Non-invasive, Multi-modal Inflammatory Biomarker Discovery for Systemic Inflammation (NOVA Study): A Feasibility Study Protocol

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Abstract

Background: Prolonged systemic inflammation is recognized as a major contributor to the development of various chronic inflammatory diseases. Daily measurements of inflammatory biomarkers can significantly improve disease monitoring of systemic inflammation, thus contributing to reducing the burden on patients and the entire healthcare system. There exists, however, no scalable, cost-efficient, and non-invasive biomarker for remote assessment of systemic inflammation. To this end, we propose a novel multi-modal, non-invasive approach for measuring inflammatory biomarkers.

Objective: We aim to evaluate the relationship between the levels of inflammatory biomarkers in serum (gold standard) and those measured non-invasively in urine, sweat, saliva, exhaled breath, stool, and core body temperature in patients with systemic inflammation.

Methods: This study is a single-center, cross-sectional study and includes a total of 20 participants (10 systemic inflammation patients and 10 control patients). Eligible participants provide serum, urine, sweat, saliva, exhaled breath, and stool samples for biomarker analyses. Core body temperature is measured using a sensor. The primary endpoint is the level of C-reactive protein. The secondary endpoints are interleukin (IL)-1?, IL-6, IL-8, IL-10, and tumor necrosis factor-? levels. The tertiary endpoints are fractional exhaled nitric oxide, calprotectin, and core body temperature. Shapiro-Wilk test will be used to evaluate the normality of the distribution in each variable. We will perform the two-tailed t-test (or Wilcoxon rank-sum test, if non-normal) to compare the levels of inflammatory biomarkers between patients with systemic inflammations and controls. Pearson and Spearman correlation coefficients will assess the relationship between non-invasive methods and the serum.

Results: A total of 20 participants participated in the study measurements between February and September 2023. Participants were on average 52.8 (range 24-82, SD 14.4) years of age, and 70% (n=14) of them were women. The analysis results reporting findings are expected to be published in the fall of 2024.

Conclusions: This is a proof-of-concept study evaluating the feasibility of non-invasive, multi-modal inflammatory biomarker discovery. Promising results could lead to the creation of non-invasive and digital biomarkers for systemic inflammation, which could be implemented for a larger, prospective, longitudinal cohort study.

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Abstract

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Conclusions: This is a proof-of-concept study evaluating the feasibility of non-invasive, multi-modal inflammatory biomarker discovery. Promising results could lead to the creation of non-invasive and digital biomarkers for systemic inflammation, which could be implemented for a larger, prospective, longitudinal cohort study.

Keywords: systemic inflammation; chronic inflammation; inflammatory biomarkers; biofluids; serum; urine; sweat; saliva; exhaled breath; stool; C-reactive protein; IL-1β; IL-6; IL-8; IL-10; TNF-α; fractional exhaled nitric oxide; calprotectin; core body temperature; noninvasive biomarker; multi-modal biomarker; remote monitoring; surrogate biomarker; rheumatology; chronic inflammatory disease

Introduction

Chronic systemic inflammation is a persistent upregulation of inflammatory activity as a result of prolonged exposure to a pathogen or autoimmune dysfunction. Prolonged systemic inflammation is associated with the disease onset and progression of various chronic inflammatory diseases such as rheumatoid arthritis, spondyloarthritis, or systemic lupus erythematosus [1].

Current standardized practice to assess the presence of systemic inflammation and inflammatory disease activity is based on serum biomarkers obtained from a blood test [2]. Increased levels of pro-inflammatory markers such as the C-reactive protein (CRP) and interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α are closely associated with active inflammation and disease progression in cardiovascular and autoimmune diseases [3]. The serum CRP is found to be the most precise and objective measurement and is regularly assessed at routine hospital visits to confirm the clinical diagnosis, classification of disease activity, and treatment decisions in chronic inflammatory diseases. In the case of rheumatoid arthritis, for instance, clinicians determine the current state of disease activity and treatment response based on aggregated information of the inflammatory biomarker levels from a blood test (e.g., CRP or ESR), physical assessment (e.g., joint counts) and composite score (e.g., Disease Activity Score 28) obtained during a routine visit [4,5]. While blood tests provide valuable information to diagnose, screen, and monitor various inflammatory biomarkers and clinical conditions, this procedure has three distinct disadvantages such as 1) it is invasive and burdensome to patients; 2) it requires trained personnel and is not feasible for self-administration; 3) real-time monitoring or at-home measurements are not available. In addition, there are a limited number of studies and insufficient evidence to support the validity of detecting CRP or cytokines non-invasively.

Existing studies assess only specific disease populations, biofluids, or biomarker types, limiting the generalizability of findings to a wide patient population of systemic inflammation. Urinary CRP, for example, is mostly researched in patients with urinary tract infections, other urologic conditions or rejection of transplanted kidneys [6,7]. Likewise, the fractional exhaled nitric oxide (FeNO) – a product of metabolic and inflammatory processes in the human body – is largely investigated in cancers and respiratory diseases such as asthma, but rarely in other systemic inflammatory condition [8,9]. Fecal calprotectin is a well-established biomarker for inflammatory bowel disease, yet its clinical relevance to other inflammatory diseases remains largely unexplored [10]. Recent studies have shown encouraging results including significant associations with serum calprotectin in spondyloarthritis patients compared to matched controls and increased serum calprotectin levels correlated with disease activity in both rheumatoid arthritis and axial spondyloarthritis [11,12]. Furthermore, chronic inflammation contributes to the disruption of homeostatic control mechanisms, such as core body temperature, and results in increased vulnerability to homeostatic dysregulation and diseases [13]. Yet the underlying mechanisms responsible for this connection are not yet fully understood.

Non-invasive assessment of inflammatory biomarkers could overcome the limitations of the invasive blood draw and improve the lives of patients with chronic inflammatory diseases by enabling continuous disease monitoring. This study demonstrates the potential to utilize non-invasive methods for developing accessible and less invasive diagnostic tools. These advancements would alleviate multiple burdens from blood tests, contribute to patients' self-management, and add significant value to clinicians and caregivers by enabling meaningful treatment and effective prevention of future events, a step towards population-wide measurement and personalized prevention.

To date, however, there exists no scalable solution that allows patients to gauge one’s inflammatory state remotely. As a result, many patients might postpone contacting their physicians until late during a disease progression or even until the next routine visit to evaluate inflammatory symptoms and the CRP level. This delay may not only impact negatively on patients’ quality of life but also cause unanticipated consequences or harm in some cases. An option of remotely monitoring symptoms and inflammatory activity could empower patients toward self-management, increase comprehension of the disease, and thus, ensure engagement in treatment adherence and timely intervention.

To address this research gap, we propose a proof-of-concept feasibility study (NOVA study) to evaluate the relationship between the levels of serum inflammatory biomarkers and those measured non-invasively in urine, sweat, saliva, exhaled breath, core body temperature, and stool in patients with systemic inflammation. We hypothesize that the levels of inflammatory biomarkers measured non-invasively in various biofluids can be utilized to detect and monitor disease activity and are associated with inflammatory biomarkers measured in serum via a blood test. Once this link is established, it would pave the way to the development of digital biomarkers for real-time disease activity prediction and monitoring of the effectiveness of treatments via scalable, cost-effective, and patient-oriented digital solutions. The proposed study aims at targeting the following three main research questions:

1. To what extent is systemic inflammation measured by serum CRP associated with the CRP measured non-invasively in urine, sweat, and saliva?

2. To what extent is systemic inflammation measured by serum cytokines (i.e., IL-1β, IL-6, IL-8, IL-10, and TNF-α) associated with cytokines measured non-invasively in urine and sweat?

3. To what extent is systemic inflammation measured by serum CRP and cytokines associated with non-invasive measures of FeNO from exhaled breath, calprotectin from stool, and core body temperature?

Methods

Study Design & Participants

The NOVA study is a two-group, cross-sectional, single-center observational study aiming to recruit 20 individuals among the hospitalized patients from the Rheumatology ward of University Hospital Zurich (USZ). The systemic inflammation group and the control group will include 10 participants each. The inclusion criteria are adult persons (aged 18 years or older) who have provision of written informed consent. Systemic inflammation patients are defined as patients with inflammatory syndrome (i.e., CRP > 5 mg/L). The control patients are patients who do not have an inflammatory syndrome (i.e., CRP ≤ 5 mg/L). Exclusion criteria are active psychiatric or mental disorders (i.e., clinically relevant depression, dementia, Alzheimer’s disease), alcohol abuse, or other substance abuse, patients needing immediate treatment with glucocorticoids or other drugs for suppression of the inflammation until study procedures can be performed, language barriers or incapable of understanding and signing an informed consent form, and other factors which impair daily functions and make adherence to the study protocol impossible.

Procedures

Recruitment

Figure 1 shows the study procedures of the NOVA study. A clinician will conduct a pre-screening to identify planned and emergency admissions and check if they have increased CRP levels. In case a patient is deemed eligible for the NOVA study, the clinician or study nurse will contact the patient, hand out a study flyer and a patient information sheet, and ask if they are willing to participate in the study following Good Clinical Practice (GCP) guidelines. Participants will have 24 hours or overnight to consider study participation before providing informed consent.

Visit 1 (Study Measurements)

The study measurements will either take place in the patient's room or in the clinical examination room available on the ward and are estimated to take approximately 40 minutes. Participants will complete the following steps under the guidance of the study nurse and a researcher from the Centre for Digital Health Interventions (CDHI).

1) Informed consent: If the eligible patient agrees to participate in the study, a study nurse will explain the study objectives and procedures. The patient will review the informed consent form and sign it.
2) Baseline questionnaire: Participants will fill out the questionnaires (in German or English) to provide information on demographics, oral health, and prior history of surgeries and hospitalizations in the past year.
3) Blood collection: A trained nurse will perform a standard venipuncture, drawing the required blood tubes for the chemistry parameters and cytokine analysis. Upon completion, participants will answer a short questionnaire assessing their experience and impression of the blood draw. This questionnaire will be repeated after each sampling method. The blood samples will be centrifuged and aliquoted into cryovial vials according to the laboratory instruction, frozen within 1 hour of collection, and preserved in sterile tubes at −80 °C until analysis.
4) Urine collection: The midstream urine samples will be collected in sterile containers. Immediately after collection, we will perform a dipstick test (i.e., Roche Combur Test Strip) to exclude samples with relevant bacterial contamination. The suitable samples will be centrifuged and aliquoted into cryovial vials according to the laboratory instruction, frozen within 1 hour of collection, and preserved in sterile tubes at −80 °C until analysis.
5) Core body temperature sensor placement: The CDHI researcher will attach a core body temperature sensor (e.g., greenTEG CORE) to the upper body/chest area approximately 20 cm below the armpit using a heart rate monitor strap or medical patch. The sensor will be collected at the follow-up meeting.
6) Saliva collection: Saliva samples are collected using an absorbent swab. Participants will place an absorbent swab (i.e., Salimetrics SalivaBio Oral Swab) underneath the tongue for 3 minutes followed by direct disposal to a collection tube. Salivary samples will be transported to a storage unit within one hour of measurement and refrigerated at -80 °C until the analysis.
7) Sweat patch placement: The CDHI researcher will attach two sweat patches (i.e., PharmChek Sweat Patch) on the participants' arms or abdomen for sweat collection following the manufacturer's instructions. Participants will be informed of the dos/don'ts for the next 48 hours. The sweat patches will be collected at the follow-up meeting.
8) Exhaled breath test: participants will complete FeNO measurements in exhaled breath using the 'Bosch Vivatmo me' device. All measurements will be repeated twice per participant. 'Bosch Vivatmo me' is a breathalyzer designed to measure airway inflammation based on the FeNO level detected in exhaled breath.
9) Stool sampling instruction: Participants will be provided a guide for collecting stool samples.

Visit 2 (Follow-up Meeting)

Participants will be scheduled for a 20-minute follow-up meeting after 48 hours from study measurements. At this meeting, the CDHI researcher will remove the sweat patch and the core body temperature sensor, collect the stool sample, and conduct an interview on overall experience and preferences comparing sampling methods. Collected sweat patches and stool samples will be refrigerated at -80°C until the analysis. The follow-up meeting will occur either in the patient's room or the clinical examinations room available on the ward.

Patient Instructions

If participants agree to participate in this research project, they are asked to adhere to the specifications and requirements of the research project through the protocol as follows:

- (From two hours before study measurement to saliva collection) Refrain from eating, drinking coffee or acidic/sweet liquids, chewing gums, brushing teeth, using mouthwash and smoking.
- (From study measurements to follow-up meeting) Avoid hot tubs, tanning booths, prolonged exposures in a sauna, or sweat lodges. Normal activities such as bathing, showering, swimming, and exercising are permitted. When drying the area where the sweat patch is applied, gently pat the area with a towel instead of rubbing the area.

Measurements

Endpoints

The primary endpoint of this study is the level of CRP. Secondary endpoints are the levels of inflammatory cytokines such as IL-1β, IL-6, IL-8, IL-10, and TNF-α. Tertiary endpoints are FeNO, calprotectin, and core body temperature (Figure 2).

Additional Information

As described in Textbox 1, we will collect additional information on patient characteristics and demographics, disease characteristics (e.g., disease diagnosis, age at diagnosis, disease duration, comorbidities), medications (e.g., type, duration, dose), measures of disease activity/functional outcomes (e.g., Disease Activity Score-28 with CRP (DAS28-CRP) for rheumatoid arthritis, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) for spondyloarthritis, Disease Activity in PSoriatic Arthritis (DAPSA) for psoriatic arthritis, and Systemic Lupus Erythematosus Disease Activity (SLEDAI) for systemic lupus), oral health (e.g., fasting, teeth status, presence of oral diseases), and prior histories of surgeries and hospitalizations will be collected. To standardize the results of measured biomarkers, we will measure creatinine, albumin, and total protein levels in serum and urine and report the excretion of each urinary protein as a ratio between the concentration of the respective protein and urinary creatinine in a similar manner to albumin/creatinine ratio.

Textbox 1. Additional information.

Baseline characteristics
- Demographics: age, sex, race, marital status, employment status, education, weight, height, body mass index, blood pressure, smoking status, comorbidities

Disease characteristics
- Disease diagnosis, age at diagnosis, disease duration, disease activity/functional outcomes

Medications
- Type, duration, dose

Oral health
- Status of fasting, brushing teeth, chewing gums, smoking, drinking
- Bleeding gums, presence of oral diseases

Prior history
- Hospitalization within 1 year
- Surgical operation within 1 year

Sample standardization
• Creatinine, albumin, total protein levels in serum • Creatinine, albumin, total protein levels in urine

Statistical Analysis

Sample Size Calculation

Since there is no prior knowledge or evidence of the outcome measures in this context, we calculate the sample size based on the following assumptions and considerations. First, we compute the sample size based on the serum CRP level to make sure that differences in CRP level are significant between both study groups. Second, we refer to studies of adult patients diagnosed with rheumatoid arthritis, which is one of the most prevalent systemic inflammatory diseases. The CRP also plays a pivotal role in disease assessment and inflammatory activity in rheumatoid arthritis, which makes it a suitable choice of disease for exploring the sample size [14,15].

Bechman et al. reported that the serum CRP levels in rheumatoid arthritis patients in systemic inflammatory states (i.e., medium and high disease activity) range from 5 to 9 mg/L (SD=3.0) [16]. Lillegraven et al. reported that the serum CRP levels in the non-systemic inflammatory state (i.e., remission) subjects range from 2 to 3 mg/L [17]. Based on these results, it is plausible to conclude that we will observe a 3 to 6 mg/L difference in serum CRP levels comparing systemic inflammation vs. non-systemic inflammation among rheumatoid arthritis patients. On this basis, and assuming 80% of power, 5% of type I error, and equal variance in groups [18], a sample size of 10 participants per group is required to detect a difference of 4 mg/L in serum CRP levels with SD=3.0 (Table 1). Considering dropout and missing data, the sample size required may be increased by 10-20%.

Table 1. Sample size calculation.

	Pooled Standard Deviation (SD)				
Difference of means between groups	SD: 1.0	SD: 2.0	SD: 3.0	SD: 4.0	SD:5.0
2mg/L	6	17	37	64	100
3mg/L	4	9	17	29	45
4mg/L	3	6	10	17	26
5mg/L	3	4	7	12	17

Statistical Analysis

Descriptive analysis will report the mean (SD) for continuous variables and the count (%) for categorical variables. The two-tailed t-test and the Chi-square test (or Fisher’s exact test if expected counts < 5) will be used for univariable analysis. In addition, the Shapiro-Wilk test will be used to evaluate the normality of the distribution in each variable. We will perform the two-tailed t-test (or Wilcoxon rank-sum test, if non-normal) to compare the levels of inflammatory biomarkers between patients with systemic inflammations and controls. Subsequently, analyses of covariance or multiple regressions will be conducted to examine whether the mean biomarker level differs significantly between systemic inflammation vs. controls adjusting for confounding factors such as baseline characteristics and oral health. Secondary and tertiary endpoints will be analyzed analogously. We will compute Pearson and Spearman correlation coefficients to assess the relationship between non-

invasive methods and the serum. For core body temperature data, the repeated measures correlation method (rmcorr) will be performed to assess the association [18]. Statistical analysis will be performed with R or Python. Statistical significance is set at two-sided $p < 0.05$.

Biomarker Analysis

Due to the pilot nature of this study, it is difficult to estimate the precise time required to complete study recruitment and data collection. To overcome this limitation and enable preliminary data analysis, samples will be collected and submitted for biomarker analysis in two batches as described in Figure 3. Samples from the first half of the participants (i.e., Patients 1-5 from each group) will be collected and submitted to laboratories for the biomarker analyses (Batch 1). Batch 2 will consist of samples from the second half of the participants (i.e., Patients 6-10 from each group). This will provide us with information on the accuracy of our estimated sample size and allow us to make corrections with regard to the planned number of patients to be included. Full data analysis will be conducted including the entire samples. Serum CRP, serum cytokines, and fecal calprotectin will be analyzed in the in-house laboratories of USZ according to the laboratory procedures. The levels of urinary CRP, urinary cytokines, salivary CRP, sweat CRP, and sweat cytokines will be determined at Swiss BioQuant (Reinach, Switzerland). Non-invasive CRP levels will be quantified by ELISA according to instructions provided by the manufacturer. Non-invasive cytokines will be analyzed using electrochemiluminescence immunoassay.

Data Management

Project data will be handled with the uttermost discretion and is only accessible to authorized personnel who require the data to fulfill their duties within the scope of the research project. Participant data will be encrypted. The participant identification list will be stored separately from the study records and only accessible by a dedicated data manager, who was not involved in the study. On the case-report forms and other project-specific documents, participants are only identified by a unique participant number. Access and changes to documents and data servers will be logged and traced. Data will further be backed-up on encrypted drives and safeguarded in a key-locked cupboard.

Ethics Approval & Informed Consent

This study received ethical approval from the independent research ethics committee of Canton Zurich in October 2022 (BASEC-Nr: 2022-01386). This research project will be conducted in accordance with the protocol, the Declaration of Helsinki, the principles of GCP, the Human Research Act (HRA), and the Human Research Ordinance (HRO) as well as other locally relevant regulations. All participants will provide written informed consent before they participate in the study. Participation in the study is fully voluntary, and withdrawal from the study is possible at any time.

Results

A total of 20 participants participated in the study measurements between February and September 2023. Participants were on average 52.8 (range 24-82, SD 14.4) years of age, 70% (n=14) of them were women. The analysis results reporting findings are expected to be published in the fall of 2024.

Discussion

This protocol describes a cross-sectional, proof-of-concept study aiming to establish a relationship between the levels of non-invasive inflammatory biomarkers and serum inflammatory biomarkers. Recent efforts have provided evidence that digitizing the measurement of CRP in various body fluids such as serum, saliva, and sweat is feasible [19–21]. These studies, however, have focused on a single modality of biofluids, whose detection accuracy may be highly variable by environmental factors or biomarker characteristics. This is the first study to demonstrate the feasibility of multi-modal, non-invasive measurements of inflammatory biomarkers in patients with chronic systemic inflammation and their associations with the gold standard, the serum CRP. Demonstrating a relationship with non-invasive assessments of systemic inflammation would provide the basis for digitizing a surrogate measurement for CRP in serum, and thus, contribute to the creation of a digital surrogate biomarker for CRP. This is particularly relevant to the ever-rising cases of systemic inflammatory conditions and the urgent need for rapid, precise, and remote monitoring tools for such prevalent conditions.

This study has strengths and limitations. As a strength, we collect six distinct types of body fluids in addition to blood from the same individual and perform a biomarker immunoassay assessment through comprehensive testing. A proposed multi-modal approach not only increases the precision and sensitivity of detected associations but also offers new insights on patients' experiences or preferences related to different sampling methods. Second, we include different types of inflammatory biomarkers (i.e., CRP, interleukins, calprotectin, FeNO, and core body temperature) as the sensitivity of detection varies by molecules and biofluid types. As opposed to existing research with a single outcome, we seek to contribute to the current literature by assessing multiple endpoints that have been acknowledged to have a direct or indirect association with the gold standard. Third, we minimize an unanticipated temporal or circadian influence or medication effect in the inflammatory state by obtaining blood and non-invasive samples simultaneously. To achieve simultaneous sampling, we propose an efficient workflow of study procedure that enables us to complete study measurements and the follow-up meeting within a total of one hour. Fourth, it is the first to implement non-invasive, continuous monitoring of core body temperature for 48 hours to associate the temporal pattern of body temperature with inflammatory activities in systemic inflammation. Lastly, we obtain additional information on patient and disease characteristics, disease activity, medications, comorbidities, and oral health questionnaires and adjust statistical models for potential confounding.

This study will have limitations. With the pilot nature of the study, no longitudinal measurements will be collected. Given the nature of the cross-sectional study, we are not able to establish causal relationships from observed associations. In addition, our study involves a relatively small size at a single study site among hospitalized patients at a University hospital, which may limit the generalizability of our findings to a real-world patient population. Nevertheless, this proof-of-concept study offers novel insights into the digitization of inflammatory biomarkers and directions for future research opportunities. If this proof-of-concept study yields successful results, a longitudinal, prospective cohort study is warranted.

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Authors' Contributions

- Conceptualization: JS, SM, EF, OD, FB.
- Methodology: JS, SM, EF, OD, FB.
- Project administration: JS, SM, EF, OD, FB.
- Resources: EF, OD.
- Supervision: JS, SM.
- Writing - original draft: JS.
- Writing - review & editing: JS, SM, RD, EF, OD, FB.

Conflicts of Interest

OD has/had consultancy relationship with and/or has received research funding from and/or has served as a speaker for the following companies: 4P-Pharma, Abbvie, Acceleron, Alcimed, Altavant, Amgen, AnaMar, Arxx, AstraZeneca, Blade, Bayer, Boehringer Ingelheim, Corbus, CSL Behring, Galderma, Galapagos, Glenmark, Gossamer, Horizon, Janssen, Kymera, Lupin, Medscape, Merck, Miltenyi Biotec, Mitsubishi Tanabe, Novartis, Prometheus, Redxpharma, Roivant and Topadur. Patent issued "mir-29 for the treatment of systemic sclerosis" (US8247389, EP2331143). RD has served as speakers bureau for Actelion, an advisory board for Boehringer-Ingelheim, and has received grants/research support from Pfizer, Actelion, Iten-Kohaut foundation, and congress participation support from Amgen. SM has received congress participation support from AstraZeneca. All other authors have no conflicts of interest to declare. All other authors have no conflicts of interest to declare.

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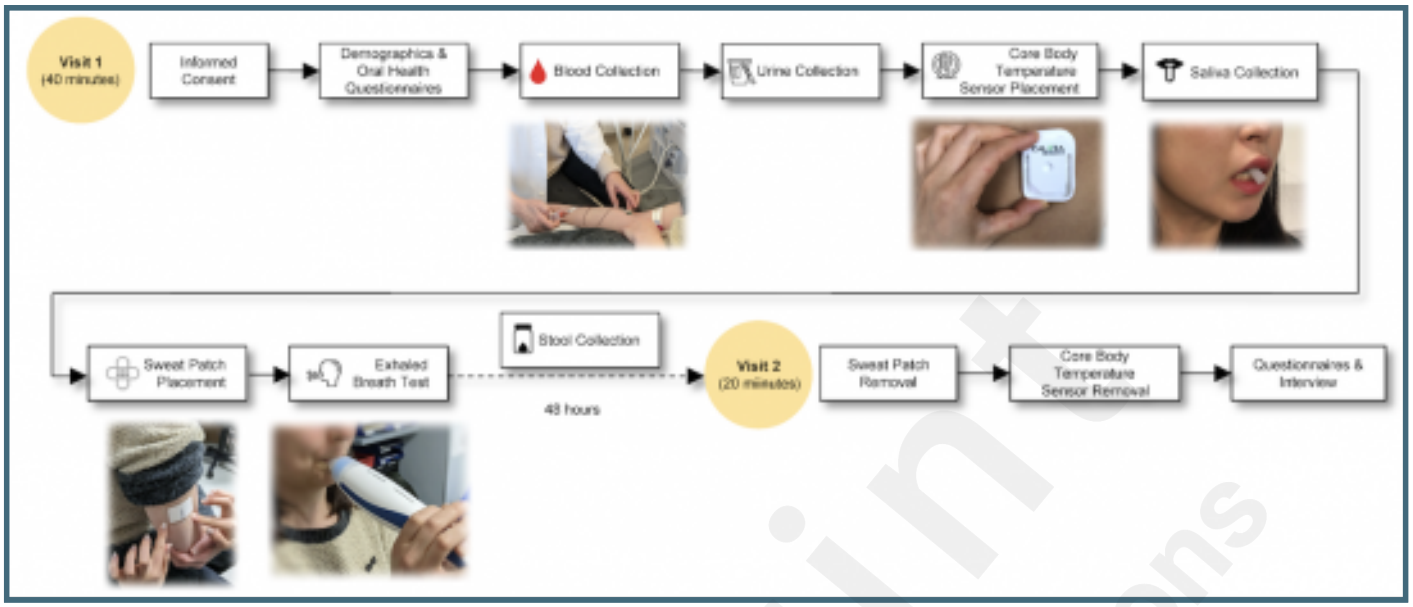
Abbreviations

BASDAI: Bath Ankylosing Spondylitis Disease Activity Index
CDHI: Centre for Digital Health Intervention
CRP: C-reactive protein
DAPSA: Disease Activity in PSoriatic Arthritis
DAS: Disease Activity Score
FeNO: fractional exhaled nitric oxide
GCP: Good Clinical Practice
HRA: Human Research Act
HRO: Human Research Ordinance
IL: interleukin
SD: standard deviation
SLEDAI: Systemic Lupus Erythematosus Disease Activity
TNF: tumor necrosis factor
USZ: University Hospital Zurich

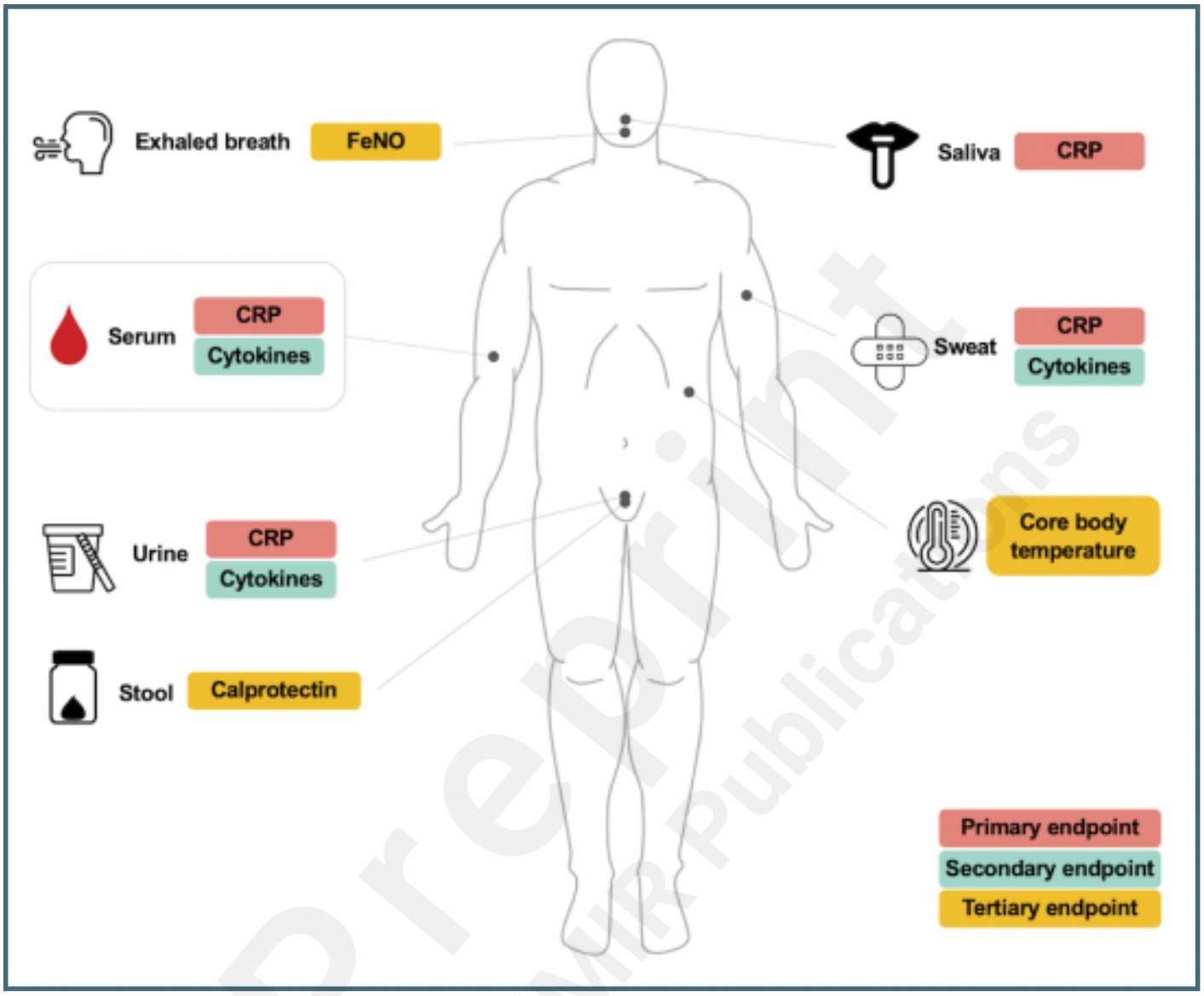
Supplementary Files

Figures

A schematic of NOVA study procedures.



An overview of study measurements and endpoints.



Proposed biomarker analysis.