs of patients with anastomotic leakage could be distinguished from control patients with high accuracy; AUC: 0.91 (95% CI: 0.81-1.00, $p<0.001$), sensitivity: 86% and specificity: 93%. Serum CRP was significantly increased in patients with a confirmed anastomotic leak but with lower diagnostic accuracy compared to VOC analysis (AUC: 0.82, 95% CI: 0.68 – 0.95, $p<0.001$). Combining VOCs and CRP did not result in a significant improvement of its diagnostic performance compared to VOCs alone.

Conclusion: Analysis by FAIMS allowed for discrimination between urinary VOC profiles of patients with a confirmed anastomotic leak and control patients following colorectal surgery. A superior accuracy compared to CRP and apparently high specificity was observed, underlining its potential as a non-invasive biomarker for the detection of colorectal anastomotic leakage.

Keywords: Colorectal surgery, Anastomotic leakage, Volatile organic compounds, Field asymmetric ion mobility spectrometry

What does this paper add to the literature

This study is the first to our knowledge to investigate urinary VOCs in the detection of colorectal anastomotic leakage. A superior accuracy compared to serum CRP was observed, underlining its potential as a novel non-invasive biomarker for the detection of colorectal anastomotic leakage.

Introduction

Major morbidity and mortality following colorectal surgery is caused almost entirely by postoperative anastomotic leakage. Studies show that anastomotic leakage has a negative impact on short-term outcomes with an increased risk of postoperative mortality (1), the need for reoperation frequently accompanied by a permanent stoma (2), hospital length of stay (3) and hospital cost (4). In addition, some data have shown that anastomotic leakage might influence long-term oncological outcomes (5-8). A recent meta-analysis concluded that leakage is associated with increased local recurrence and subsequent reduced long-term survival (9). Timely detection of anastomotic leakage could enable early intervention, possibly leading to better outcome and timely start of adjuvant treatment.

In current practice, anastomotic leakage is diagnosed based on a combination of clinical, biochemical and radiological findings. Before radiological imaging, inflammatory markers such as C-reactive protein (CRP) are used as predictive markers for the diagnosis and follow-up of anastomotic leakage. Even though studies document positive results, CRP is characterized by relatively low positive predictive value. A wide range in cut-off points is also seen (10-12). This emphasizes the need for the development of novel diagnostic approaches, preferably non-invasive, with a high degree of accuracy for diagnosing anastomotic leakage following colorectal surgery.

Volatile organic compounds (VOCs) are produced by colonic fermentation as a result of an interaction between colonocyte cells, mucosal integrity and the colonic microbiome (13). Analysis of VOCs in faeces results in a reflection of the gut microbiota composition. Microbiota composition is subjected to infectious processes leading to bacterial dysbiosis (14). VOCs also originate from pathophysiological processes such as oxidative stress and inflammation. These molecules are able to diffuse into the circulation and are excreted by the kidneys. As a result, urinary VOCs have been shown to be accurate biomarkers for the detection of gastrointestinal diseases (15-17) and pancreatic anastomotic leakage (18). Therefore, biomarkers that emerge from all conceivable bodily excrements are thought to be the future of non-invasive diagnostics (19).

The aim of this study was to determine whether urinary VOC analysis could discriminate between patients with anastomotic leakage and uneventful control patients following major colorectal surgery.

Methods

Study design and participants

This project was designed as a prospective multicentre cross-sectional study between March 2015 and December 2016 and was reported according to the STROBE guidelines (20). Eligible participants were planned to undergo open or minimally invasive colorectal surgery, consisting of hemicolectomy, low anterior resection, sigmoid resection, subtotal colectomy, resection of small intestine or restoration of intestinal continuity via closure of an ostomy. Surgery was performed in five participating centres in the Netherlands and under standardized antibiotic prophylaxis (cefuroxime and metronidazole). All patients were aged 18-90 years and had an American Society of Anaesthesiologists (ASA) physical status of three or lower.

Definitions and inclusion

A single centre ten month period between March 2015 and December 2015 was predetermined to include control patients without anastomotic leakage. This control group consisted of a prospective cohort of consecutive patients scheduled for colorectal surgery. Urine samples were collected on postoperative day three. Subsequently a multicentre twelve month time period (between January 2016 and December 2016) was started to identify all patients eligible for inclusion and with a high suspicion of anastomotic leakage, by a combination of clinical deterioration, biochemical findings and/or radiological imaging. Urine samples were collected when leakage was clinically suspected. Inclusion followed after confirmation of the leak by either CT imaging or surgical exploration. All samples were collected before initiation of treatment.

Anastomotic leakage was defined as a defect of the intestinal wall at the anastomotic site. Severity was graded, according to the International Study Group of Rectal Cancer (ISREC), based on required treatment (21). All other postoperative complications were registered. In addition, white blood cell count (WBC) and C-reactive protein (CRP) were extracted from the patients charts on the day urine samples were collected. Patient, surgery and pathology characteristics were prospectively registered.

Sample collection

Urinary samples were collected in standard 4.5ml containers (Cryopure®). Collection took place on postoperative day three for control patients, which was chosen based on the median time of urinary catheter removal and early discharge of uneventful patients. For patients with anastomotic leakage

time of collection was related to timing of its clinical suspicion. All samples were obtained from urinary catheters. Subsequently, samples were frozen at -80° C within two hours of collection for simultaneous batch analysis.

Field asymmetric ion mobility spectrometry of urinary gas

Urinary gas analysis was performed using a commercially available field asymmetric ion mobility spectrometry (FAIMS) unit (Lonestar® with ATLAS sampling system, Owlstone, UK). This unit separates VOCs based on their mobility in high electric fields. It uses a radiation source (Ni-63) to ionize VOCs before they pass between parallel plates. Onto these plates an asynchronous high electric field is applied (22). This results in ions either being attracted/repelled by one of the plates or having no effect. If an ion touches a plate, it loses its charge and only ions that exit the plates with their charge are detected. To remove the effect of this ion drift, a compensation voltage is added and therefore, by scanning through different compensation voltages (in this case from +6V to -6V), it is possible to scan through a range of mobilities. As mobility can be a function of electric field strength, the magnitude of the electric fields is also scanned through a range of values (typically 51 steps from 0 to 100 on the instrument, with each step having a compensation voltage sweep). Each urine sample was analysed in triplicate and both positive and negative modes were used, producing 52.224 data points per sample per test. Once the data was obtained, the test data was exported and visualized using the Advanced Viewer software (version 4.2, Owlstone® Ltd, UK). A more detailed description and practical application of FAIMS has been reported previously (23).

Urinary analysis

Urine samples were analysed after a median storage period of six months, ranging between one and twelve months. Esfahani et al. observed no significant loss in VOCs within nine months of storage and proposed to analyse urine samples before twelve months (24). Before analysis, samples were first removed from the freezer to defrost at room temperature for a maximum of two hours. After defrosting, 4.5 ml of urine sample was aliquoted into a 22 ml glass vial and placed in the ATLAS sample system (Owlstone®, UK). The system heats the urine samples to 38° C and controls the flow of dry clean air over the top of the sample and into the instrument. Room air was compressed (0.1 MPa) and cleaned before being blown over the sample at a flow rate 500 millilitre per minute and with a total flow into the

instrument of two litre per minute. Temperatures were pre-set at 38° C for the sample holder, 70° C for the lid and 100° C for the filter region. All urine samples were measured in triplicate. In between urine samples a sample of clean tap water was analysed in triplicate to ensure that the baseline response was returned. Glass vials were sanitized at the department of microbiology after each sample.

Statistical analysis

IBM SPSS statistics (version 23) was used for standard statistical analysis and "R" for urinary VOC data analysis. Continuous variables were expressed as median (range) or mean ± standard deviation and frequency percentages were calculated for dichotomous variables. Between-group comparisons were done with an independent sample t test or Mann-Whitney U test for continuous variables and a chi-square test or Fisher's exact test (in case of small cell counts) for categorical variables. A p-value of <0.05 was considered statistically significant. Urinary VOCs of patients with anastomotic leakage were compared to control patients who had an uncomplicated postoperative course. Each sample resulted in 52.224 data points in a 2D matrix. Figure 1 shows visualized urinary VOC data, the samples are chosen closest to the mean of each group and therefore representative for the entire dataset. Data was processed using an existing pipeline (17, 23). A 2D wavelet transform (using Daubechies D4 wavelets) was utilized. Noise variables were identified by excluding the bottom 65% of variables from the analysis, ranked by standard deviation. The remaining variables were passed as input to a Random Forests classifier (25). Area under the receiver operating characteristics (AUROC), sensitivity, specificity and p-values were estimated using 10-fold cross validation, with confidence intervals (CI) computed using bias-corrected and accelerated (BCa) bootstrap intervals (26). A heat map, showing the number of times different variables in the data were used by the classifier is displayed in Figure 2, indicating which parts of the FAIMS data were important for classification.

Ethical considerations

The study protocol was approved by the Medical Ethical Review Committee (METc) of the VU University Medical Center (2015.272) and by the local institutional ethical review boards of the other four participating centres (Amphia Ziekenhuis Breda, Rode Kruis Ziekenhuis Beverwijk, Spaarne Gasthuis Haarlem and Dijklander Ziekenhuis Hoorn). All participants received both verbal and written

patient information, were capable of understanding the information and provided written informed consent.

Results

Patient characteristics

Urine samples of 22 patients with anastomotic leakage and 27 patients without anastomotic leakage following colorectal surgery were analysed. Twenty-six males and 23 females had a median age of 67 years (39-87). Patients underwent surgery between March 2015 and December 2016. Procedures consisted of hemicolectomy (n=19, 38%), low anterior resection (n=16, 33%), sigmoid resection (n=6, 12%), subtotal colectomy (n=5, 10%), closure of ostomy (n=2, 4%) and ileum resection (n=1, 2%). A minimally invasive approach was chosen in 40 patients (83%), four procedures (10%) were converted to open surgery, because of adhesions (n=2), adiposity (n=1) and lack of overview (n=1). Further patient characteristics are depicted in Table 1. In patients with anastomotic leakage significantly more lymph nodes were found in the resected specimens. Also, significantly more conversions from minimally invasive to open surgery were seen in patients with anastomotic leakage. No further statistically significant differences were seen between the groups.

Anastomotic leakage

Twenty-two patients with confirmed clinical anastomotic leakage were included. The median time to confirmation of leakage was three days (Interquartile Range (IQR): 1.75). No delay in suspicion of leakage, collection of urine samples and confirmation of diagnosis was observed. Clinical suspicion was confirmed by CT imaging (n=16, 73%) or laparotomy (n=6, 27%). All patients were treated by means of relaparotomy (n=19) or relaparoscopy (n=3) (grade C), during which surgical drains were placed (n=1), a diverting ileostomy was created to salvage the anastomosis (n=3), the anastomosis was recreated (n=6) or the anastomosis was taken down and an end colostomy or ileostomy was created (n=12).

Biochemical examination

Biochemical infectious markers were determined on the day urine samples were collected. Patients with a confirmed anastomotic leak had a mean level serum CRP of 271 (SD: 86 mg/l) compared to 157 (SD: 96 mg/l) for control patients ($p<0.001$) and had an AUROC of 0.81 (95% CI: 0.68-0.95).

The mean WBC was statistically increased in the presence of anastomotic leakage ($p=0.048$). The mean WBC count was 13.9 (SD: 7.0) at the time of confirmation of anastomotic leakage, compared to

10.5 (SD: 3.5) for control patients. WBC had an AUROC of 0.66 (95% CI 0.49-0.84). The clinical course of patients is summarized in Table 2.

Volatile organic compound analysis

Analysis showed that urinary VOC profiles of patients with anastomotic leakage could be discriminated from uneventful control patients. The results are shown in a box plot of probability (Figure 3). An AUROC of 0.91 (95% CI: 0.81-1.00, $p<0.001$) was achieved, and the ROC curve is displayed in Figure 4. At the optimum cut-off point a corresponding sensitivity of 0.86 (95% CI: 0.65-0.96) and specificity of 0.93 (95% CI: 0.75-1.00) was computed.

Combined markers

A combined predictive performance for serum CRP and urinary VOC profiles was computed. To allow for a fair comparison, CRP AUROC was cross-validated on the same folds as the VOC profiles. However, serum CRP was not measured for all patients in the control group leading to an underestimation because less data was available in each fold for selecting the optimum classification threshold. This led to an AUROC of 0.75 (95% CI 0.60-0.91) for CRP alone, which compares to an AUC of 0.92 (95% CI: 0.83-1.00) for VOC profiles alone when restricted to patients with CRP data. Combining CRP and VOCs an AUROC of 0.95 (95% CI: 0.89-1.00) was achieved. This improvement of 0.03 (95% CI: -0.028-0.13) was not significant at a 95% confidence level.

Discussion

In this study, urine samples of patients with colorectal anastomotic leakage were compared to urine samples of uneventful control patients. Analysis of FAIMS data showed that urinary VOC patterns could be used to discriminate between groups with a superior accuracy compared to serum CRP. These results suggest that urine analysis by means of FAIMS has potential as a non-invasive tool to detect postoperative anastomotic leakage.

This study is the first to our knowledge to investigate urinary VOCs, or VOCs in any bodily excrement, to detect colorectal anastomotic leakage. Previous research showed that urinary VOC patterns could predict anastomotic leakage correctly at an early stage following pancreatic surgery (AUROC 0.85), but not after oesophageal surgery (AUROC: 0.51). Present results suggest a superior accuracy of urinary VOC analysis following colorectal surgery compared to pancreatic surgery, however it must be noted that this study was designed as a cross-sectional study while in the pancreatic surgery study a longitudinal design was applied (18).

In previous decades, several specific biomarkers for infectious complications following colorectal surgery have been proposed (27, 28). Among surgeons, serum CRP level is the best known and is frequently determined biomarker during the postoperative period. Recent studies found that patients who developed anastomotic leakage could be discriminated from control patients with only modest accuracy following colorectal surgery (postoperative day five, AUROC: 0.79) (12). An increased accuracy was observed for infectious complications following rectal surgery (postoperative day four, AUROC: 0.88) (10). Next to CRP, procalcitonin has been proposed as a biomarker for severe anastomotic leakage following colon surgery. At the optimal cut-off point on postoperative day five an AUROC of 0.74 was found (12).

Other urinary biomarkers for colorectal anastomotic leakage have been proposed. Dusek et al. (29) evaluated the use of neopterin, kynurenine and tryptophan as predictors of anastomotic leakage following rectal surgery. Results show that high preoperative and postoperative levels of urinary neopterin were seen in patients with colorectal anastomotic leakage. It was suggested that urinary neopterin could be a useful marker for leakage.

The apparently high specificity of urinary VOCs compared to other markers underlines the potential of this novel method to serve as a complementary routine non-invasive biomarker in the diagnosis of anastomotic leakage. It must be noted that in the present study urinary analysis was performed in patients with a high suspicion of anastomotic leakage and observed leaks were early and severe (median postoperative day 3 and requiring surgical intervention). Hence, extrapolating the current data to diagnostic value of VOC analysis in the detection of subtle or delayed anastomotic leakage must be done cautiously. Neither does it elucidate the place of urinary VOC analysis in screening for leakage, since urine samples were collected once anastomotic leakage was already highly suspected. Future longitudinal studies should preferably collect urine on certain pre- and postoperative time points to determine the ability of urinary VOCs to predict anastomotic leakage. Obviously, upon externai validation of presented results in a longitudinal study, implementation of VOC analysis could possibly facilitate earlier recognition of anastomotic leakage and has the potential to avert surgical management and allow treatment in a less invasive manner.

As VOC analysis by FAIMS has yet to make it into clinical practice, only specialized research facilities have access to this technology. Even though actual sampling is quick and inexpensive, methodologies utilized in medical studies are costly and time-consuming. Samples are frozen and stored for batch sampling followed by extensive data analysis, which is not feasible in clinical practice. It appears that freezing and storage of urine samples does not result in a significant loss of VOCs, however real-time sampling of unfrozen urine samples requires further investigation. Likely future developments are necessary for this technique to be of clinical value, instant sample analysis is a prerequisite and data-processing should preferably be incorporated in the instrument itself (23).

Apart from urine, faeces and exhaled breath have also been proposed as biological media to detect human VOCs. Metabolites of intestinal pathophysiological processes are primarily discharged in faeces. Faecal VOCs have shown to be highly accurate biomarkers for gastrointestinal diseases (30-32). Application in colorectal surgery patients however is limited due to temporary impairment of bowel mobility in postoperative patients. Collection of urine is “patient friendly” and can be provided on

demand. Urine collection was preferred over exhaled breath, due to high costs and logistical challenges of collection, storage and analysis of exhaled breath.

In this study FAIMS was used. Although gas chromatography combined with mass spectrometry (GC-MS) is considered the gold standard of VOC analysis, FAIMS has much higher sensitivity, requires less infrastructure and has a shorter analysis time. This may make it more realistic as a routine diagnostic tool. However, GC-MS allows for identification of individual VOCs based on their physiochemical properties. In the near future, GC-MS will be used to identify individual VOCs to further understand the pathophysiology behind colorectal anastomotic leakage.

A limitation of this pilot study is its cross-sectional design, which resulted in a variable timing of urine collection in the anastomotic leakage cohort and the inability to assess the potential influence of other factors. However, this design was favoured over a longitudinal design, since the incidence of anastomotic leakage following colorectal surgery is low and therefore a cross-sectional approach is the most feasible first step to identify specific VOC markers without needing a large number of patients. Secondly, our pilot design resulted in a small sample size and inability to match groups on specific baseline characteristics. Future studies should preferably be performed in a multicentre, prospective, longitudinal design, and collect urine on certain pre- and postoperative time points to allow for detailed assessment of the intra-individual course of specific urinary VOC patterns and to investigate its ability to predict anastomotic leakage.

In conclusion, urinary VOC analysis by FAIMS allowed for discrimination between patients with anastomotic leakage and uneventful postoperative patients after colorectal surgery. A superior accuracy compared to serum CRP was observed, underlining its potential as a non-invasive biomarker for the detection of colorectal anastomotic leakage.

Figure 1. Visualized urinary VOC data generated using FAIMS, which measures ion counts by altering compensation voltages (+6 to -6 V in 512 steps) and magnitude of electric field (the dispersion field, 0 to 100% in 51 steps) to produce 52.224 data points per sample per test. This figure shows the negative/positive ion counts for an patient with anastomotic leakage and a control patient. The specific plumes indicate the presence of specific VOCs in the urine sample. VOCs: volatile organic compounds, FAIMS: field asymmetric ion mobility spectrometry.

Figure 2. Heat map of the number of times different variables in the data were used by the classifier, indicating which parts of the FAIMS data were important for classification. The grey background shows all the plumes in the data set. The red overlay highlights which variables were used by the classifier, brightness indicated frequency. FAIMS: field asymmetric ion mobility spectrometry**.**

Figure 3. Analysis of urinary VOCs resulted in a predicted probability of anastomotic leakage for each sample. This figure shows the predicted probability for both groups (anastomotic leakage and control patients). AL: anastomotic leakage VOCs: volatile organic compounds.

Figure 4. ROC-curve with 95% confidence interval for diagnosis of anastomotic leakage compared to control patients. AUC: 0.91 (95% CI: 0.81-1.00, $p<0.001$). ROC: Receiver operating characteristics, AUC: area under the curve.

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Characteristic	AL (n = 22)	Controls (n = 27)	P-value
Female gender	9 (41)	14 (52)	0.318
Age (year)	67.3 ± 11.1	67.0 ± 11.1	0.930
BMI (kg/m^2)	27.1 ± 3.7	27.5 ± 8.0	0.849
ASA	2 (1 – 3)	2 (1 – 3)	0.705
Comorbidities			
Hypertension	11 (50)	14 (52)	0.897
Heart failure	2 (9)	4 (15)	0.438
COPD	4 (18)	4 (15)	0.618
Diabetes Mellitus	4 (18)	8 (30)	0.279
Chronic kidney disease	2 (9)	1 (4)	0.422
Indication for surgery			0.231
Cancer	19 (86)	26 (96)	
Diverticulitis	3 (14)	1 (4)	
Tumour pathology			
T-stadium	3 (0 – 4)	2 (0 – 4)	0.642
N-stadium	0 (0 – 2)	0 (0 – 2)	0.909
Minimally invasive surgery	17 (77)	22 (81)	0.635
Conversion	4 (24)	0 (0)	0.029*
Type of surgery			0.888
Hemicolectomy	8 (36)	11 (41)	
Sigmoid resection	3 (14)	3 (11)	
Low anterior resection	6 (27)	10 (37)	
Subtotal colectomy	3 (14)	2 (7)	
Small intestine	1 (5)	0 (0)	
Ostomy closure	1 (5)	1 (4)	
Lymph nodes resected	19 (9 – 45)	13 (2 – 40)	0.020*

Data are n (%), mean ± standard deviation and median (range)

Anastomotic leakage (AL), Body mass index (BMI), American Society of Anaesthesiologists (ASA), Chronic obstructive pulmonary disease (COPD). * Significant

Table 1. Summary of demographic and clinical characteristics

Parameter	AL (n = 22)	Controls (n = 27)	P-value
Leakage grade (ISREC)			
Grade A	0 (0)	-	
Grade B	0 (0)	-	
Grade C	22 (100)	-	
Sample collection (POD)	3 (1 – 10)	3	
Storage time (months)	5 (1 – 12)	6 (1 – 12)	0.571
Serum CRP	271 ± 86	157 ± 96	<0.001*
WBC	13.9 ± 7.0	10.5 ± 3.5	0.048*
Other complications			
Pneumonia	1 (5)	1 (4)	0.702
Gastroparesis	1 (5)	1 (4)	0.702
Atrial fibrillation	2 (9)	1 (4)	0.422
Ileus	2 (9)	1 (4)	0.422
Wound infection	2 (9)	0 (0)	0.196
Urinary retention	3 (14)	0 (0)	0.084
Respiratory insufficiency	2 (9)	0 (0)	0.196
Hospital stay (days)	24 (11 – 67)	5 (3 – 34)	<0.001*
ICU stay (days)	2 (0 – 40)	0 (0 – 1)	0.035*
30-day mortality	0 (0)	0 (0)	

Data are n (%), mean ± standard deviation and median (range)

Anastomotic leakage (AL), International Study Group of Rectal Cancer (ISREC), Postoperative day (POD), C-reactive protein (CRP), White blood cell count (WBC), Intensive care unit (ICU). * Significant

Table 2. Summary of clinical course

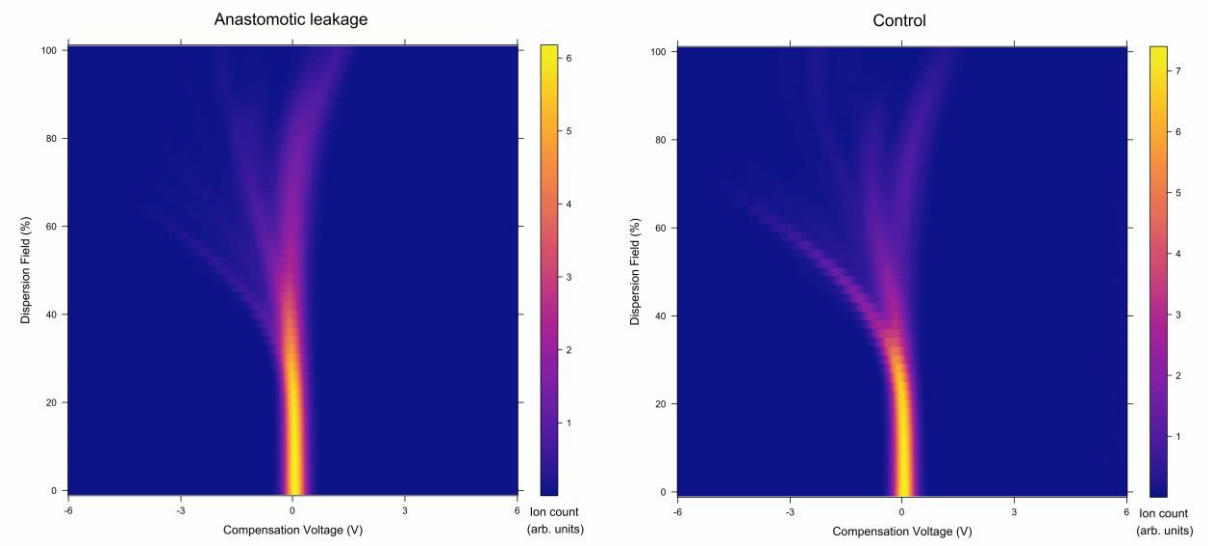

Figure 1. Visualized urinary VOC data generated using FAIMS, which measures ion counts by altering compensation voltages (+6 to -6 V in 512 steps) and magnitude of electric field (the dispersion field, 0 to 100% in 51 steps) to produce 52.224 data points per sample per test. This figure shows the negative/positive ion counts for an patient with anastomotic leakage and a control patient. The specific plumes indicate the presence of specific VOCs in the urine sample. VOCs: volatile organic compounds, FAIMS: field asymmetric ion mobility spectrometry.

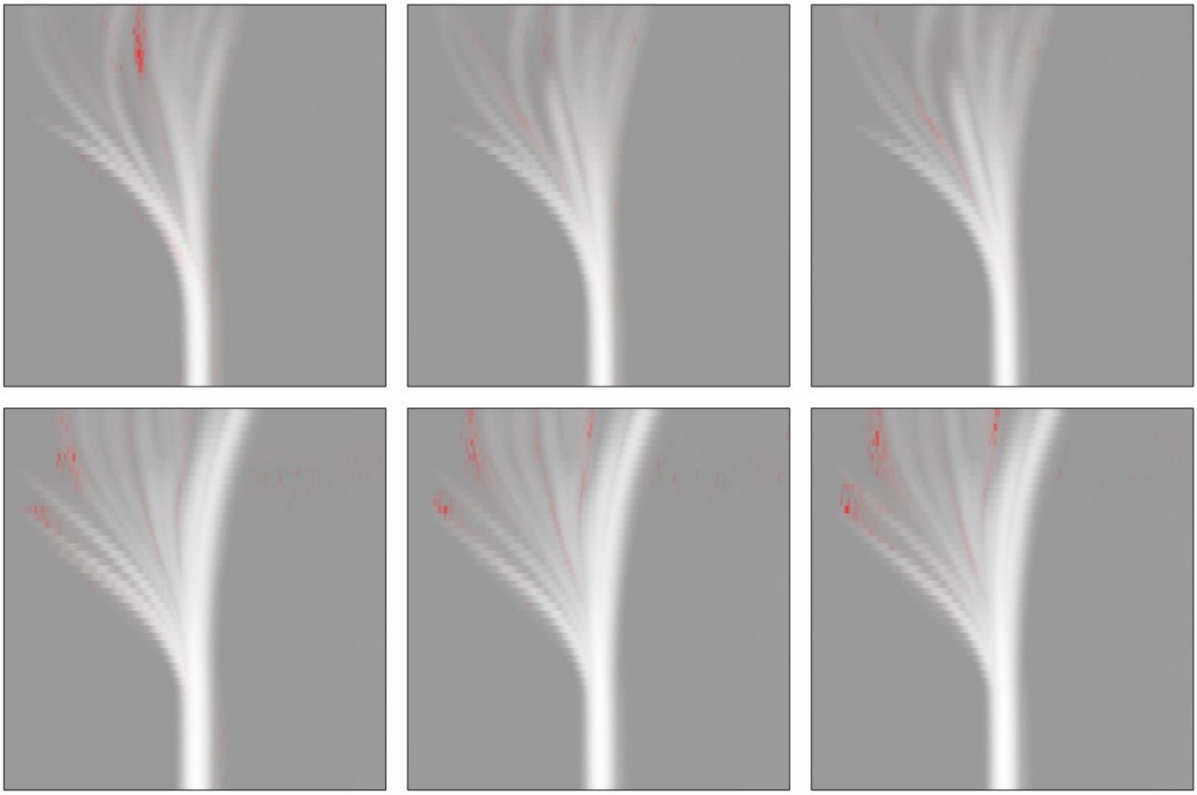

Figure 2. Heat map of the number of times different variables in the data were used by the classifier, indicating which parts of the FAIMS data were important for classification. The grey background shows all the plumes in the data set. The red overlay highlights which variables were used by the classifier, brightness indicated frequency. FAIMS: field asymmetric ion mobility spectrometry.

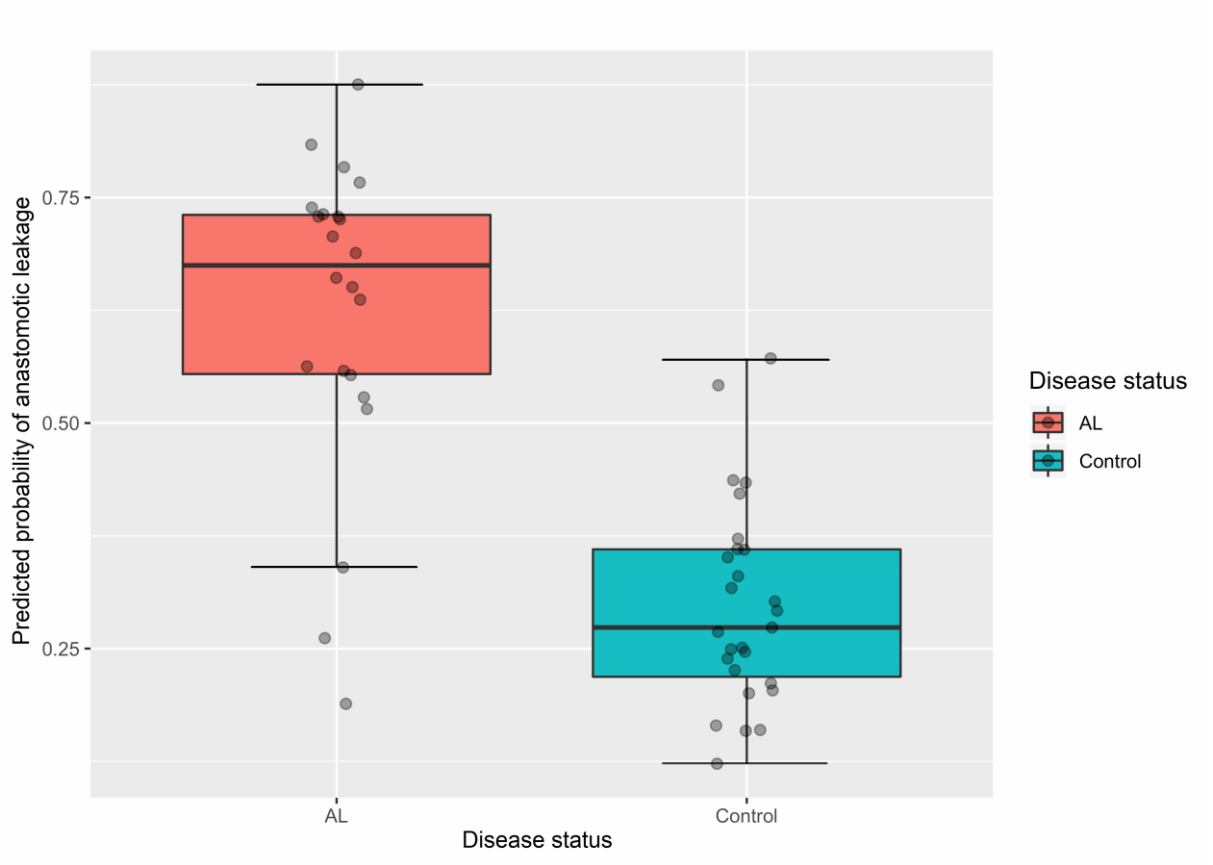

Figure 3. Analysis of urinary VOCs resulted in a predicted probability of anastomotic leakage for each sample. This figure shows the predicted probability for both groups (anastomotic leakage and control patients). AL: anastomotic leakage VOCs: volatile organic compounds.

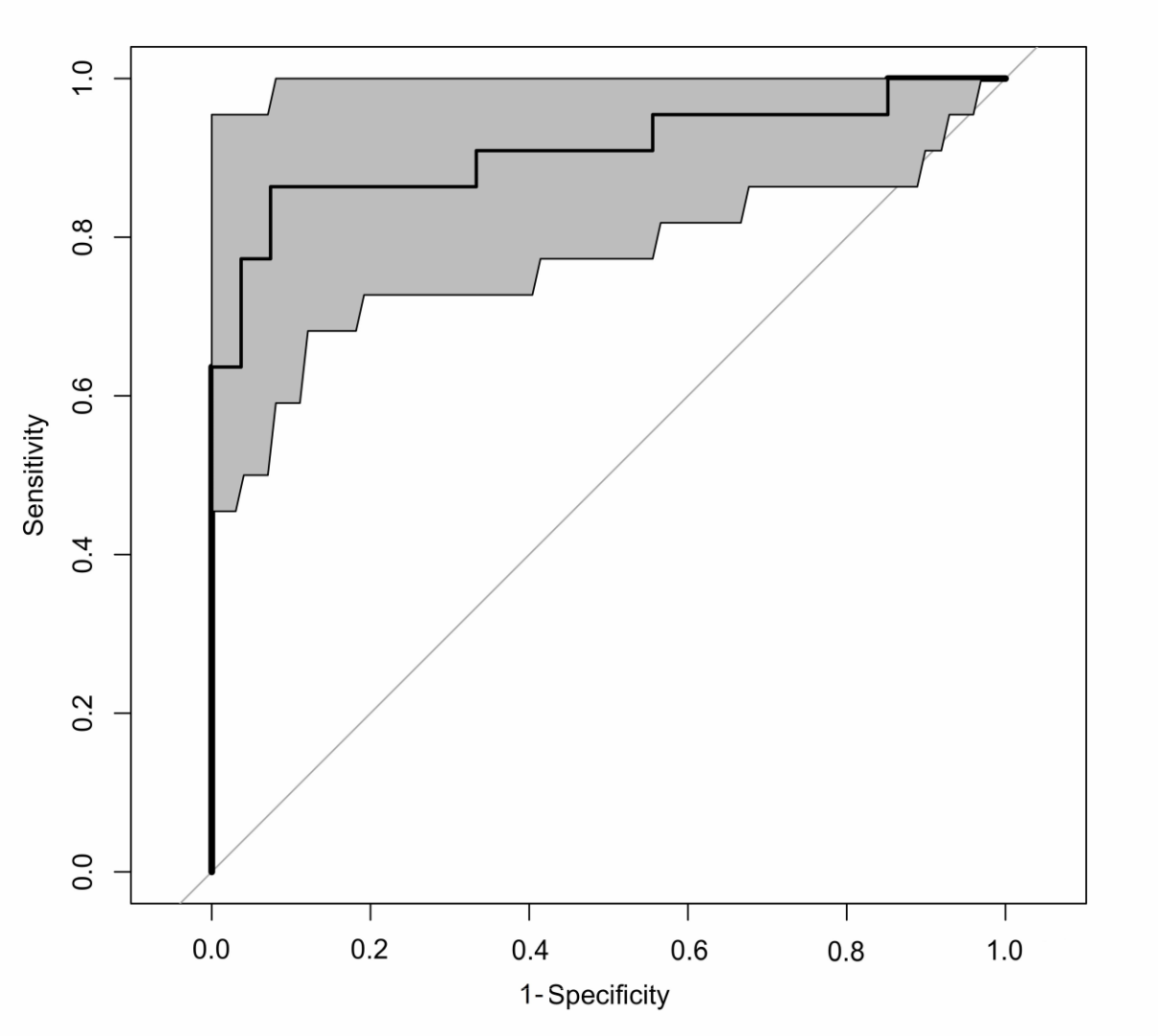

Figure 4. ROC-curve with 95% confidence interval for diagnosis of anastomotic leakage compared to control patients. AUC: 0.91 (95% CI: 0.81-1.00, p<0.001). ROC: Receiver operating characteristics, AUC: area under the curve.