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STUDY PROTOCOL

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Biomarkers of Airway Disease, Barrett's and Underdiagnosed Reflux Noninvasively (BAD-BURN) in World Trade Center exposed firefighters: a case–control observational study protocol

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Abstract

Background Particulate matter exposure (PM) is a cause of aerodigestive disease globally. The destruction of the World Trade Center (WTC) exposed first responders and inhabitants of New York City to WTC-PM and caused obstructive airways disease (OAD), gastroesophageal reflux disease (GERD) and Barrett's Esophagus (BE). GERD not only diminishes health-related quality of life but also gives rise to complications that extend beyond the scope of BE. GERD can incite or exacerbate allergies, sinusitis, bronchitis, and asthma. Disease features of the aerodigestive axis can overlap, often necessitating more invasive diagnostic testing and treatment modalities. This presents a need to develop novel non-invasive biomarkers of GERD, BE, airway hyperreactivity (AHR), treatment efficacy, and severity of symptoms.

Methods Our observational case-cohort study will leverage the longitudinally phenotyped Fire Department of New York (FDNY)-WTC exposed cohort to identify *Biomarkers of Airway Disease, Barrett's and Underdiagnosed Reflux Noninvasively (BAD-BURN)*. Our study population consists of $n = 4{,}192$ individuals from which we have randomly selected a sub-cohort control group ($n = 837$). We will then recruit subgroups of *i.* AHR only *ii.* GERD only *iii.* BE *iv.* GERD/BE and AHR overlap or *v.* No GERD or AHR, from the sub-cohort control group. We will then phenotype and examine non-invasive biomarkers of these subgroups to identify under-diagnosis and/or treatment efficacy. The findings may further contribute to the development of future biologically plausible therapies, ultimately enhance patient care and quality of life.

Discussion Although many studies have suggested interdependence between airway and digestive diseases, the causative factors and specific mechanisms remain unclear. The detection of the disease is further complicated by the invasiveness of conventional GERD diagnosis procedures and the limited availability of disease-specific biomarkers. The management of reflux is important, as it directly increases risk of cancer and negatively impacts quality

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of life. Therefore, it is vital to develop novel noninvasive disease markers that can effectively phenotype, facilitate early diagnosis of premalignant disease and identify potential therapeutic targets to improve patient care.

Trial registration Name of Primary Registry: "Biomarkers of Airway Disease, Barrett's and Underdiagnosed Reflux Noninvasively (BADBURN)". Trial Identifying Number: NCT05216133. Date of Registration: January 31, 2022.

Keywords Air pollutants, Airway hyperreactivity, Ambient particulate matter, Barrett's esophagus, Gastro-esophageal reflux disease, Particulate, Aerodigestive

Background

Particulate matter (PM) exposure is a risk factor for aerodigestive disease and mortality [1–3]. On September 11, 2001 (9/11), first-responders and inhabitants of New York City were exposed to World Trade Center (WTC)-PM [4–35]. Many subsequently developed aerodigestive diseases including obstructive airways disease (OAD), gastroesophageal reflux disease (GERD) and Barrett's Esophagus (BE) [23, 34, 36–42]. By 2005, approximately 44% of WTC rescue and recovery workers had developed GERD, which is 8.2-fold higher than the pre-9/11 prevalence, and more than double the general US population [43–46]. After WTC-PM exposure, GERD occurred more often in asthmatics [42]. Comorbid aerodigestive disease affected 51.4% of firefighters [47].

GERD and BE are risk factors for esophageal adenocarcinomas (EAC) [48]. Patients with BE face at least 30-fold higher risk of developing EAC than the general population [49, 50]. Complications of GERD extend beyond malignancy and can adversely affect quality of life (QoL), impair productivity, and lifespan [46, 51–53]. GERD can incite or exacerbate co-morbidities such as allergies, sinusitis, chronic bronchitis, and asthma [54]. There is a 59.2% prevalence of GERD symptoms in patients with asthma compared to 38.1% in controls [55]. GERD treatment in WTC responders with proton pump inhibitors (PPIs) have been found to increase risk of severe cognitive impairment [56]. Cognitive decline with PPI use has also been reported in the general population [57].

Despite numerous studies suggesting potential interdependence between airway and digestive diseases, the underlying causative factors and mechanisms remain unclear [55]. Biomarkers are often key to identifying causative pathways and mechanistic targets. While some studies have investigated serum, salivary, and microbial biomarkers of GERD, they are often not focused on the contribution of respiratory disease [58–60].

The availability of clinical longitudinal phenotyping makes the WTC-PM exposed Fire Department of New York (FDNY) first responders cohort ideal for biomarker discovery [10, 22, 28–31, 61–65]. Notably, we have successfully identified biomarkers associated with GERD and BE in a pilot population with respiratory disease, facilitating the identification of biologically relevant immune pathways [3].

The diagnosis of GERD itself is a complex process that relies on subjective clinical symptoms and often necessitate objective but invasive testing such as endoscopy and 24-h pH monitoring [66]. Those with endoscopic evidence of reflux may be entirely asymptomatic, potentially leading to under-diagnosis of patients at risk of BE and EAC [67, 68]. Even with the most invasive procedures, the diagnosis of GERD can be elusive and plagued by poor sensitivity [69].

In light of this, we propose to explore noninvasive biomarkers that could identify a population of aerodigestive disease, enabling better phenotyping of FDNY-WTC cohort with aerodigestive disease. In addition to their diagnostic utility, noninvasive biomarkers may direct future research into mechanisms and their downstream effects. GERD/BE biomarkers are also important to identify in the clinically silent presentations [69]. Additionally, we will identify novel non-invasive biomarkers of aerodigestive disease through a multi-OMIC approach. We will profile not only the metabolome and microbiome, but also exhaled, secreted, and blood biomarkers of aerodigestive disease Fig. 1 [70].

To address a critical gap in the current literature, we will 1. *Quantify noninvasive measures* of aerodigestive disease (salivary pepsin, serum biomarkers/metabolome, fractional exhaled nitric oxide (FeNO), exhaled breath condensate (EBC), microbiome, cognitive measures and aerodigestive QoL/disease severity measures to phenotype and assess treatment efficacy. 2. *Develop and optimize* a noninvasive biomarker model of aerodigestive disease and also 3. Determine the effect of aerodigestive disease on QoL, cognition and symptom phenotype.

Methods/design

Study design and participants

The FDNY WTC-health program (WTC-HP) electronic medical record (EMR) will be used to obtain clinical variables such as age, gender, years of FDNY service, WTC site exposure level, and lung function measures, as previously described [22, 27, 62–65, 71]. Our observational study is NYU IRB Approved # 21–00679 and available at

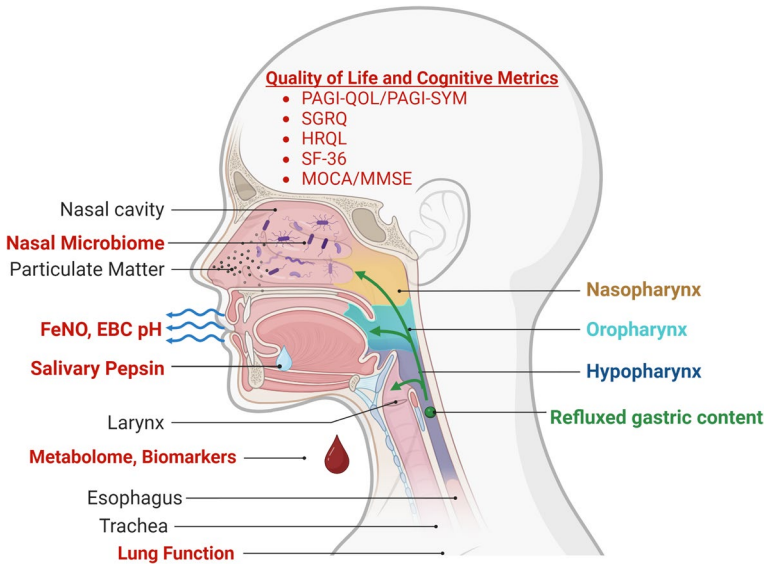

Fig. 1 Overview of planned biomarker assessments

clinicaltrials.gov #NCT05216133. Study Definitions and Inclusion/Exclusion Criteria can be found in Table 1.

Study oversight

It will be the responsibility of the principal investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events, as well as the construction and implementation of a site data and safety-monitoring plan (Study Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of adverse events. All modifications will be communicated to the IRB and will be reviewed.

Data safety monitoring

The principal investigator will be responsible for overall data safety monitoring. The following data points will be monitored: Adverse events (AE) will be monitored. Data safety monitoring reviews will be conducted yearly to ensure the safety of subjects. There are no predefined halting rules in place. We do not foresee temporary suspension of enrollment and/or study intervention due to the intent to treat nature of the study intervention. Data Monitoring Committee is not needed due to minimal risk study.

Study population

Source cohort

All participants in the WTC-HP ($n = 14{,}976$) were screened, Fig. 2. *Inclusion Criteria:* i. Actively consented and enrolled member of the WTC-HP. ii. Pre-9/11 spirometry with Forced Expiratory Volume in 1 s (FEV_1) ≥ Lower Limit of Normal (LLN) iii. Male Firefighter status on 9/11 with exposure at the WTC-site and entry into WTC-HP before the site closure on 7/24/2002. *Exclusion Criteria:* i. lung disease prior to 9/11 as defined by positive methacholine or bronchodilator test, or $FEV_1 <$ LLN. ii. Not part of initial cohort in data extraction from August 1, 2017 [72]. After all inclusion/exclusion criteria applied, the baseline cohort consists of $n = 4{,}192$. *Sub-cohort Development.* A representative cohort of 20% was randomly selected ($n = 837$; SPSS v. 28) from the above baseline cohort, Fig. 2.

Recruited Cohort will be developed to assess for noninvasive biomarkers. We will recruit a subset $N = 40$/group (*i.* AHR only *ii.* GERD only *iii.* BE *iv.* GERD/BE and AHR overlap or *v.* No GERD nor AHR) from the sub-cohort, Fig. 2. Recruitment strategies will include: i. Direct mailings; ii. Email (potential participants will be sent the same IRB-approved recruitment message to their personal emails using end-to-end encryption; iii. Study website will include recruitment messages providing general information on the study and answers to frequently-asked questions. No direct communications will be made with participants through the website, and no PHI will be used or available within the study website; iv. Telephone contact. A description of the study will be provided to potential participants and, upon their expression of interest, the investigator will perform an eligibility screening. In addition to meeting the inclusion criteria as outlined above, participants should: i. have available serum from their first post 9/11 WTC-HP ii. Not currently be receiving treatment for malignancy iii. Have no limitations to

Table 1 Inclusion and exclusion criteria

Inclusion			Exclusion
Demographics	Age 37-90.		Not enrolled in the WTC-Health Program
	FDNY rescue and recovery worker.		
	Male		Female
	Documented WTC exposure.		Unwilling to complete an informed consent.
	Consented/Enrolled in the FDNY WTC Health Program		
	Subjects are willing and able to consent for themselves to study enrollment		
	Subjects willing and able to participate in study procedures		Have pre-existing and documented conditions or concurrent diagnoses, including (and not necessarily limited to) active cancer, severe heart disease, significant cognitive impairment, eating disorders, significant psychiatric illness, end-stage COPD, severe pulmonary hypertension, or organ transplant.
	Spirometry available longitudinally.		High dose steroid (>20mg prednisone or equivalent) or other hormonal treatments/chemotherapy use in the last month, including testosterone supplementation.
	Are able to attend a single in-person visit		Life-expectancy < 6 months
	Pre-9/11 spirometry with $FEV_1\%_{predicted}$ ≥LLN and if not available 1st -post 9/11 spirometry with an FEV_1 >80% predicted.		Did not have serum available in the biorepository from the first post 9/11 WTC-HP visit that was previously assayed for biomarkers.
	Exposure at the WTC-site within 2 weeks of the 9/11/2001		
	Entered WTC-HP before the site closure on 7/24/2002.		
	Serum from their first post 9/11 WTC-HP visit is available		
	Are not currently being treated for malignancy		
Definitions	**AHR**	At least once post-9/11 • A methacholine (PC_{20}<16) and/or • positive bronchodilator response (ATS/ERS guidelines: improvement of FEV_1 by 12% and at least 200mL) OR • OR EMR diagnosis • No recorded positive AHR testing pre-9/11	
	GERD	• Erosive esophagitis LA grade C or D (as described on endoscopy), OR • Stricture or Barrett's esophagus on endoscopy, OR • Esophageal acid exposure time >6% on a pH or pH impedance study.	Esophageal acid exposure time less than 4% on a pH or pH impedance test (if available).
	BE	• Columnar epithelium lining ≥1 cm of the distal esophagus AND • Histologic examination of biopsy specimens from that columnar epithelium reveal intestinal metaplasia (goblet cells).	

FDNY New York City Fire Department, *WTC* World Trade Center, *COPD* Chronic Obstructive Pulmonary disease, *FEV1* Forced expiratory volume in the first second, *LLN* lower limit of normal, *WTC-HP* World Trade Center Health Program, *PC20* provocative concentration of Methacholine, *AHR* airway hyperresponsiveness, *ATS/ERS* American Thoracic Society/European Respiratory Society, *EMR* Electronic Medical Record, *GERD* Gastroesophageal Reflux Disease, *BE* Barrett's Esophagus

a minimal risk blood draw iv. Be willing and able to sign consent; and v. be able to attend a single-visit.

All co-investigators have received training from the principal investigator in how to obtain consent and answer questions that may arise during the consent process. The consent and letter have been written to comply with the requirement that they be written at a 5th grade reading level, evaluated by the Flesch–Kincaid readability test. In addition, subject will be asked to provide their understanding of what the study is

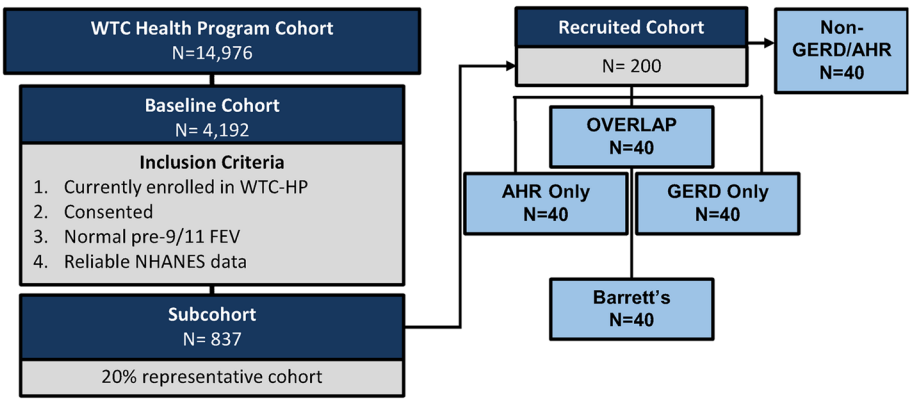

Fig. 2 Study design

about at the time of the consenting process. English is the primary language of all FDNY rescue workers, see Appendix.

Participant-related study information will be identified through the Patient Identification Number (PID) on all participant Case Report Forms (CRFs). Participant names or other personally-identifying information will not be used on any study documents. All study-related documents will be kept in double-locked, limited access areas at each study site. A log that links the names of participants to their PID numbers will also be kept under double locks separate from all other research records, accessible only to the study staff. Original source documents for individual participants will be maintained at the FDNY-BHS and will be accessible only to study staff.

Case status

WTC-AHR will be defined as having a positive methacholine ($PC_{200} < 16$), or a positive bronchodilator response (by ATS/ERS guidelines with improvement of FEV_1 by 12% and at least 200 mL) at least once post-9/11 [73, 74] and/or EMR diagnosis. *GERD* will be defined as: biopsy-proven erosive esophagitis LA grade C or D; stricture or Barrett's esophagus on endoscopy; and/or esophageal acid exposure time > 6% on a pH or pH impedance study. GERD will also be defined on EMR diagnosis and/or PPIs, H_2 blockers, antacid, or surface agent use [75]. *BE*, as a subset of GERD, will have any of the following additional inclusion criteria: biopsy-proven columnar epithelium lining ≥ 1 cm of the distal esophagus with intestinal metaplasia characterized with goblet cells on histology; diagnosis on EMR, Tables 2 and 3 [75]. The recruited participants will be consented prior to any research activity and measurement visit via REDCap software or in person.

Measurement visit

Participant demographic information, medical history and medication history will be obtained. A physician will perform the physical examination, and verify that inclusion/exclusion criteria are met. Enrolled participants will undergo the following assessments.

Blood sampling

After at least an 8 h fast, serum and plasma will be obtained, aliquoted and banked. Each stored specimen will be assigned a unique code to ensure proper identification and linkage to the respective participant. Aliquots from the fresh samples will be assayed for complete blood count (with differential) and chemistry panel. These data are already available for the banked samples. For all samples, lipid profile, metabolomics, and protein biomarker profiling will be performed [10, 28–30, 76, 77].

Salivary pepsin assessment

30 mL sterile plastic tubes with 0.5 ml of 0.01 M citric acid, adjusted to a pH of 2.5 (RD Biomed Ltd., Hull, UK), will be used by the participants to collect saliva in the AM (prior to brushing teeth, drinking or eating), 1 h after finishing lunch, and 1 h after finishing dinner [78, 79]. Participants will be instructed to cough a few times prior to spitting into the tube to clear saliva from the back of the throat and then spit into the tube. The collected samples will be stored at 4 °C and analyzed within 2 days. Salivary Pepsin will be analyzed using Peptest (RD Biomed Ltd., Hull, UK) as previously described [79]. Briefly, plastic tubes will be centrifuged at 4,000 rpm for 5 min, and 80µL of supernatant will be added to 240µL of migration butter solution for 10 s. 80µL of the mixture will be added to the well of the Peptest, which contains two unique human monoclonal antibodies that detect and capture pepsin protein (specific to pepsin-3), with

Table 2 Schedule of study related activities

TIMEPOINT (Visit)		Enrollment	REDCAP*	Measurement	Close-Out
Eligibility screen		✓			
Informed consent			✓		
Physical exam				✓	
Spirometry				✓	
Phlebotomy				✓	
Microbiome				✓	
FeNO				✓	
EBC				✓	
Saliva Collection **				✓	
Questionnaires	SF-36		✓		
	HRQL		✓		
	SGRQ		✓		
	PAGI-SYM		✓		
	PAGI-QoL		✓		
	Post Study Follow-up				✓
	MOCA			✓	
	MMSE			✓	

FeNO Fractional Exhaled Nitric Oxide, *EBC* Exhaled Breath Condensate, *SF-36* Short-Form 36, *HRQL* Health-Related Quality of Life, *SGRQ* St. George's Respiratory Questionnaire, *PAGI-SYM* Patient Assessment of Upper Gastrointestinal Disorders- Symptoms Severity, *PAGI-QoL* Patient Assessment of Upper Gastrointestinal Disorders- Quality of Life, *MOCA* Montreal Cognitive Assessment, *MMSE* Mini Mental State Examination

* Consent and questionnaires may be obtained in person if subjects prefer

** Samples collected prior to in person visit

a lower limit of detection of 16 ng/mL and an upper limit of 500 ng/mL. A salivary pepsin level of ≥ 16 ng/mL will be considered positive. The sample will be processed in a Pepcube reader to quantify the pepsin concentration [78].

Spirometry will be assessed using a KoKo PFT spirometer (nSpire Health Inc), and lung function assessment will be considered acceptable as per the ATS/ERS guidelines [80]. We will select the largest acceptable measures for electronic archiving. Each participant's predicted percentage (%) will be calculated by NHANES III equations based on their age at examination, height, sex, and race [80, 81].

Table 3 Endpoints of the BADBURN trial

Outcome Measure		Description
Blood		Aliquots of (serum/plasma) will be stored for studies such as: • Analytes such as (C-peptide, TNF-α, Fractalkine, and IP-10) • Metabolomics
Saliva		• Pepsin is a digestive enzyme whose precursor pepsinogen is produced by gastric chief cells • Salivary pepsin was the most studied non-invasive biomarker for diagnosing reflux. • Subjects are sent saliva collection kits (3 sample tubes and instructions). – Sample 1: prior to breakfast – Sample 2: 1 hour after lunch – Sample 3: 1 hour after dinner • Samples processed and aliquoted • PepCube reader quantifies pepsin content in ng/mL (Lateral Flow Device)
EBC		pH, Histamine, adenosine, ammonia, hydrogen peroxide, isoprostanes, leukotrienes, nitrogen oxides, peptides, cytokines. • May identify novel non-invasive biomarkers for multiple coexisting pathologies. • EBC collected during in-person subject visit with Rtube – 10 minutes of quiet breathing – Yields 0.5-1.5 ml of condensate. • Samples are aliquoted. • pH measured in each sample pre & post Argon gas – Argon is an inert gas, allowing for deaeration of samples from CO_2, thus enhancing stability of pH measurements.
Spirometry		Usual spirometric technique with reproducibility and acceptability based on ATS/ERS guidelines will be assessed. This will include measures of FEV_1 and FVC.
FeNO		NIOX VERO will be used to assess airway inflammation. • NO is a marker of airway inflammation and epithelial damage • Clinically utilized biomarker of lung disease • Levels may increase in obstructive airway disease • May be used to differentiate airway inflammation from obstructive airway disease and non-pulmonary causes like GERD • Identify underlying AHR exacerbating symptoms of GERD
Microbiome		GenoTek Oragene-OMR110 oropharyngeal swab collection kit will be used to assess microbiome of aerodigestive disease. • Collected using OMNIgene OMR-110 (DNA genotek) • Combining Oropharyngeal and Nasopharyngeal sampling may serve as a surrogate for bronchoalveolar lavage sample microbiome. • Swabs are placed in vial with stabilizing liquid, Proteinase K. • Samples incubated at 50°C for 1 hr in a water bath and aliquoted at -80 °C
Questionnaires		
Respiratory	**SGRQ**	**St. George's Respiratory Questionnaire for COPD Patients (SGRQ-C)** Derived from the original SGRQ following analysis or large COPD data sets only. Standardized/validated airways disease-specific survey assessing symptoms, activity hindrance, and overall impact.
		• Consists of two parts with 3 subscores Part 1: Frequency of respiratory symptoms and produces one subscore (Questions 1-7) – Symptoms Subscore • Severity of symptoms Part 2: Patient's current state and produces two subscores (Questions 8-14) – Activity Subscore • Effect of respiratory impairment on activity performance – Impact Subscore • Effect of impairment on personal/social life **Scores range from 0-100**
GERD	**PAGI-SYM**	**Patient Assessment of Upper Gastrointestinal Symptom Severity Index (PAGI-SYM)** Developed in parallel with and often used in conjunction with PAGI-QOL. • Measures symptom severity in patients with GERD, dyspepsia, and gastroparesis • Intended to cover the main symptom groupings for said diseases. Upper gastrointestinal disorders-symptom severity index (PAGI-SYM) assessing symptoms of GERD and related disorders. • 20 items • 6-point Likert Scale from 0-5 (none or absent – very severe) • Consists of 6 subscales – Heartburn/Regurgitation – Fullness/Early Satiety – Nausea/Vomiting, Bloating – Upper Abdominal Pain – Lower Abdominal Pain **Final score = average of all subscale scores and can range from 0-5** **Higher score indicates higher severity of symptoms.**
	PAGI-QOL	**Patient Assessment of Upper Gastrointestinal Disorders- Quality of Life (PAGI-QOL)** • Specific to Upper Gastrointestinal disorders. • Validation studies observed a significantly lower QOL score in individuals with impairment in daily activities from upper GI symptoms than those who did not (p<0.001) • Assesses quality of life in GERD, dyspepsia, and gastroparesis Measures quality of life outcomes of patients with upper gastrointestinal disorders. • Available in multiple languages. • Consists of 30 items • 6 point Likert scale (0 - 5; all of the time – none of the time) • Items are grouped into 5 dimensions – Daily Activities – Clothing – Diet and Food Habits – Relationship – Psychological Well-being and Distress • Total score = average of all dimension sub scores. **Final scores can range from 0 (lowest QOL) to 5 (highest QOL)**
QoL	**SF-36**	**36 Item Short Form Survey** 36 self-administered QoL measures • Physical Health Measure – Physical Function – Role Limitations due to Physical Health – Pain – General Health • Mental Health Measure – Role Limitations due to Emotional Health – Energy – Emotional Wellbeing – Social Functioning • Each sub-score is average of respective weighted answers. **Scores range from 0-100**
	HRQL	**Health Related Quality of Life (HRQL)** Assesses perceived physical and mental health. • 14-item questionnaire • Consists of three modules: – **Core Healthy Days Module (Quantitative):** produces an Unhealthy Days Score that describes the number of physically and mentally unhealthy days in the past month. – **Activity Limitations Module:** how and what kind of health impairment has limited one's daily activities – **Healthy Days Symptoms Module:** how many days in the past month one has felt different ways (e.g., worried, tense, anxious, healthy, full of energy, etc.) **Scores range from 0-30**
Cognitive	**MOCA**	**Montreal Cognitive Assessment (MoCA)** • 11 question that evaluate seven areas: – Executive and Visuospatial Function – Naming – Attention – Language – Abstraction – Delayed Recall – Orientation • Better at distinguishing between normal cognition and mild cognitive impairment. **Normal is considered ≥26 out of 30**
	MMSE	**Mini Mental Status Examination (MMSE)** Screening tool that provides a quantitative assessment of the cognitive impairment. • 11 question measure that tests five areas: – Orientation – Registration – Attention and Calculation – Recall – Language • Is less sensitive to patients with mild cognitive impairment. – Better for patients with known dementia. – Can detect subtle changes in cognition even if certain domains are unaffected. **Normal is considered ≥24 out of 30**

Table 3 (continued)

TNF-α Tumor Necrosis Factor, *C peptide* Connecting peptide, *IP-10* Interferon-gamma-induced protein 10, *FEV1* Forced Expiratory Volume in 1 s, *EBC* Exhaled Breath Condensate, *FeNO* Fractional Exhaled Nitric Oxide, *SGRQ St.* George's Respiratory Questionnaire, *GERD* Gastroesophageal Reflux Disease, *PAGI-SYM* Patient Assessment of Upper Gastrointestinal Disorders- Symptoms Severity, *PAGI-QOL* Patient Assessment of Upper Gastrointestinal Disorders- Quality of Life, *SF-36* Short-Form 36, *HRQL* Health-Related Quality of Life measures, *MOCA* Montreal Cognitive Assessment, *MMSE* Mini-Mental State Examination

FeNO will be quantified using NIOX VERO® (Aerocrine) [82, 83]. Participants will be instructed to inhale to their total lung capacity via mouthpiece for 2–3 s. Then, they will exhale at a flow rate of 0.05L/second. The device will provide results in parts per billion (ppb).

Exhaled breath condensate (EBC) will be collected using RTubes (Respiratory Research, Inc., USA) [84]. Approximately 1-2 mL of EBC sample will be obtained after 10 min of quiet normal breathing [85]. *PH measurement.* EBC pH assay is extremely simple to perform, inexpensive, and robust, and can be easily processed on the day of collection [86]. EBC will be de-aerated of CO_2 by bubbling free argon gas (350 ml/min) under a micro-pH reader (Orion PerpHecT micro-pH electrode) and stabilized pH will be recorded after approximately 3–5 min [87]. Aliquots are then stored at -80^{0}C and thawed only once prior to histamine and biomarker assessment.

Naso/oropharyngeal microbiome

Collection

Trained study team members will collect naso/oropharyngeal samples using commercially available kits (OMR-110 by DNA Genotek, Canada). Each naris will be swabbed in a circular fashion 10 times. The oropharyngeal sample will be collected by swabbing in the back of the throat in 10 circular motion to ensure sufficient swab collection. Each absorbent swab will be placed into a vial containing 1 mL of stabilizing liquid using aseptic technique. The sample will be treated with lyophilized Proteinase K, and incubated in the original vial at 50^{0}C for 1 h in a water bath prior to aliquoting for long-term storage at -80^{0}C.

Quality of life, aerodigestive disease and end-organ effect questionnaires

Gastrointestinal impact will be assessed using with the Patient Assessment of Upper Gastrointestinal Disorders – Quality of Life (*PAGI-QoL*) and the Patient Assessment of Upper Gastrointestinal Disorders Symptom Severity Index (*PAGI-SYM*). Both questionnaires use a 6-point Likert scale (MAPI Research Trust) [88–91].

Respiratory and QoL assessment will utilize the Health-Related Quality of Life measures (*HRQL*) [92], St. George's Respiratory Questionnaire (*SGRQ*), and the Short-form-36 (*SF-36*). HRQL assesses an individual's

perceived physical and mental health. The SGRQ is a standardized, self-administered airways disease-specific questionnaire divided into three subscales- symptoms, activity, and impact [93]. SF-36 will capture supplemental information about their mental health, general health perception, emotional, and social role functioning [94].

Cognition will be assessed using the Montreal Cognitive Assessment (*MoCA*; version 8.1) and the Mini-Mental State Examination (*MMSE*). MMSE is a cognitive test used to evaluate early dementia [95, 96]. Combining MoCA and MMSE can improve diagnostic utility [97]. The MoCA will be administered by a trained/certified investigator. Members of our research team have completed MoCA training and certification through a validated MoCA cognition portal [98] (https://mocacognition.com/). Similar to the MoCA, the MMSE assesses orientation, memory, visuospatial and language domains. Additionally, the MMSE evaluates comprehension, reading and writing [99]. The PI will thoroughly review all scores.

Outcomes

Levels of salivary pepsin, pH Levels from EBC, Histamine Concentration from EBC, Score on PAGI-QOL Questionnaire, Score on PAGI-SYM Questionnaire, Score on SGRQ-C Questionnaire, and SF-36.

Power analysis

A sample size of 40 cases for each group of GERD, AHR, AHR/GERD overlap, BE, and non-GERD/non-AHR Controls (all will be subsets of AIM 1 $N = 898$ randomly selected cohort) achieves 80% power to detect difference as small as 0.78 SD with two-sample t-test at 0.01 significance level to account for multiple comparisons. This will allow us to achieve 80% power and significance of 0.05, based on prior studies with salivary pepsin test (personal communication with Dr. Peter Dettmar of Peptest), Fig. 2.

Statistical Analysis SPSS 28 (IBM) will facilitate database management and statistics. Continuous variables expressed as mean, standard deviation (SD) if normally distributed, and as median, inter-quartile range (IQR) if skewed. Two-sample t-test and ANOVA will compare continuous data. Count and proportions will summarize categorical data and Pearson- χ^2 will compare categorical data. Multivariate binary logistic regression will estimate biomarker-disease relationship for case status as a binary outcome while adjusting for confounding. Cox proportional hazards model will evaluate the effects of biomarkers, smoking, and exposure on the hazard of developing WTC-GERD or BE over time. The maximum potential effectiveness of a biomarker will be calculated by Youden Index [100]. Goodness of fit, using the Hosmer–Lemeshow test. Survival curves compared by Log-rank test. Pearson χ^2-test will compare SABA and LABA usage between GERD, AHR, AHR/GERD overlap, BE, and non-GERD or AHR controls. Significance will be assessed by $p < 0.05$ for all statistical tests. Graphs will be created using Prism (v.10, GraphPad Software).

Missing data

Variables with missing values in a small proportion of participants will be imputed using multiple imputation methods. To assess the missing at random assumption, we will evaluate the comparability between samples with missing data and those without. Sensitivity analysis will be performed by comparing the results obtained from the complete data analysis to the results obtained from multiple imputation.

Model building

We have previously identified key biomarkers using a machine learning approach [10, 28–30]. We have further refined this analysis pipeline and will utilize this methodology to identify AHR, GERD, AHR/GERD overlap, and BE biomarkers**.** Specifically, we will utilize random forests (RF) of the filtered, normalized biomarkers. Models assessed via a modified hamming distance between variable importance rankings of models with identical hyperparameters. A refined profile of the top 5% of important biomarkers by MDA will be included in a gradient-boosted tree model (xgboost package, R-Project) to build a classifier of AHR, GERD, AHR/GERD overlap, and BE. A random hyperparameter space search determined a final model that maximized AUC_{ROC}.

We will also use linear mixed-effects models will be used to assess the temporal trend of biomarkers with time adjusting for confounders. The longitudinal biomarkers processes will be associated to risk of developing WTC-GERD/BE using the joint modeling technique [101]. The joint-modeling approach has become the primary method for analysis of longitudinal biomarker process and time-to-event outcome, and multiple R packages are available to implement the models. We will also consider a single index longitudinal model which enables us to reduce the dimensionality of multiple biomarkers and to evaluate joint effects of multiple biomarkers together to identify key risk factors. The single-index model incorporates longitudinal data to calculate hazard of each parameter as well as personalized dynamic risk for prognostication. Specifically, this will allow us to use a patient's data from a single clinical exam to identify risk of GERD, AHR, overlap, or BE. Furthermore, this will allow the identification of false negatives and undertreated cases in the entire FDNY cohort.

Discussion

PM exposure, a significant component of ambient and occupational exposures is a risk factor for aerodigestive disease (such as GERD and AHR) and is associated with approximately 7-million deaths annually [1–3, 11, 102–104]. GERD is the most prevalent gastrointestinal disorder in the US, with an estimate as high as 30% [66]. Globally, the prevalence of GERD ranges from 10–25%, with an increased risk in firefighters [52, 66]. GERD is an independent risk factor in the development of BE which can lead to malignancy [66].

Despite the similar risks, the understanding of the underlying pathophysiological interrelatedness between the aerodigestive diseases (AHR, GERD and BE) remains limited. Furthermore, GERD diagnosis and treatment has been invasive and costly. Therefore, our work is focused on identifying non-invasive biomarkers which may help identify at risk populations who may benefit from earlier intervention, targeted therapies and a further understanding of how their AHR is impacted by co-morbid GERD. The identification of non-invasive biomarkers of GERD/BE and the overlapping aerodigestive disease is crucial.

Our work will address the existing *knowledge gap* in aerodigestive overlap and validate biomarkers of WTC-aerodigestive disease. Biomarkers of BE may also identify individuals at risk for neoplastic disease. These findings may have broader implications for populations with GERD and PM exposure. In contrast to currently used invasive testing, noninvasive testing offers diagnostic utility with reduced risk and can direct future research into mechanisms/downstream effects. We also systematically studied biomarkers of GERD/BE and defined some of the lacunae in the non-invasive aerodigestive biomarker literature [105]. Therefore, our Case–Control Observational Study is designed to sample a broad biomarker profile, Table 3.

Microbiome of the gut/lung axis

Asthma susceptibility is influenced by the gut microbiome [106–111]. Noninvasive collection sites that can approximate the pulmonary environment are of key interest. Studies have failed to show that the microbiomes of induced sputum were similar to the lung [112, 113]. Noninvasively collected oropharyngeal and nasopharyngeal swabs in conjunction could approximate the lung microbiome [114]. Research has revealed that the esophageal microbiome undergoes alteration in individuals with GERD, BE, and other motility disorders [115, 116]. Although these findings highlight the potential role of the microbiome studies in the diagnosis and therapeutic approaches for aerodigestive disease, further studies are needed and will be one of the key readouts planned in our study.

EBC analysis holds great promise in addressing unmet medical needs by expanding the portfolio of noninvasive assays for the multiple coexisting pathological mechanisms underlying respiratory disorders and GERD. Compounds identified in EBC include histamine, adenosine, ammonia, hydrogen peroxide, isoprostanes, leukotrienes, nitrogen oxides (NOx), peptides, cytokines, protons and various ions [85]. Histamine plays a vital role in digestion but elevated levels can contribute to the development of GERD [117, 118].

Salivary pepsin has been studied in several GERD biomarker studies [105]. Due to the overlap of various reflux symptoms with other GI pathologies, the diagnosis of GERD can be challenging. However, salivary pepsin test offers a simple and convenient way for detecting reflux through salivary sample collection, providing quick and non-invasive results. Compared to other diagnostic modalities, this approach is time-efficient and requires much less effort [119]. Moreover, pepsin measurements can identify pathologic reflux even in the absence of symptoms, and remain unaffected by the concurrent use of PPI. Several studies have demonstrated that pepsin detection in the sputum and/or saliva can be regarded as a sensitive, non-invasive method for the diagnosis of the proximal reflux of gastric contents, with a sensitivity ranged from 41.5% to 73% and high specificity of 86.2 to 98.2% [78, 79]. Despite these findings little is known about pepsin in the context of aerodigestive co-morbid disease.

FeNO, a biomarker of lung disease activity, will be a valuable measure in our population. FeNO is associated with airway hyperreactivity, and several studies demonstrated that FeNO is increased during obstructive exacerbations [120]. In our population with the aerodigestive overlap, FeNO levels can serve as an indicator of potential underlying AHR exacerbating symptoms of GERD. Thus, our work will also contribute to understand the role of FeNO in GERD, which remains inconclusive as only a limited number of studies have examined AHR/GERD [121, 122]. The detrimental impact of even once-weekly episodes of GERD on quality of life [123] highlighted the importance of assessing aerodigestive disease quality of life and disease activity, therefore we will quantify the effects of GERD on these aspects through a validated set of questionnaires that will assess QoL, GERD specific symptoms and also cognitive involvement.

Non-invasive biomarkers of GERD, BE, AHR, treatment efficacy, and severity of symptoms will also be assessed in serum. This will allow us to measure Tumor Necrosis Factor (TNF-α), C-peptide, Fractalkine and Interferon-gamma-induced Protein 10 (IP-10) in our

case cohort study to validate our prior pilot study [1–3]. Serum samples will also be used to perform metabolomic profiling that will allow us to investigate metabolic correlates of aerodigestive disease. In addition, by validating serum biomarkers (proteins and metabolome) of GERD/BE, we seek to provide a biologically plausible target that enables early detection and facilitates therapeutic intervention in the PM exposed populations. Moreover, non-invasive phenotyping of WTC aerodigestive disease holds promise in improving the sensitivity and specificity of GERD diagnosis, enabling earlier identification of BE and facilitate the development of personalized therapy, thus to improve both the quality of life and overall health outcomes.

Limitations and potential study concerns

We envision there are several limitations of our study. It is possible that no significant association exists between noninvasive biomarkers and aerodigestive diseases in the second decade after WTC exposure. The generalizability of our study could be impacted because the FDNY source cohort had no aerodigestive disease prior to 9/11 and had their serum samples banked within six months of 9/11, therefore making it less comparable to other cohorts. There may also be a subset of patients without history of GERD, but could still receive a clinical diagnosis of GERD based on questionnaires and/or elevated pepsin/biomarkers. For these patients, further follow-up with a gastroenterologist will be recommended. Additionally, we may use FeNO levels to identify the potential underlying AHR exacerbating symptoms associated with GERD. We will also account for the potential risk of loss to follow-up regarding the completion of the questionnaires and attendance of the in-person visit.

Further investigation into the overlap of GERD/BE and AHR is envisioned to provide valuable insights in distinguishing disease phenotypes, demonstrating that biomarkers can predict GERD and/or BE. This work will have clinical implications for the diagnosis and treatment of WTC associated disease, as well as for the management of other patients in the WTC monitoring programs, and for the general population as intense PM exposures are occurring more frequently, for example through wild fire related PM. Our research will contribute to the development of a robust biomarker set with optimal explanatory power when applied to diverse cohorts.

Abbreviations

AHR	Airway Hyper reactivity
BADBURN	Biomarkers of Airway Disease, Barrett's and Underdiagnosed Reflux Noninvasively
BMI	Body Mass Index (kg/m^2)
COPD	Chronic Obstructive Pulmonary Disease
DBP	Diastolic Blood Pressure
EAC	Esophageal adenocarcinomas
ECG	Electrocardiogram
FDNY	Fire Department of New York
FE_{NO}	Fractional Exhaled Nitric Oxide
FEV_1	Forced Expiratory Volume in 1 s
FVC	Forced Vital Capacity
HRQL	Health-Related Quality of Life
HP	Health Program
IRB	Internal Review Board
LI	Lung Injury
LLN	Lower Limit of Normal
LoCalMed	Low Calorie Mediterranean Diet
MetSyn	Metabolic Syndrome
MoCA	Montreal Cognitive Assessment
MMSE	Mini-Mental State Examination
MOP	Manual of Procedures
MND	MyNetDiary
N	Number
NIH	National Institutes of Health
NYU	New York University
OAD	Obstructive Airways Disease
PAGI-QOL	Patient Assessment of Upper Gastrointestinal Disorders- Quality of Life
PAGI-SYM	Patient Assessment of Upper Gastrointestinal Disorders- Symptoms Severity
PI	Principal Investigator
PM	Particulate Matter
PWV	Pulse Wave Velocity
QoL	Quality of Life
RCT	Randomized Clinical Trial
SBP	Systolic Blood Pressure
SF-36	Short-Form 36
SGRQ	St. George's Respiratory Questionnaire
SOP	Standard Operating Procedure
US	United States
WTC	World Trade Center
9/11	September 11, 2001

Supplementary Information

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Supplementary Material 1.

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Dissemination policy

Results will be disseminated by publication.

Authors' contributions

AN was the primary investigator, had full access to all of the data in the study and takes responsibility for the integrity and the accuracy of the data analysis. UJ, SK, SP, FF, ARK and AN participated in study conception and design; UJ, SP, SK, RZO, TS, DP and AN were responsible for data collection; SK and AN were responsible for data validation; UJ, SP, SK, GG, and ML participated in data analysis. All authors including (DHK, AFZ, YL, AV, JZ, and GC) participated in data interpretation, writing and revision of the report and approval of the final version.

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Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Participants signed informed consent at the time of enrollment allowing analysis of their information and samples for research. This study was approved by the Institutional Review Boards of Montefiore Medical Center (#07–09-320) and New York University (NYU IRB # 21–00679).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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