Inflammatory biomarker profiling in classical orthostatic hypotension: Insights from the SYSTEMA cohort☆

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ABSTRACT

Objective: To investigate the inflammatory biomarker signature associated with classical orthostatic hypotension (OH).
Methods: A cross-sectional study including 778 patients with unexplained syncope and/or orthostatic intolerance undergoing head-up tilt test (HUT) and supine blood sampling. Of these, 98 met diagnostic criteria of classical OH and 181 demonstrated normal haemodynamic response during HUT. Blood plasma samples were analysed by antibody-based Proximity Extension Assay technique simultaneously measuring 57 inflammatory and cancer-related human protein biomarkers. The discovery algorithm was a sequential two-step process of biomarker signature identification by multivariate principal component analysis (PCA), and verification by univariate ANOVA with Bonferroni correction.
Results: Patients with classical OH were older (68 vs. 60 years; $p < 0.001$) and more likely to be men (58 vs. 41%; $p < 0.001$). PCA and Bonferroni-adjusted ANOVA identified midkine (MK), immunoglobulin-like transcript 3 (ILT-3), regenerating islet-derived protein 4 (REG-4), and tartrate-resistant acid phosphatase type 5 (TR-AP) as the most robust targeted biomarker signature for OH. In multivariate regression analysis adjusting for age, sex, cardiovascular disease and risk factors, the results remained significant for ILT-3 ($p = 0.036$), MK ($p = 0.008$) and REG-4 ($p = 0.024$), but not for TR-AP.
Conclusions: Targeted protein profiling in classical orthostatic hypotension reveals a biomarker signature associated with immunoregulatory functions and vascular inflammation. Circulating levels of midkine, immunoglobulin-like transcript-3, regenerating islet-derived protein-4 are elevated in orthostatic hypotension, suggesting a complex interplay among inflammation, autonomic dysfunction and atherothrombosis.

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1. Introduction

Orthostatic hypotension (OH) is a hallmark sign of autonomic failure frequently observed in patients with neurodegenerative diseases and comorbidities, such as diabetes and hypertension [1–3]. Presence of OH may cause debilitating symptoms and indicates higher risk of cardiovascular disease (CVD) and premature death [2,4,5]. Nevertheless, OH is frequently overlooked in cardiovascular screening programmes, epidemiological studies, and diagnostic work-up of patients with symptoms potentially related to this condition [2].

Traditionally, OH is divided into two main categories: neurogenic and non-neurogenic [2]. Neurogenic OH is a primary manifestation of chronic autonomic failure in neurodegenerative disorders, such as pure autonomic failure, multiple system atrophy and Parkinson disease [6]. Orthostatic hypotension can also be secondary to various inflammatory and non-inflammatory conditions such as multiple myeloma, paraneoplastic syndrome, autoimmune diseases or amyloidosis [4], with a presumable affection of autonomic nervous system, although in many cases the aetiology remains unknown [1]. On the other hand, non-neurogenic OH can be caused by conditions that impair the compensatory mechanisms governed by the autonomic nervous system, such as diabetes and chronic cardiovascular disorders [2], but overlap between neurogenic and non-neurogenic factors in secondary OH may exist [2].

The most severe form of OH, often referred to as classical [7], implies a significant blood pressure reduction within the first 3 min of upright standing [8]. The majority of cases related to neurogenic OH belong

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to this category [2,7]. However, in at least one third of cases, the aetiology of OH remains elusive, even after an extensive diagnostic work-up [1].

Both neurogenic and non-neurogenic forms of OH may potentially involve activation of inflammatory pathways, as components of the underlying pathological process eventually leading to autonomic failure [9]. Notably, a cholinergic anti-inflammatory pathway that reflexively adjusts macrophage activation via parasympathetic outflow has recently been described [10]. Further, the immune system has been shown to modulate autonomic activity, hence completing the wiring of the so called "inflammatory reflex" [11]. Thus, it is important to explore the expression of inflammatory mediators in OH, as a potential diagnostic tool and therapeutic target in this understudied and difficult-to-treat condition.

In this study, we sought to discover inflammatory biomarkers associated with OH in order to identify a signature which could be potentially useful to understand the pathophysiological pathway underlying the link between classical OH and CVD, as observed in epidemiological studies.

2. Methods

2.1. Study population

The study was carried out from September 2008 to May 2014 as a part of the Syncope Study of Unselected Population in Malmö (SYSTEMA) [12]. Patients with unexplained syncope and/or symptoms of orthostatic intolerance were referred to the tertiary syncope unit at Skåne University Hospital in Malmö from outpatient care and hospitals in southern Sweden. Additional tests were performed, if indicated, to eliminate any cardiac and neurological causes of the symptoms, e.g. exercise and ambulatory prolonged electrocardiogram (Holter ECG), 2D transthoracic echocardiography, coronary and pulmonary angiography, brain imaging and encephalography. During the study period, 994 patients were examined by head-up tilt test (HUT) according to current European syncope guidelines [7]; of these, 778 patients had blood samples collected during HUT examination (Fig. 1). All patients gave written informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and has been approved by The Regional Ethical Review Board of Lund University (No. 82/2008).

The PICO model was as follows: patients with unexplained syncope or orthostatic intolerance (Population), blood samples and HUT (Intervention), classical OH versus controls (Comparison), targeted protein biomarker discovery and hemodynamic response (Outcome).

2.2. Examination protocol

Patients were taking their regular medications, fasted for 2 h prior to examination but were allowed to drink water at will. They were asked to fill out a questionnaire about past medical history. The patients were placed on a tilt table and rested for at least 10 min before blood samples were collected through a venous cannula inserted in the forearm. Subsequently, patients rested for another 10 min to obtain haemodynamically stable parameters; thereafter the standardized 70°HUT was carried out for 20 min followed by nitroglycerine provocation according to the Italian protocol if passive HUT was negative, or until syncope/pre-syncope or pronounced symptoms of orthostatic intolerance occurred [13]. Beat-to-beat blood pressure and ECG was monitored continuously by a validated non-invasive photoplethysmographic method (Nexfin monitor; BMEYE, Amsterdam, Netherlands) with a wrist unit and finger cuff of appropriate size [14].

2.3. Multiplex protein analysis

Plasma biomarkers were measured from supine blood samples (total volume: 30 ml) that had been first centrifuged, then stored as $16 \times 250\,\mu l$ aliquots of EDTA plasma in plastic thermotubes, and frozen at −80 °C. For biomarker analysis, the samples were thawed and examined by the Proximity Extension Assay technique using the Olink Proteomics Proseek Multiplex Oncology I v1 96×96 reagents kit, which simultaneously measures 57 inflammatory and cancer-related human protein biomarkers in plasma (Table S1). In short, a pair of oligonucleotide-labelled antibodies, Proseek probes, binds to the target protein in the plasma sample. When the two Proseek probes are in close proximity, a new polymerase-chain reaction (PCR) target sequence is formed by a proximity-dependent DNA polymerization event. This complex is subsequently detected and quantified using standard real-time PCR. The generated Normalized Protein Expression (NPX) unit is on a log2 scale, which means that a larger number represents a higher protein level in the sample. Additional information about limit of detection, reproducibility and validation is available at the Olink Proteomics website (http://www.olink.com/products/document-download-center).
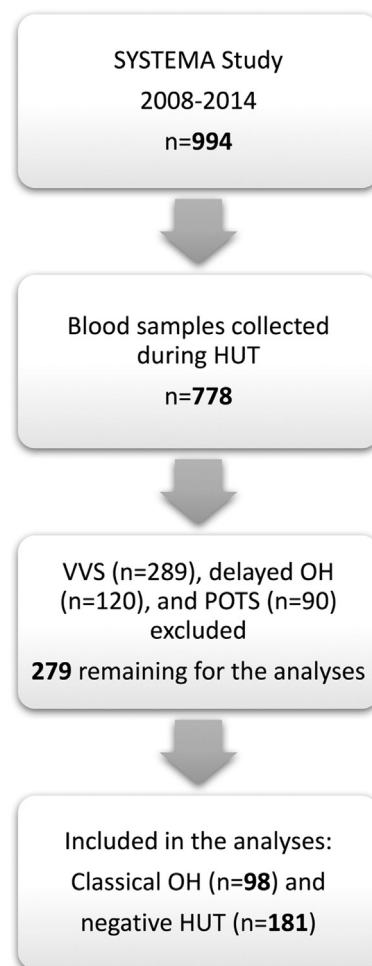

Fig. 1. Flow-chart summarising the selection process of study population. HUT, head-up tilt; OH, orthostatic hypotension; POTS, postural orthostatic tachycardia syndrome; SYSTEMA, Syncope Study of Unselected Population in Malmö; VVS, vasovagal syncope.

2.4. Data analysis

Supine and 3-min HUT BP were calculated over an averaged 30-s period. The supine BP was calculated during a stable period between 1 and 5 min prior to HUT. The 3-min HUT value was calculated after 3 min of HUT.

We defined classical orthostatic hypotension as a sustained drop in systolic BP ≥20 mm Hg and/or drop in diastolic BP (DBP) ≥10 mm Hg after 3 min of passive HUT [8]. A significant drop in BP occurring after 3 min of HUT was defined as delayed OH [15], and these patients were excluded from the analyses. Vasovagal syncope (VVS) was defined as a reproduction of syncope associated with a characteristic pattern of pronounced hypotension, bradycardia or asystole [7], while postural orthostatic tachycardia syndrome (POTS) as a reproduction of symptoms of orthostatic intolerance (lightheadedness, dizziness or discomfort) with heart rate increase >30/min or tachycardia >120/min during HUT [7,8]. Patients with VVS and POTS were excluded from the analyses.

The baroreflex sensitivity (BRS; ms/mm Hg) index was calculated according to the formula: (60 / highest HR during HUT − 60 / supine HR) × 1000 ms / (lowest SBP during HUT − supine SBP), and compared between OH-positive and OH-negative patients. Valsalva maneuver was also performed to further assess nonpostural hemodynamic responses and BRS. Adrenergic BRS failure was featured by clear V-shaped SBP response, as previously reported [16].

We used the Modification of Diet in Renal Disease (MDRD) study equation to calculate the glomerular filtration rate.

2.5. Statistical analysis

The main characteristics of study population are presented as mean and standard deviation for continuous variables and as percentages for categorical variables.

The discovery algorithm for the identification of potentially relevant biomarkers associated with the presence of OH was a sequential two-step process of i) biomarker signature identification by supervised, multivariate, principal component analysis, and ii) verification by univariate ANOVA with Bonferroni correction.