Postural Orthostatic Tachycardia Syndrome. The role of cardiovascular dysautonomia and inflammation

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The role of cardiovascular dysautonomia and inflammation

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Postural Orthostatic Tachycardia Syndrome

The role of cardiovascular dysautonomia and inflammation

JASMINA MEDIC SPAHIC, M.D.

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden. To be defended at Agardh Lecture Hall, Skåne University Hospital, Malmö, on November 18, 2022, at 9 a.m.

Faculty opponent
Professor Lennart Bergfeldt, Sahlgrenska Academy, Gothenburg

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Title and subtitle: Postural Orthostatic Tachycardia Syndrome: The role of cardiovascular dysautonomia and inflammation.

Background:

Postural Orthostatic Tachycardia Syndrome (POTS) is a chronic autonomic nervous system dysfunction affecting predominantly younger females of childbearing years with great impact on life quality. In developed countries, the prevalence of POTS has been estimated at 0.2 - 1.0%. Various pathophysiological pathways are believed to interact causing great symptom diversity, prompting mandatory further exploration.

Aims and methods:

Project I: The aim was to explore and compare clinical and neuroendocrine characteristics in patients among those presenting with orthostatic intolerance (orthostatic hypotension, OH, vasovagal syncope, VVS and POTS). Patients underwent head-up tilt (HUT) for autonomic testing, answered a questionnaire for symptom evaluation and had blood samples. Project II and III: The aim was to discover biomarker footprints related to POTS using an antibody-based proteomics technique, Olink, measuring simultaneously 57 inflammatory and respectively 92 cardiovascular biomarkers. Three-hundred-and-ninety-six POTS patients were included. Project IV: The aim was to develop a novel, questionnaire -based symptom scoring system, Malmö POTS Score (MAPS), including 12 most prevalent symptoms experienced by patients with POTS. Scores in 62 POTS patients were compared with 50 healthy individuals and symptom burden related to haemodynamic changes during HUT. Project V: The aim was to investigate the renin-angiotensin-aldosterone system (RAAS) in POTS patients compared with healthy controls without orthostatic intolerance symptoms. All participants performed active standing test for autonomic testing and blood sampling after 10 minutes supine rest.

Results:

Project I: Patients with POTS were more often younger females and had higher HR during passive HUT compared with other groups, orthostatic intolerance, and negative HUT. A significant rise in norepinephrine (NE) was observed in POTS and lower resting MR-proANP in relation to VVS and OH but not to negative HUT. Project II: Patients with POTS were predominantly females with lower BMI. Maximum HR was significantly higher compared with negative HUT patients. Proconvertase Furin, a protein that promotes proteolytic maturation of other proproteins was downregulated in POTS but not in healthy individuals. Project III: Higher levels of growth hormone in females with POTS were observed whereas males with POTS have decreased plasma myoglobin levels compared with healthy controls. Project IV: POTS patients reported a 5-fold higher symptom burden on MAPS score compared with controls (mean; 78±20 vs. 14±12, *p<0.001).* Correlation between total MAPS score and haemodynamic changes was observed in both groups though more prominent in POTS. Project V: Renin activity was decreased in POTS patients but not in healthy controls. Aldosterone levels were intact in both groups. There was an inverse correlation between renin activity and blood pressure in controls but absent in POTS patients.

Conclusions:

Patients with POTS were predominantly younger females with maximal standing heart rate on average above 110 bpm during orthostatic provocation. Abnormal neuroendocrine and enzymatic responses in POTS patients were found potentially explaining symptoms experienced during orthostasis and confirming inflammatory involvement. Finally, POTS patients had significantly higher symptom scores in the newly developed questionnaire MAPS confirming their great burden of symptoms affecting life quality and offering simpler clinical diagnosis.

Key words

Biomarkers, Postural orthostatic tachycardia syndrome, Malmö POTS score.

Classification system and/or index terms (if any)

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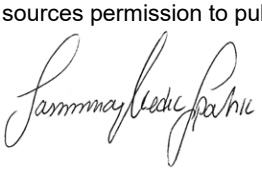
Signature  Date 2022-10-11

Postural Orthostatic Tachycardia Syndrome

The role of cardiovascular inflammation and dysautonomia

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Above all, don´t fear difficult moments.
The best comes from them.
Rita Levi-Montalcini

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List of papers

This thesis is based on following papers.

I. Hamrefors V, Medic Spahic J, Nilsson D, Senneby Martin, Sutton R, Melander O, and Fedorowski A. *Syndromes of orthostatic intolerance and syncope in young adults.* Open Heart 2017; 4:e000585. PMID: 28674628

II. Spahic JM, Ricci F, Aung N, Axelsson J, Melander O, Sutton R, Hamrefors V and Fedorowski A. *Proconvertase Furin Is Downregulated in Postural Orthostatic Tachycardia Syndrome.* Frontiers Neuroscience. 2019;13:301. PMID: 31001074

III. Medic Spahic J, Ricci F, Aung N, Hallengren E, Axelsson J, Hamrefors V, Melander O, Sutton R, Fedorowski A. *Proteomic analysis reveals sex-specific biomarker signature in postural orthostatic tachycardia syndrome.* BMC Cardiovasc Disorder 2020;20(1):190. PMID: 32321428

IV. Spahic JM, Hamrefors V, Johansson M, Ricci F, Melander O, Sutton R, Fedorowski A. *New symptom score for postural orthostatic tachycardia syndrome*. Journal of Internal Medicine 2022. Published online. PMID: 36111700

V. Spahic JM, Mattisson IY, Hamrefors V, Johansson M, Ricci F, Nilsson J, Melander O, Sutton R, Fedorowski A. *Evidence for impaired renin activity in Postural Orthostatic Tachycardia Syndrome.* Submitted 2022

Abbreviations

95% CI	95% Confidence interval
ABP	Arterial Blood Pressure
ACE	Angiotensin Converting Enzyme
ACh	Acetylcholine
ANOVA	Analysis of variance
ANS	Autonomic Nervous System
ANP	Atrial Natriuretic Peptide
BMI	Body Mass Index
BP	Blood Pressure
BPM	Beats per minute
cAMP	Cyclic adenosine monophosphate
CFS	Chronic Fatigue Syndrome
CT-proAVP	Terminal Pro-Arginine Vasopressin
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
EEG	Electroencephalogram
ESC	European Society of Cardiology
FUR	Furin
GH	Growth Hormone
GPCR	G-protein coupled receptors
HR	Heart Rate
HUT	Head-up Tilt Test
IQR	Interquartile range

JGC	Juxtaglomerular cells
MAPS	Malmö POTS Score
MB	Myoglobin
NE	Norepinephrine
NO	Nitric Oxide
OH	Orthostatic Hypotension
OHQ	Orthostatic Hypotension Questionnaire
PCA	Principal Component Analysis
PCR	Polymerase-Chain reaction
POTS	Postural Orthostatic Tachycardia Syndrome
PRA	Plasma Renin Activity
RAAS	Renin-Angiotensin-Aldosterone System
ROC	Receiver Operating Characteristics
SA	Sinoatrial
SBP	Systolic Blood Pressure
SD	Standard Deviation
SYSTEMA	Syncope Study of Unselected Population in Malmö
VVS	Vasovagal Syncope

Introduction

The autonomic nervous system (ANS) is a component of the peripheral nervous system that regulates involuntary physiological processes including respiration, digestion, heart rate and blood pressure (1). The ANS is comprised of sympathetic, parasympathetic, and enteric nervous system providing neuronal control of all body parts except skeletal muscles (2, 3). The sympathetic division emerges from the spinal cord in the thoracic and lumbar areas whereas the parasympathetic division emerges via cranial nerves and sacral spinal cord (4) **(Figure 1).**

Sympathetic nervous system releases mainly norepinephrine (NE) (5) and prepares the body for stressful situations. It increases heart rate (positive chronotropic effect), contractility (positive inotropic effect) and promotes vasoconstriction through activation of adrenergic receptors (6). Parasympathetic nervous system is most active in restful conditions through activation of muscarinic receptors by acetylcholine (ACh) (4) **(Table 1).**

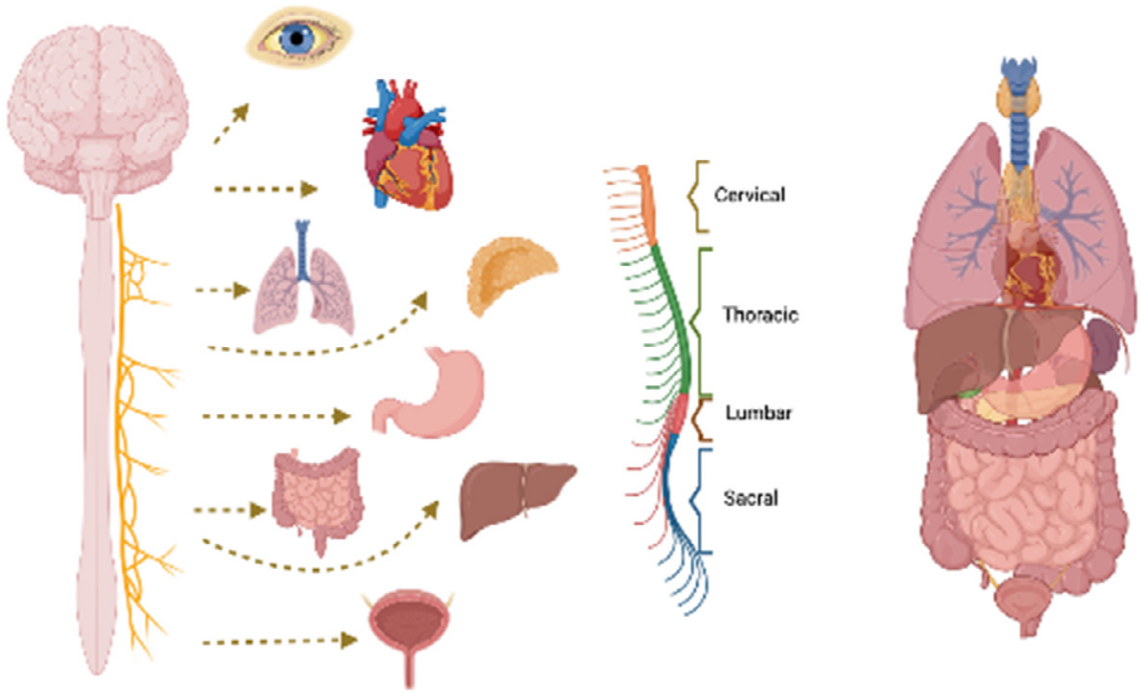

Figure 1. Autonomic nervous system illustrated using biorender.

Tablel 1. Components of the autonomic nervous system and their effect on different organs when stimulated

Organ	Sympathetic	Parasympathetic
Pupils	Dilatation	Constriction
Respiratory tract	Dilatation of airways	Constriction of airways
Heart	↑ Heart rate	↓ Heart rate
Vessels	Vasoconstriction	No effect*
Digestive system	↓Peristalsis	↑ Peristalsis
Liver	↑ Glucose level	No effect
Adrenal medulla	↑ Norepinephrine / epinephrine	No effect
Urinary tract	↑ Urination frequency	↑ Urinary retention

*Most blood vessels in the body do not have parasympathetic innervation. However, parasympathetic nerves do innervate salivary glands, gastrointestinal glands, and genital erectile tissue where they cause vasodilatation.

Heartbeats originate from the rhythmic pacing discharge from the sinoatrial (SA) node within the heart itself regulated by the nervous system, hormones and other factors (4). The adrenergic receptors present in the myocardium that are involved in cardiovascular autonomic function belong to the family of G-protein coupled receptors (GPCR) (7). GPCR are transmembrane proteins present on the cell surface that mediate most cellular responses to external stimuli. They detect molecules outside the cell which activate a cellular response through either the cAMP signal or the phosphatidylinositol pathway (8). There are different groups and receptor types in the GPCR family that are active in various physiological processes. Beta-adrenergic receptors and Angiotensin II receptors play a crucial role in the regulation of cardiovascular function. Dysregulation in their activity may cause hypertension, coronary artery disease or heart failure (9).

Dysregulation of the autonomic nervous system, dysautonomia, affects involuntary physiological processes which may involve functioning of the heart, blood vessels, intestine, respiration and sweat glands. Dysfunction of the autonomic compensatory mechanisms during orthostatic stress is termed orthostatic intolerance.

Orthostatic Intolerance

Orthostatic Intolerance is clinically a group of different conditions causing cardiovascular and non-cardiovascular disabilities when upright due to impaired ANS function. There are three subgroups: Orthostatic Hypotension (OH), Postural Orthostatic Tachycardia syndrome (POTS) and Orthostatic vasovagal syncope (10). POTS is the most common form of orthostatic intolerance in young females (11) while orthostatic hypotension affects predominantly the elderly population. The common link between these three disorders is ANS dysfunction and neuroendocrine responses to accompanying haemodynamic changes.

Orthostatic hypotension is defined as a fall in blood pressure of at least 20 mmHg in systolic blood pressure and/or at least 10 mmHg in diastolic blood pressure within 3 minutes of standing or head-up tilt test (HUT) (12, 13).

Vasovagal syncope (VVS) occurs due to cerebral hypoperfusion from progressive fall in blood pressure followed by bradycardia and/or asystole (14).

When upright, gravitational effect causes approximately 1 litre of blood to descend from the chest to lower abdomen, splanchnic vasculature, buttocks, and legs. The subsequent increase in hydrostatic pressure leads to a 10% shift of plasma volume from the intravascular space to the interstitial space (15). Consequently, there is a reduction in venous return which decreases stroke volume, arterial blood pressure and cerebral blood flow. Symptoms most frequently reported in orthostatic intolerance are due to cerebral hypoperfusion (dizziness, blurred or tunnel vision, concentration difficulties) and sympathetic overactivity including epinephrine release (palpitations, tremor, and syncope) (16, 17).

Baroreceptor reflex

Alterations in arterial blood pressure (ABP) are sensed by stetch-sensitive arterial baroreceptors present in the aortic arch and carotid sinuses which constitute the mechanism for maintenance of perfusion pressure when circulatory homeostasis is disturbed (18). Stimulated baroreceptors by arterial pressure changes modulate both sympathetic and vagal activity affecting heart rate, contractility, and peripheral vascular resistance (19). Baroreceptors respond to both blood pressure increase or decrease. When blood pressure falls i.e., during orthostatic provocation or hypovolemia due to bleeding, autonomic neurons react with increasing sympathetic outflow and decreasing parasympathetic outflow which effect vasoconstriction, tachycardia and positive inotropy. These mechanisms aim to restore cardiac output and blood pressure (20) .

Postural Orthostatic Tachycardia Syndrome

Postural orthostatic tachycardia syndrome (POTS) is a chronic condition of the autonomic nervous system (ANS) affecting predominantly younger females of childbearing years (11). Diagnostic criteria for POTS, endorsed by all major autonomic, neurological and cardiological societies, outlined by the European Society of Cardiology (ESC) Guidelines for Syncope Management, include symptoms of orthostatic intolerance for at least 3-6 months together with orthostatic heart rate (HR) increase > 30 bpm (HR increase >40 bpm in patients <19 years) or HR exceeding 120 bpm when upright in the absence of orthostatic hypotension (21-24). The aetiology of POTS is not fully understood though its wide symptom

diversity implies a multifactorial mechanism causing both cardiac (palpitations, chest pain and dyspnoea) and non-cardiac symptoms (insomnia, headache, and concentration difficulties). Prevalence in developed countries is estimated at 0.2 and 1% (21, 25).

The onset of POTS is usually precipitated by immunological stressors such as viral infections, surgery or trauma (21, 26, 27) but it can also be related to psychosocial stress (28). Furthermore, a constellation of other conditions related to autoimmunity and chronic diseases, such as Ehlers-Danlos Syndrome, mastocytosis and chronic fatigue syndrome (CFS) can sometimes accompany POTS (29-32). The aetiology remains obscure though it is considered multifactorial with the autonomic nervous system providing the link between body systems. Thus, the pathophysiology of POTS includes autoimmune disorder, sympathetic denervation and hypovolemia, reflex tachycardia, and catecholamine excess (21, 31, 33, 34).

To date, there is no specific treatment for POTS but only mechanisms for symptom relief. The approach to treatment depends on the pathophysiological mechanisms at play in each patient. Stockings, abdominal binders, and vasoconstrictors are used to enhance venous return in patients with peripheral denervation. Exercise, increased salt, and volume intake are recommended in patients with hypovolemia while betablockers can be used in hyperadrenergic conditions (35). Long term prognosis has not been fully appreciated, , though 50% of patients may experience symptom reduction or recover spontaneously within 1-3 years (21). Patients with POTS are often disabled due to their great symptom burden adversely affecting quality of life with societal and economic impacts.

Malmö POTS Score

Malmö POTS score (MAPS) is a questionnaire developed as a self-assessment of symptom burden using a visual analogue scale graded from 0 (no symptoms) to 10 (very pronounced symptoms). Selection of the twelve questions included were based on the available literature from a large international POTS survey, expert opinion, and the authors ‘own clinical experience. Our intention with MAPS was to include symptoms from both cardiovascular and non-cardiovascular systems which can be used not only for diagnosis but also as a follow-up tool over longer periods. **(Appendix 1)**

To date, no specific questionnaire is developed for symptom grading in patients with POTS. Previous studies using Composite Autonomic Symptom Scale 31 (COMPASS-31) (36) and Orthostatic hypotension questionnaire (OHQ) do not cover the wide symptom diversity in POTS rendering them inadequate.

Five cardiac symptoms (palpitations, dizziness, presyncope, dyspnoea, and chest pain) and seven non-cardiac symptoms (gastrointestinal symptoms, insomnia, concentration difficulties, headache, myalgia, nausea, and fatigue) during the previous seven days were incorporated **(Table 2).** Symptom severity was assessed through calculation of the total score that ranged from 0 to a maximum score of 120 points. The optimal cut-off-point value of MAPS scores to discriminate between POTS and healthy controls was calculated to be ≥42 using the by Youden method.

Table 2. Five cardiovascular and seven non-cardiovascular symptoms included in MAPS

Cardiovascular Symptoms	Non-cardiovascular symptoms
Palpitations	Gastrointestinal Symptoms
Dizziness	Insomnia
Presyncope	Concentration difficulties
Dyspnoea	Headache
Chest pain	Myalgia
	Nausea
	Fatigue

Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone system (RAAS) is one of the most prominent endocrine, paracrine and intracrine vasoactive systems (37) that regulates arterial blood pressure, plasma sodium concentration, and extracellular volume through activation of hormonal cascade (38, 39). When blood flow through renal arteries is reduced, juxtamedullary epithelioid cells in the kidneys convert angiotensinogen into angiotensin I by secretion of renin (12). Further on, angiotensin-converting enzyme (ACE), released from endothelial cells in the lungs, converts angiotensin I to angiotensin II and degrades active bradykinin. This process play an important role in blood pressure regulation through promotion of vasoconstriction (40, 41). Angiotensin II is a potent vasoconstrictor exerting its effect by binding to AT1 and AT 2 receptors and by stimulating aldosterone release from the adrenal cortex (42). Aldosterone enhances sodium and water reabsorption increasing plasma volume and blood pressure **(Figure 2).**

Angiotensin-converting enzyme 2 (ACE2) is an important enzyme regulating RAAS. Moreover, it is also a functional receptor in cell surface allowing SARS-CoV-2 virus to enter the host cell causing an ACE/ACE2 balance disruption and COVID-19 progression (43, 44). Previous studies have observed an increase of patients diagnosed with POTS three months after covid-19 infection (45) suggesting a correlation between virus infection and altered activity of ACE2.

Renin

Renin is a hormone responsible for the control of blood pressure and other physiological functions. The main source of renin comes from the juxtaglomerular cells (JGC) described as specialized smooth muscle cells located mainly in afferent arterioles. Juxtaglomerular cells synthesize, store and release renin from storage granules as a response to decreased blood pressure but renin is also released through formation of cyclic adenosine monophosphate (cAMP) via activation of Beta-adrenoreceptors. (46).

Aldosterone

Aldosterone, a mineralocorticoid synthesized and secreted by in the glomerulosa zone of the adrenal cortex, has a fundamental role in electrolyte and blood pressure regulation (47-49). It regulates plasma sodium and water reabsorption and potassium excretion through activation of mineralocorticoid receptors in the distal tubules and collecting ducts of the nephron. Aldosterone increases the transport of sodium across the cell in exchange for potassium and hydrogen ions (48). It exerts effect on the vascular system by inducing oxidative stress, inflammation, remodeling, fibrosis, and endothelial dysfunction (50).

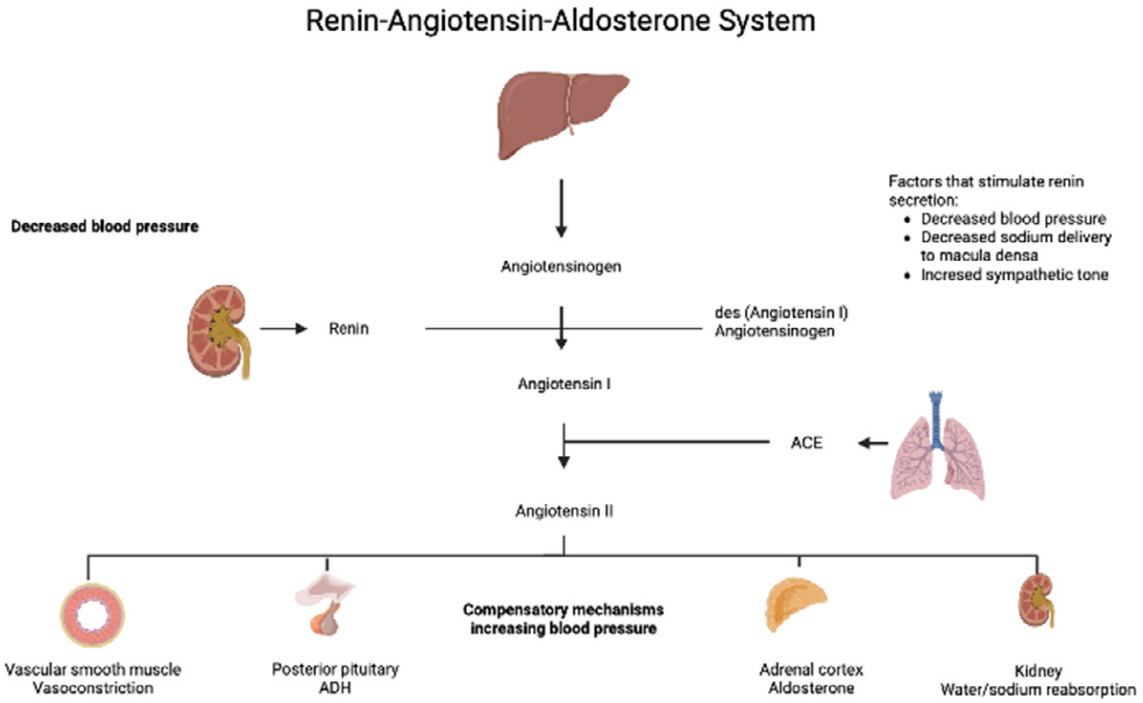

Figure 2. Renin-angiotensin-aldosterone system illustrated using biorender.

Biomarkers

A biomarker is a naturally occurring molecule that is measured as an indicator of normal biological processes, pathological processes or response to an exposure or intervention (51). They can be used to screen and characterise diseases and inform prognosis, identify cell types, and yield other important information. There are different kinds of biomarkers used in various clinical assessments, disease screening, research, and therapeutic development (52).

Biomarkers are relatively inexpensive and easily measured in urine, serum, stool, or tissue through minimally (or non-) invasive techniques (53). Different biomarkers relate to different body processes. Hormones indicate metabolic states or disorders. Cytokines, chemokines, and growth factors reveal intercellular signalling while phosphoproteins concern intracellular signalling.

In cardiology, biomarkers have become valuable tools for diagnosis and risk stratification (54). Most commonly used cardiovascular biomarkers are natriuretic peptides and cardiac troponins, which are today used widely in diagnosis of heart failure and acute myocardial infarction respectively (55). Inflammatory biomarkers can be detected in several chronic inflammatory diseases including cancer and immunological conditions (56).

Growth Hormone

Growth hormone (GH). is a peptide hormone secreted from the anterior hypophysis and plays an important role in growth stimulation and development. GH secretion is regulated by two hypothalamic hormones, growth hormone releasing hormone (GHRH) excreting a stimulatory effect and somatostatin, excreting an inhibitory effect (57). GH secretion is controlled by many factors like age, gonadal steroids, body composition and time of the day (58) but also hormones like oestrogen, thyroid hormone and insulin-like growth factor 1 (IGF-1) (59). The hormone is released in a pulsatile manner with significant circadian rhythm and peak discharge occurring at night-time (60).

Previous studies have shown that individuals with either excess or deficiency of growth hormone levels have an increased risk for cardiovascular morbidity and mortality (61). This may be related to increased prevalence of cardiovascular risk factors such as hypertension, diabetes mellitus, insulin resistance and dyslipidemia which all, together or separately, increases risk for vascular atherosclerosis (62).

Growth hormones exert two major effects on the cardiovascular system, peripheral vasodilation, and increased myocyte growth where many of the effects are mediated by IGF-1. IGF-1 stimulates endothelial nitric oxide (NO) synthesis inducing endothelium-dependent vasodilation (63). NO produced by endothelial NO synthase

is an important factor in blood flow regulation. Genetic polymorphism of the NO synthase affects enzyme activity and have been associated with several cardiovascular diseases, among those POTS (64). In patients with low levels of GH, endothelial dysfunction, decreased endothelium-dependent vasodilation and depletion of endothelial nitric oxide production have been observed (65). Moreover, in POTS previous findings suggest increased flow-mediated vasodilatation (66) and reduced nitric oxide release (67) suggesting involvement of endothelial dysfunction in pathogenesis of POTS.

Multiplex protein analysis

Both cardiovascular and inflammatory biomarkers may play a crucial role in patients with POTS, but further research is required. In this thesis, biomarkers, using the Proximity Extension Assay technique that analyse a patient´s protein profile from a small blood sample, have been assessed. A pair of oligonucleotide-labelled antibodies, Proseek probes, binds to the target protein in the plasma sample. When the two Proseek probes are in proximity, a new polymerase-chain reaction (PCR) target sequence is triggered by a proximity-dependent DNA polymerisation event. This complex is detected and quantified using a standard real-time PCR. In case of non-specific binding, they cannot hybridize ensuring the high specificity of this method **(Figure 3).**

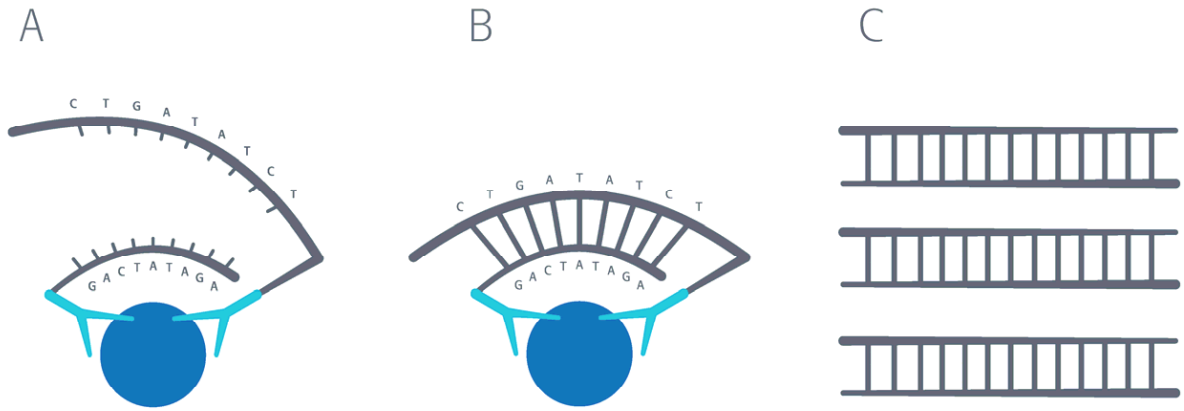

Figure 3. Proximity Extension Assay technique. Image courtesy of Olink Proteomics..

Aims

The overall aim in this PhD thesis project was to explore inflammatory involvement in postural orthostatic tachycardia syndrome through biomarkers and neuroendocrine changes. Moreover, to improve understanding of the clinical complications experienced by these patients using a newly developed grading scale, the Malmö POTS score.

Specific aims:

I. The clinical and neuroendocrine characteristics of syndromes of orthostatic intolerance and syncope in young adults were studied in order to increase knowledge of neuroendocrine changes during orthostatic stress.

II. Fifty-seven inflammatory biomarkers and their association with postural orthostatic tachycardia syndrome were investigated using an antibody-based Proximity Extension Assay Technique (Olink).

III. Ninety-two cardiovascular biomarkers with possible involvement in postural orthostatic tachycardia syndrome were also investigated using the same technique as in Project II.

IV. The clinical picture was examined, and the symptom burden was objectively measured in POTS patients by developing a self-grading questionnaire, Malmö POTS score (MAPS). Subsequently, possible correlation between symptom burden and haemodynamic changes were examined during orthostasis.

V. The renin-angiotensin-aldosterone system in POTS was investigated by measuring renin activity and aldosterone using the ELISA technique and the data from age-and sex matched healthy controls was used for comparison. Subsequently, renin activity during orthostatic stress in POTS and healthy controls were compared.

Materials and Methods

Cohorts and Study population

SYSTEMA cohort

The Syncope Study of Unselected Population in Malmö (SYSTEMA) is a prospective cohort initiated in 2008 at Skåne University Hospital, Malmö (68, 69). Patients from primary care and hospital outpatient clinics including other hospitals in southern Sweden with unexplained syncope or symptoms of orthostatic intolerance were referred to the Syncope Unit at Skåne University Hospital for autonomic testing. Prior to autonomic testing other assessments such as electrocardiogram (ECG), echocardiography, electroencephalogram (EEG) and blood sample were performed as seemed appropriate to the investigating physician. Cardiovascular autonomic testing is performed at the Syncope Laboratory in Malmö in the presence of a physician employing careful monitoring of haemodynamic parameters using beat-to-beat blood pressure and heart rate monitoring throughout the examination. Head-up tilt test (HUT) is used as a diagnostic tool for symptom reproduction and autonomic testing.

POTS Sub-Cohort

POTS sub-cohort is a subgroup of SYSTEMA including patients with POTS and healthy controls. Control participant are volunteers without symptoms of orthostatic intolerance and absence of cardiovascular disease. This cohort was formed in September 2017 and to date included 91 patients with POTS and 84 healthy controls.

Study Population

In project I, a study was conducted involving 236 patients, aged 18-40 years with orthostatic intolerance and/or syncope from the SYSTEMA cohort included between August 2008 and October 2013. Head-up- tilt test was performed in all participants as well as blood sampling supine and upright at 3 minutes HUT.

In project II and III, a case-control study was performed including 396 patients with symptoms of orthostatic intolerance, aged 15-50 years from the SYSTEMA cohort

with either POTS (*n*=113) or normal haemodynamic response during passive head-up-tilt test (*n*=283). The study was performed between September 2008 and May 2014. Blood samples were collected before autonomic testing for biomarker analysis in all participants.

In project IV, a case-control study was conducted in 62 POTS patients and 50 healthy individuals without symptoms of orthostatic intolerance. All participants were included at the clinical research unit in Malmö, Skåne University Hospital. POTS patients had positive POTS findings on HUT and a history of orthostatic intolerance for at least 6 months. Control subjects had no symptoms of orthostatic intolerance and a normal active standing test.

In project V, the relationship between renin-angiotensin-aldosterone system (RAAS) and haemodynamic parameters was investigated in 46 patients with POTS and 48 heathy controls. All participants underwent autonomic testing during their visit for blood sampling at the Clinical Research Unit in Malmö, Skåne University Hospital between September 2017, and June 2019.

In project I, II and III, all participants included were referred to Skåne University Hospital for autonomic testing due to symptoms of orthostatic intolerance, however, not all patients had positive (for syncope) head-up tilt test. Participants with a normal haemodynamic response during the tilt-test and no cardiovascular disease (ischaemic heart disease, heart failure, and stroke) or hypertension were included as healthy controls.

In project IV and V, healthy controls, including myself, had no symptoms of orthostatic intolerance and had a normal active standing test. These participants were volunteers from among hospital staff or medical students.

Project-specific Methods

Project I

In this study, 836 patients with unexplained syncope and/or orthostatic intolerance referred to Syncope Unit of Skåne University Hospital between August 2008 and October 2013 were examined. 671 of these patients underwent HUT according to the Italian protocol (70) and had blood sampling during the test. Blood samples were collected through peripheral vein cannulation after 10 minutes supine rest and after 3 minutes upright. Their ages spanned 18-40 years prompted by previous epidemiological studies on POTS incidence. Thus, 236 patients were included in the study **(Figure 4).** Plasma levels of epinephrine, norepinephrine, renin, C-terminal-pro-arginine-vasopressin (CT-proAVP), C-terminal-endothelin-1 and mid-regional-fragment of pro-atrial-natriuretic-peptide (MR-proANP) were analysed and

compared between four groups: POTS, OH, VVS and normal haemodynamic response during HUT.

POTS was defined for these adults as heart rate increase ≥ 30 bpm or tachycardia > 120 bpm when upright accompanied by symptoms of orthostatic intolerance (dizziness, palpitations, nausea).

Orthostatic hypotension (OH) was defined as decrease in systolic blood pressure ≥20 mmHg and/or decrease in diastolic blood pressure ≥ 10 mm Hg during passive HUT (12, 13).

Vasovagal syncope (VVS) was defined as pronounced hypotension, bradycardia or asystole accompanied by reproduction of symptoms (14).

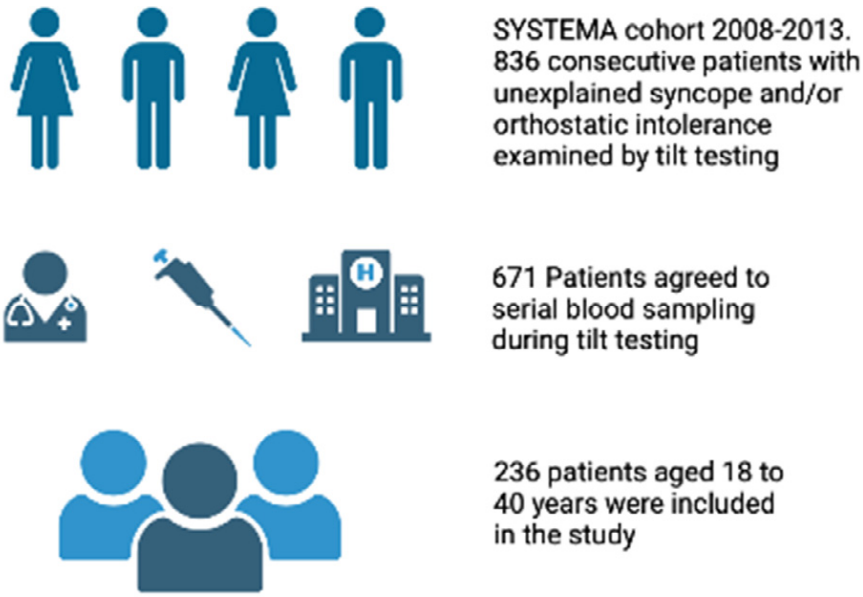

Figure 4. Inclusion of study population in Project I. Illustration by Biorender.

Project II and III

In project II and III, case-control studies were undertaken from the same patient group but analysed for different types of biomarkers and their possible association with POTS. 994 patients from the (SYSTEMA) cohort were assessed between 2008 and 2014. All patients were referred to the Syncope unit at Skåne University Hospital from primary and hospital outpatient clinics due to syncope and/or symptoms of orthostatic intolerance for autonomic testing. A total of 396 patients, aged 15-50 years with confirmed POTS or negative HUT and available proteomic data were included in the study **(Figure 5).** All patients performed HUT and had

blood sampling after 10 minutes supine rest during their Syncope unit visit. Fifty-seven inflammatory and, respectively, ninety-two cardiovascular biomarkers in the two projects were analysed using multiplex protein analysis.

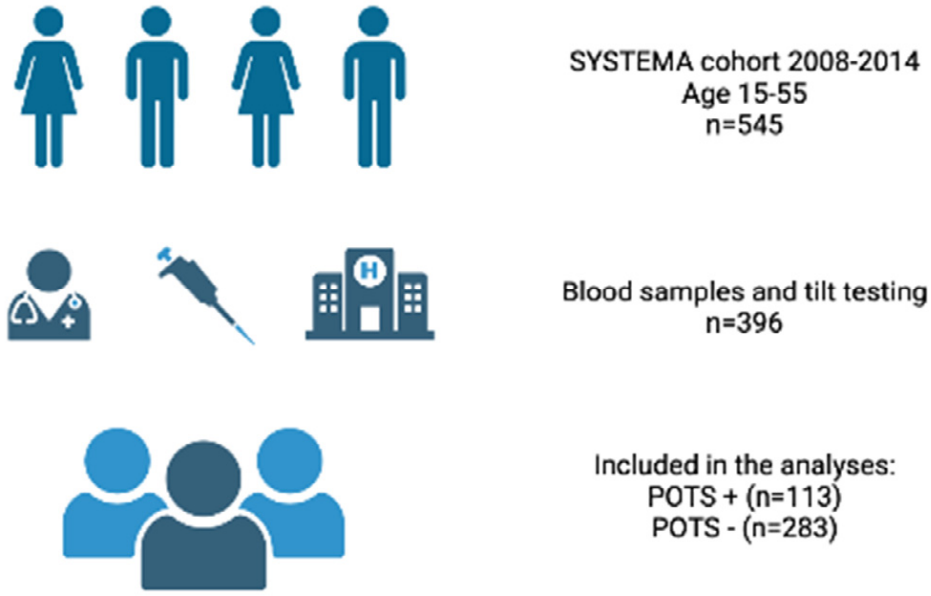

Figure 5. Inclusion of study population in project II and III. Illustration by Biorender.

Project IV

In project IV, a case-control study was conducted including 62 patients with POTS and 50 healthy controls from our SYSTEMA cohort subgroup, (Clinical Research Unit cohort). Symptom burden, over the previous 7 days, was compared between the groups using a newly developed self-grading scale, the Malmö POTS Score. POTS patients performed autonomic testing with head-up tilt test whereas healthy controls performed an active standing test at the Clinical Research Unit (Department of Internal Medicine) Skåne University Hospital, Malmö. Sweden **(Figure 6).** Healthy controls had no symptoms of orthostatic intolerance and were closely age-and-sex matched to the POTS group. Before the active standing test, all participants had to rest supine in a quiet room for 10 minutes before baseline blood pressure and heart rate was measured using an autonomic BP monitor (Omron M6, Kyoto, Japan). Later, blood pressure measurements were made during the active standing test at 1,3,5 and 10 minutes. Two measurements were averaged per time interval for group comparisons.

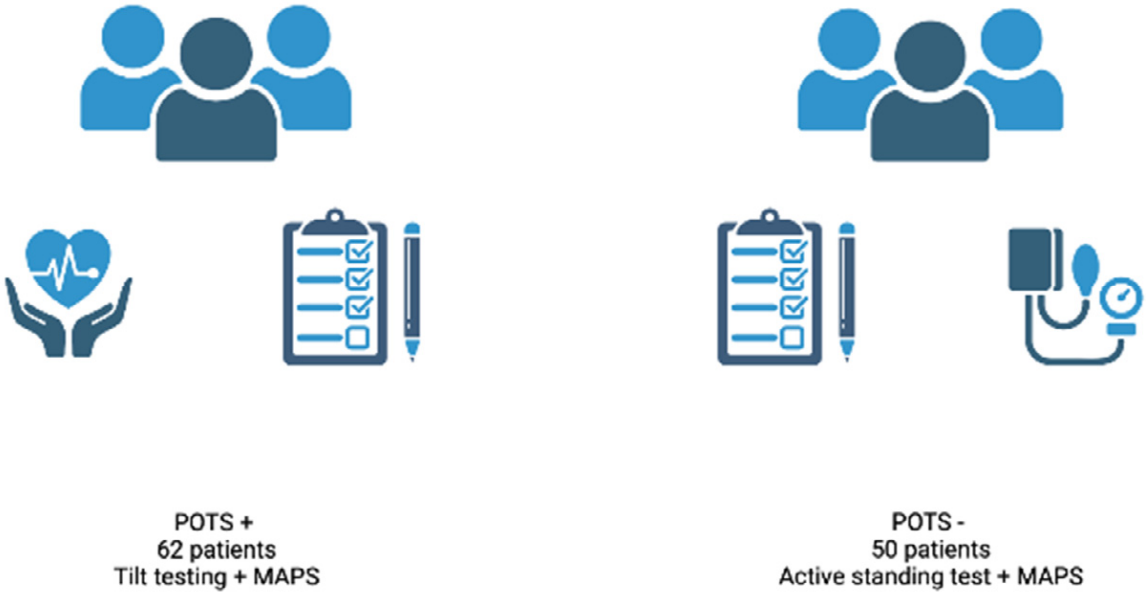

Figure 6. Inclusion of study population in project IV: Illustration by Biorender.

Project V

A case-control study including 46 POTS patients and 48 healthy individuals with no symptoms of orthostatic intolerance or cardiovascular disease from the Clinical Research Unit cohort was conducted. All patients were examined at the Clinical Research Unit in Malmö, Skåne University Hospital between September 2017, and June 2019. Controls had blood sampling from venous cannulae and performed active standing test for autonomic assessment (**Figure 7).** All participants were asked to fast from 10pm the night before and, for patients, discontinuation of cardiovascular and salt retaining medications 48 hours before the visit was also requested. However, low salt diets were not imposed. Later, blood samples were collected into EDTA vacutubes and centrifuged within 4 hours. Centrifuged samples were transferred into new tubes and refrigerated. Frozen samples were transported to the laboratory and enzyme-linked immunosorbent assay (ELISA) of human intact angiotensinogen and total angiotensinogen were performed. Angiotensin I, interpreted as plasma renin activity, was calculated as total angiotensinogen – intact angiotensinogen **(Figure 10).**

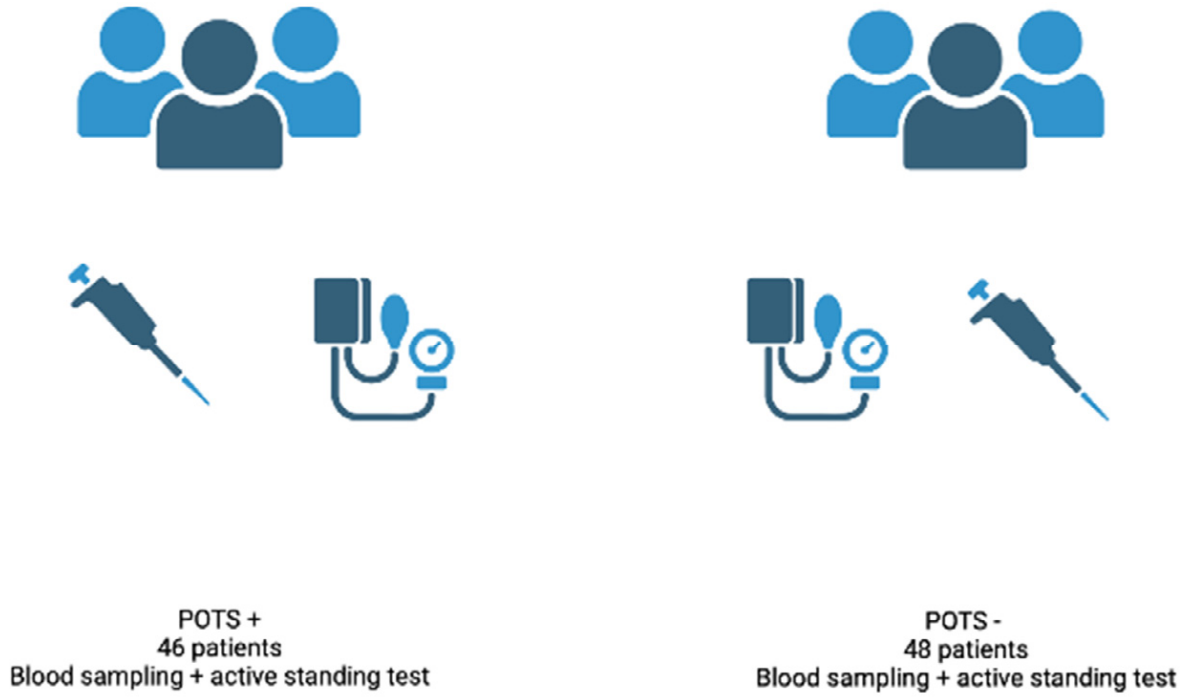

Figure 7. Inclusion of study population in project IV: Illustration by Biorender.

Head-up tilt test (HUT)

Head-up tilt test (HUT) is commonly used for diagnosis and evaluation of syndromes of orthostatic intolerance, dysautonomia and transient loss of consciousness (TLOC) (71). This test consists of 10–15-minute rest supine followed by elevation of the tilt table to 60-70° head up while simultaneously monitoring blood pressure and heart rate responses. If the patients do not develop any symptoms during passive HUT, drug provocation with sublingual nitroglycerine administration (400-500mcg) is indicated when stable haemodynamic parameters are maintained during the passive phase of the tilt-test (72).

Statistical analysis

Project I

Differences between means of neuroendocrine biomarkers and haemodynamic parameters were compared and study population characteristics between patients with negative HUT and patients within the groups of orthostatic intolerance (OH, VVS and POTS) were also compared. If appropriate, further exploration was made between different patient categories within the orthostatic intolerance group. This was accomplished using one-way analysis of variance (ANOVA) with Tukey´s post

hoc test. If Levene´s test indicated a violated assumption of homogeneity of variances ($p<0.05$), a Welch test with Games Howell post hoc was run instead to compare all possible combinations of group differences. Pearson´s $\chi 2$ test was used for dichotomous variables. A special vasovagal syncope score "VVS score" including male sex, resting HR < 70 bpm, and resting MR-proANP levels > 45 pm/L was created for comparison with other examined groups (OH, POTS and neg HUT) in a logistic regression model using age as a covariate.

The main characteristics of the study population and haemodynamic parameters were displayed as mean and standard deviation (SD). Neuroendocrine biomarkers at rest and at 3 minutes HUT were displayed as median and interquartile range (IQR) in pm/L. OR and 95% CI was used for reporting VVS score in relation to male sex, resting heart rate and resting MR-proANP levels.

All tests were two-sided and a *p-value* <0.05 was considered to be statistically significant. All analyses were performed using IBM SPSS Statistics V.23 (SPSS, Chicago, Illinois, USA).

Project II and III

In project II and III, the same method and statistical analysis for biomarker calculation in patients with POTS and controls, as defined above, was used. The study population and haemodynamic results were reported as mean and standard deviation for continuous variables and as percentages for categorical variables. Box plots were used to display the distribution of biomarker levels between the groups. Univariate and multivariate ordinary least square linear and logistic regression models were applied for bivariate correlation between plasma levels of selected biomarkers and maximum orthostatic heart rate change or POTS status respectively. Age, BMI, and sex adjustments were made in multivariate regression in Project III while only age and sex in Project II.

Missing data was imputed with multiple imputations by chained equations (MICE) approach to create 10 complete datasets. Predictive mean matching was used for continuous variables, logistic regression for binary variables and polytomous regression for categorical variables.

Identification of relevant biomarkers associated with POTS was explored using a two-step process.

I. Biomarker identification by supervised multivariate, principal component analysis (PCA). Markers with much missing data i.e., > 35% were excluded.

II. Verification of the selected biomarkers by univariate ANOVA with Bonferroni correction. A Bonferroni-adjusted significance level of $p<0.05$/number of PCA-selected biomarkers was used for confirmation.

Since the principal component analysis (PCA) requires pairwise complete data, markers with missingness i.e., above 35% were excluded. In project II, this resulted in removal of 9 biomarkers: erythropoietin, interlukin-2, interferon-gamma, tumour necrosis factor, carcinoembryonic antigen, vascular endothelial statin, lipopolysaccharide-induced tumour necrosis factor (TNF)-alpha factor, myeloid differentiation primary response protein, MHC class I polypeptide-related sequence A. Univariate logistic regression was performed for each of the 48 biomarkers. The regression coefficients were then standardized by dividing the coefficient with its standard error. All possible thresholds (Standardized coefficient (θ) ranging from minimum to maximum with 0.05 increments) were used to select groups of biomarkers and construct principal components (PCs). The outcome variable (POTS status) was then regressed onto the 1st two PCs from each group of biomarkers using the binominal link function. This step identified the group of biomarkers which gave the best accuracy (POTS+ versus controls) was selected by ten-fold cross-validation.

Further on, in project III, the threshold providing the best classification accuracy (POTS versus controls) was selected by ten-fold cross-validation identifying 23 biomarkers: TIM, PSGL1, MB, VEGFD, PIGF, MMP1, GDF15, FAS, TF, AM, UPAR, TNFR2, TRAIL, MCP1, TRAILR2, OPG, CASP 8, HGF, CD40L, GH and PTX3. Nine PCA-selected proteins differed significantly at the stage of biomarker verification analysis however only GH and myoglobin attained significance after Bonferroni correction.

Statistical analyses were carried out using IBM SPSS Statistics version 25 (SPSS Inc., Chicago, Illinois, United States) and R Statistical Software (version 3.4.4; R Foundation for Statistical Computing, Vienna, Austria).

Project IV

In project IV, a linear regression analysis was performed with individual symptom scores (n=12) and total score as dependent variables. Age, sex, duration of symptoms, and haemodynamic parameters obtained during active standing tests were used as independent variables in univariable and multivariable-adjusted models. The multivariable model was adjusted by entering age and sex as covariates. Study population characteristics were reported as mean and standard deviation or median and interquartile range for continuous variables. Categorical variables were described as counts and percentages. Between-group comparison was performed using independent samples t-test and Mann Whitney U test, as appropriate.

The optimal cut-off-point value of MAPS to discriminate between POTS and healthy individuals was calculated by the Youden method, a summary measure of the Receiver Operating Characteristic (ROC) curve constructed to test the ability of MAPS to predict POTS. P-value <0.05 was considered statistically significant.

Data were analysed using SPSS software version 27 (SPSS, Chicago, Illinois, USA), and easyROC: An Interactive Web-tool for ROC Curve Analysis Using R Language Environment, and OptimalCutpoints R package available from the Comprehensive R Archive Network (CRAN).

Project V

In project V, group characteristics were reported as mean and standard deviation or median and interquartile range, as appropriate for continuous variables. Categorical values were described as counts and percentages. Independent samples t-test were used for group comparisons in normally distributed variables whereas non-parametric tests (Mann-Whitney) were used for non-normally distributed continuous variables. Spearman's rank correlation coefficients were used to examine relationships among continuous variables. Angiotensin I level, interpreted as plasma renin activity, was calculated as total angiotensinogen – intact angiotensinogen. P-value <0.05 was considered statistically significant

IBM SPSS Statistics for Macintosh, Version 27.0 (IBM Corp, Armonk, NY, USA) was used for statistical analyses.

Results

Project I

In project I, 836 patients with unexplained syncope or symptoms of orthostatic intolerance were examined by HUT. Later, 671 had blood sampling supine and upright, of these 236 patients, aged 18-40 years were included in the study **(Figure 4)**.

Depending on the haemodynamic changes during HUT, patients were divided into subgroups of orthostatic intolerance, VVS (n=103), OH (n=22) and POTS (n=72). Those patients with normal haemodynamic response to tilt - negative HUT (n=39) were considered as a reference group.

The results showed female predominance in all categories. Patients with POTS were significantly younger (27±6 vs 30±6, *p=0.030)* when compared with those with negative HUT. The proportion of male subjects was highest in VVS (41,7%). The median duration of syncope-related symptoms was 3 years, without difference between groups. Surprisingly, patients with POTS reported less palpitation compared with those with negative HUT **(Table 3).**

Patients with VVS had significantly lower resting diastolic blood pressure *(*69±7 vs 73±8, *p=0.034)* and HR *(*66±11 vs 74±11, *p=0.001)* when compared with negative HUT. Those with OH had lower systolic and diastolic blood pressure (*p<0.001*) whereas POTS patients had significantly higher HR (*p<0.001*) at 3 minutes of HUT when compared with normal haemodynamic response patients (negative HUT) **(Table 4).**

Neuroendocrine analysis showed significantly higher rise in norepinephrine (NE) at 3 minutes of HUT in patients with POTS. CT-proAVP was also increased in POTS when compared with negative HUT (*p=0.033*) but not with OH (*p=0.693*) or VVS (*p=0.768*). Resting MR-proANP in POTS was decreased in relation to VVS (*p=0.039*) and OH (*p=0.030*) but not with negative HUT (*p=0.96*) **(Table 5).**

Some predisposing factors in the study population seem to increase OR for VVS which prompted the VVS score including male sex, resting HR <70 and resting MRproANP levels > 45 pm/L.

Table 3. Baseline characteristics.

	All (n=236)	**No Dx** (n=39)	**VVS** (n=103)	**P***	**POTS** (n= 72)	**P***	**OH** (n=22)	**P***	**P****
Age, years	28.1 (6.7)	30.2 (6.3)	28.2 (6.8)	0.335	26.6 (6.4)	0.030	28.2 (6.7)	0.632	0.053
Sex, % male	32.6	38.5	41.7	-	20.8	-	18.2	-	0.011
BMI, kg/m^2	23.7 (3.9)	24.7 (4.9)	24.0 (3.5)	0.785	22.8 (3.4)	0.074	23.9 (4.8)	0.852	0.076
Symptoms reported by the patients									
Duration of symptoms, years, (median, [IQR])	3 (9)	4 (8)	5 (10)	0.698	3 (9)	0.998	2.5 (5)	0.813	0.247
Total no of syncope (median, [IQR])	5 (18)	5 (22)	5 (7)	0.734	5 (28)	1.000	9 (18)	0.979	0.266
Prodrome (nausea, perspiration etc.), %	72.4	65.5	77.6	-	65.0	-	88.9	-	0.128
Palpitations, %	39.1	51.7	32.8	-	31.7	-	66.7	-	0.017
Traumatic fall, %	55.1	53.8	52.0	-	60.6	-	54.5	-	0.731
Dizziness on standing, %	73.2	82.1	61.2	-	81.7	-	86.4	-	0.003

No Dx = no diagnosis; VVS = vasovagal syncope; POTS = postural tachycardia syndrome; OH = orthostatic hypotension. Values displayed as mean (SD) if not otherwise stated; IQR = interquartile range.

* P-value for Tukey's or Games-Howell post-hoc test in relation to No dx (reference group) for continuous variables.

** ANOVA or Welch test P-value for continuous variables and Pearson chi^2 P-value for dichotomous variables.

Table 4. Haemodynamic parameters at rest and during HUT

	All (n=236)	**No Dx ref** (n=39)	**VVS** (n=103)	***P****	**POTS** (n=72)	***P****	**OH** (n=22)	***P****	***P *****
SBP rest	121.6 (13.0)	122.9 (13.3)	120.0 (12.2)	0.660	123.0 (12.4)	1.00	121.7 (17.6)	0.987	0.437
DBP rest	70.7 (7.6)	73.2 (7.8)	69.3 (6.8)	0.034	71.5 (7.6)	0.671	70.0 (9.9)	0.378	0.035
HR rest	69.9 (11.8)	74.2 (10.9)	66.0 (11.3)	0.001	72.5 (11.0)	0.880	71.6 (13.1)	0.818	<0.001
SBP 3'	122.1 (16.0)	128.7 (14.9)	121.5 (13.8)	0.068	122.6 (15.4)	0.192	111.0 (22.7)	<0.001	<0.001
DBP 3'	77.3 (10.8)	81.1 (11.5)	76.6 (9.2)	0.096	78.7 (10.0)	0.648	68.6 (14.1)	<0.001	<0.001
HR 3'	86.7 (15.5)	82.6 (11.6)	79.6 (11.9)	0.600	99.9 (13.6)	<0.001	83.1 (15.2)	0.999	<0.001
SBP min	108.7 (15.3)	116.1 (11.5)	110.1 (12.3)	0.111	107.7 (16.8)	0.015	91.8 (16.4)	<0.001	<0.001
DBP min	70.8 (10.8)	75.1 (9.6)	71.0 (9.2)	0.125	72.0 (10.8)	0.418	58.0 (10.8)	<0.001	<0.001
HR max	93.3 (18.2)	85.8 (14.9)	84.1 (12.8)	0.920	111.0 (14.6)	<0.001	91.5 (13.6)	0.404	<0.001

Displayed as mean (SD). HUT = head-up TILT

No Dx = No diagnosis; VVS = vasovagal syncope; POTS = postural tachycardia syndrome; OH = orthostatic hypotension. SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate

* P-value for Tukey's or Games-Howell post-hoc test in relation to No dx (reference group) for continuous variables.

** ANOVA or Welch test P-value for continuous variables and Pearson chi^2 P-value for dichotomous variables.

Table 5. Neuroendocrine biomarkers at rest (0') and at 3 minutes head-up-TILT (3')

	All (n=163-187)	No Dx ref (n=24-30)	VVS (n= 79-87)	*P**	POTS (n=44-55)	*P**	OH (n=16-17)	*P**	*P **
P-renin 0'	15.0 [13]	17.0 [12]	15.5 [12]	0.573	14.0 [14]	0.550	10.5 [38]	0.030	0.054
P-renin 3'	15.5 [13]	16.5 [15]	16.0 [14]	0.816	15.0 [13]	0.658	10.0 [9]	0.074	0.106
Δrenin	0.0 [2.0]	0.0 [2.5]	0.0 [3.0]	0.369	0.0 [2.0]	0.993	0.0 [1.5]	0.995	0.101
P-epinephrine 0'	0.10 [0.1]	0.09 [0.1]	0.10 [0.1]	0.999	0.085 [0.1]	0.998	0.10 [0.2]	0.976	0.937
P-epinephrine 3'	0.19 [0.2]	0.17 [0.2]	0.21 [0.2]	0.846	0.18 [0.2]	0.825	0.14 [0.2]	0.932	0.457
Δepinephrine	0.07 [0.13]	0.06 [0.09]	0.09 [0.2]	0.690	0.07 [0.1]	0.628	0.04 [0.11]	1.00	0.452
P-NE 0'	1.40 [0.9]	1.70 [1.0]	1.30 [0.8]	0.071	1.48 [0.9]	0.137	1.30 [0.6]	0.524	0.097
P-NE 3'	2.40 [1.4]	2.45 [1.4]	2.30 [0.9]	0.513	2.90 [1.5]	0.744	2.00 [1.1]	0.744	0.018
ΔNE	1.00 [0.7]	0.85 [0.5]	0.90 [0.5]	0.733	1.40 [1.2]	0.008	0.90 [0.9]	0.987	0.013
MR-proANP 0'	45.0 [23.9]	37.9 [30.2]	48.0 [25.4]	0.349	40.1 [23.0]	0.955	52.7 [25.8]	0.145	0.009
MR-proANP 3'	45.7 [24.7]	40.4 [33.1]	50.6 [24.5]	0.122	41.0 [18.5]	0.999	53.7 [38.0]	0.168	0.015
ΔMR-proANP	2.25 [4.2]	1.18 [4.4]	2.22 [2.8]	0.992	2.55 [5.0]	1.00	3.63 [7.3]	0.999	0.958
CT-proET1 0'	43.3 [13.5]	47.4 [23.2]	41.9 [11.8]	0.998	43.8 [14.0]	1.00	43.3 [8.7]	0.991	0.891
CT-proET1 3'	42.5 [14.2]	39.4 [22.1]	42.3 [11.2]	0.996	41.7 [15.5]	1.00	43.3 [12.8]	0.791	0.769
ΔCT-proET1	0.40 [4.6]	0.80 [6.2]	0.50 [4.3]	0.796	0.40 [4.7]	0.932	-0.18 [3.6]	0.712	0.723
CT-proAVP 0'	6.14 [5.9]	6.68 [4.8]	5.65 [5.0]	0.834	6.75 [6.9]	0.933	5.04 [5.6]	0.859	0.824
CT-proAVP 3'	6.77 [6.7]	6.16 [5.5]	6.13 [6.8]	0.576	7.24 [8.2]	0.641	6.79 [7.1]	0.62	0.615
ΔCT-proAVP	0.16 [2.3]	-0.29 [1.5]	0.34 [2.5]	0.098	0.18 [3.9]	0.033	0.07 [1.3]	0.533	0.052

Displayed as median (IQR) in pm / L.
* P-value for Tukey's or Games-Howell post-hoc test in relation to No dx (reference group) for continuous variables.
** ANOVA or Welch test P-value for continuous variables and Pearson chi2 P-value for dichotomous variables.
No Dx = No diagnosis; VVS = vasovagal syncope; POTS = postural tachycardia syndrome; OH = orthostatic hypotenson. Number of patients displayed as range of available samples in the diagnosis groups.

Project II

We included 396 patients with either POTS (n=113) or negative HUT (n=283) **(Figure 5).** Patients with POTS were younger (age, 26±8 vs 31±10, $p<0.001$) and had a lower BMI (22.7±3.5 vs 24.3±4.1, $p<0.001$). There was no reported difference in resting blood pressure and HR between the groups. Patients with POTS had a significantly lower systolic blood pressure (108±16 vs 112±13, $p=0.003$) and higher HR (112±16 vs 85±14, $p<0.001$) during HUT when compared to patients with intact haemodynamic response **(Table 6).**

Table 6. Baseline characteristics and haemodynamic parameters.

Characteristic	POTS- (n=283)	POTS+ (n=113)	P-value
Age	31.47 (9.85)	26.27 (8.41)	<0.001
Female sex, n (%)	189 (66.8)	83 (73.5)	0.241
BMI, Kg/m^2	24.33 (4.14)	22.69 (3.50)	<0.001
SBP supine, mmHg	120.07 (14.16)	120.41 (14.20)	0.833
DBP supine, mmHg	69.98 (8.21)	70.22 (8.22)	0.788
HR supine, bpm	68.86 (11.87)	71.13 (11.57)	0.084
SBP HUT min, mmHg	112.34 (13.35)	107.58 (16.24)	0.003
DBP HUT min, mmHg	71.83 (9.11)	72.46 (10.58)	0.552
HR HUT max, bpm	84.77 (13.77)	112.41 (15.63)	<0.001
Smoking, n (%)	58 (20.5)	16 (14.2)	0.188

The following four biomarkers reached the determined thresholds following PCA described in method section: carbonic anhydrase IX; receptor tyrosine-protein kinase erbB-2; Fms-related tyrosine kinase 3 ligand; and proconvertase Furin.

All PCA-selected biomarkers except carbonic anhydrase IX differed significantly in pairwise comparison, but only proconvertase furin attained significance after Bonferroni correction **(Table 7).**

Table 7 High throughput multiplex analysis biomarkers selected by supervised PCA.

Biomarker	POTS+ (n=113)	POTS- (n=283)	P-value
FUR	6.38 (0.05)	6.58 (0.03)	0.000276*
CAIX	1.53 (0.09)	1.73 (0.06)	0.050109
Flt3L	7.76 (0.05)	7.89 (0.03)	0.012979
ErbB2HER2	7.79 (0.04)	7.92 (0.03)	0.026045

Plasma concentrations of the assessed proteins are expressed on a log2-scale. Inter group differences were assessed using analysis of variance method. *Bonferroni-corrected significant values (p<0.012)

In multivariate regression analysis, both a POTS diagnosis and maximum orthostatic ΔHR were significantly associated with proconvertase furin **(Table 8 and 9 and Figure 8).**

Table 8. Relationship between POTS status and selected biomarker in univariate and multivariate regression

Biomarker	Univariate			Multivariate*		
	OR	95% CI	P-value	*OR*	95% CI	P-value
FUR	0.81	0.73 - 0.92	<0.001	0.86	0.77 - 0.96	0.009

Table 9. Relationship between changes in heart rate during head-up tilt test and selected biomarker in univariate and multivariate regression.

Biomarker	Univariate			Multivariate*		
	β	95% CI	P-value	β	95% CI	P-value
FUR	-7.0	-10.6 - -3.38	<0.001	-4.2	-7.9 - -0.5	0.03

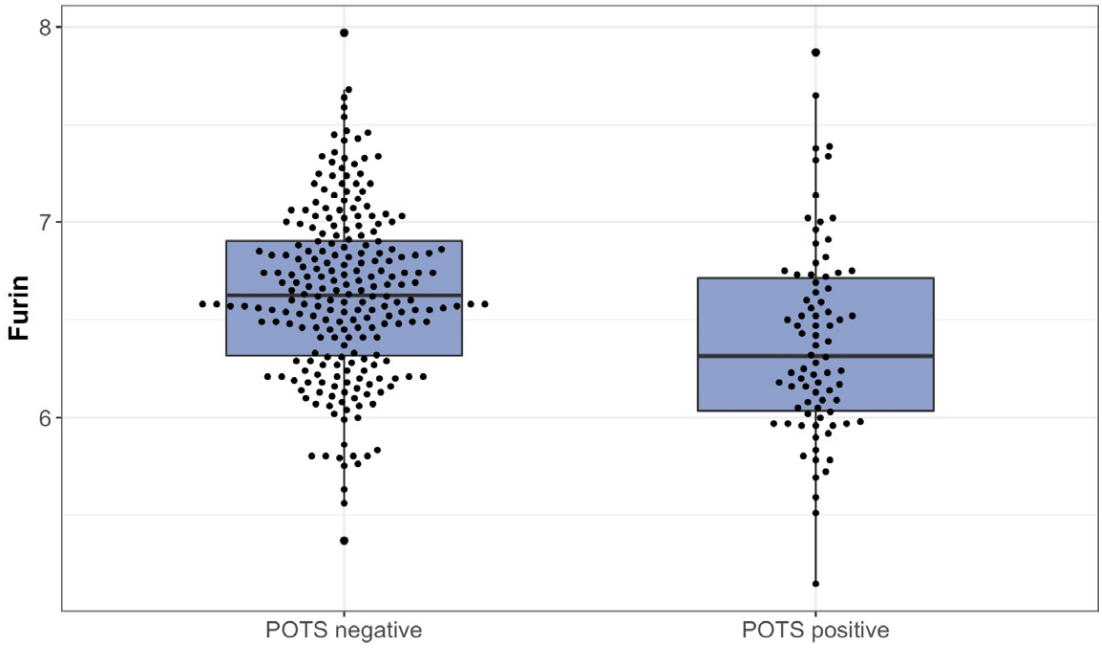

Figure 8. The plasma levels of proconvertase Furin, expressed on a log2 scale, presented in relation to POTS status. Data are shown as a box and whisker plot with median in the box and the whiskers representing the 5th and 95th percentiles in relation to plasmatic biomarker levels.
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Furin

Furin is a proprotein convertase regulating the maturation of a wide range of proproteins involved in various physiological and pathophysiological processes (73). It has been reported to process a variety of secreted factors including cytokines and chemokines such as anti-inflammatory transforming growth factor (TGF)-ß1 and secreted TNF-family receptors (74). According to previous studies, proconvertase furin inhibition may result in a breakdown of peripheral tolerance (75) and, also, development of systemic autoimmune disease (75). This stands in line with prior findings reporting high proconvertase furin levels and lower systemic activity disease in primary Sjögren´s syndrome (76).

Project III

In project III, study population characteristics showed that patients with POTS were younger (age, 26±8 vs 32±10, $p<0.001$) and had lower BMI (22.7±3.5 vs 24.3±4.1, $p<0.001$). There was no reported difference in resting blood pressure and HR between the groups. Patients with POTS had a significantly lower systolic blood pressure (108±16 vs 112±13, $p=0.003$) and higher HR (112±16 vs 85±14, $p<0.001$)

during HUT when compared with patients with intact haemodynamic response (negative HUT) **(Table 10).**

Table 10. Baseline characteristics and haemodynamic parameters..

Characteristics	POTS- (n=283)	POTS+ (n=113)	P-value
Age (years)	31.5 (9.8)	26.3 (8.4)	<0.001
Female sex, n (%)	189 (66.8)	83 (73.5)	0.241
BMI, Kg/m^2	24.3 (4.1)	22.7 (3.5)	<0.001
SBP baseline, mmHg	119.9 (14.2)	120.4 (14.2)	0.833
DBP baseline, mmHg	69.9 (8.2)	70.2 (8.2)	0.788
HR baseline, bpm	68.7 (11.9)	71.1 (11.6)	0.084
SBP HUT min, mmHg	112.2 (13.4)	107.6 (16.2)	0.003
DBP HUT min, mmHg	71.8 (9.1)	72.5 (10.6)	0.552
HR HUT max, bpm	84.7 (13.8)	112.4 (15.6)	<0.001
Smoking, n (%)	58 (20.5)	16 (14.2)	0.188

Plasma levels of GH were significantly higher in female patients with POTS when compared with males with POTS (p=0.0002) and both male (p<0.0001) and female controls (p=0.003). Plasma level of MB was, on the contrary, significantly lower in males with POTS when compared with male controls (p=0.0009) **(Figure 9).**

In multivariate regression analysis adjusted for age and BMI and stratified by sex both POTS status and maximum orthostatic ΔHR were significantly associated with lower MB level in males and higher GH level in females.

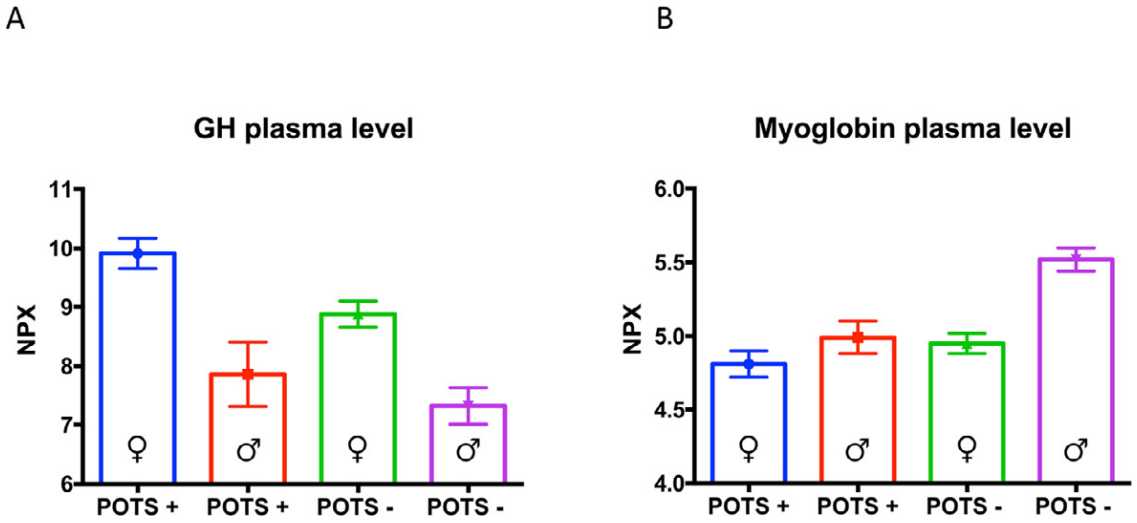

Figure 9 The plasma levels of growth hormone (GH) (panel A) and myoglobin (panel B), expressed on Normalised Protein Expression (NPX) on a log2 scale, are presented in relation to POTS and sex status. Data are shown as a box and whisker plot with median in the box and the whiskers representing the 5th and 95th percentiles in relation to plasmatic biomarker level. Reprinted from Paper III under Open Access CC by 4.0

Project IV

Patients with POTS reported a significantly higher symptom burden compared with controls (mean total MAPS score, 78±20 vs. 14 ±12, *p<0.001*). The three most prominent symptoms reported by patients with POTS were palpitations (7.7±1.7), fatigue (7.6±2.5) and concentration difficulties 7.2±2.1). All twelve symptom categories were noticeably elevated in POTS with an average score of at least 5 per category. In contrast, healthy controls reported headache (1.9±2), concentration difficulties (1.8±1.7) and insomnia (1.8±2.6) as most prominent **(Table 11).** The optimal cut-off value to discriminate between POTS and healthy controls was a total MAPS score of ≥42, yielding exceptional (97%;95%CI 0.89-0.99) and specificity (98%; 95%CI 0.89-0.99).

Table 11. Most prominent symptoms reported on MAPS in POTS and control group.

POTS	Controls
Palpitations	Headache
Fatigue	Concentration difficulties
Concentration difficulties	Insomnia

Patients with POTS were younger (age 28±9 years vs. age 32±10 years, *p=0.016)* and showed a higher baseline systolic blood pressure (125±12 vs. 114±10), diastolic blood pressure (76±8 vs. 69±8) and heart rate (82±15 vs. 64±11) *(p<0.001)* when compared with controls. There was a female predominance in both POTS (88,7%) and controls (82%) **(Table 12).**

Table 12. Study population and baseline characteristics..

Baseline Characteristics*	POTS (n = 62)	Controls (n = 50)	P-value
Age (years)	28 (9)	32 (10)	0.016
Female sex, n (%)	55 (89)	41 (82)	0.067
SBP supine, mmHg	125 (12)	114 (10)	<0.001
DBP supine, mmHg	76 (8)	69 (8)	<0.001
HR supine, bpm	82 (15)	64 (11)	<0.001
SBP standing 3 min, mmHg	127 (14)	113 (10)	<0.001
DBP standing 3 min, mmHg	89 (11)	75 (8)	<0.001
HR standing 3 min, bpm	115 (19)	81 (14)	<0.001

Values are reported as mean (standard deviation) for continuous variables and as count (percentage) for categorical variables. P-values for differences between the groups are shown.

* HR and BP values here presented and compared were collected from head-up tilt test for POTS patients and from active standing for controls.

DBP, diastolic blood pressure; HR, heart rate; POTS, postural orthostatic tachycardia; SBP, systolic blood pressure.

Project V

Patients with POTS had a higher baseline heart rate (69±11 bpm vs. 63±11 bpm, *p=0.006)* and diastolic blood pressure (73±9 vs 69±9 mmHg, *p=0.008*). During the active standing test, both heart rate and diastolic blood pressure were higher in POTS at 1, 3 and 5 minutes **(Table 13).**

Renin activity was decreased in patients with POTS (median, 3406 ng/ml; interquartile range (IQR), 2275-4767) when compared with healthy controls (median, 9949 ng/ml; IQR 3937-17175; *p<0.001*) whereas aldosterone concentration did not differ between the groups (median, 218pmol/L; IQR,397) vs. 218 pmol/L; IQR 135-407; *p=0.26).*

Table 13. Baseline characteristics and study population.

	POTS + (n=46)	POTS - (n=48)	p-value
Age, years	27±9	30±9	0.053
Female sex n, (%)	39 (85)	36 (75)	0.238
SBP, baseline	117±13	115±11	0.221
DBP, baseline	73±9	69±9	0.008
HR, baseline	69±11	63±11	0.006
SBP, standing 1 min	121±18	114±11	0.13
DBP, standing 1 min	84±13	75±8	<0.001
HR, standing 1 min	93±19	79±13	<0.001
SBP, standing 3 min	117±16	112±14	0.080
DBP, standing 3 min	81±13	72±10	0.001
HR, standing 3 min	96±18	83±12	<0.001
SBP, standing 5 min	117±18	112±13	0.08
DBP, standing 5 min	80±15	74±11	0.015
HR, standing 5 min	93±18	84±13	0.005

Renin activity demonstrated an inverse correlation with blood pressure both supine and upright in controls (*p<0.05)* but not in POTS, except for DBP after 5 minutes standing. There was a weak correlation between aldosterone and supine heart rate in POTS (*p=0.049*).

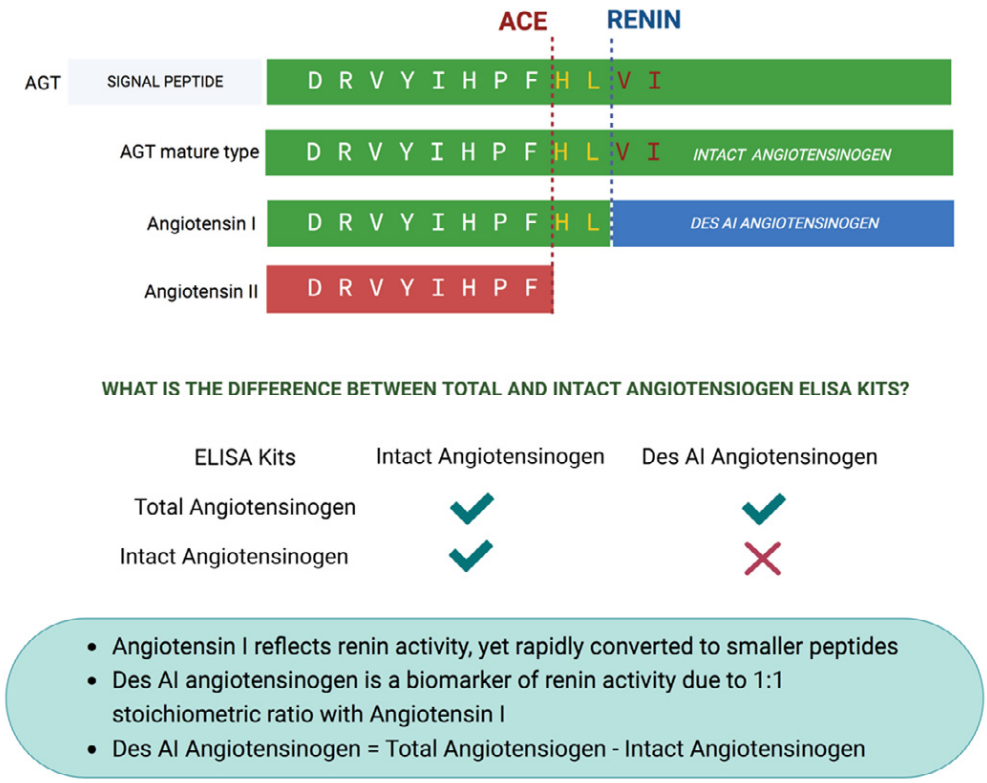

Figure 10. Assessment of renin activity. Reprinted from Paper IV.

Discussion

In this thesis, the role of inflammation and cardiovascular dysautonomia in postural orthostatic tachycardia syndrome was investigated by analyzing biomarkers, neuroendocrine hormones, and developing a questionnaire for symptom evaluation and correlation with haemodynamic parameters.

In Project I, an overall female predominance in both patients with orthostatic intolerance and negative head-up tilt test was reported. Patients with POTS were younger which correlates well with previous findings (21, 27) . Patients with orthostatic hypotension (OH) had a significantly lower SBP and DBP while patients with POTS had a significant HR at 3 minutes of HUT, as expected by definition. Blood pressure drop during orthostatic stress in patients with OH is often a consequence of multiple factors including hypovolemia and the autonomic disturbance of Parkinson´s. However, most often they are a side-effect of antihypertensive medications (12, 17). In POTS, increased heart rate may be a consequence of different mechanisms working separately or together. In this project, we found an increase in norepinephrine during HUT which corresponds to a previous hypothesis of catecholamine excess as potential aetiology in POTS. Increased norepinephrine levels stimulate beta-receptors in the heart causing increased heart rate (21, 24).

Atrial natriuretic peptide (ANP) is derived from the cleavage of its precursor pro-hormone , MR-proANP, which is more stable in the circulation than the mature peptide (77). It is believed that MR-proANP may be a more specific marker than ANP due to greater analytic stability, longer half-life and lack of receptor binding and interactions (78). It is released in an equimolar ratio to ANP (79). MR-proANP is responsible for various physiological and pathophysiological pathways including natriuresis and diuresis, an important role in the regulation of water balance and blood pressure in heart failure (80, 81). ANP is synthesised and released into the circulation by cardiac muscle cells in the atria as result of stretching in the atrial wall due to increased blood volume (78). In patients with POTS, resting MR-proANP was decreased when compared to VVS and OH but not to negative HUT. Possible explanation for this finding may be hypovolemia or decreased venous return to the heart due to sympathetic denervation in lower extremities. Patients with POTS are known to have small heart volumes.

In Project II, biomarker verification demonstrated lower circulating levels of proprotein convertase subtilisin/kexin type (PCSK)-3 i.e., proconvertase Furin in patients with POTS compared with individuals with negative HUT. Furthermore, the younger the patient, the lower proconvertase furin level. Furin is a proprotein convertase regulating the proteolytic maturation of other proproteins involved in various physiological and pathophysiological processes (82). It is also involved in activation of surface proteins in viruses including the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), implying increased susceptibility and higher severity of covid-19 infection in cancer patients (83-85). Our results indicate a downregulation of Furin in POTS suggesting involvement of the immune system, but the exact mechanism is unknown. Further research is mandatory before Furin may be used as a potential biomarker in POTS.

In project III, we found increased levels of growth hormone (GH) in female patients while myoglobin was decreased in male patients with POTS when compared with individuals with negative HUT. These results are in contrast to previous work showing decreased levels of GH in patients with POTS (86). A possible explanation for these opposing results of GH levels may be explained by different methods of analysis. Hypothetically, it is possible that Proximity Extension Technique bind to GH receptors with greater affinity and on several binding sites resulting in higher concentration. Generally, GH is related to increased prevalence of cardiovascular risk factors which emphasizes its importance (62).

Male patients with POTS had significantly lower levels of myoglobin when compared with healthy individuals. MB, an oxygen and iron binding protein characteristic of skeletal and cardiac muscle cells (87) usually increased when muscle tissue damage occurs i.e. myocardial infarction. MB is also used as a biomarker in patients with suspected myocardial infarction due to early release into plasma if myocardial micro-injuries occur. Results in this study are difficult to interpret because the method used for protein detection provides only relative values within the analysed sample. Hypothetically, lower myoglobin levels might be a result of immobilisation and limited physical activity in POTS, possibly a consequence of cardiovascular deconditioning or chronic fatigue (21).

In project IV the focus was on the heavy symptom burden patients with POTS experience prompting a new self-assessment score, Malmö POTS score (MAPS). Results obtained show a 5-fold higher symptom burden in POTS when compared with controls (healthy individuals without symptoms of orthostatic intolerance and negative standing test). Previous questionnaires used for symptom evaluation in POTS (OHQ and COMPASS-31) did not include the wide variety of symptoms experienced which is the strength of the MAPS for both diagnostic and follow-up purposes. Subsequently, by correlating scores obtained in MAPS with haemodynamic parameters, a strong positive correlation was found between total MAPS score and orthostatic heart rate at 3 minutes of HUT in POTS which may also prove to be valuable for patient assessment.

In project V, we observed decreased renin activity in patients with POTS when compared to control group. Potential explanation for these findings may be related to disruption in the feedback loop of the renin-angiotensin-aldosterone system or a consequence of inappropriate activation or blockade of angiotensin II, type I receptors. Aldosterone levels did not differ between POTS patients and controls with normal active standing test.

In summary, this work has shown clear pathological abnormalities in patients with a clinical diagnosis of POTS including deranged biomarkers and significant abnormalities in neuroendocrine system behaviour under test conditions. There must be links across body systems to account for the wide variety of symptoms presenting which may be best explained by affection of the autonomic nervous system. Thus, it is reasonable to consider POTS as a disease despite its unknown aetiology. The symptom burden in POTS is the pointer toward accurate diagnosis and assessment of the severity. Use of the MAPS and tilt-testing provide a better way to diagnose and assess progress in POTS.

Limitations

Although we have tried to design the papers to the best of our ability, some issues can be raised concerning the methods and designs.

- Overall, a larger cohort in project IV and V would be preferable for result confirmation. The present studies, especially project IV, are limited by being single-centre, observational in nature with potential selection and referral bias.
- Patients that were included as controls in projects I, II and III had a negative HUT but were referred for Syncope unit investigation due to symptoms of orthostatic intolerance and/or syncope. Thus, they cannot be considered true controls.
- Blood sampling did not occur throughout the whole autonomic testing examination but were performed only early in testing which could contribute to false interpretation of the results.
- In project V, patients were asked to stop cardiovascular medications and salt tablets 48 hours prior to examination, however they were not on a low sodium diet which may contribute to altered hormonal activity. Further, no check was made on compliance with the request to cease medication.

Conclusions

- Patients with POTS are predominantly younger females in childbearing years.
- We observed characteristic haemodynamic changes with increased heart rate above 110 bpm on average during head-up tilt test (HUT) in patients with POTS.
- Norepinephrine (NE), an endocrine biomarker and adrenergic mediator is more increased in patients with POTS during orthostatic stress.
- Proconvertase Furin, is decreased in POTS insinuating involvement of immunological system. Further research is mandatory before Furin may be used as a potential biomarker in POTS. Myoglobin (MB) was decreased in men with POTS which could be explained by deconditioning due to symptom burden.
- Growth Hormone (GH) analysed using Multiplex protein analysis is increased in patients with POTS which in turn could increase risk of future cardiovascular events. However, the long-term effects of increased GH in POTS patients should be further explored.
- Newly developed POTS symptom score, Malmö POTS score (MAPS) has been successively used as self-grading symptom assessment in patients with POTS. Total MAPS score was 5-fold higher in POTS when compared to healthy individuals indicating a heavy symptom burden in this patient category.
- Palpitations, fatigue, and concentration difficulties were most prominent symptoms reported by patients with POTS while headache, concentration difficulties and insomnia were reported in control group.
- Palpitations and concentration difficulties were associated with haemodynamic changes in POTS.
- Renin activity was decreased in patients with POTS when compared individuals having normal haemodynamic response during active standing test. Potential explanation for these findings may be related to disruption in the feedback loop of the renin-angiotensin-aldosterone system or a consequence of inappropriate activation or blockade of angiotensin II, type I receptors.
- Renin activity is dissociated from supine and standing blood pressure levels in POTS but not in healthy individuals.
- Aldosterone levels did not differ between patients with POTS and controls who had normal active standing test.

Swedish Summary

Posturalt ortostatiskt takykardsyndrom (POTS) är ett kroniskt kardiovaskulärt tillstånd sekundärt till en dysfunktion i det autonoma nervsystemet. POTS drabbar främst yngre kvinnor i fertil ålder. Diagnostiska kriterier för POTS inkluderar symptom på ortostatisk intolerans i minst 3–6 månader tillsammans med hjärtfrekvensökning på mer än> 30 slag per minut (mer än> 40 slag per minut hos patienter <19 år) eller en hjärtfrekvens som överstiger 120 slag per minut vid stående i frånvaro av blodtrycksfall. Etiologin bakom POTS är inte helt utredd även om dess breda symptomdiversitet talar för multifaktoriell mekanism som bidrar till kardiovaskulära (hjärtklappning, bröstsmärtor och andningsbesvär) samt icke-kardiovaskulära symptom (sömnlöshet, huvudvärk och koncentrationssvårigheter). Det förekommer olika hypoteser om bakomliggande mekanismer som autoimmun avvikelse, sympatisk denervering, hypotoni och reflextakykardi samt överskott av katekolaminer. Debuten av POTS sker inte sällan efter exponering för olika immunologiska stressfaktorer som virusinfektioner, graviditet eller trauma.

Det finns ingen specifik behandling för POTS utan fokus ligger primärt på symptomlindring som kompressionsstrumpor, ökad salt-och vätskeintag samt läkemedel som sänker hjärtfrekvensen. POTS ger i svårare fall en betydande morbiditet, med både stort individuellt lidande för patienten och stora samhällsekonomiska kostnader. Långtidsprognosen vid POTS är inte fullt utforskad, dock har man noterat i vissa fall symptomlindring och spontan återhämtning inom 1–3 år.

Denna avhandling inkluderar fem delprojekt med fokus på olika aspekter av POTS.

I det första delprojektet, undersökte vi kliniska och hormonella (neuroendokrina) egenskaperna hos patienter med olika typer ortostatisk intolerans och synkope, både POTS och andra diagnoser. Vi inkluderade 236 patienter i åldern 18–40 från vår SYSTEMA- kohort, vilka genomgått tilt-test på synkopeenheten på Skånes Universitet sjukhus i Malmö, lämnat blodprover samt fyllt i frågeformulär. Utifrån de observerade hemodynamiska förändringarna under tilt-testet delades alla inkluderade patienter in i undergrupper av ortostatisk intolerans; vasovagal synkope, VVS (n=103), ortostatisk hypotension, OH (n=22) och POTS (n=72). Resterande patienter med normal hemodynamisk respons under tilt (n=39) betraktades som referensgrupp. Resultaten visade en kvinnlig dominans i samtliga grupper. Patienter med POTS var signifikant yngre (27±6 vs 30 ±6, $p=0.030$) och

hade högre hjärtfrekvens vid 3 minuters tilt test (83±12 vs 100±14, *p <0.001)* i jämförelse med referensgruppen. Neuroendokrina analyser visade signifikant ökning av noradrenalin hos POTS patienter efter 3 minuter stående vilket stödjer hypotesen att det finns förekomst av katekolaminer överskott hos vissa POTS patienter.

I delprojekt II och III analyserade vi immunologiska och kardiovaskulära biomarkörer och deras eventuella association med POTS med hjälp av så kallad multiplex protein analys från Olink. Vi utförde en fall-kontroll studie med 113 POTS patienter samt 283 kontroller i åldern 15 – 55 år. Alla deltagare hade genomgått tilt och lämnat blodprover. Våra resultat visade en nedreglering av proproteinet Furin, som aktiverar andra proproteiner genom klyvning. Tidigare studier har visat att Furin kan påverka aktivering eller hämning av andra proteiner inom immunsystemet vilket talar för en sådan påverkan även i POTS. Vidare hittade vi förhöjda värden av tillväxthormonet GH i POTS men inte i kontrollgruppen. Vid både underskott och överskott av tillväxthormonet har man kunnat påvisa förhöjd risk för kardiovaskulära händelser. Det är oklart vad detta kan betyda hos POTS patienter på sikt dock är det viktigt att man driver forskning kring detta vidare. I analysen fann vi också att myoglobin var lägre hos män med POTS när vi jämförde med friska kontroller. Detta är rimligtvis sekundärt till dekonditionering, som ofta ses vid POTS.

I delprojekt IV utformade vi en egen självskattningsskala, Malmö POTS score (MAPS) där vi inkluderade 62 POTS patienter samt 50 kontroller som svarade på formuläret och utförde ortostatisk provokation med tilt-test respektive så kallad active standing test. Vi jämförde resultatet mellan grupperna men undersökte även eventuella korrelationer mellan symptomen och hemodynamiska parametrar inom grupperna. Patienter med POTS fick i genomsnitt 78±20 poäng medan kontrollerna fick endast 14 ±12 poäng, *p <0.001.* De tre mest förekommande symptomen vid POTS var hjärtklappning (7.7±1.7), svaghet (7.6±2.5) och koncentrationssvårigheter (7.2±2.1) medan kontrollerna rapporterade huvudvärk (1.9±2), koncentrations-svårigheter (1.8±1.7) samt sömnbesvär (1.8±2.6). Det optimala gränsvärdet för att skilja mellan POTS och friska kontroller var en total summa på ≥42. Hjärtklappning och koncentrationssvårigheter var relaterade till hemodynamiska förändringar i POTS men inte i kontrollgruppen.

I projekt V undersökte vi sambandet mellan renin-angiotensin-aldosteron systemet (RAAS), ett viktigt blodtrycksreglerande system, och POTS. Vi utförde en fall-kontroll studie där vi inkluderade 46 patienter med POTS och 48 friska kontroller utan några symptom på ortostatisk intolerans. Alla inkluderade fick genomgå blodprovstagning efter 10 minuters vila. Resultatet visade en nedreglering av renin aktivitet hos POTS patienter (median, 3406 ng/ml) men inte i kontrollgruppen. (median, 9949 ng/ml, *p <0.001*). Samtidigt såg man ingen skillnad i aldosteronkoncentrationen (median, 218pmol/L vs. 218 pmol/L, *p=0.26*) mellan grupperna.

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Appendix 1

LUND UNIVERSITY
Faculty of Medicine

Malmo POTS Score (MAPS)

Date: ……..

Name: ……

Date of birth: ……

Study number: …….

Dear Mr/Mrs/Ms,

This questionnaire concerns symptoms related to postural orthostatic tachycardia syndrome (POTS). It will help us to evaluate how affected you are by symptoms originating from this syndrome. We kindly ask you to fill in this questionnaire as thoroughly as possible.
Please circle the number on following scale that corresponds to your average symptoms for the past week. You should only answer once per question. If you haven't experienced symptoms described below, circle zero (0).

No symptoms **Pronounced symptoms**

1. Dizziness in upright position or while standing up

0 1 2 3 4 5 6 7 8 9 10

2. Dizziness, feeling that you are going to faint

0 1 2 3 4 5 6 7 8 9 10

3. Palpitations, high pulse, or feeling heart beating irregularly

0 1 2 3 4 5 6 7 8 9 10

4. Difficult breathing/dyspnoea, both at effort and rest

0 1 2 3 4 5 6 7 8 9 10

5. Chest pain

0 1 2 3 4 5 6 7 8 9 10

6. Headache

0 1 2 3 4 5 6 7 8 9 10

7. Concentration difficulties and/or problems with thinking

0 1 2 3 4 5 6 7 8 9 10

8. Muscle pain

0 1 2 3 4 5 6 7 8 9 10

9. Nausea

0 1 2 3 4 5 6 7 8 9 10

10. Gastrointestinal problems (stomach-ache, diarrhoea, constipation)

0 1 2 3 4 5 6 7 8 9 10

11. Abnormal tiredness that persists after rest

0 1 2 3 4 5 6 7 8 9 10

12. Insomnia

0 1 2 3 4 5 6 7 8 9 10

Paper I

openheart

Syndromes of orthostatic intolerance and syncope in young adults

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ABSTRACT

Objective To explore the clinical and neuroendocrine characteristics of syndromes of orthostatic intolerance and syncope in young adults.

Methods Two hundred and thirty-six patients aged 18–40 years with orthostatic intolerance and/or syncope were examined by head-up tilt test (HUT). Plasma levels of epinephrine, norepinephrine, renin, C-terminal-pro-arginine-vasopressin (CT-proAVP), C-terminal-endothelin-1 and mid-regional-fragment of pro-atrial-natriuretic-peptide (MR-proANP) were analysed. Patients' history, haemodynamic parameters and plasma biomarkers were related to main diagnoses such as vasovagal syncope (VVS), postural tachycardia syndrome (POTS), orthostatic hypotension (OH) and negative HUT.

Results No self-reported symptom of orthostatic intolerance was highly specific for any diagnosis. Patients with VVS (n=103) were more likely to be men (p=0.011) and had lower resting heart rate (HR; 66±11) compared with POTS (73±11; n=72; p=0.001) and negative HUT (74±11; n=39; p=0.001). Patients with POTS demonstrated greater rise in norepinephrine (p=0.008) and CT-proAVP (p=0.033) on standing compared with negative HUT, and lower resting MR-proANP compared with VVS (p=0.04) and OH (p=0.03). Patients with OH had lower resting renin (p=0.03). Subjects with a resting HR <70 and MR-proANP >45 pm/L had an OR of 3.99 (95 % CI 1.68 to 9.52; p=0.002) for VVS compared with subjects without any of these criteria; if male sex was added the OR was 21.8 (95% CI 3.99 to 119; p<0.001).

Conclusions Syndromes of orthostatic intolerance and syncope share many characteristics in younger persons. However, patients with VVS are more likely to be men, have lower HR and higher MR-proANP at rest compared with POTS, which might be taken into account at an early stage of evaluation.

INTRODUCTION

Syncope, a common clinical problem affecting between 30% and 40% of all humans during their lifetime,[1] is clearly dominated by reflex aetiology in the first four decades of life.[2] The vasovagal reflex, by far the most common mechanism of loss of consciousness, is frequently related to orthostatic intolerance.[3 4] Within syndromes of orthostatic intolerance, three distinct syncope-related conditions are traditionally defined on the grounds of haemodynamic response to orthostatic challenge: orthostatic hypotension (OH),[5] postural tachycardia syndrome (POTS)[6] and orthostatic (vasovagal) reflex syncope, the latter showing no haemodynamic signs of the two former conditions during the presyncopal phase.[3] While POTS is a condition typically observed in younger patients, especially women,[6] the prevalence of OH in the younger population is <5% and increases with advancing age.[5]

The treatment of reflex syncope and orthostatic intolerance poses a challenge for clinicians, especially when symptoms are frequent and pronounced.[3 4] Recent reports have suggested that syndromes of orthostatic intolerance may have antiadrenergic autoimmune background[7 8] and that they demonstrate different neuroendocrine patterns,[9 10] especially in children.[11] In particular, abnormalities in resting and orthostatic levels of catecholamines, vasopressin, renin-angiotensin system, endothelin and natriuretic peptides were detected, however, with partially contradicting results in regard to vasopressin in VVS versus OH.[11 12] Consequently, there is a need for more data to define typical clinical and neuroendocrine features of the main syncope-related syndromes of orthostatic intolerance in younger populations, both as a possible diagnostic tool and therapeutic guide.

In the present study, we determined patients' history, haemodynamic parameters and neuroendocrine biomarkers in a consecutive series of young adults (aged 18–40 years) who were investigated for suspected syncope and/or orthostatic intolerance with a standardised head-up tilt test (HUT).

METHODS

Study population

The Syncope Study of Unselected Population in Malmö cohort has been previously described.[9] In brief, 836 consecutive patients with unexplained syncope and/or symptoms of orthostatic intolerance were

KEY QUESTIONS

What is already known about this subject?

Syncope and orthostatic intolerance are common clinical problems. The vasovagal reflex, the most common mechanism of loss of consciousness in young adults, is frequently related to orthostatic intolerance. Within syndromes of orthostatic intolerance, three distinct syncope-related conditions are traditionally defined: orthostatic hypotension, postural tachycardia syndrome and orthostatic (vasovagal) reflex syncope, the latter showing no haemodynamic signs of the two former conditions during the presyncopal phase. The treatment of reflex syncope and orthostatic intolerance in young adults poses a clinical challenge, especially when symptoms are severe. Since the treatment strategies for common diagnoses of orthostatic intolerance may differ, an accurate diagnosis is essential in order to alleviate symptoms and prevent syncope recurrence.

What does this study add?

In this study, young patients with unexplained syncope and/or orthostatic intolerance were investigated with head-up tilt testing non-invasive beat-to-beat monitoring in specialised syncope unit. Surprisingly, none of the clinical features reported by the patients, such as palpitations or prodromal symptoms of syncope, was highly specific for any diagnosis. Furthermore, this study demonstrates that patients diagnosed with vasovagal syncope and postural tachycardia syndrome are different regarding sex (higher proportion of men among the patients with vasovagal syncope) and seem to show opposite patterns of both haemodynamic factors (resting heart rate lower among patients with vasovagal syncope) and neuroendocrine markers (resting mid-regional-fragment of pro-atrial-natriuretic-peptide (MR-proANP) lower in postural tachycardia syndrome.

How might this impact on clinical practice?

When diagnosing syncope and orthostatic intolerance, the uncertainty of the final diagnosis if based on patient's history must be accepted with caution. While it has been shown that a level of accuracy when an expert takes history is very high, this study emphasises the utility of head-up tilt testing with non-invasive beat-to-beat monitoring as a method of diagnosis in unexplained syncope, especially in the absence of a syncope expert. The study also suggests that sex, resting heart rate and MR-proANP, the latter easily assessed through commercially available test kits, may be valuable as additional tools in the initial evaluation of young patients with unexplained syncope.

referred to and investigated at the Syncope Unit of Skåne University Hospital between August 2008 and October 2013. Of these, we identified 671 patients who underwent HUT according to the Italian protocol[13] and accepted serial blood sampling during the test. For the current study, we selected participants aged 18–40 years, yielding a series of 236 eligible patients (figure 1). These patients were managed post-test according to the current European Society of Cardiology syncope guidelines.[3]

Examination protocol

The patients were asked to take their regular medication and fast for 2 hours before HUT, although they were allowed to drink water ad libitum. Prior to examination, the patients were asked to fill a questionnaire, which explored past medical history, as well as duration, frequency and features of syncope-related symptoms. Time from the first-ever syncope to examination <6 months was assigned symptom duration equal to 0 years in the database and the values were rounded up to 1 year.

The HUT protocol included peripheral vein cannulation, supine rest for 10 min, blood sampling both at supine rest and in the upright position 3 min after elevation of the table at an angle of 60–70° and optional nitroglycerin provocation according to the Italian protocol.[13] Nitroglycerin (400 µg spray sublingually) was administrated first after 20 min of passive HUT if syncope had not occurred and the haemodynamic parameters were stable , that is, no significant hypotension (systolic blood pressure (SBP) <90 mm Hg) or orthostatic intolerance due to sinus tachycardia >120 beats per minute (bpm) were observed. Thus, this nitroglycerin phase played no part in any of the neuroendocrine measurements, but contributed to the ultimate diagnosis of VVS. Beat-to-beat blood pressure (BP) and ECG were recorded using a non-invasive validated method (Nexfin monitor, BMEYE, Amsterdam, The Netherlands),[14] and subsequently analysed offline using dedicated software provided by the manufacturer. Mean BP and heart rate (HR) in supine position, after 3 min of HUT, and at the lowest BP/highest HR during passive orthostasis were calculated as an average of a 30 s period. The predefined point for the second haemodynamic assessment and blood sampling assigned to 3 min of HUT was selected to comply with the time point when postural haemodynamic stability is usually achieved in normal individuals.[15]

The third assessment of the haemodynamic parameters between 3 and 20 min of HUT, corresponding to lowest SBP/highest HR prior to either activation of vasovagal reflex and/or syncope or end of the passive HUT, was intended to identify those with delayed haemodynamic instability, that is, if significant haemodynamic changes were observed beyond the first 3 min of HUT. The onset of vasovagal reflex was identified by typical prodrome and/or an abrupt change in haemodynamic parameters such as bradycardia and/or pronounced hypotension.

VVS was defined as a reproduction of syncope associated with a characteristic pattern of pronounced hypotension, bradycardia or asystole. For the current study, patients were classified as VVS only if they had no signs of POTS or OH during the test. OH was defined as a sustained decrease in SBP ≥20 mm Hg and/or decrease in diastolic BP (DBP) ≥10 mm Hg, while POTS as reproduction of symptoms of orthostatic intolerance (lightheadedness, dizziness or discomfort) with HR increase >30/min or tachycardia >120/min during HUT.[3]

The Regional Ethical Review Board in Lund, Sweden accepted the study protocol (ref no 82/2008), and all study participants gave their written informed consent.

Neuroendocrine biomarkers

As neuropeptides, in particular atrial natriuretic peptide, endothelin-1 and vasopressin, are characterised by

Table 1 Patient characteristics

	All (n=236)	No Dx (n=39)	VVS (n=103)	p Value*	POTS (n=72)	p Value*	OH (n=22)	p Value*	p Value†
Age, years	28.1 (6.7)	30.2 (6.3)	28.2 (6.8)	0.335	26.6 (6.4)	**0.030**	28.2 (6.7)	0.632	0.053
Sex, % male	32.6	38.5	41.7	–	20.8	–	18.2	–	**0.011**
BMI, kg/m^2	23.7 (3.9)	24.7 (4.9)	24.0 (3.5)	0.785	22.8 (3.4)	0.074	23.9 (4.8)	0.852	0.076
Symptoms reported by the patients									
Duration of symptoms, years, (median, (IQR))	3 (9)	4 (8)	5 (10)	0.698	3 (9)	0.998	2.5 (5)	0.813	0.247
Total no of syncope (median, (IQR))	5 (18)	5 (22)	5 (7)	0.734	5 (28)	1.000	9 (18)	0.979	0.266
Prodrome (nausea, perspiration, etc), %	72.4	65.5	77.6	–	65.0	–	88.9	–	0.128
Palpitations, %	39.1	51.7	32.8	–	31.7	–	66.7	–	**0.017**
Traumatic fall, %	55.1	53.8	52.0	–	60.6	–	54.5	–	0.731
Dizziness on standing, %	73.2	82.1	61.2	–	81.7	–	86.4	–	**0.003**

Values displayed as mean (SD) if not otherwise stated.
*p Value for Tukey's or Games-Howell post hoc test in relation to no Dx (reference group) for continuous variables.
†ANOVA or Welch test p value for continuous variables and Pearson's χ^2 p value for dichotomous variables.
ANOVA, analysis of variance; no Dx, no diagnosis; OH, orthostatic hypotension; POTS, postural tachycardia syndrome; VVS, vasovagal syncope. The use of medications in the study population was generally very low. Blood pressure-increasing medication was used in seven patients (one negative HUT, two VVS, three POTS, one OH). β-Blockers were used by two patients (both POTS); calcium antagonists was used in one patient (VVS) as were angiotensin receptor blockers (VVS), levothyroxine was used by two patients (POTS; OH) and anti-EP medications were used by four patients (two negative HUT, one POTS, one OH). Antidepressants in the form of selective serotonin-reuptake inhibitors were used by 14 patients (3 negative HUT, 5 VVS, 2 POTS, 4 OH) and other antidepressants were used by two patients (both POTS). Symptomatic drugs including sedatives, analgetics and sleep agents were used in no more than five patients for each class of drugs. No patient used antidiabetic medication, platelet inhibitors, oral anticoagulants, diuretics, ACE-inhibitors, α-blockers, long-acting nitroglycerine, lipid-lowering drugs, digoxin, opiates, anti-Parkinson medication or cytostatics.

a short half-life of a few minutes, we applied newly developed laboratory assays to detect their stable fragments, thus allowing better estimation of neurohormone biosynthesis. Blood samples collected in the supine position before HUT and at 3 min of HUT were used for determination of epinephrine, norepinephrine, renin, C-terminal-pro-arginine-vasopressin (CT-proAVP), C-terminal-endothelin-1 (CT-proET-1) and

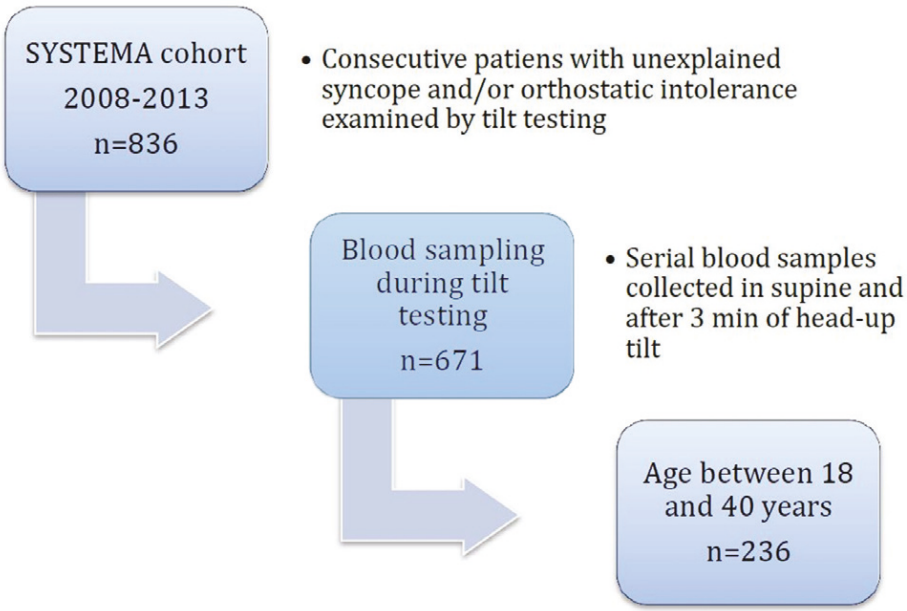

Figure 1 Flow chart of patient selection. The selection of patients for the current study. SYSTEMA, Syncope Study of Unselected Population in Malmö.

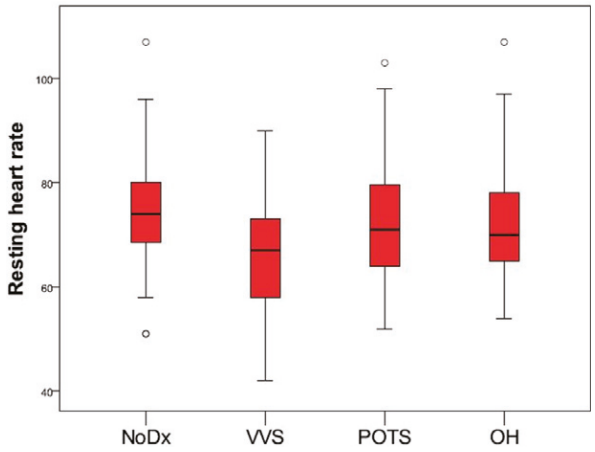

Figure 2 Resting heart rate according to diagnosis. Resting heart rate stratified according to final diagnosis at head-up tilt test. NoDx, no diagnosis; VVS, vasovagal syncope; POTS, postural tachycardia syndrome; OH, orthostatic hypotension.

mid-regional-fragment of pro-atrial-natriuretic-peptide (MR-proANP). The total amount of blood drawn for the analyses was 60 mL (30+30 mL), and no fluid substitution was given. Plasma biomarkers were measured from blood samples (16×250 µL aliquots of EDTA plasma in plastic thermotubes) that had been frozen at −80°C after collection.

CT-proAVP, CT-proET-1 and MR-proANP were measured using the assays provided by the manufacturer: Thermo Fisher Scientific BRAHMS CT-proAVP KRYPTOR, Thermo Fisher Scientific BRAHMS CT-proET-1 KRYPTOR and Thermo Fisher Scientific BRAHMS MR-proANP KRYPTOR (BRAHMS GmbH, part of Thermo Fisher Scientific, 16761 Hennigsdorf, Germany). Concentrations of epinephrine and norepinephrine were determined by high-performance liquid chromatography with fluorescence detection.[16] Plasma renin concentrations were analysed using an immunoradiometric assay (Renin III Generation; Cisbio Bioassays International, 30200 Codolet, France).

Statistics

One-way analysis of variance (ANOVA) with Tukey's post hoc test was used to determine the difference in baseline characteristics, haemodynamic parameters and neuroendocrine biomarkers between the patients with negative HUT and those diagnosed with VVS, POTS or OH, respectively, or between the diagnostic groups, if appropriate. If the assumption of homogeneity of variances was violated (indicated by Levene's test $p<0.05$), a Welch test with Games Howell post hoc was run instead. For dichotomous variables, Pearson's χ^2 test was used. Any continuous variables with skew deviation were log-transformed in the statistical analyses. When appropriate, significant findings from the χ^2, ANOVA and Welch models were further explored by testing the relation between those variables and diagnosis in logistic regression models yielding OR with 95% CI. All analyses were performed using IBM SPSS Statistics V.23 (SPSS, Chicago, Illinois, USA). All tests were two-sided, whereby $p<0.05$ was considered to be statistically significant.

RESULTS

Study population characteristics

The proportions of final diagnoses and patients' characteristics are displayed in table 1. There was a predominance of females. The median duration of syncope-related symptoms was 3 years with no difference between the diagnostic groups. Patients diagnosed with POTS were more often female and younger compared with patients with negative HUT. In contrast, the proportion of male subjects was highest among patients diagnosed with VVS. Moreover and somewhat surprisingly, patients with POTS tended to report palpitations

Table 2 Haemodynamic parameters at rest and during HUT

	All (n=236)	No Dx ref (n=39)	VVS (n=103)	p Value*	POTS (n=72)	p Value*	OH (n=22)	p Value*	p Value†
SBP rest	121.6 (13.0)	122.9 (13.3)	120.0 (12.2)	0.660	123.0 (12.4)	1.00	121.7 (17.6)	0.987	0.437
DBP rest	70.7 (7.6)	73.2 (7.8)	69.3 (6.8)	**0.034**	71.5 (7.6)	0.671	70.0 (9.9)	0.378	**0.035**
HR rest	69.9 (11.8)	74.2 (10.9)	66.0 (11.3)	**0.001**	72.5 (11.0)	0.880	71.6 (13.1)	0.818	**<0.001**
SBP 3'	122.1 (16.0)	128.7 (14.9)	121.5 (13.8)	0.068	122.6 (15.4)	0.192	111.0 (22.7)	**<0.001**	**<0.001**
DBP 3'	77.3 (10.8)	81.1 (11.5)	76.6 (9.2)	0.096	78.7 (10.0)	0.648	68.6 (14.1)	**<0.001**	**<0.001**
HR 3'	86.7 (15.5)	82.6 (11.6)	79.6 (11.9)	0.600	99.9 (13.6)	**<0.001**	83.1 (15.2)	0.999	**<0.001**
SBP min	108.7 (15.3)	116.1 (11.5)	110.1 (12.3)	0.111	107.7 (16.8)	**0.015**	91.8 (16.4)	**<0.001**	**<0.001**
DBP min	70.8 (10.8)	75.1 (9.6)	71.0 (9.2)	0.125	72.0 (10.8)	0.418	58.0 (10.8)	**<0.001**	**<0.001**
HR max	93.3 (18.2)	85.8 (14.9)	84.1 (12.8)	0.920	111.0 (14.6)	**<0.001**	91.5 (13.6)	0.404	**<0.001**

Displayed as mean (SD).
*p Value for Tukey's or Games-Howell post hoc test in relation to no Dx (reference group) for continuous variables.
†ANOVA or Welch test p value for continuous variables and Pearson's χ^2 p value for dichotomous variables.
ANOVA, analysis of variance; DBP, diastolic blood pressure; HR, heart rate; HUT, head-up tilt test; no Dx, no diagnosis; OH, orthostatic hypotension; POTS, postural tachycardia syndrome; SBP, systolic blood pressure; VVS, vasovagal syncope.

to a less extent than patients with negative HUT, whereas the proportion of patients reporting palpitations were similar among the VVS and POTS groups. Orthostatic dizziness was less common among patients with VVS, even though it was not very specific for any diagnosis (table 1). In the group who tolerated tilt testing without significant haemodynamic changes (negative HUT), five patients demonstrated psychogenic pseudosyncope, and four other patients were subsequently monitored with implantable loop recorder without diagnostic findings, that is, no syncope during monitoring or fainting episodes recorded with normal heart rhythm only. Patients with psychogenic pseudosyncope did not significantly differ in haemodynamic parameters and biomarkers from the rest of HUT-negative patients.

Haemodynamic parameters

At rest, patients with VVS showed significantly lower HR compared with negative HUT (table 2, figure 2), and with POTS (p=0.001). In agreement with the predefined diagnostic criteria, the small number of patients with OH showed significantly lower SBP and DBP during HUT, whereas those with POTS showed higher HR during HUT (table 2), both at 3 min of HUT and at the point of lowest BP.

Neuroendocrine biomarkers

Patients with POTS showed a significantly higher rise in norepinephrine at 3 min of HUT (table 3) and had lower resting MR-proANP in relation to VVS (p=0.039) and OH (p=0.030), but not to negative HUT (p=0.96). Patients with POTS also had a greater increase in CT-proAVP compared with negative HUT (table 3) but not VVS (p=0.768) or OH (p=0.693). Patients with OH also had lower resting renin level compared with negative HUT (p=0.030). There were no other significant differences between patients with negative HUT and those with VVS, POTS or OH, respectively (table 3). Furthermore, resting MR-proANP was inversely related to resting HR in a linear model (p=0.009).

Multivariable models for diagnosis

Based on our findings of variables associated with VVS in the study population, we constructed a 'VVS score' including sex (male), resting HR (<study population median of 70 bpm) and resting MR-proANP levels (>study population median of 45 pm/L). The score was then related to a diagnosis of VVS (compared with any other diagnosis, including negative HUT) in a logistic regression model, with age as a covariate. Subjects that had all of the three characteristics male sex, resting

Table 3 Neuroendocrine biomarkers at rest (0') and at 3 min head-up tilt (3')

	All (n=163–187)	No Dx ref (n=24–30)	VVS (n=79–87)	p Value*	POTS (n=44–55)	p Value*	OH (n=16–17)	p Value†	
P-renin 0'	15.0 (13)	17.0 (12)	15.5 (12)	0.573	14.0 (14)	0.550	10.5 (38)	**0.030**	0.054
P-renin 3'	15.5 (13)	16.5 (15)	16.0 (14)	0.816	15.0 (13)	0.658	10.0 (9)	0.074	0.106
ΔRenin	0.0 (2.0)	0.0 (2.5)	0.0 (3.0)	0.369	0.0 (2.0)	0.993	0.0 (1.5)	0.995	0.101
P-epinephrine 0'	0.10 (0.1)	0.09 (0.1)	0.10 (0.1)	0.999	0.085 (0.1)	0.998	0.10 (0.2)	0.976	0.937
P-epinephrine 3'	0.19 (0.2)	0.17 (0.2)	0.21 (0.2)	0.846	0.18 (0.2)	0.825	0.14 (0.2)	0.932	0.457
ΔEpinephrine	0.07 (0.13)	0.06 (0.09)	0.09 (0.2)	0.690	0.07 (0.1)	0.628	0.04 (0.11)	1.00	0.452
P-NE 0'	1.40 (0.9)	1.70 (1.0)	1.30 (0.8)	0.071	1.48 (0.9)	0.137	1.30 (0.6)	0.524	0.097
P-NE 3'	2.40 (1.4)	2.45 (1.4)	2.30 (0.9)	0.513	2.90 (1.5)	0.744	2.00 (1.1)	0.744	**0.018**
ΔNE	1.00 (0.7)	0.85 (0.5)	0.90 (0.5)	0.733	1.40 (1.2)	**0.008**	0.90 (0.9)	0.987	**0.013**
MR-proANP 0'	45.0 (23.9)	37.9 (30.2)	48.0 (25.4)	0.349	40.1 (23.0)	0.955	52.7 (25.8)	0.145	**0.009**
MR-proANP 3'	45.7 (24.7)	40.4 (33.1)	50.6 (24.5)	0.122	41.0 (18.5)	0.999	53.7 (38.0)	0.168	**0.015**
ΔMR-proANP	2.25 (4.2)	1.18 (4.4)	2.22 (2.8)	0.992	2.55 (5.0)	1.00	3.63 (7.3)	0.999	0.958
CT-proET1 0'	43.3 (13.5)	47.4 (23.2)	41.9 (11.8)	0.998	43.8 (14.0)	1.00	43.3 (8.7)	0.991	0.891
CT-proET1 3'	42.5 (14.2)	39.4 (22.1)	42.3 (11.2)	0.996	41.7 (15.5)	1.00	43.3 (12.8)	0.791	0.769
ΔCT-proET1	0.40 (4.6)	0.80 (6.2)	0.50 (4.3)	0.796	0.40 (4.7)	0.932	−0.18 (3.6)	0.712	0.723
CT-proAVP 0'	6.14 (5.9)	6.68 (4.8)	5.65 (5.0)	0.834	6.75 (6.9)	0.933	5.04 (5.6)	0.859	0.824
CT-proAVP 3'	6.77 (6.7)	6.16 (5.5)	6.13 (6.8)	0.576	7.24 (8.2)	0.641	6.79 (7.1)	0.62	0.615
ΔCT-proAVP	0.16 (2.3)	−0.29 (1.5)	0.34 (2.5)	0.098	0.18 (3.9)	**0.033**	0.07 (1.3)	0.533	0.052

Displayed as median (IQR) in pm/L.
Number of patients displayed as range of available samples in the diagnosis groups.
*p Value for Tukey's or Games-Howell post hoc test in relation to no Dx (reference group) for continuous variables.
†ANOVA or Welch test p value for continuous variables and Pearson's χ^2 p value for dichotomous variables.
ANOVA, analysis of variance; no Dx, no diagnosis; OH, orthostatic hypotension; POTS, postural tachycardia syndrome; VVS, vasovagal syncope.

HR <70 and resting MR-proANP levels >45 pm/L had an OR of 21.8 (95 % CI 3.99 to 119; p<0.001) for being diagnosed with VVS compared with subjects that lacked all of these characteristics (table 4). When excluding male sex as a criteria, patients with fulfilling the criteria of resting HR <70 bpm plus MR-proANP >45 pm/L had an OR of 3.99 (95 % CI 1.68 to 9.52; p=0.002) of being diagnosed with VVS compared with any other diagnosis in relation to subjects without these two criteria in a sex-adjusted and age-adjusted logistic regression model. On the contrary, the patients with HR ≥70 bpm plus MR-proANP <45 pm/L had an increased probability of being diagnosed with POTS (OR 3.66; 95 % CI 1.40 to 9.58; p=0.008).

DISCUSSION

In this study, we report that resting HR in patients with vasovagal reflex syncope during tilt testing was lower compared with those who had postural orthostatic tachycardia syndrome and with those whose tests were negative. Furthermore, MR-proANP was significantly higher among those with VVS compared with POTS, and MR-proANP was inversely related to supine HR. When these variables were combined, patients with both a resting HR <70 bpm plus MR-proANP levels >45 pm/L had an OR of approximately four times for reflex syncope compared with subjects without any of these criteria; the OR increased to 22 if male sex was also included as a criteria in the model. We also showed that patients with OH had lower resting renin, while patients with POTS demonstrated pronounced increase in norepinephrine. Patients with POTS also demonstrated greater increase in CT-proAVP during HUT than patients with negative HUT, however, not compared with any other group. Finally, we have observed that patient's history and symptoms during syncope may not be specific for any diagnosis.

Our previous reports suggested that lower values of MR-proANP were predictive of both VVS[12] and orthostatic tachycardia[9] in the general syncope population. In this study, the head-to-head comparison between age-matched younger patients with VVS and patients with POTS demonstrated that lower MR-proANP is more suggestive of POTS.

Table 4 Markers of vasovagal syncope in relation to diagnosis

Number of markers	OR for VVS	95 % CI	p Value
0	1.00		
1	1.89	0.77 to 4.63	0.164
2	2.47	0.99 to 6.15	0.052
3	21.8	3.99 to 119	<0.001
Model trend	1.98	1.37 to 2.87	<0.001

OR and 95% CI for vasovagal syncope in relation to occurrence of the following markers in individual subjects: male sex, resting heart rate <70 bpm; resting plasma MR-proANP levels >45 pm/L.

The finding of lower resting HR among patients with VVS implies higher vagal tone and/or a lower sympathetic tone affecting the heart at rest compared with patients with a negative test. Interestingly, this difference in HR seems to be attenuated during orthostatic challenge, which may be explained by either a marked vagal withdrawal or a more pronounced increase in adrenergic drive. For obvious reasons, patients with POTS demonstrated greatest increase in HR during HUT, outperforming that of VVS positive and negative HUT but the difference between patients with VVS and negative HUT was also significant. However, although orthostatic increase in HR in POTS is pathognomonic for this syndrome, less is known about chronotropic response in patients with VVS compared with normal subjects. Our reference group with negative HUT do not represent normal subjects, but they are autonomically more integrated and do not demonstrate the hypotensive tendency usually detected by tilt testing.[17] Consequently, the attenuation of difference in HR between patients with VVS positive and negative HUT during orthostatic challenge may be due to counteracting the hypotensive tendency in standing in the former by increasing HR and cardiac output. Interestingly, epinephrine elevation during early HUT phase did not differ between the groups, an observation that suggests a baroreceptor-mediated vagal withdrawal as the main mechanism of HR increase in patients with positive VVS.

Compared with patients with POTS, MR-proANP was significantly higher among those with VVS, which corroborates our previous findings of decreased ANP in postural tachycardia.[9] Also, we found that higher MR-proANP was inversely related to resting HR. Thus, patients with VVS show lower HR, which is in turn associated with higher ANP. The most important stimulus for ANP secretion is stretching of atrial walls, which takes place with a high blood volume and raised atrial pressure.[18 19] One could hypothesise that the lower resting HR of pateints with VVS would lead to greater filling of these cardiac chambers during the cardiac cycle, which in turn would trigger increased release of ANP in these patients. On the contrary, patients with POTS may have underfilling of the atria, leading to reduced ANP. Whether higher resting MR-proANP levels may in itself also predispose to a vasovagal reaction during orthostatic stress remains to be determined.

Current clinical guidelines[3] emphasise the need for careful history taking when evaluating patients with unexplained syncope. VVS is by far the most common cause of syncope in young patients[2 3] and it is suggested by some authors that VVS may be diagnosed solely by careful history taking.[20] In this study, none of the clinical features such as the total number of attacks, how many years ago the first syncope occurred, dizziness on standing and palpitations or typical prodrome preceding syncope was highly specific for any diagnosis. Of interest, the proportion of patients with POTS that reported palpitations was low (3 of 10), and this

proportion was the same among patients with VVS. Furthermore, even though the proportion of patients reporting dizziness on standing was slightly lower among VVS compared with other diagnoses, 6 of 10 patients with VVS still reported this symptom. The history is without doubt a powerful tool in diagnosing syncope, in particular when taken by a trained expert.[21] In this study, patients were asked to fill a standard questionnaire prior to tilt testing. Self-reported history obtained by filling a questionnaire is similar to history taking by a non-expert yielding around 60% accuracy.[22] While it has been shown that a level of accuracy when an expert takes history is very high, as much as 90%.[23] We believe that these results indicate that tilt testing with non-invasive beat-to-beat monitoring should be considered in unexplained syncope associated with symptoms of orthostatic intolerance, especially in the absence of a syncope expert. When an expert is available, tilt testing may be seen as a diagnostic tool for confirmation of diagnosis and an additional test in unresolved cases.

VVS and POTS are diagnoses that are very common in young subjects, yet the treatment strategies for these diagnoses may differ[6] and an accurate diagnosis is essential when attempting to prevent syncope recurrence. Since patients with VVS and POTS seem to show opposite patterns of both haemodynamic factors (resting HR lower among patients with VVS) and neuroendocrine markers (resting MR-proANP lower in POTS), we suggest the possibility that resting HR and MR-proANP, easily assessed through commercially available test kits, may be considered as additional tools in the evaluation of young patients with unexplained syncope. Moreover, the lower HR in patients with VVS would tend to make them unsuitable for treatment with β-blockers, a strategy, which has failed in randomised trials on prevention of syncope recurrences.[3] The lower resting HR in patients with VVS may be closer to 'normal' as corroborated by other data from the Malmö population. In the Malmö Preventive Project, 8370 healthy individuals aged 27–40 years had resting HR of 67±10 bpm,[24] similar to VVS in our study. Consequently, patients with VVS can be considered normal except when they are having reflex syncope. In contrast, POTS and OH are patients with persistent manifestations of their condition, expressed by abnormal adrenergic activation or vagal withdrawal at rest and higher HR. Patients with negative HUT are, on the other hand, a non-homogenous group, possibly with over-representation of anxiety disorders, which would explain the higher pretest HR. The fact that the proportion of men is higher among patients with VVS than among patients with POTS is consistent with the well-known fact that POTS is more often diagnosed in women.[6]

Among other observations, lower renin in OH is supported by earlier studies in diabetic patients with OH,[25] but has not been recently confirmed[11] in very young subjects and thus warrants further study, possibly including other components of renin-angiotensin system. In contrast, a significant increase in norepinephrine during HUT in patients with POTS can be considered confirmatory, as it has been previously demonstrated in several studies.[9 11 26] The finding of a greater increase in AVP during orthostasis in patients with POTS may have physiological basis in that a low blood volume is a well-known strong stimulus for AVP release. Of possible clinical relevance relating to these findings, copeptin levels have been suggested to predict the outcome of treatment with midodrine hydrochloride[27] and β-blockers in children with POTS.[28]

Our study has some limitations that should be mentioned. First, no control subjects without a history of syncope and/or orthostatic intolerance were included. However, the fact that only patients with unexplained syncope and/or orthostatic intolerance referred for further evaluation were included also makes the results clinically relevant. Second, the neuroendocrine data were measured at rest and at 3 min during HUT only. Further changes in these parameters might have occurred later during HUT, as well as after syncope. Third, there is an overlap between POTS and VVS in that many patients with POTS also experience syncope by VVS. In our study, 47 out of the 72 patients with POTS (65%) also had VVS during HUT. However, as treatment in these patients should probably be directed to the precipitating factors in form of their main diagnosis including the postural tachycardia and orthostatic intolerance rather than the VVS per se, we still find the distinction between 'pure VVS' and POTS (±VVS) important for successful treatment outcome. Fourth, the strict cut-off for HR in the diagnosis of POTS means that some patients with haemodynamic findings that are suggestive of, but not diagnostic for, POTS may be classified as either VVS or negative HUT. Finally, even though the use of medications influencing HUT results was very low in the study population should not affect results on the group level, such medications may of course have affected test results for a small number of individual patients.

In conclusion, we have shown that among young patients with unexplained syncope, patients with VVS are more likely to be men, have lower HR and higher MR-proANP at rest than patients with POTS. We propose that these parameters might be taken into account during the initial evaluation of unexplained syncope in young patients.

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Contributors All authors (VH, JMS, DN, MS, RS, OM, AF) participated in 1) conception and design or analysis and interpretation of data; 2) drafting of the manuscript or revising it critically and 3) final approval of the manuscript submitted.

Competing interests AF and OM are listed as co-inventors on a patent application 'Biomarkers for the diagnosis, prognosis, assessment and therapy stratification of

syncope' (PCT/EP2013/001081) for the use of BRAHMS CT-proAVP, CT-proET-1, MR-proADM and MR-proANP for diagnosis of syncope. RS is a consultant to Medtronic.

Patient consent All patients signed an informed consent, however not a specific BMJ form. All data were unidentified and results are presented on group level only.

Ethics approval The Regional Ethical Review Board in Lund, Sweden accepted the study protocol (ref no 82/2008).

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Paper II

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Proconvertase Furin Is Downregulated in Postural Orthostatic Tachycardia Syndrome

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Background: Postural Orthostatic Tachycardia Syndrome (POTS) is a cardiovascular autonomic disorder characterized by orthostatic intolerance and high prevalence among young women. The etiology of POTS is uncertain, though autoimmunity and inflammation may play an important role. We aimed to identify novel inflammatory biomarkers associated with POTS.

Methods and Results: In the Syncope Study of Unselected Population in Malmö (SYSTEMA) cohort, we identified 396 patients (age range, 15–50 years) with either POTS (n = 113) or normal haemodynamic response during passive head-up-tilt test (n = 283). Blood samples were analyzed using antibody-based Proximity Extension Assay technique simultaneously measuring 57 inflammatory protein biomarkers. The discovery algorithm was a sequential two-step process of biomarker signature identification by supervised, multivariate, principal component analysis and verification by univariate ANOVA with Bonferroni correction. POTS patients were younger (26 vs. 31 years; $p < 0.001$) and there was no significant difference in sex distribution (74% vs. 67% females, $p = 0.24$). PCA and Bonferroni-adjusted ANOVA identified proconvertase furin as the most robust biomarker signature for POTS. Plasma level of proconvertase furin was lower (6.38 vs. 6.58 of normalized protein expression units (NPX); $p < 0.001$ in POTS, compared with the reference group. Proconvertase furin met Bonferroni-adjusted significance criteria in both uni- and multivariable regression analyses.

Conclusion: Patients with POTS have lower plasma level of proconvertase furin compared with individuals with normal postural hemodynamic response. This finding suggests the presence of a specific autoimmune trait with disruption of immune peripheral tolerance in this hitherto unexplained condition. Further studies are needed for external validation of our results.

Keywords: postural orthostatic tachycardia syndrome, inflammation, biomarkers, proteomics, proconvertase furin

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INTRODUCTION

Postural orthostatic tachycardia syndrome (POTS) is a complex condition featuring signs of autonomic dysfunction with both cardiovascular and non-cardiovascular symptoms (Benarroch, 2012). Although typically multi-symptomatic, POTS is by definition characterized by an abnormally increased heart rate upon standing and symptoms of orthostatic intolerance without significant blood pressure decrease (Sheldon et al., 2015). The syndrome affects predominantly young women (70–80%) with increasing incidence in developed countries, but its etiology has not been established (Sheldon et al., 2015; Brinth et al., 2018). Aside from orthostatic intolerance, patient frequently report headache, palpitations, brain fog and fatigue, which is believed to be a consequence of both hyperadrenergic state and decreased blood flow to the brain (Benarroch, 2012; Li et al., 2014; Arnold et al., 2018).

As etiology of POTS is still unknown, effective treatment for this syndrome is yet to be developed. Therapeutic options available today have modest efficacy and focus only on alleviating symptoms, thus, further research is mandatory. It has been observed that some patients develop POTS after experiencing a febrile illness, presumably viral (Grubb, 2008; Li et al., 2014). This has led to the hypothesis of an autoimmune-mediated etiology of POTS, and recent case series of POTS following immune triggers like infection or vaccination (Blitshteyn, 2015; Brinth et al., 2015; Watari et al., 2018) support this hypothesis. It has already been established that activating autoantibodies (AAb) to the α1-adrenergic (α1AR), β1/2-adrenergic receptors (β1/2AR), and angiotensin-receptor type 1 can be found in serum from POTS patients but not in controls (Fedorowski et al., 2017; Yu et al., 2018). In current knowledge of the presence of autoantibodies and possible immunological triggers, exploration of expression of inflammatory mediators in POTS is required, both as a potential diagnostic tool and therapeutic target in this ill-understood condition for which there is no effective treatment.

In this study, we sought to discover inflammatory biomarkers associated with POTS in order to identify a signature, which could potentially be useful to understand the pathophysiology underlying the syndrome. We applied a new method of multiple-protein screening using oligonucleotide-labeled antibodies against selected serum proteins.

MATERIALS AND METHODS

Study Population

The study was performed between September 2008 and May 2014 on a series of 545 consecutive patients aged 15–50 years enrolled in the ongoing Syncope Study of Unselected Population in Malmö (SYSTEMA) (Fedorowski et al., 2010). The age range was based on previous epidemiological studies on POTS incidence (Goodman, 2018). The recruited patients were referred to the tertiary syncope investigation unit at Skåne University Hospital in Malmö from primary and outpatient care clinics as well as hospitals in the southern region of Sweden due to unexplained syncope and/or symptoms of orthostatic intolerance. The referred patient underwent an initial diagnostic workup, typically including clinical history, resting, exercise and ambulatory prolonged electrocardiogram (Holter-ECG), transthoracic echocardiography, coronary and pulmonary angiography, brain imaging and encephalography, if requested by the referring physician. In the Syncope Unit, the patients were investigated by cardiovascular autonomic tests including head-up tilt testing (HUT), according to the European syncope guidelines available during this period (Moya et al., 2009). Blood samples were collected during the examination. The final study population included 113 POTS individuals and 283 controls with normal hemodynamic response during HUT as well as without prevalent cardiovascular disease (ischemic heart disease, heart failure, and stroke) or hypertension.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by The Regional Ethical Review Board of Lund University (No 82/2008). All patients gave their written informed consent.

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

Examination Protocol

Patients discontinued cardiovascular drugs 48 h before the test, fasted for 2 h prior to examination and were allowed to drink water at will. The past medical history was explored using a standard study questionnaire. The patients were placed on a tilt table and a venous cannula was inserted in the forearm after which a rest period for at least 10 min was allowed before blood samples were collected through the cannula. As soon as the haemodynamic parameters were stable, a standard 70°HUT was carried out according to the Italian protocol recommended by ESC (Bartoletti et al., 2000; Moya et al., 2009). Beat-to-beat blood pressure and ECG were monitored continuously by a validated non-invasive photoplethysmographic method (Nexfin monitor; BMEYE, Amsterdam, Netherlands) with a wrist unit and finger cuff of appropriate size (Eeftinck Schattenkerk et al., 2009). POTS was defined as reproduction of symptoms of orthostatic intolerance (lightheadedness, dizziness, or discomfort) along with heart rate increase >30 beats/min or sinus tachycardia >120 beats/min during first 10 min of HUT; or increase >40 beats/min for those under 18 years of age, with a history of orthostatic intolerance for at least 6 months (Sheldon et al., 2015).

Multiplex Protein Analysis

Plasma biomarkers were measured from supine blood samples (total volume: 30 ml) that had been first centrifuged, then stored as 16 × 250 μL 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 (**Supplementary Table S1**). Briefly, a pair of oligonucleotide-labeled 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 Normalised 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 concerning limit of detection, reproducibility and validation is available at the Olink Proteomics website[1].

Statistical Analysis

For the statistical analyses, patients with available proteomics data and definitive diagnosis of POTS (n = 113) or normal hemodynamic response during HUT (n = 283), i.e., without orthostatic hypotension (Moya et al., 2009; Freeman et al., 2011) and abnormal postural tachycardia, as well as without prevalent cardiovascular disease (ischemic heart disease, heart failure, and stroke) or hypertension were selected. Missing data was imputed with multiple imputations by chained equations (MICE) approach to create 10 complete datasets. We used predictive mean matching for continuous variables, logistic regression for binary variables, and polytomous regression for categorical variables. All covariates were included in the imputation models. The maximum iteration was set at 20 and convergence was confirmed by visual examination of trace plots. The main characteristics of study population were calculated as mean and standard deviation for continuous variables and as percentages for categorical variables, both for the total study population, and separately for POTS-positive and -negative patients.

The discovery algorithm for the identification of potentially relevant biomarkers associated with the presence of POTS was a sequential two-step process of (i) biomarker signature identification by a supervised, multivariate, principal component analysis, and (ii) verification by univariate ANOVA with Bonferroni correction. This method has been previously validated in our hands (Johansson et al., 2018a,b).

After defining a minimal call rate <75%, we screened the biomarker panel through supervised principal component analysis, according to the algorithm first described by Hastie et al. (2013), which includes the following steps:

(1) For each biomarker, compute the standardized univariate logistic regression coefficient which represents the effect size for the outcome (presence or absence of POTS).
(2) Using an arbitrary effect size threshold θ from the list $0 \leq \theta_1 < \theta_2 < \cdots < \theta_K$.
    (a) Form a reduced data matrix consisting of only those biomarkers whose univariate coefficient exceeds θ in absolute value, and compute the principal components of this matrix.
    (b) Use these principal components in a multivariate logistic regression model to predict POTS status.
(3) Select the threshold θ which gives the best predictive accuracy by 10-fold cross-validation.

[1]http://www.olink.com/products/document-download-center

Thereafter, for the verification of the selected biomarkers we applied a conservative univariate ANOVA approach, using a Bonferroni-adjusted significance level of $p < 0.05$/number of PCA-selected biomarkers. Box plots were generated to display the distribution of biomarker levels between groups.

Furthermore, we performed both univariate and multivariate ordinary least square linear regression and logistic regression models for bivariate correlation between plasma level of selected biomarkers and maximum orthostatic heart rate change (ΔHR) or POTS status, respectively. In multivariate regression models we adjusted for age and sex. Finally, we performed a quantile-regression analysis in order to identify differing relationships at different quartiles of HR changes during HUT. The mean estimates and standard errors of the beta-coefficients for the imputed datasets were combined with Rubin's rules (Rubin, 1987). Statistical analyses were carried out using IBM SPSS Statistics version 25 (SPSS Inc., Chicago, IL, United States) and R Statistical Software (version 3.4.4; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Biomarker Signature Discovery

The dataset consisted of 396 patients (113 POTS and 283 controls). Baseline characteristics of the study population by POTS status are shown in **Table 1**. There was no significant difference in characteristics between the whole cohort and complete cases without missing data (**Supplementary Table S2**). Since the principal component analysis requires pairwise complete data, we did not include markers with high missingness, i.e., above 35%. This filter resulted in removal of 9 biomarkers: erythropoietin, interleukin-2, interferon-gamma, tumor necrosis factor, carcinoembryonic antigen, vascular endothelial statin, lipopolysaccharide-induced tumor necrosis factor (TNF)-alpha factor, myeloid differentiation primary response protein, MHC class I polypeptide-related sequence A. Univariate logistic

TABLE 1 | Baseline characteristics of the study population.

Characteristic	POTS− (n = 283)	POTS+ (n = 113)	P-value
Age	31.47 (9.85)	26.27 (8.41)	<0.001
Female sex, *n* (%)	189 (66.8)	83 (73.5)	0.241
BMI, Kg/m^2	24.33 (4.14)	22.69 (3.50)	<0.001
SBP supine, mmHg	120.07 (14.16)	120.41 (14.20)	0.833
DBP supine, mmHg	69.98 (8.21)	70.22 (8.22)	0.788
HR supine, bpm	68.86 (11.87)	71.13 (11.57)	0.084
SBP HUT min, mmHg	112.34 (13.35)	107.58 (16.24)	0.003
DBP HUT min, mmHg	71.83 (9.11)	72.46 (10.58)	0.552
HR HUT max, bpm	84.77 (13.77)	112.41 (15.63)	<0.001
Smoking, *n* (%)	58 (20.5)	16 (14.2)	0.188

P-values for differences between the groups are shown as mean and SD for continuous variables and as percentages for categorical variables; DBP, diastolic blood pressure; HR, heart rate; HUT min/max, lowest/highest value during passive head-up tilt test; IHD, ischemic heart disease; POTS, postural orthostatic tachycardia syndrome; SBP, systolic blood pressure.

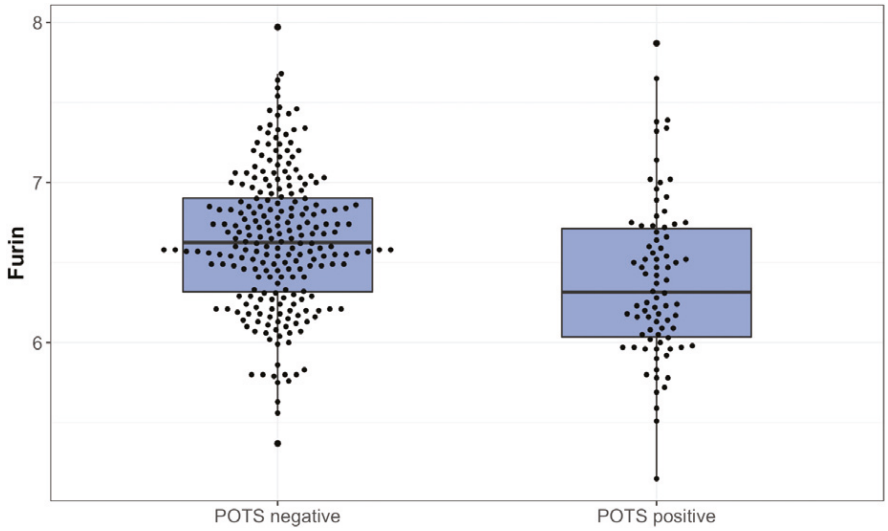

FIGURE 1 | The plasma levels of proconvertase furin, expressed on a log2 scale, presented in relation to POTS status. Data are shown as a box and whisker plot with median in the box and the whiskers representing the 5th and 95th percentiles in relation to plasmatic biomarker levels.

regression was performed for each of the 48 biomarkers. Heatmap representation of the data showing the hierarchical clustering of the 48 biomarkers by POTS status is shown in **Supplementary Figure S2**.

The regression coefficients were then standardized by dividing the coefficient with its standard error. All possible thresholds (Standardized coefficient (θ) ranging from minimum to maximum with 0.05 increments) were used to select groups of biomarkers and construct principal components (PCs). The outcome variable (POTS status) was then regressed onto the 1st two PCs from each group of biomarkers using the binomial link function. This step identified the group of biomarkers which gave the best classification accuracy. The threshold that gave the best classification accuracy (POTS+ vs. controls) was selected by ten-fold cross-validation. The following four biomarkers reached this threshold: carbonic anhydrase IX; receptor tyrosine-protein kinase erbB-2; Fms-related tyrosine kinase 3 ligand; and proconvertase furin.

Biomarker Verification

All PCA-selected biomarkers except carbonic anhydrase IX differed significantly in pairwise comparison, but only proconvertase furin attained significance after Bonferroni correction (**Figure 1** and **Table 2**). In multivariate regression analysis both POTS status and maximum orthostatic ΔHR were significantly associated with proconvertase furin (**Tables 3**, **4**). Quantile regression analyses investigating the relationships between proconvertase furin and the quartiles of ΔHR did not reveal any obvious threshold effect or step function (**Supplementary Figure S1**). Finally, tertiles of proconvertase furin were inversely proportional to duration of symptoms (ANOVA, *p*-value for multiple comparisons = 0.021), defined as the time elapsed from the first symptom manifestation (presyncope or syncope) in patient's history and the time of diagnostic examination (HUT).

DISCUSSION

In this study, we explored the inflammatory proteomic signature of POTS in order to elucidate pathophysiological mechanisms underlying this particular phenotype of cardiovascular autonomic dysfunction. We demonstrated that POTS is

TABLE 2 | High throughput multiplex analysis biomarkers selected by supervised multivariate principal component analysis.

Biomarker	POTS+ (*n* = 113)	POTS− (*n* = 283)	*P*-value
FUR	6.38 (0.05)	6.58 (0.03)	0.000276*
CAIX	1.53 (0.09)	1.73 (0.06)	0.050109
Flt3L	7.76 (0.05)	7.89 (0.03)	0.012979
ErbB2HER2	7.79 (0.04)	7.92 (0.03)	0.026045

*Plasma concentrations of the assessed proteins are expressed on a log2-scale. Inter-group differences were assessed using analysis of variance method. *Bonferroni-corrected significant values (p < 0.012). CAIX, Carbonic anhydrase IX; ErbB2HER2, Receptor tyrosine-protein kinase erbB-2; Flt3L, Fms-related tyrosine kinase 3 ligand; FUR, furin.*

TABLE 3 | Relationship between POTS status and selected biomarker in univariate and multivariate regression.

Biomarker	Univariate			Multivariate*		
	OR	95% CI	*P*-value	*OR*	95% CI	*P*-value
FUR	0.81	0.73–0.92	<0.001	0.86	0.77–0.96	0.009

**Adjusted for age, sex.*

TABLE 4 | Relationship between changes in heart rate during head-up tilt test and selected biomarker in univariate and multivariate regression.

Biomarker	Univariate			Multivariate*		
	β	95% CI	*P*-value	β	95% CI	P-value
FUR	−7.0	−10.6 to −3.38	<0.001	−4.2	−7.9 to −0.5	0.03

Adjusted for age, sex.

associated with lower circulating levels of proprotein convertases subtilisin/kexin type (PCSK)-3, i.e., proconvertase furin. Interestingly, the earlier the disease starts, the lower the proconvertase furin level.

Our findings confirm and further expand the growing body of evidence pointing to an immune dysregulation as the primary pathophysiological mechanism underlying POTS. However, it is noteworthy that pro-inflammatory cytokines and chemokines, i.e., interleukin-7, interleukin-12 and CXC motif chemokine 13, associated with systemic inflammatory diseases such as systemic lupus erythromatosus or rheumatoid arthritis were not increased among POTS-positive patients.

The Role of Immune System in POTS

The presence of immunoglobulins activating and modulating multiple G-protein coupled receptors (GPCR) linked to the autonomic nervous system has been previously demonstrated in POTS patients (Fedorowski et al., 2017; Yu et al., 2018), as well as their ability to alter dose-response to the natural ligands *in vitro*. Patients suffering from POTS are most frequently young females of childbearing age (Li et al., 2014). Its onset is occasionally preceded by or associated with a viral-like illness or post vaccination which leads to the suspicion that autoimmunity may have an important role in these patients (Li et al., 2014; Brinth et al., 2015). Studies performed recently indicate that patients with POTS have higher prevalence of autoimmune markers and co-morbid autoimmune disorders compared with the general population (Blitshteyn, 2015). Accordingly, nearly one fourth of patients with POTS have positive ANA and one in three have some type of autoimmune markers (Blitshteyn, 2015). Moreover, a high proportion of POTS patients are seropositive for circulating antiganglionic acetylcholine receptor antibodies (Watari et al., 2018).

Finally, presence of antibodies against adrenergic and cholinergic receptors were confirmed in patients suffering from POTS in a number of studies, which could explain the adrenergic-related symptoms including exaggerated heart rate during orthostatic challenge (Ruzieh et al., 2017).

Proconvertase Furin

Proconvertase furin is one of seven proprotein convertase family members that promote proteolytic maturation of proproteins (Barr et al., 1991). Ubiquitously expressed it has been reported to process a variety of secreted factors including cytokines and chemokines such as anti-inflammatory transforming growth factor (TGF)-β1 and secreted TNF-family receptors (Loetscher et al., 1990). Interestingly, previous evidence has suggested that proconvertase furin inhibition may result in a breakdown of peripheral tolerance (Pesu et al., 2008) and development of systemic autoimmune disease (Lisi et al., 2010). In experimental models of rheumatoid arthritis, exogenous proconvertase furin has been successfully used to harness autoimmunity (Lin et al., 2012). This is consistent also with recent findings reporting the association between high proconvertase furin levels and lower systemic activity disease in primary Sjögren's syndrome (Ranta et al., 2018). Considering that proconvertase furin appears to be involved in maintaining immune homeostasis, it is important to note that only one POTS patient in our series had overt autoimmune comorbidity. Consequently, we may reasonably exclude that we have tracked down the effect of parallel autoimmune disorders on proconvertase furin in POTS population.

While the exact source of circulating levels of this protein in POTS patients remains unclear, we speculate that they may reflect a so far undetected viral activity. Indeed, cleavage of the human papilloma virus capsid protein L2 by proconvertase furin is necessary for infection (Richards et al., 2006), while the HIV-1 protein *Nef* is known to bind furin in order to sequester human leukocyte antigen-family receptors in the *trans*-Golgi network (Piguet et al., 2000). Presumably as a countermeasure, proconvertase furin is down-regulated during inflammation in a suppressor of cytokine signaling (Sox)-dependent manner (Guimont et al., 2007).

Taken together, further studies are warranted to confirm these findings in independent populations before proconvertase furin can be considered as an objective serum marker of POTS.

Limitations

There is a number of important limitations that should be addressed. Firstly, inflammatory biomarkers have been sampled only at the time of POTS diagnosis; the lack of serial measurements prevent us to understand whether the alterations seen are a cause or a consequence of POTS, and whether there is any temporal correlation between proconvertase furin levels and the progression of the disease.

Secondly, this is a hypothesis-generating study aimed to discover alternative pathophysiological pathways involved in POTS, requiring external validation in another cohort.

Thirdly, data on important pro-inflammatory cytokines like IL-2, TNF-a, IFN is lacking.

Finally, in order to rule-out possible false positive signals, our findings should be verified and validated with alternative technologies enabling as much sensitive and robust detection and quantification of biomarkers; However, the use of a proximity assay with the requirement for a dual binding event which ensures minimal background signal, and the robust discovery algorithm, based on a sequential two-step process including principal component analysis and a very strict Bonferroni correction, would make a false positive result less likely.

CONCLUSION

Proteomic profiling by proximity extension technique revealed an inflammation-specific biomarker fingerprint in

POTS patients. Circulating levels of proconvertase furin are downregulated in POTS suggesting a complex and intriguing interplay between autoimmune activity and cardiovascular autonomic dysfunction.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of local ethics guidelines, the Local Ethic Committee of Lund University, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by The Regional Ethical Review Board of Lund University (No. 82/2008).

AUTHOR CONTRIBUTIONS

FR, AF, JS, and NA had full access to all the data in the study and took responsibility of the data and accuracy of the data analysis. OM, AF, JS, VH, and JA contributed to the study conception and design. OM and AF contributed to the acquisition of data. All authors analyzed and interpreted the data. AF was the study supervisor. NA and FR did the statistical analysis. JS, FR, AF, and VH drafted the manuscript with critical revision for important intellectual content from all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2019.00301/full#supplementary-material

FIGURE S1 | Heatmap visualization of the proteomics data showing the hierarchical clustering of 48 biomarkers by POTS status.

FIGURE S2 | Quantile regression analysis. Furin regressed on 25th, 50th and 75th quantiles of delta HR max. The x axis is the quantile of delta HR max (black dots in the plots represent the regression coefficient at 0.25, 0.5 (median) and 0.75). The gray bands are the 95% CI of the quantile regression coefficient. The horizontal red and the two horizontal dotted lines are the ordinary least square (OLS) linear regression lines. What you can see here is that 95% CI of the coefficients from quantile regression overlaps widely with OLS lines indicating that Furin does not have differing effects on different quantiles of delta HR max.

TABLE S1 | Immuno-oncology panel: biomarker list.

TABLE S2 | Comparison of baseline characteristics between the whole cohort and complete cases without missing data.

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Paper III

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Proteomic analysis reveals sex-specific biomarker signature in postural orthostatic tachycardia syndrome

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Abstract

Background: Postural orthostatic tachycardia syndrome (POTS) is a variant of cardiovascular (CV) autonomic disorder of unknown etiology characterized by an excessive heart rate increase on standing and orthostatic intolerance. In this study we sought to identify novel CV biomarkers potentially implicated in POTS pathophysiology.

Methods: We conducted a nested case-control study within the Syncope Study of Unselected Population in Malmö (SYSTEMA) cohort including 396 patients (age range, 15–50 years) with either POTS ($n = 113$) or normal hemodynamic response during passive head-up-tilt test ($n = 283$). We used a targeted approach to explore changes in cardiovascular proteomics associated with POTS through a sequential two-stage process including supervised principal component analysis and univariate ANOVA with Bonferroni correction.

Results: POTS patients were younger (26 vs. 31 years; $p < 0.001$) and had lower BMI than controls. The discovery algorithm identified growth hormone (GH) and myoglobin (MB) as the most specific biomarker fingerprint for POTS. Plasma level of GH was higher (9.37 vs 8.37 of normalised protein expression units (NPX); $p = 0.002$), whereas MB was lower (4.86 vs 5.14 NPX; $p = 0.002$) in POTS compared with controls. In multivariate regression analysis, adjusted for age and BMI, and stratified by sex, lower MB level in men and higher GH level in women remained independently associated with POTS.

Conclusions: Cardiovascular proteomics analysis revealed sex-specific biomarker signature in POTS featured by higher plasma level of GH in women and lower plasma level of MB in men. These findings point to sex-specific immune-neuroendocrine dysregulation and deconditioning as potentially key pathophysiological traits underlying POTS.

Keywords: Postural orthostatic tachycardia syndrome, Autonomic nervous system diseases, Syncope, Biomarker, Cardiovascular disease

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Background

Postural orthostatic tachycardia syndrome (POTS) is an autonomic disorder characterized by orthostatic intolerance and high prevalence among young women [1]. As etiology of POTS is largely unknown, effective therapeutic intervention for this syndrome has yet to be developed. Beyond genetic norepinephrine transporter deficiency, several theories have been proposed for the syndrome's etiology, including antiadrenergic autoimmunity [2–4], baroreflex dysfunction [5], deconditioning [6], abnormal mast cell activation [1], excessive sympathetic drive and/or sympathetic denervation [7].

Targeted proteomics, yielding broad screening of circulating proteins with presumed role in cardiovascular pathology, offer promise as a tool for biomarker discovery. High-throughput multiplex protein arrays that rely on common methods such as polymerase chain reactions, require small sample volumes and are available at a fraction of the cost of large-scale platforms. Such a solution may provide an effective resource to discover disease pathways and identify novel therapeutic targets for individualised treatment based on biomarker profiling [8]. The proximity extension assay has been shown to be useful for biomarker discovery in cardiometabolic disease [9], immunology [10], cardio-oncology [11] and neuroscience research [12]. Moreover, multiprotein assays have been used to discover key mechanisms by which CV autonomic dysfunction is associated with increased CV morbidity and mortality [13, 14].

By applying multiple-protein screening based on proximity extension assay technology, we aimed to discover CV disease biomarkers associated with POTS in order to understand better the pathophysiology underlying this unexplained condition.

Methods

Study population

We analysed 994 consecutive patients from the Syncope Study of Unselected Population in Malmö (SYSTEMA). All patients were referred to our specialized syncope unit at Skåne University Hospital in Malmö due to unexplained syncope and/or symptoms of chronic orthostatic intolerance, and have been investigated by CV autonomic tests including head-up tilt testing (HUT), according to existing European guidelines [15]. From the cohort of 994 patients, we selected those age 15–50 years with available proteomics data and either diagnosis of POTS or normal hemodynamic response during passive head-up tilt test (Fig. 1).

The age filter was selected on previous epidemiological studies on POTS incidence [16, 17].As the study cohort (SYSTEMA) is basically a patient cohort and enrollment in the study demands an obligatory examination by tilt testing, normal asymptomatic controls were not available. Thus, in this situation, those, who were tilt-negative i.e. without hemodynamic changes corresponding to overt autonomic dysfunction including vasovagal syncope, orthostatic hypotension (OH) or POTS, were considered normal on the day tests were performed and were taken as 'controls'.

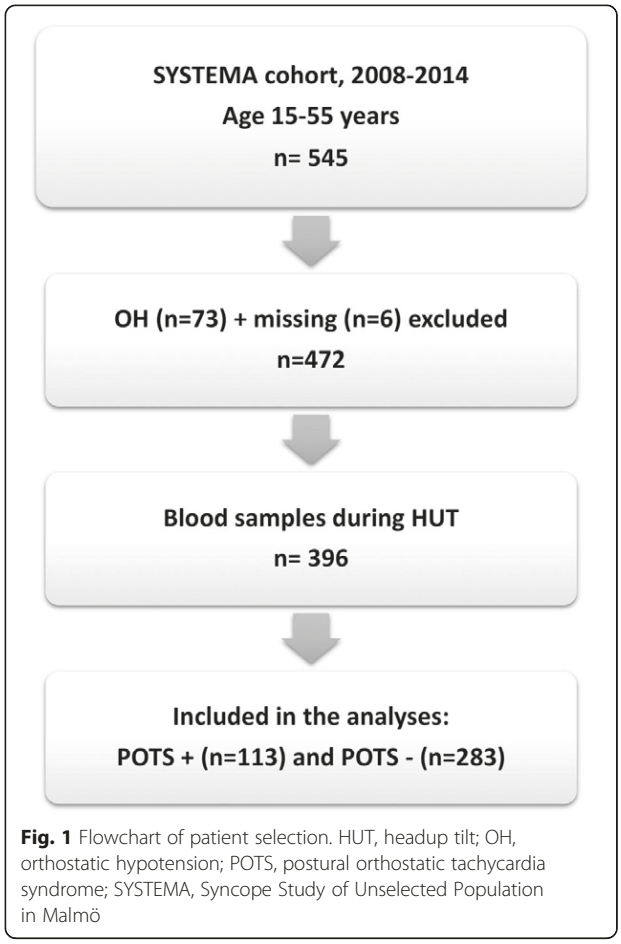

Fig. 1 Flowchart of patient selection. HUT, headup tilt; OH, orthostatic hypotension; POTS, postural orthostatic tachycardia syndrome; SYSTEMA, Syncope Study of Unselected Population in Malmö

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by The Regional Ethical Review Board of Lund University (No 82/2008).

All patients provided their written informed consent.

Examination protocol

Patients were on their regular medication, except for CV drugs, which were discontinued at least 48 h prior to examination. Patients were told to fast for 2 h prior to examination but were allowed to drink water at will. Previous medical history was explored using a standard study questionnaire. During examination performed in the morning, the patients were placed on a tilt table and after a rest period for at least 10 min blood samples were collected through a venous cannula inserted in the forearm. As soon as the hemodynamic parameters were

stable, a standard 70°HUT was carried out according to the Italian protocol recommended by European Society of Cardiology [18]. Beat-to-beat blood pressure and ECG were monitored by a validated non-invasive photoplethysmographic method (Nexfin monitor; BMEYE, Amsterdam, the Netherlands) with a wrist unit and finger cuff of appropriate size [19]. POTS was defined as the reproduction of symptoms of orthostatic intolerance associated with heart rate increase > 30 bpm; or sinus tachycardia > 120 bpm during first 10 min of HUT; or increase > 40 beats/min for those < 18 years of age, with history of orthostatic intolerance for at least 6 months [20]. Patients with signs of OH i.e. with systolic blood pressure (BP) drop ≥20 mmHg or diastolic BP drop ≥10 mmHg [21] during tilt testing were excluded.

Proteomics analysis

Plasma biomarkers were measured from blood samples (total volume: 30 ml) that had been first centrifuged, then stored as 16 × 250 μL aliquots of EDTA plasma in plastic thermotubes, and frozen at − 80 °C. Samples were thawed and examined by the Proximity Extension Assay technology enabling high-throughput, multiplex immunoassays that measure 92 CVD-related human proteins across 96 samples simultaneously using only one microliter of plasma.

Concisely, 92 pairs of oligonucleotide-labeled antibodies (probes) were used to detect the corresponding target proteins in the plasma sample. When the two antibodies are brought in proximity, a new polymerase-chain reaction (PCR) target sequence is formed. The complex is subsequently detected and quantified by standard real-time PCR. Quantitative PCR quantification cycles corrected for technical variation by the Inter-plate Control generate Normalized Protein Expression (NPX) values, which are arbitrary units on log2 scale. A higher NPX value corresponds to a higher protein level. More information about PEA technology, assay performance, quality control and validation is available at the Olink webpage (http://www.olink.com).

Statistical analysis

Patients with available proteomics dataset and a definitive diagnosis of POTS ($n = 113$) or normal hemodynamic response to HUT ($n = 283$), i.e. without vasovagal syncope, OH and abnormal postural tachycardia, and importantly without prevalent cardiovascular disease or hypertension, were selected. Missing data was imputed with multiple imputation by chained equations (MICE) approach. We used predictive mean matching for continuous variables, logistic regression for binary variables, and polytomous regression for categorical variables. All covariates were included in the imputation models. The maximum iteration was set at 20 and convergence was confirmed by visual examination of trace plots (Online Fig. S1).

We explored the change in cardiovascular proteomics associated with POTS through a sequential two-stage process including supervised principal component analysis and univariate ANOVA with Bonferroni correction, as previously described [13, 14, 22].

Univariate and multivariate ordinary least square linear regression and logistic regression models were conducted for bivariate correlation between plasma level of biomarkers and maximum orthostatic heart rate change (ΔHR) or POTS status, respectively. Multivariate regression models were adjusted for age, sex and body-mass index (BMI). We also performed sensitivity analyses stratified by sex and quantile-regression analysis in order to identify differing relationships at different quartiles of HR changes during HUT. The mean estimates and standard errors of the beta-coefficients for the imputed datasets were combined under Rubin's rules [23, 24]. Statistical analysis was performed using IBM SPSS Statistics version 25 (SPSS Inc., Chicago, IL, USA) and R Statistical Software (version 3.4.4; R Foundation for Statistical Computing, Vienna, Austria).

Results

We analyzed 396 patients (female, 69%; age range, 15–50 years) including 113 POTS and 283 controls. Basic characteristics of the study population stratified by POTS status are shown in Table 1. Biomarkers with > 35% missing values, i.e. leptin, were excluded (see Online Table S1), inasmuch pairwise complete data are required for running principal component analysis.

At the stage of biomarker signature discovery, univariate logistic regression was performed for each biomarker and regression coefficients were standardised by dividing

Table 1 Baseline characteristics of the study population

Characteristic	POTS- ($n = 283$)	POTS+ ($n = 113$)	*P*-value
Age (years)	31.5 (9.8)	26.3 (8.4)	< 0.001
Female sex, n (%)	189 (66.8)	83 (73.5)	0.241
BMI, Kg/m^2	24.3 (4.1)	22.7 (3.5)	< 0.001
SBP supine, mmHg	119.9 (14.2)	120.4 (14.2)	0.833
DBP supine, mmHg	69.9 (8.2)	70.2 (8.2)	0.788
HR supine, bpm	68.7 (11.9)	71.1 (11.6)	0.084
SBP HUT min, mmHg	112.2 (13.4)	107.6 (16.2)	0.003
DBP HUT min, mmHg	71.8 (9.1)	72.5 (10.6)	0.552
HR HUT max, bpm	84.7 (13.8)	112.4 (15.6)	< 0.001
Smoking, n (%)	58 (20.5)	16 (14.2)	0.188

P-values for differences between the groups are shown as mean and SD for continuous variables and as percentages for categorical variables.; *DBP* Diastolic blood pressure; *HR* Heart rate; *HUT* min/max, lowest/highest value during passive head-up tilt test; *IHD* Ischemic heart disease; *POTS* Postural orthostatic tachycardia syndrome; *SBP* Systolic blood pressure

the coefficient with its standard error. All possible thresholds (standardised coefficient (θ) ranging from minimum to maximum with 0.05 increments) were used to select groups of biomarkers and build principal components (PCs). POTS status was then regressed onto the first two PCs from each group of biomarkers using the binomial link function. The threshold providing the best classification accuracy (POTS+ vs controls) was selected by ten-fold cross-validation and the following 23 biomarkers were identified: TIM, PSGL1, CTSL1, MB, VEGFD, PIGF, MMP1, GDF15, FAS, TF, AM, UPAR, TNFR2, TRAIL, MCP1, TRAILR2, OPG, CASP 8, HGF, CD40L, GH, PTX3 (see Online Table S2 for acronyms).

At the stage of biomarker verification analysis, nine PCA-selected proteins differed significantly in pairwise comparison, but only GH and MB attained significance after Bonferroni correction (Table 2). Plasma levels of GH were significantly higher in POTS women compared with POTS men ($p = 0.0002$), and both male ($p < 0.0001$) and female controls ($p = 0.003$) (Fig. 2). Conversely, plasma level of MB were significantly lower in POTS men compared with male controls ($p = 0.0009$).

Table 2 High throughput multiplex analysis of biomarkers selected by supervised multivariate principal component analysis. Plasma concentrations of the assessed proteins are expressed on a log2-scale. Inter-group differences were assessed using analysis of variance method. *Bonferroni-corrected significant values ($p < 0.0022$)

Biomarker	POTS+ (***n*** = 113)	POTS- (***n*** = 283)	***P***-value*
TIM	4.32 (0.08)	4.49 (0.05)	0.078
PSGL1	0.69 (0.04)	0.8 (0.02)	0.009
CTSL1	5.3 (0.04)	5.46 (0.03)	0.004
MB	4.86 (0.07)	5.14 (0.06)	**0.0020***
VEGFD	6.7 (0.06)	6.82 (0.03)	0.062
PlGF	7.1 (0.05)	7.21 (0.04)	0.131
MMP1	3.04 (0.14)	3.41 (0.09)	0.029
GDF15	8.22 (0.07)	8.42 (0.05)	0.058
FAS	6.93 (0.04)	7.02 (0.03)	0.045
TF	5.53 (0.04)	5.61 (0.03)	0.120
AM	5.83 (0.09)	6.05 (0.05)	0.029
UPAR	9.66 (0.03)	9.72 (0.03)	0.127
TNFR2	4.82 (0.04)	4.9 (0.03)	0.116
TRAIL	8.44 (0.04)	8.52 (0.03)	0.183
MCP1	3.07 (0.05)	3.24 (0.05)	0.014
TRAILR2	1.21 (0.03)	1.28 (0.02)	0.080
OPG	9.16 (0.04)	9.25 (0.03)	0.095
CASP8	1.45 (0.07)	1.33 (0.05)	0.126
HGF	6.22 (0.04)	6.34 (0.04)	0.030
CD40L	8.57 (0.11)	8.6 (0.07)	0.647
GH	9.37 (0.26)	8.37 (0.2)	**0.0019***
PTX3	1.38 (0.06)	1.28 (0.04)	0.124
SRC	7.66 (0.07)	7.77 (0.03)	0.087

In multivariate regression analysis adjusted for age (Fig. S1) and BMI and stratified by sex both POTS status and maximum orthostatic ΔHR were significantly associated with lower MB level in men and higher GH level in women (Tables 3, 4, and Online S3, S4).

Quantile regression analyses investigating the relationships between selected biomarkers and quartiles of ΔHR did not reveal any obvious threshold effect or step function.

Discussion

Using a novel high-throughput proteomics platform, we examined 92 cardiovascular plasma biomarkers in patients diagnosed with postural orthostatic tachycardia syndrome and controls with normal orthostatic response. We identified higher plasma levels of growth hormone and lower plasma levels of myoglobin in patients with POTS, whereas other biomarkers did not significantly differ between the two groups. We also documented sex-specific patterns of significance where lower MB level in men and higher GH level in women were independently associated with both binary POTS status and changes in heart rate during head-up tilt test, even after adjustment for age and BMI.

Growth hormone

Higher levels of growth hormone in patients with postural orthostatic tachycardia syndrome, notably in females, was an unexpected finding that deserves detailed commentary. GH is an anabolic neuropeptide regulating carbohydrate and lipid metabolism via complex interactions with insulin and insulin-like growth factor-1 (IGF-1). The secretion of GH from the anterior pituitary gland is stimulated by growth hormone releasing hormone (GHRH) and inhibited by somatostatin and a negative feedback loop of IGF-1 [25]. The hormone is released in a pulsatile manner with significant circadian rhythm and peak discharge occurring at night-time, approximately one hour after sleep onset [26]. Previous studies reported higher incidence of CV events related to increased levels of GH [26]. Since long-term prognosis in POTS patients is still unknown, the effects of increased GH levels on CV outcome in this patient population remain to be explored.

There is a number of possible mechanistic explanations for higher levels of GH observed in POTS patients. Firstly, an increase in plasma GH concentration could be the result of proinflammatory cytokines acting as negative regulatory signals fine-tuning the action of hormones and growth factors. Tumour necrosis factor alpha

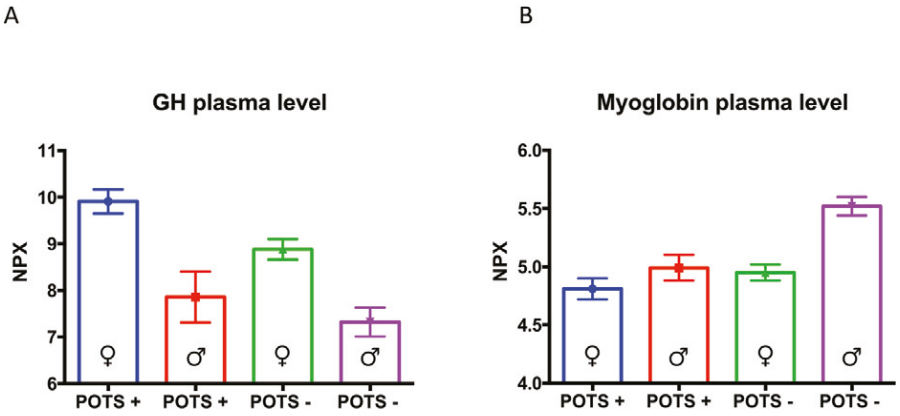

Fig. 2 The plasma levels of growth hormone (GH) (panel A) and myoglobin (panel B), expressed on Normalised Protein Expression (NPX) on a log2 scale, are presented in relation to POTS and sex status. Data are shown as a box and whisker plot with median in the box and the whiskers representing the 5th and 95th percentiles in relation to plasmatic biomarker level

and interleukin-1 beta are believed to cause IGF-1 resistance by weakening downstream signalling in myoblasts and this could eventually cause increased release of GH due to negative feedback [27]. This hypothesis could be tested in future studies comparing the levels of inflammatory mediators in POTS with healthy subjects. The current body of knowledge is very sparse and with only IL-6 reported to be elevated in POTS [28].

Secondly, it has been shown that POTS patients present with autoantibodies against alpha- and beta-receptors, which belong to the G-protein coupled receptor (GPCR) family of rhodopsin type [3]. Receptors for GHRH (GHRHr) are distributed on the anterior pituitary gland, which also belong to GPCR family, though of slightly different secretin type. Hypothetically, higher levels of GH might be the result of abnormal stimulation of GHRHr by circulating anti-GPCR antibodies. Nevertheless, the presence of such specific and functionally active anti-GHRHr autoantibodies has not been yet demonstrated in POTS patients.

Thirdly, patients with POTS usually have a lean body type with lower BMI which could be related to increased lipolysis due to higher GH [29]. Unfortunately, plasma insulin levels or HbA1c were not tested in this group of patients.

Fourthly, octreotide - a somatostatin analogue classically used to control hypersomatotropism in acromegaly through the inhibition of GH action and GH secretion [30] – has been reported to be an effective treatment for POTS patients by reducing upright tachycardia and symptoms of orthostatic intolerance due to its splanchnic vasoconstrictor effect [31]. Splanchnic blood flow has been shown indeed to be increased in the supine posture and to progressively increase during incremental tilt in POTS patients [32]. In the absence of peripheral sympathetic denervation, locally mediated vasodilation - involving vasoactive autacoids such as vasoactive intestinal polypeptide, substance P, calcitonin gene-related peptide, and nitric oxide - has been proposed as a possible explanation for splanchnic pooling. Interestingly, insulin-like growth factor-1 (IGF-1), that is synthesized in the liver, is secreted into the blood under the control of GH, and is known to induce peripheral vasodilation via NO synthase and/or potassium channel activity [33]. It may, therefore, provide a possible mechanistic link among the observed high levels of GH, splanchnic pooling and octreotide efficacy in reducing symptoms in POTS patients.

Finally, the relative syndrome-dependent inactivity among POTS patients might hypothetically lead to a reversal or gross disturbance in circadian patterns of GH release. However, deconditioning as an underlying pathophysiology of POTS and to possibly related syndromes such as chronic fatigue syndrome has not been supported by recent studies, and other mechanisms such as low ventricular filling have been proposed [34, 35]. It remains to be demonstrated if physical training may reverse GH level abnormality.

Table 3 Sex-specific relationship between POTS status and selected biomarkers in univariate and multivariate regression analysis

Biomarker	Women					
	Univariate			Multivariate[a]		
	OR	95% CI	***P*-value**	***OR***	95% CI	***P*-value**
MB	0.92	0.81–1.04	0.193	0.94	0.83–1.07	0.378
GH	1.04	1.01–1.06	0.003	1.03	1.00–1.06	0.022
	Men					
MB	0.77	0.67–0.89	0.001	0.80	0.70–0.93	0.004
GH	1.01	0.98–1.04	0.422	1.00	0.97–1.03	0.981

[a]Adjusted for age and body mass index

Table 4 Sex-specific relationship between changes in heart rate during head-up tilt test and selected biomarker in univariate and multivariate regression analysis

Biomarker	Women					
	Univariate			Multivariate[a]		
	β	95% CI	***P***-value	***β***	95% CI	***P***-value
MB	−1.81	−5.35 to 1.72	0.317	−0.66	−4.35 to 3.03	0.726
GH	1.28	0.48 to 2.08	0.002	0.95	0.13 to 1.76	0.024
	Men					
	β	**95% CI**	**P-value**	***β***	**95% CI**	**-value**
MB	−7.44	−12.05 to −2.82	0.003	−5.0	−9.46 to − 0.54	0.03
GH	0.52	−0.53 to 1.58	0.331	0.06	−0.94 to 1.06	0.908

[a]Adjusted for age and body mass index

The influence of gender on serum concentrations of GH has been the object of previous investigations [36, 37]. In many species, including rats, mice, and humans, the temporal pattern of pituitary GH secretion is sex-specific (episodic in males, more frequent in females) and leads to sex differences in downstream signaling pathways in target tissues [38]. In our study blood samples were obtained in the morning after two-hour fasting, which ascertained stabilisation of GH concentration, but it has been also reported as a possible explanation for the observed high concentrations of GH in women, as if something in the morning - which may be regarded as very mild stress of fasting - could trigger a GH burst in almost all of the women but in very few of the men [37]. This could be because of gender differences in the sensitivity of the pituitary or hypothalamus to the GH-releasing effects of mild stress. Furthermore, there is quite robust evidence in the literature to suggest that endogenous estrogens play a major role in increased GH secretion in women compared with men [39]. Interestingly, in our study we observed POTS women presenting with significantly higher levels of GH than women without POTS. This could be the result of (i) complex interactions amongst sex-related and sex-unrelated immune-neuroendocrine mechanisms, (ii) sexually dimorphic patterns of GHRH secretion (iii) impaired cerebrovascular autoregulation [40], (iv) chronic deconditioning [41], and/or (v) hitherto unknown pathways.

Myoglobin

Myoglobin, an oxygen and iron binding protein found in muscle cells, is usually increased when muscle tissue damage occurs though small amounts are normally present in plasma. Our analysis revealed decreased plasma myoglobin in POTS patients, notably males, compared with controls. The results are difficult to interpret as the method used for protein detection in this study provides only relative values within the analyzed sample, not absolute values that could be translated to clinically useful cut-off levels. Of note, the values reported here are likely not indicative of muscle damage, since patients were free of such clinical suspicion at inclusion. More likely, the levels detected here are linked to small amounts of myoglobin found in plasma in the absence of muscle damage. Lower myoglobin levels found in POTS might be a result of immobilisation and limited physical activity in those patients, possibly a consequence of cardiovascular deconditioning or chronic fatigue [1]. Thus, although deconditioning may not be causally related to POTS [34], such deconditioning may be the result of reduced exercise tolerance experienced by many POTS patients [42]. In addition, the lower myoglobin levels found in POTS may be seen to parallel reduced iron stores, which is, in turn, associated with POTS [43]. Unfortunately, data on iron status was not available in our study population. Moreover, considering the role of myoglobin in muscle metabolism [44], one may also hypothesize that myoglobin may, indeed, also have a role in the pathophysiology of POTS, even if such hypotheses are highly speculative at this stage.

Even though we did not measure lean body mass, POTS patients in our study did have lower BMI. It remains to be explored why this association is limited to men only.

Beyond growth hormone and myoglobin, we could observe aberrations in other proteomics biomarkers, although not achieving the adjusted significance level. However, the overall impression was that there were only slight differences in the analysed biomarkers. It may indicate that POTS is an inflammatory condition involving hitherto unknown pathophysiological mechanisms deserving further explorative and experimental studies.

Final remarks

This study points the way toward application of other proteomics technology in POTS research. For instance, it might be useful to use similar technology to screen a large number of antibody variable region epitope

sequences for coexisting antibodies that might be directed toward candidate G-protein coupled receptors considered relevant to POTS [3, 45] and related conditions such as chronic fatigue syndrome.

Limitations

There are some limitations that must be addressed. Firstly, our control group included symptomatic individuals, who had normal hemodynamic response to tilt testing, but were referred to our center due to unexplained syncope and/or symptoms of orthostatic intolerance.

Secondly, this is a single-center experience with limited generalisability, also due to uneven sex distribution and lack of age matching; accordingly, and in the need of an external validation cohort, our findings have to be interpreted as hypothesis-generating.

Thirdly, our findings are based on one-off measurement precluding information about causality and temporal correlation of selected biomarkers with the progression of the disease, onset and burden of clinical symptoms.

Fourthly, we acknowledge the lack of information about menstrual cycle and use of hormonal contraceptives, as previous studies demonstrated that the hormonal fluctuations that occur during the normal menstrual cycle may alter autonomic regulation of blood pressure during various environmental stimuli [46], and the intake of exogenous estrogen has been shown to increase plasma levels of GH [37].

Fifthly, we recognize the lack of plasma GH determination on clinically validated high-sensitivity chemiluminescence sandwich immunoassay platforms for direct correlation with GH levels measured with PEA technology.

Finally, in order to rule-out false positive signals, our findings should be validated with alternative technologies enabling sensitive and robust detection and quantification of biomarkers. However, the use of a proximity extension assay technique, with the requirement for a dual binding event ensuring minimal noise signal, and the robust discovery algorithm would make a false positive result very unlikely.

Conclusions

Our study confirms and extends the concept that high throughput multiplex analysis for protein profiling may considerably improve the understanding of POTS. Targeted cardiovascular biomarkers profiling based on proximity extension assay technology identified higher plasma level of growth hormone and lower plasma level of myoglobin respectively in women and men with POTS compared with subjects presenting normal hemodynamic response during head-up tilt test. This observation would be in keeping with the presence of distinct and sex-specific pathophysiological pathways underlying this unexplained syndrome.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12872-020-01465-6.

Additional file 1.

Additional file 2: Table S1. Absolute and percentage missingness data. **Table S2.** Multiplex Cardiovascular Disease Panel: Biomarker List

Additional file 3.

Additional file 4.

Abbreviations

ANOVA: Analysis of variance; BMI: Body mass index; CV: Cardiovascular; GH: Growth hormone; GHRH: Growth hormone releasing hormone; GHRHr: Growth hormone releasing hormone receptors; GPCR: G-protein coupled receptor; HUT: Head-up tilt testing; IGF-1: Insulin-like growth factor-1; HR: Heart rate; MB: Myoglobin; NPX: Normalised protein expression units; PICO: Problem/patient/population intervention/indicator comparison outcome; POTS: Postural orthostatic tachycardia syndrome; SYSTEMA: Syncope study of unselected population in Malmö

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Preliminary results of the current work submitted and accepted for poster presentation at the 2019 European Heart Rhythm Association (EHRA) Congress.

Authors' contributions

FR, AF, JS, NA had full access to all the data in the study and take responsibility of the data and accuracy of the data analysis. OM, AF, JS, VH, JA contributed to the study conception and design. OM, AF contributed to the acquisition of data. All authors analysed and interpreted the data. AF was the study supervisor. NA, FR did the statistical analysis. JS, FR, AF, VH, RS, EH drafted the manuscript with critical revision for important intellectual content from all authors. All authors have read and approved the manuscript.

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Availability of data and materials

Supporting data will be made available upon request to the corresponding authors.

Ethics approval and consent to participate

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by The Regional Ethical Review Board of Lund University (No 82/2008). All patients gave their written informed consent. Written informed consent was obtained from a parent or guardian for participants under 16 years old.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Malmö POTS symptom score: Assessing symptom burden in postural orthostatic tachycardia syndrome

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Abstract. Spahic JM, Hamrefors V, Johansson M, Ricci F, Melander O, Sutton R, et al. Malmö POTS symptom score: Assessing symptom burden in postural orthostatic tachycardia syndrome. *J Intern Med*. 2022;**OO**:1–9.

Background. Postural orthostatic tachycardia syndrome (POTS) is a common cardiovascular autonomic disorder characterized by excessive heart rate (HR) increase on standing and symptoms of orthostatic intolerance, posing significant limitations on functional capacity. No objective tool exists to classify symptom burden in POTS.

Methods. We conducted a case–control study in 62 POTS patients and 50 healthy controls to compare symptom burden between groups using the newly developed, self-rating, 12-item, Malmö POTS Score (MAPS; 0–10 per item, total range 0–120) based on patients own perception of symptoms through visual analogue scale assessment. We have also explored correlations between symptom severity assessed by MAPS, basic clinical parameters and postural haemodynamic changes.

Results. POTS patients showed significantly higher total MAPS score (78 ± 20 vs. 14 ± 12, $p < 0.001$), higher baseline systolic blood pressure (BP), diastolic BP and HR ($p < 0.001$) compared with healthy controls. The most prominent symptoms in POTS were palpitations, fatigue and concentration difficulties. Haemodynamic parameters on standing were significantly correlated with palpitations in POTS after adjustment for age and sex (lower systolic and diastolic BP, and higher HR) ($p < 0.001$ for all). Orthostatic HR was significantly associated with concentration difficulties and total MAPS score. The optimal cut-point value of MAPS to differentiate POTS and healthy controls was ≥42 (sensitivity, 97%; specificity, 98%).

Conclusions. Symptom severity, as assessed by MAPS score, is fivefold higher in POTS compared with healthy individuals. The new MAPS score can be useful as a semiquantitative system to assess symptom burden, monitor disease progression and evaluate pre-test likelihood of disease.

Keywords: autonomic dysfunction, postural orthostatic tachycardia, POTS, scoring system, symptoms

Introduction

Postural orthostatic tachycardia syndrome (POTS) is a common multifaceted condition, characterized by autonomic dysfunction and an exaggerated adrenergic response in the upright position [1]. Prior to COVID-19, POTS affected an estimated 1–3 million individuals in the United States [2], predominantly young females aged 15–45 at diagnosis [3–5]. Current diagnostic criteria for POTS, outlined by the European Society of Cardiology (ESC) Guidelines on Syncope, include symptoms of orthostatic intolerance for at least 3–6 months together with orthostatic heart rate (HR) increase >30 bpm (HR increase > 40 bpm in patients <19 years) or HR exceeding 120 bpm in upright position in the absence of orthostatic hypotension (ESC recommendation: Class IIa, level C) [4, 6].

The precise aetiology of POTS remains unknown, although the haemodynamic changes are thought

to reflect convergence of multiple pathophysiological processes including peripheral autonomic neuropathy, hypovolemia, elevated sympathetic tone, deconditioning and auto-antibodies to cardiovascular receptors [7]. Previous studies have shown that certain immunological stressors such as viral infections, trauma, surgery and pregnancy predispose to the onset of POTS, which suggest an autoimmune component in the pathophysiological pathway [3, 5]. Long-term prognosis is poorly explored, though around 50% of patients may experience symptom reduction or recover spontaneously within 1–3 years [3]. Recently, POTS has been indicated as a possible form of post-acute-COVID-19 sequelae [8–10].

Symptoms often include both cardiac symptoms (rapid palpitations, light-headedness, presyncope, chest discomfort, and dyspnoea) and non-cardiac symptoms (reduced concentration, brain fog, headache, nausea, fatigue, blurred vision, and exercise intolerance) [11]. Cardiovascular symptoms experienced by POTS patients are thought to reflect haemodynamic changes of upright posture [3]. Although the primary symptoms of POTS occur when standing and improve when sitting or lying, patients often have other chronic symptoms and comorbidities that cannot be explained by orthostatic intolerance including chronic fatigue, gastrointestinal disorders, nausea, fibromyalgia and joint hypermobility [12], contributing to the complexity of symptoms in POTS [5, 13–15].

To date, no objective tool exists to classify symptom burden and assess disease progression in POTS. Integrating the best evidence available with our clinical expertise, we aimed to design a symptom scoring system and test it in the setting of an established cohort of POTS patients.

Further, we explored the correlation between haemodynamic postural changes and symptoms at upright posture.

Methods

Study design and population

Patients with POTS were recruited from the Syncope Study of Unselected Population in Malmö (SYSTEMA) cohort, including over 2200 patients with unexplained syncope and/or orthostatic intolerance syndromes examined in a tertiary care centre in Malmö, Sweden between 2008 and 2021 [16]. The study population included 62 symptomatic patients with disease history ≥6 months [3] and confirmed POTS diagnosis by head-up tilt test (HUT) on their first evaluation according to current European Society of Cardiology syncope guidelines [17]. Patients with POTS were excluded in the setting of POTS following COVID-19, iron deficiency, sleep disorders, endocrine disorders, arrhythmias, volume depletion and psychiatric illness.

Fifty healthy controls were recruited among staff members or volunteers at the study site and underwent an active standing test at the Clinical Research Unit of the Department of Internal Medicine, Skåne University Hospital, Malmö, Sweden. Healthy controls reported no symptoms of orthostatic intolerance and no history of syncope and were age- and sex-matched with the POTS group as closely as possible.

Investigation protocol

All participants were asked to discontinue cardiovascular medications (beta-blockers, ivabradine and midodrine) 48 h prior to testing. Fasting was started during the evening before, with unrestricted water consumption.

POTS patients were investigated with head-up tilt test. The protocol included supine rest for 10 min preceding table elevation to 60–70° for 20 min, and optional nitroglycerine provocation according to the Italian protocol, exclusively for patients with unexplained syncope and negative unmedicated phase of head-up tilt [18]. Beat-to-beat blood pressure and ECG were recorded continuously using a non-invasive validated method (Nexfin monitor, BMEYE, Amsterdam or Finapres Nova, Finapres Medical Systems, Enschede, The Netherlands) [19]. POTS diagnosis was defined by symptoms of orthostatic intolerance lasting for ≥6 months associated with pathological head-up tilt test or active standing test showing HR increase >30 bpm (HR increase > 40 bpm in patients <19 years) or HR exceeding 120 bpm in upright position (prior to nitroglycerine administration), within 10 min and in the absence of orthostatic hypotension [3].

Healthy controls were examined with active standing. Before initiating the active standing test, each participant had to lie quietly in a supine position for 10 min, after which baseline supine blood pressure and HR were measured. The subject was then asked to perform active standing for 10 min. Blood pressure was measured at 1, 3, 5 and 10 min

using an automatic BP monitor (Omron M6, Kyoto, Japan). An average of two measurements was used for group comparisons.

Malmö POTS symptom score

We developed a novel, questionnaire-based, symptom scoring system, the Malmö POTS Symptom Score (MAPS), for self-assessment of symptom burden using a visual analogue scale graded from 0 (no symptoms) to 10 (very pronounced symptoms), based on patients' own perception of 12 commonly reported symptoms: five cardiac symptoms (palpitations, dizziness, presyncope, dyspnoea and chest pain) and seven non-cardiac symptoms (gastrointestinal symptoms, insomnia, concentration difficulties, headache, myalgia, nausea and fatigue) during the previous 7 days (Supporting Information: MAPS questionnaire in original Swedish language and English translation). The selection of questions included in MAPS was based on available literature on self-reported symptomatology in POTS, expert opinion, and authors' own clinical experience [2, 3, 11, 12, 20–22]. Three co-authors (JS, VH and AF) analysed data from a large international POTS survey based on 4835 individual online questionnaires [20] and selected the most prevalent symptoms reported by at least 75% of participants. The symptoms were grouped into 12 main categories. Gastrointestinal symptoms were merged into one category (pain in the stomach, diarrhoea or constipation) as majority of POTS patients report varying and alternating symptoms from gastrointestinal tract. The questionnaire was preliminary tested on 10 consecutive newly diagnosed POTS patients who confirmed an adequate coverage of their POTS-related symptoms. The score ranges from 0 to a maximum score of 120 points(see Graphical Abstract).

All study participants were asked to complete the MAPS questionnaire. The total score was calculated for evaluation and comparison between POTS patients and healthy controls. Frequency distribution graphs for each symptom reported in the MAPS questionnaire are available in the Supporting Information.

Statistical analysis

Group characteristics were reported as mean and standard deviation or median and interquartile range, as appropriate, for continuous variables, and as counts and percentages for categorical variables. Between-groups comparison was performed using independent samples *t*-test and Mann–Whitney *U* test, as appropriate.

Construct validity

Linear regression analysis was performed with individual symptom scores ($n = 12$) and total score as dependent variables, and following variables as independent variables: age, sex, duration of symptoms and haemodynamic parameters obtained during standing test in univariable and multivariable-adjusted models. The multivariable-adjusted model was created by entering age and sex as covariates. Receiver operating characteristics (ROC) curves were constructed to test the ability of MAPS to predict POTS status. The optimal cut-point value of MAPS score to discriminate between POTS and healthy controls was calculated by Youden method. p-Value of <0.05 was considered statistically significant. Data were analysed using SPSS software version 27 (SPSS, Chicago, IL, USA) and easyROC: an interactive web-tool for ROC curve analysis using R language environment, and OptimalCutpoints R package available from the Comprehensive R Archive Network (CRAN).

Ethics approval

This study complies with the Declaration of Helsinki. The study was approved by the Regional Ethical Committee in Lund, Sweden (82/2008; and 2017/295), and all study participants provided informed written consent.

Results

Baseline characteristics are shown in Table 1. Patients with POTS were younger (age 28 ± 9 years vs. age 32 ± 10 years; $p = 0.016$) and had higher baseline systolic blood pressure, diastolic blood pressure and HR compared with controls ($p < 0.001$) (Table 1). Female participants were strongly overrepresented in both groups, POTS (88.7%) and controls (82%).

Correlation of MAPS with symptom severity in POTS

POTS patients reported a fivefold higher symptom burden compared with controls (mean total MAPS score, 78 ± 20 vs. 14 ± 12, $p < 0.001$) (Figures 1 and 2). The three most severe symptoms reported by POTS patients were palpitations (7.7 ± 1.7), fatigue (7.6 ± 2.5) and concentration difficulties (7.2 ± 2.1) (Figure 2), although all 12 symptom categories were distinctly elevated in POTS patients with an average score of at least five per category

Table 1. *Baseline characteristics of the study population*

Baseline characteristics[a]	POTS ($n = 62$)	Controls ($n = 50$)	p-Value
Age (years)	28 (9)	32 (10)	0.016
Female sex, n (%)	55 (89)	41 (82)	0.067
SBP supine, mmHg	125 (12)	114 (10)	<0.001
DBP supine, mmHg	76 (8)	69 (8)	<0.001
HR supine, bpm	82 (15)	64 (11)	<0.001
SBP standing 3 min, mmHg	127 (14)	113 (10)	<0.001
DBP standing 3 min, mmHg	89 (11)	75 (8)	<0.001
HR standing 3 min, bpm	115 (19)	81 (14)	<0.001

Note: Values are reported as mean (standard deviation) for continuous variables and as count (percentage) for categorical variables. p-Values for differences between the groups are shown.
Abbreviations: DBP, diastolic blood pressure; HR, heart rate; POTS, postural orthostatic tachycardia; SBP, systolic blood pressure.
[a]HR and BP values here presented and compared were collected from head-up tilt test for POTS patients and from active standing for controls.

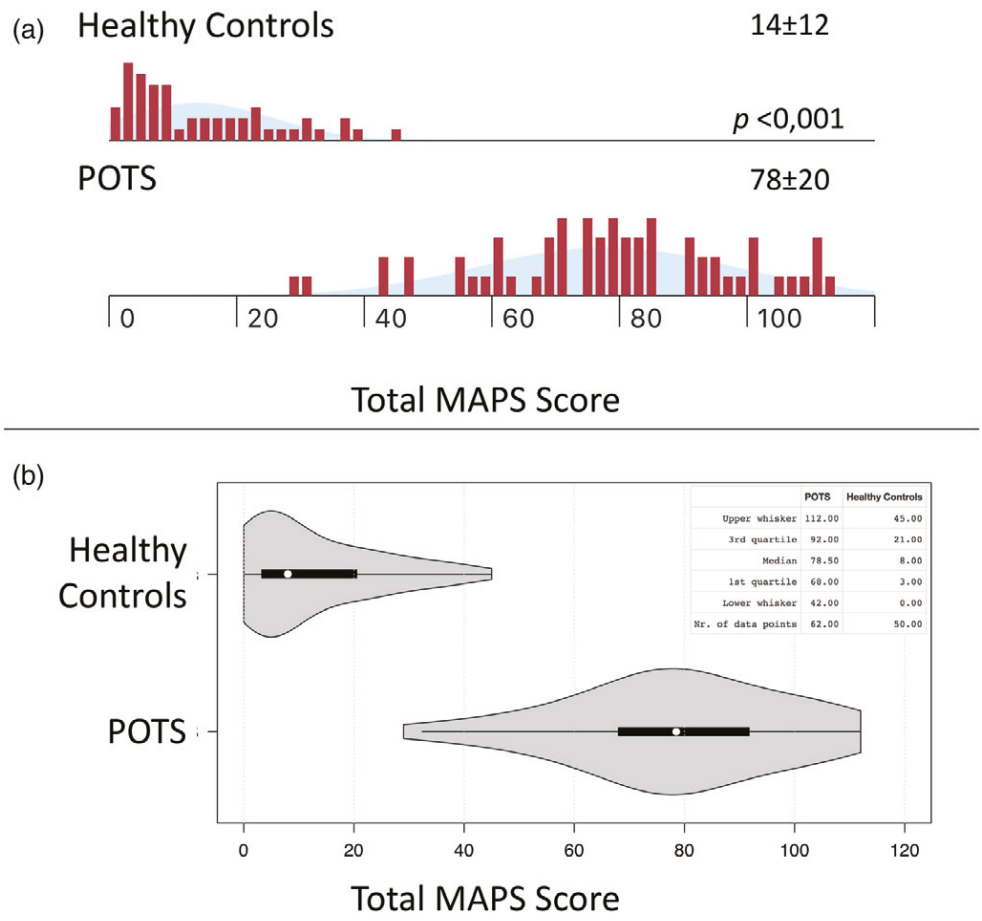


Fig. 1 *Total Malmö POTS Symptom Score (MAPS) score in healthy controls and postural orthostatic tachycardia syndrome (POTS). The maximum score is 120, and the lowest 0. Distribution of MAPS score by (a) frequency bar chart and (b) violin plots.*

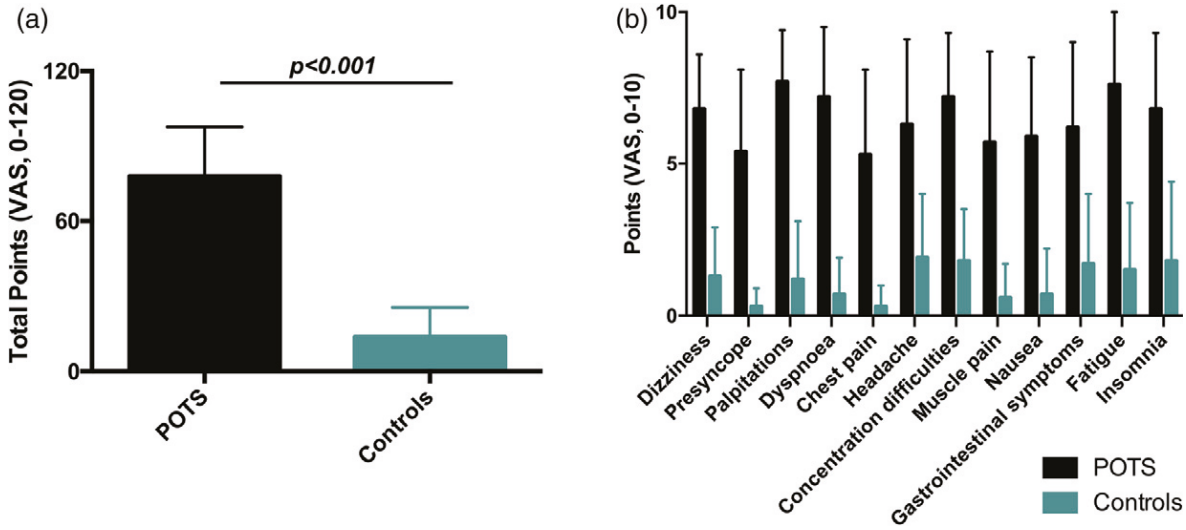


Fig. 2 *Total Malmö POTS Symptom Score (MAPS) score and individual item scores. (a) Total MAPS score; (B) individual item scores: $p < 0.001$ for all pairwise comparisons. POTS, postural orthostatic tachycardia syndrome.*

(see Graphical Abstract). In contrast, healthy controls reported the highest symptom burden due to headache (1.9 ± 2), concentration difficulties (1.8 ± 1.7) and insomnia (1.8 ± 2.6) (Figure 2). The optimal cut-off value to discriminate between POTS and healthy controls was a total MAPS score of ≥42, yielding excellent sensitivity (97%; 95% confidence interval [CI] 0.89–0.99) and specificity (98%; 95% CI 0.89–0.99) (Figure 3).

Haemodynamic parameters and symptom severity by MAPS score

We observed a strong positive correlation between total MAPS score and orthostatic HR at 3 min of head-up tilt in POTS patients and during active standing for healthy controls (Figure 4). Likewise, we observed a significant increase of total MAPS score across supine and orthostatic HR quartiles in POTS compared with controls (Figure 5).

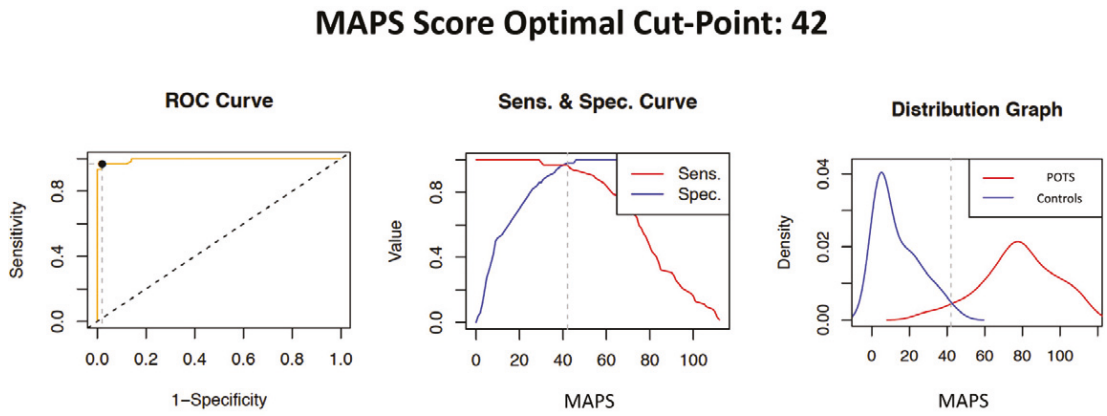
We found, furthermore, a significant correlation between all haemodynamic parameters (systolic blood pressure, diastolic blood pressure and HR) at 10 min of head-up tilt and symptoms in POTS compared with healthy controls in the univariate analysis ($p < 0.001$). After adjustment for age and sex, only palpitations remained significantly associated with all haemodynamic parameters in POTS ($p < 0.001$), whereas concentration difficulties were significantly associated with increased HR during head-up tilt in POTS patients ($p < 0.05$). Other cardiovascular and non-cardiovascular symptom categories did not demonstrate significant associations with haemodynamic parameters, neither supine nor on standing.

Discussion

In this study, we used the newly developed 12-item score Malmö Postural orthostatic tachycardia syndrome (POTS) score (MAPS) as a potential tool to assess the symptom severity in POTS patients by comparing the difference in symptom burden between POTS patients and healthy controls.

Notably, we observed a fivefold increase in symptom severity reported by patients with POTS compared with healthy controls in all symptom categories, which agrees with earlier studies confirming a decreased quality of life in POTS [5, 23, 24]. Currently, there are no clinical self-administered instruments specifically dedicated to evaluation of symptom burden in POTS. Those applied in the clinic and research are usually based on previous score systems created for other conditions such as autonomic failure or orthostatic hypotension and are inevitably associated with lesser precision.

In our study, patients with POTS were predominantly younger females, which is in line with previous studies [3–5, 25]. Also, all baseline haemodynamic parameters obtained during head-up tilt



MAPS score 42	Value	Lower Limit	Upper Limit
Sensitivity	0.968	0.888	0.996
Specificity	0.980	0.894	0.999
PPV	0.984	0.911	0.998
NPV	0.961	0.867	0.999
Positive LR	48.837	6.948	336.982
Negative LR	0.033	0.008	0.129

Fig. 3 *Optimal cut-off point identification by Youden method to discriminate between healthy controls and postural orthostatic tachycardia syndrome (POTS). Total Malmö POTS Symptom Score (MAPS) ≥ 42 likely indicates pathology. LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.*

were significantly higher in POTS patients. Interestingly, we found that elevated HR and lower systolic and diastolic blood pressure at 10 min of head-up tilt were associated with more pronounced palpitations experienced by patients with POTS, whereas concentration difficulties were associated with elevated HR on standing. These findings in accordance with sympathetic overactivity in POTS patients and may be partly explained by increased circulating norepinephrine levels in these patients as shown by previous studies [26].

Considering the pathophysiology of POTS, we know that some subtypes of POTS are suggested to be related to hypovolemia and peripheral autonomic neuropathy [7]. The observed increase in HR can be seen both as a compensatory mechanism but also as a pathophysiological pathway where catecholamine release is a priori exaggerated [26]. Moreover, reported symptoms may reflect a reduction in the capacity to autoregulate cerebral blood flow and the metabolic sequalae of persistent tachycardia [27].

A previous study in 32 POTS patients used the Composite Autonomic Symptom Scale 31 (COMPASS-31) questionnaire [28], which is a validated tool assessing autonomic symptoms across six domains, initially established to assess symptoms of neurogenic autonomic disorders, found that the COMPASS-31 did not fully ascertain key symptoms relevant for assessment of POTS, and important differences occurred when applying COMPASS-31 to POTS with domain weighting deemphasizing certain significant symptoms relevant to POTS [29].

The generalized autonomic dysfunction together with fatigue and concentration difficulties [27] observed in POTS cannot be explained by single organ dysfunction but is likely a result of multiple mechanisms [15]. In this study, we have particularly noted the association between two main symptoms, palpitations, concentration difficulties and haemodynamic parameters on standing. HR increase seems to be the only parameter that is associated with both concentration

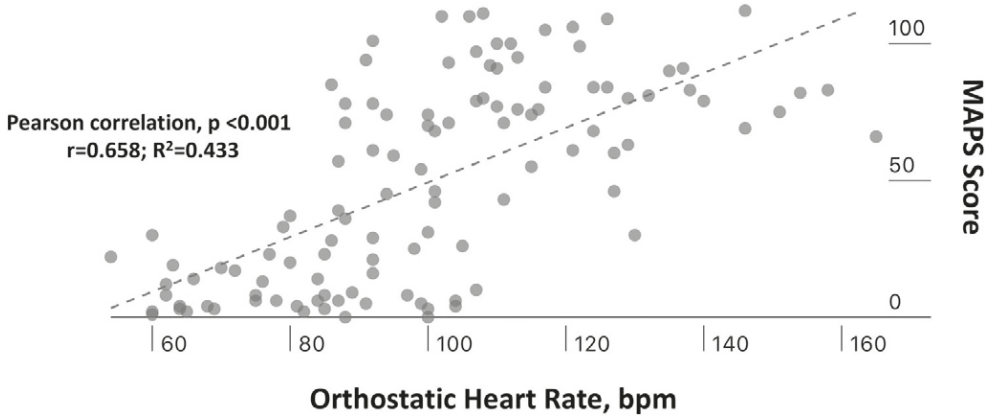


Fig. 4 *Relationship between total Malmö POTS Symptom Score (MAPS) score and orthostatic heart rate. Heart rate was measured at 3 min of standing (head-up tilt test [HUT] for postural orthostatic tachycardia syndrome [POTS] and active standing for controls).*

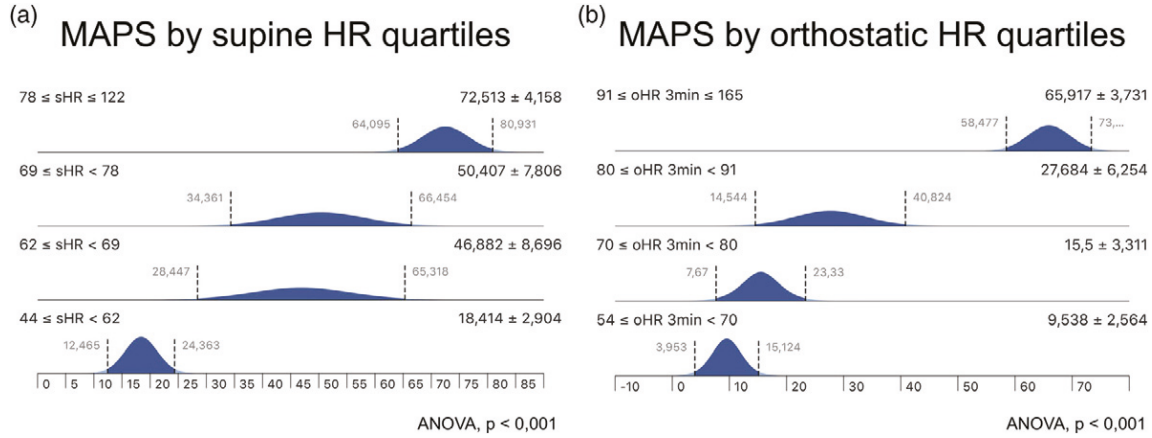


Fig. 5 *Malmö POTS Symptom Score (MAPS) score by supine (panel a) and orthostatic (panel b) heart rate (HR) quartiles in the overall study population. HR was measured at 3 min of standing. HUT, head-up tilt test; oHR, orthostatic heart rate (bpm); POTS, postural orthostatic tachycardia syndrome; sHR, supine heart rate (bpm).*

difficulties and palpitations, although blood pressure also seems to play an important part in symptom generation. We hypothesized that if vasoactive medications were added as a treatment strategy in patients showing signs of lower blood pressure during head-up tilt, compensatory tachycardia could possibly decrease and concentration difficulties improve. Thus, future studies assessing serial assessments using the MAPS symptom score in relation to treatment of POTS would be of interest.

Strengths and limitations

To date, no objective tool exists to gauge symptom burden, assess disease progression and response to treatment in POTS. This is the first study to derive a questionnaire-based scoring system to assess severity of symptom in POTS. Further, MAPS score yields excellent specificity and sensitivity for classifying POTS. Moreover, the self-administered nature of the MAPS questionnaire eliminates possible interviewer bias associated with physician-assisted or health worker-assisted administration. This study is limited by its single-centre, observational nature and selection and referral biases. The score warrants external validation and replication in a larger multicentre setting. The group of POTS patients was highly selected, meaning that the results may not represent symptoms

presented by POTS patients with less pronounced disease.

Perspectives

POTS is a complex syndrome with symptoms of orthostatic intolerance significantly impacting and limiting the functional capacity. The newly developed MAPS score may be useful for the structured evaluation of symptom severity in patients with POTS. The observed increase in individuals reporting POTS-like symptoms following COVID-19 emphasizes the urgent need for an easily self-administered questionnaire, such as MAPS, to assess the severity of the syndrome [30]. Future prospective studies should aim to test the utility of MAPS score to evaluate pre-test likelihood of disease, to assess health-related quality of life, to track symptom progression/regression over time, to monitor the outcome of patients and to measure the effect of the therapies either for clinical purposes or for research. Additional studies are also necessary to validate the score in other populations and clinical settings. Finally, comparative studies using validated autonomic symptom scores such as COMPASS-31 and non-disease-specific questionnaires assessing the total functional capacity are warranted.

Conclusions

Patients with POTS report a broad-spectrum symptom burden significantly exceeding predefined normality when compared with healthy individuals, posing significant limitations on functional capacity. The newly developed Malmö POTS symptom score may be useful for the structured evaluation of symptom severity in patients with POTS, offering excellent specificity and sensitivity. Future studies are warranted to test the utility of MAPS score in other populations and clinical settings, and also to track symptom progression/regression and to monitor treatment response over time.

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Conflict of interest

Artur Fedorowski receives lecture fees from Medtronic Inc., Biotronik, and Finapres Medical Systems. Richard Sutton reports acting as a consultant to Medtronic Inc. and membership of the Speakers bureau of Abbott Laboratories (SJM) Corp. and holds stock in Boston Scientific Corp. and Edwards Lifesciences Corp. All other authors declare that there is no conflict of interest.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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Supporting Information

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Supplement Material ■

