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Gastrointestinal aspects on postural orthostatic tachycardia syndrome

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Gastrointestinal aspects on postural orthostatic tachycardia syndrome

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Hanna Tufvesson



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DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine, Lund University, Sweden

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Faculty opponent

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Abstract:

Postural orthostatic tachycardia syndrome (POTS) is a disorder characterized by cardiovascular autonomic dysfunction and a broad range of symptoms, in which gastrointestinal (GI) complaints are common. The pathophysiology of POTS remains incompletely understood. Peripheral neuropathic mechanisms have been proposed, but much of the existing literature is retrospective, uncontrolled, and lacks comparison with matched healthy populations. Neuropathic features have also been described in primary GI dysmotility disorders.

The overall aim of this thesis was to explore potential links between cardiovascular autonomic dysfunction, GI manifestations, and peripheral neuropathic features in POTS, through comparisons with healthy control participants, to contribute to a more integrated understanding of POTS.

In papers I–IV, 43 patients with POTS, previously investigated with orthostatic tests, were recruited. Participants completed questionnaires covering socioeconomic factors, lifestyle habits, dietary patterns, and GI- and neuropathic symptoms. Clinical routine examinations as well as sensory neurological tests were performed, and 3-mm skin punch biopsies were obtained for assessment of intraepidermal nerve fiber density (IENFD). Blood samples were analyzed for nutritional parameters, inflammatory markers, autoantibodies associated with systemic autoimmune diseases, markers of mast cell activity, long-term glycemic control, and gut hormones involved in GI motility, secretion, blood flow, and glucose homeostasis. In Paper III, biobank material, including fasting blood samples, from an additional cohort of 42 patients with POTS was analyzed. The results were compared with matched healthy controls.

POTS was associated with a high symptom burden across multiple organ systems, with impact on socioeconomic participation and lifestyle factors. GI symptoms were frequent, with nausea being reported by 79%, and bloating and flatulence being the most severe symptoms. Chronic GI symptom burden was inversely associated with the magnitude of orthostatic heart rate increase and was significantly linked to reduced psychological well-being. Fasting insulin levels were strongly associated with blood pressure levels in POTS but not in healthy controls, which was interpreted as sympathetic nervous system activation. In contrast, objective evidence of nutritional deficiency, chronic mast cell disorders, classical systemic autoimmunity, metabolic disturbances, gut-hormonal abnormalities, or generalized peripheral neuropathy was scarce and did not meaningfully differ from healthy controls.

In conclusion, POTS is characterized by substantial GI and neuropathic symptom burden that is not readily explained by measurable structural pathology in an unselected cohort. A multifactorial and potentially centrally mediated disease model may be considered.

Key words: postural orthostatic tachycardia syndrome, gastrointestinal, peripheral nervous system, glycated hemoglobin, insulin, autoimmunity, incretins, nutrition, sociodemographic factors

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MADE IN SWEDEN 

To my family: Mats, Karin and Eva.

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- I. **Tufvesson H**, Hamrefors V, Roth B, Fedorowski A, Ohlsson B. Sociodemographic factors, nutritional status, and inflammatory markers in patients with postural orthostatic tachycardia syndrome. *Acta Neurol Scand*. 2023 March 17: 2023:3992718. doi:10.1155/2023/3992718.
- II. **Tufvesson H**, Hamrefors V, Fedorowski A, Hansson M, Ohlsson B. Gastrointestinal symptoms in patients with postural orthostatic tachycardia syndrome in relation to hemodynamic findings and immunological factors. *Front Physiol*. 2024 Jan 29: 15:1342351. doi:10.3389/fphys.1342351.
- III. **Tufvesson H**, Roth B, Johansson M, Hamrefors V, Fedorowski A, Lindholm E, Ohlsson B. Gut hormones in POTS and their relation to hemodynamic parameters and gastrointestinal symptoms. *Submitted manuscript*.
- IV. Ekman L*, **Tufvesson H***, Englund E, Dahlin LB, Ohlsson B. Symptoms and objective signs of peripheral sensory neuropathy in POTS and correlations to gastrointestinal symptoms. *PLoS One*. 2025 Jul 3: 20(7):e0327549. doi: 10.1371/journal.pone.0327549.

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Related publications by the author

Tufvesson H, Hamrefors V, Ohlsson B. Mechanisms behind diffuse idiopathic peripheral neuropathy in humans – a systematic review. *Scand J Gastroenterol*. 2023 Jun 3:58(6): 572-582.

Tufvesson H, Dreja J, Ekberg O, Leander P, Månsson S, Ohlsson B. Quantified small bowel motility in patients with ulcerative colitis and gastrointestinal symptoms: a pilot study. *Acta Radiol*. 2020 Aug 17:62(7): 858-866.

Errata

In Paper I, the 95% confidence interval (CI) for the odds ratio (OR) regarding educational level was misprinted in Table 1 of the original published article. The OR for lower educational level in patients, after adjustment for age and sex, was reported as **0.386 [0.216–689]; p for trend = 0.001** but should read **0.386 [0.216–0.689]**.

Abbreviations

ΔHR	Change in heart rate
ANA	Antinuclear antibodies
Anti–Scl-70	Anti–topoisomerase 1 antibodies
ANS	Autonomic nervous system
BMI	Body mass index
BP	Blood pressure
Bpm	Beats per minute
CI	Confidence interval
CNS	Central nervous system
CRP	C-reactive protein
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
ENS	Enteric nervous system
GI	Gastrointestinal
GIP	Glucose-dependent insulintropic polypeptide
GLP-1	Glucagon-like peptide-1
HbA1c	Glycated hemoglobin
HR	Heart rate
HRV	Heart rate variability
HSD/EDS	Hypermobility spectrum disorder/Ehlers-Danlos syndrome
Hz	Hertz
IBS	Irritable bowel syndrome
IBS-SSS	Irritable bowel syndrome severity scoring system
ICD	International classification of diseases
IENFD	Intraepidermal nerve fiber density
IQR	Interquartile range
MCAS	Mast cell activation disorder
MFV	Multifrequency vibrometry

NSS	Neuropathy symptom score
OR	Odds ratio
PYY	Peptide YY
PNS	Peripheral nervous system
POTS	Postural orthostatic tachycardia syndrome
SFN	Small fiber neuropathy
SYSTEMA	Syncope study of unselected population in Malmö
VAS-IBS	Visual analogue scale for irritable bowel syndrome
VPT	Vibration perception threshold

Preface

I am a gastroenterologist and a consultant in internal medicine. I began my clinical work at Skåne University Hospital in Malmö in 2013. From the very beginning, senior colleagues encouraged me to consider research as a complement to clinical practice. At that time, I was hesitant, as I wanted to focus on clinical practice.

As the years passed and my clinical experience grew, I found myself increasingly interested in trying to understand symptoms reported by a particular group of patients, most often women. They presented unexplained gastrointestinal complaints, histopathological signs of inflammation that did not fit into the classical inflammatory bowel diseases, food intolerance, and other widespread symptoms. I read about their complaints, and investigated the patients I met, but rarely found satisfactory explanations for their problems.

During this period, I also conducted a small research project as part of my training in internal medicine, together with my future doctoral supervisor. Small bowel motility was examined using magnetic resonance imaging in patients with ulcerative colitis and was found to be reduced compared with healthy controls. This finding puzzled me, as ulcerative colitis is traditionally considered a disease restricted to the large bowel. While writing the discussion of the resulting manuscript, I encountered literature on the enteric and autonomic nervous system that gave me a deeper interest. Gradually, I began to see connections between autonomic nervous system physiology and the diffuse, seemingly unrelated symptoms I struggled to explain in clinical practice.

I shared my growing interest with my supervisor, who in turn told me about a pilot study that caught my attention: could there be a link between dysfunction of the cardiovascular autonomic nervous system, the enteric nervous system, and measurable neuropathic changes in peripheral skin? I was instantly drawn to this idea, and the results of that work form the foundation of this thesis.

The doctoral journey has been challenging, and there were moments when I considered giving up. However, step by step, I have gained insight into a patient group I previously knew little about. Looking back, I realize that this work has deepened my understanding of the “mysterious” group of patients that first captured my interest earlier in my career. My efforts to understand the different components of the nervous system have not only contributed to this thesis but have also strengthened my clinical confidence and inspired me to learn more.

Introduction

Postural orthostatic tachycardia syndrome (POTS) is a chronic disorder of orthostatic intolerance characterized by an excessive heart rate (HR) increase upon standing, in the absence of hypotension. The symptom complex has been described with several names throughout history, but the current definition was introduced by Low and colleagues at the Mayo Clinic in 1993 (1). Following the COVID-19 pandemic, the awareness of POTS has increased due to its overlap with the post-COVID syndrome (2). In parallel, improved diagnostic coding, including adoption of a unique ICD-10 code in the United States and an upcoming ICD-11 classification internationally, may facilitate epidemiological research in POTS (Fig 1) (3, 4).

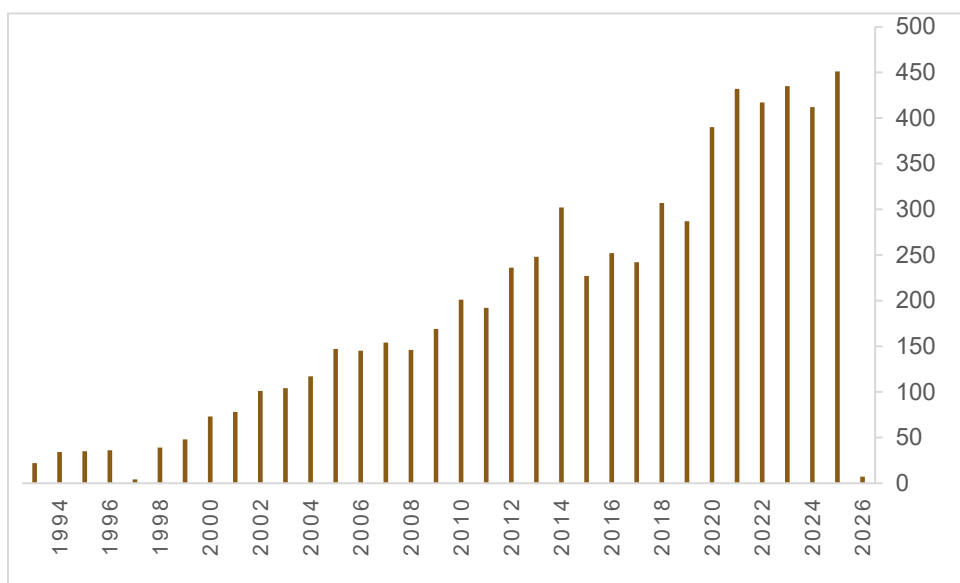


Figure 1. POTS publications on PubMed

Number of publications per calendar year matched by the search terms “postural orthostatic tachycardia syndrome” or “postural tachycardia syndrome” on PubMed.

Typical symptoms include palpitations, light-headedness, and fatigue upon standing. Additionally, non-cardiovascular complaints, particularly gastrointestinal (GI) symptoms, are frequently described, and may overlap or co-occur with other

non-cardiovascular disorders. This multisystem presentation may contribute to diagnostic delay, excessive or insufficient diagnostic work-up, and other inadequate management strategies (5, 6). In any case, the disorder has substantial limitations on quality of life.

Over the past decades, advances in autonomic testing and clinical research have drawn increased attention toward the systemic nature of POTS. However, the pathophysiology remains incompletely understood, and much of the existing literature is based on retrospective or uncontrolled studies, often lacking comparison with matched healthy populations.

The conceptual background for this thesis originates from a double case study conducted in Malmö in 2018. Two patients with well-characterized neuropathy of the GI tract also presented with symptoms of autonomic and peripheral neuropathy. Cardiovascular autonomic testing demonstrated orthostatic intolerance in both patients, where one of them fulfilled criteria for POTS. Reduced intraepidermal nerve fiber density (IENFD), obtained by skin punch biopsies, was observed in both patients (7). These observations raised the question whether a shared neuropathic mechanism could explain symptoms reported by patients who present in different clinical settings, with POTS serving as a clinical model of cardiovascular autonomic dysfunction.

The purpose of this thesis is to explore potential common denominators linking cardiovascular autonomic dysfunction, GI disturbances, and peripheral neuropathic features in POTS, using systematic comparisons with matched healthy controls.

Pathophysiological framework – the nervous system

Since POTS is proposed to be a disorder of the cardiovascular autonomic nervous system, it is essential to understand the basic structure and function of the nervous system, and normal autonomic cardiovascular regulation.

The nervous system comprises the central and peripheral nervous system (CNS and PNS), with different anatomical locations, protective structures, and functions (8, 9). The somatic nerves of the PNS convey sensory information from muscles, skin and sensory organs to the CNS, and carries motor commands from the CNS to skeletal muscle. In contrast, the autonomic nervous system (ANS) regulates involuntary functions of the smooth muscles of visceral organs, vasculature, and glands through afferent input and efferent output, subdivided into the sympathetic and parasympathetic divisions (10-12).

Preganglionic sympathetic neurons synapse with postganglionic neurons in the para- or prevertebral ganglia, or they may project directly on the adrenal medulla. Postganglionic sympathetic neurons provide output to visceral organs, vasculature

and skin structures throughout the body, including the limbs. In contrast, postganglionic parasympathetic neurons reside within plexuses closer to, or within the target organs. The postganglionic parasympathetic nerve axons are short, and they do not innervate limbs (13).

Preganglionic neurons in both divisions of the ANS release acetylcholine, activating nicotinic receptors (10, 14). Acetylcholine is also mainly secreted by postganglionic parasympathetic neurons, acting on muscarinic receptors, whereas the principal transmitter of postganglionic sympathetic neurons is norepinephrine, acting on α - and β -adrenergic receptors. In the adrenal medulla, epinephrine and norepinephrine are secreted directly into the bloodstream (13).

Very simplified, the parasympathetic nervous system is activated during rest, enhancing GI motility and secretion, while sympathetic activity supports “fight or flight” responses during stress, with major cardiovascular effects and increased blood flow to skeletal muscles (Fig 2) (12, 15).

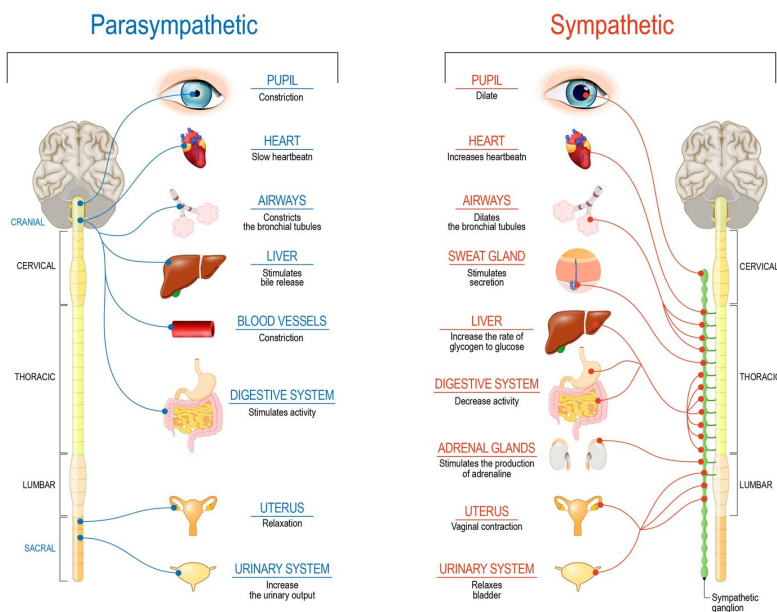


Figure 2. Schematic illustration of the autonomic nervous system

Schematic illustration of the autonomic nervous system. *Image adapted from iStock; © [ttsz]/iStock.*

The nervous system's influence on the gastrointestinal tract

The third major component of the ANS is the enteric nervous system (ENS), which is located within the GI tract and can operate independently of central autonomic control (8). The neurons of the ENS secrete many different neurotransmitters and neurohumoral substances and communicate internally via reflex pathways. Nerves within the myenteric plexus regulate the circular and longitudinal muscles of the gut, while the submucosal plexus regulate glands, blood flow, and absorption (12, 13). Although intrinsically organized, the ENS is heavily modulated by the ANS, and deliver extensive visceral afferent input to the CNS (8, 11, 16).

The CNS processes and integrates all somatic and autonomic information and coordinates appropriate responses. The hypothalamus, structures of the limbic system, and various cortical areas are considered the most important structures regarding regulation of the ANS, in turn regulating the ENS function (10, 13, 17).

Autonomic response to postural changes in health and disease

The cardiovascular system maintains adequate blood flow to the brain, internal organs, skin, and skeletal muscles under varying physiological demands. Arterial baroreceptors, located primarily in the aortic arch and carotid sinuses, respond to changes in arterial pressure and signal through visceral afferent neurons to the vasomotor center in the brain stem medulla (5, 14).

When moving from supine to a standing position, gravity causes 500–1000 mL of blood to pool in the lower limbs and the pelvic region. Prolonged standing maintains gravitational blood pooling by increased distal hydrostatic pressure, with fluid movements from intravascular to interstitial spaces (18, 19). Consequently, venous return to the heart decreases, with reduced cardiac output and reduced BP. This triggers the baroreceptor reflexes, leading to the following sympathetically coordinated outputs:

- Arteriolar constriction, to increase total peripheral resistance.
- Venous constriction, to mobilize venous blood toward the thorax and increase preload.
- Increased myocardial contractility, to increase stroke volume.
- Reduced vagal influence on the sinoatrial node, combined with direct sympathetic stimulation, to increase HR.

Together, these responses rapidly work to restore blood pressure (BP) and stabilize cerebral perfusion following the postural orthostatic change (14, 15). After returning to supine position, the BP eventually increases, and the opposite reactions as described above occur. After prolonged standing, the slower working renin-

angiotensin-aldosterone system is activated to increase blood volume and vascular resistance, as an additional compensatory mechanism (20).

Altered hemodynamic and symptomatic response to postural changes is caused by abnormalities in any of the autonomic reflex pathways peripherally or centrally. The severity of the orthostatic disturbances depends on the underlying etiology (10). Common disorders of orthostatic intolerance and cardiovascular autonomic dysfunction include orthostatic hypotension, orthostatic vasovagal syncope, and POTS (21, 22).

Postural orthostatic tachycardia syndrome

Clinical presentation

Orthostatic symptoms

Common orthostatic symptoms include sustained palpitations, blurred vision, fatigue, nausea, and light-headedness. These symptoms occur when the patient moves from supine to standing position, and conversely, the patient experience symptom relief when moving back to supine (23).

Non-orthostatic symptoms

Apart from orthostatic complaints, widespread non-orthostatic symptoms and overlapping comorbidities are commonly reported in POTS, including migraine, fibromyalgia, chronic fatigue, and hypermobility spectrum disorders/Ehlers-Danlos syndrome (HSD/EDS) (6, 23). GI complaints and irritable bowel syndrome (IBS) are very common in POTS (24, 25). In addition, autoimmune, inflammatory, and allergic comorbid disorders have been reported (6, 26-29). Finally, symptoms of somatic peripheral sensory small and large fiber neuropathy have been reported (6). Non-orthostatic symptoms do not resolve when the patient moves from standing to supine.

Definition

Diagnostic criteria include sustained HR acceleration ≥ 30 beats per minute (bpm), or HR ≥ 120 bpm, within 10 minutes after transition from supine to standing position, with no significant drop in BP. To fulfil diagnosis, the patient must experience chronic symptoms of orthostatic intolerance as described above, present for at least 3–6 months (23, 30). Alternative causes of sinus tachycardia and orthostatic symptoms must be ruled out, including anemia, infections, endocrine disorders, medication effects, and primary psychiatric conditions. In addition,

severe deconditioning due to prolonged bed rest should be considered and excluded (23, 31).

Epidemiology

A typical POTS patient is female (80–95%) and between 15 and 45 years of age (32, 33). The symptoms may evolve gradually, or acute, often precipitated by a viral infection (in 20–50%), or other stressors such as pregnancy or surgery (23). In a large survey of 3835 patients with POTS, the median diagnostic delay from symptom onset was 24 months, but for some respondents up to 10 years. In the same cohort, 42% reported some improvement, but 44% reported gradual worsening of symptoms (6).

The true prevalence of POTS is not completely known due to lack of proper ICD-code, unawareness and discrepancies in interpretations of diagnostic criteria among clinicians (5, 34). However, previous studies have suggested a prevalence of 0.1–1% in the USA (32).

Socioeconomic consequences and lifestyle implications

According to international studies, a POTS diagnosis is associated with substantial consequences regarding employment rates, education, economic income loss, job modifications, as well as sick leave days. Additionally, the diagnosis may have social impact on relationships and the patient's ability to move out from the parents (33, 35). Patients often report exercise intolerance, and physical activity is reduced (36, 37). Dietary habits, smoking, and alcohol intake have been sparsely studied in POTS.

Pathophysiological mechanisms

The integrated CNS-ANS-ENS network is essential for maintaining homeostasis. Disturbances at any level may therefore contribute to the multisystem symptoms observed in POTS, including orthostatic intolerance, GI dysfunction, and sensory disturbances.

Defects in autonomic cardiovascular regulation with alterations along the baroreflex arc with reduced cardiovagal modulation and altered sympathetic activation have been described in POTS, but the underlying mechanisms are still not fully known. Several mechanisms have been proposed, which probably overlap with each other. None of the mechanistic pathways below can alone adequately explain all of the systemic symptoms, and the evidence supporting some of these mechanisms are scarce, with lack of control groups (5, 23).

Hyperadrenergic states

Norepinephrine levels are in general higher in POTS than in controls, although more pronounced in a subset of patients, which represents the hyperadrenergic phenotype. These patients have elevated BP upon hemodynamic testing and increased sympathetic activity, possibly reflecting impaired norepinephrine reuptake, dysfunctional peripheral sympathetic nerves, and/or compensatory activation to counteract venous pooling (23, 38). Increased sympathetic activity affect GI function as well, with reduced motility and glandular secretion, but also pro-inflammatory GI effects (39).

Autonomic neuropathy

Peripheral sympathetic denervation of small unmyelinated nerve fibers has been estimated in ~ 50% of POTS patients, possibly with associations to peripheral sensory small fiber neuropathy (SFN). Indirect and direct tests have shown signs of denervation, including sympathetic super-sensitivity. Denervation of distal sympathetic neurons may lead to inadequate vascular constriction, resulting in excess pooling of blood in the legs upon standing with compensatory sympathetic response (36, 40). Neuropathic changes may be widespread, with evidence of disturbances of enteric nerves in POTS (41).

Hypovolemia and blood distribution abnormalities

A physiologic reduction of cardiac mass and blood volume can be seen after prolonged bedrest (“deconditioning”). Hence, deconditioning is not the primary cause of POTS but may exert an additive effect on chronic orthostatic intolerance (5, 42). Low relative blood volume has been associated with abnormalities in the renin-angiotensin-aldosterone system and increased post-prandial blood pooling in the splanchnic bed in POTS, with secondary activation of baroreflex pathways and sympathetic response (18, 23, 42-45). It is not known whether splanchnic blood-pooling, or factors regulating GI blood flow, are associated with GI symptoms in POTS.

Autoimmunity

Autoimmunity has been suggested as underlying mechanism, due to female predominance, and the tendency of POTS to follow infections or other precipitating events (32). Some systemic autoimmune diseases are associated with autonomic dysfunction, including enteric dysmotility (46) and conversely, autoimmune diseases and associated antibodies have been described in the POTS population (26, 27). Additionally, specific antibodies directed at nicotinic, muscarinic, and sympathetic receptors in the ANS have been found in POTS but results are conflicting and there is still no evidence of one causative specific antibody (47-49).

Mast cell activation and other inflammatory mechanisms

Mast cell activation has been proposed in POTS, particularly in patients with atypical symptoms including increased GI symptoms and skin rashes, and a co-morbid link between allergic disorders and POTS has been described (28, 50, 51).

Collectively, these mechanistic frameworks suggest a multifactorial disorder involving both neural and vascular components, leading to dysregulation across several organ systems.

Multisystemic neuropathic manifestations in POTS

Gastrointestinal manifestations

GI complaints are common in POTS and are consistently reported across several cohorts (25, 52, 53). In a large survey, approximately 30% of the patients with POTS reported a diagnosis of IBS (6). A pooled analysis of six studies found that nausea and abdominal pain were the most common non-cardiovascular symptoms in POTS (25), highlighting the importance of dedicated GI investigations.

GI symptoms in POTS can be orthostatic and/or non-orthostatic. Previous studies have shown that the typical orthostatic symptoms nausea and vomiting may improve in supine position and potentially respond to cardiovascular-focused therapies (29, 52, 54, 55). In contrast, most other GI symptoms are non-orthostatic and typically require alternative management strategies (29).

Similarities with irritable bowel syndrome

Symptom complexes including chronic abdominal pain, bloating, and bowel habit disturbances are diagnostic of IBS (56), which share similarities with GI symptoms in POTS (25). One major pathophysiological explanation to IBS is disturbances along the communication axis between the enteric nerves, within the nerve circuits of the ANS, and finally higher centers of the CNS, called “the gut-brain axis” (16, 56). To learn more about GI symptoms in POTS, validated questionnaires commonly used in IBS and other disorders of gut-brain interaction were evaluated in Paper II.

Since POTS is characterized by dysfunction of the cardiovascular autonomic nervous system (23), it is plausible that autonomic disturbances extend to the ENS and contribute to GI symptomatology. Dysfunction of the ENS may lead to enteric dysmotility, presenting with abnormal gastric or intestinal transit, abdominal pain, and irregular bowel habits (57). Enteric dysmotility, including altered intestinal and/or colonic myoelectric activity, and alterations in GI or colonic transit time has been demonstrated in IBS (56). Disturbances in GI motor function are increasingly

recognized in POTS, where delayed *or* rapid gastric emptying have been demonstrated in 18% and 48%, respectively (41). Other abnormalities in GI motility have been shown as well (41, 58, 59). Whether orthostatic tachycardia severity reflects overall autonomic dysfunction and predicts chronic GI symptom burden remains unclear. This potential association was explored in Paper II.

Poor dietary habits have been documented in IBS, including low consumption of fruits and vegetables and high intake of fast food, sweets, and soft drinks (60). These dietary patterns correlate with symptom severity, and dietary improvement significantly reduces symptoms (61, 62). Nutritional deficiencies may also contribute to autonomic neuropathy. Vitamin B12 and iron deficiency are established causes of peripheral neuropathy and orthostatic hypotension (63-65). In POTS, dietary habits have not been explored. Low B12 levels have been reported in adolescents with POTS (66), but the extent of nutritional deficiencies in adult POTS patients remains unexamined. Dietary habits and micronutrient status in POTS was investigated in Paper I.

Another proposed mechanism for GI symptoms in IBS is low-grade inflammation with increased permeability and prevalence of inflammatory cells in the gut mucosa (56, 67). Increased mast cell infiltration and local tryptase levels have been found in colonic mucosa in IBS, with a correlation between mast cell proximity to small enteric nerve fibers and GI symptoms (68). In POTS, prevalence of autoantibodies and abnormal cytokine profiles have been found, suggesting immune involvement (27, 49, 69), and mast cell activation has been proposed (50). This raises the possibility that immune activation or inflammation contributes to autonomic dysfunction and GI symptoms in POTS. Prevalence of autoantibodies otherwise associated with systemic disorders with known connections to enteric dysmotility was explored in Paper II, as well as levels of systemic tryptase, released upon mast cell activation.

Gut hormones represent an additional area of interest. In IBS, altered expression of enteroendocrine cells has been demonstrated throughout the GI tract (70-72), raising the question of whether similar abnormalities may occur in POTS. A previous study demonstrated abnormal gut hormone responses during oral glucose tolerance testing in POTS, with correlations to reduced stroke volume upon standing. The same study also found evidence of insulin resistance. These findings suggest that abnormalities in gut hormonal regulation may have a pathogenetic role in POTS (44). Moreover, increased splanchnic and pelvic blood flow during orthostatic challenge has been reported in POTS (43), but the relationship of these hemodynamic changes to GI symptoms remains unclear. Gut hormones influence motility, secretion, permeability, and visceral sensitivity, acting locally in the gut and through autonomic and central pathways (70). Levels of gut hormones and glycated hemoglobin (HbA1c) were explored in Paper III, where also potential associations to GI symptoms as well as hemodynamic findings were examined.

Peripheral neuropathy and sensory dysfunction

Symptoms that resemble peripheral somatic large and small fiber neuropathy were frequently reported by POTS patients in a large international survey, including coldness in hands and feet (84 and 82%), tingling in hands and feet (76 and 67%), and numbness in hands and feet (65 and 58%) (6). As mentioned above, one pathophysiologic mechanistic theory in POTS includes involvement of sympathetic neuropathy, with signs of peripheral denervation and sympathetic super-sensitivity, with reduced norepinephrine spill-over rate in the lower limbs. These symptoms and findings suggest SFN in a subset of POTS patients (40).

SFN is characterized by degeneration and/or dysfunction of thinly myelinated A δ -nerve fibers and unmyelinated C-fibers in the PNS. Both peripheral somatic and/or autonomic nerve fibers can be affected (73). SFN can be caused by a variety of underlying disorders, including diabetes mellitus, hereditary disorders, infectious diseases, and disorders with excess tissue deposits (46). Also disorders of the CNS can show signs of peripheral neuropathy (57). Systemic autoimmune rheumatic disorders are associated with SFN, as well as primary autoimmune disorders with antibodies directed at structures in the nervous system, such as autoimmune autonomic ganglionopathy (10, 46). The peripheral mechanisms behind peripheral neuropathies are complex and difficult to study. In humans, lymphocytic infiltrates can be seen in the microvasculature surrounding peripheral nerves in autoimmune systemic disorders, but the most common mechanisms include pathways activated in response to alterations in glucose homeostasis (74). Therefore, we investigated antibodies common in systemic autoimmune disorders, and levels of insulin and HbA1c in POTS in Paper II and III, respectively.

Previous studies have reported objective signs of SFN in some patients with POTS, where assessments of intraepidermal nerve fiber density (IENFD) in skin biopsies have shown reduced nerve fiber density in up to 56% of the POTS population (75-79). Quantitative sudomotor axon reflex test reflects the function of the sympathetic cholinergic synapses, where reduced sweat production suggests post-ganglionic sudomotor dysfunction. This finding is present in various autonomic neuropathies (80) and has been demonstrated in some studies on POTS (75, 81-84).

It is not clear whether the findings of neuropathy in POTS correlate with symptoms. In addition, some of the reported neuropathic symptoms are classically sensed by larger, A β -nerve fibers (i.e. numbness), but only one study has previously studied the function of larger sensory nerves in POTS, with retrospective correlation to symptoms (77). Gibbons et al reported that GI symptoms were more common among POTS patients with objective signs of sudomotor dysfunction, but not in POTS patients with low IENFD (75). No study has investigated objective signs of both large and small nerve fiber neuropathy in POTS with simultaneous documentation and correlation to clinical neuropathic symptoms, including GI symptoms. In addition, few studies have compared data on SFN in POTS with

healthy controls (75). This issue was addressed in Paper IV, where objective signs of peripheral neuropathy were explored in relation to subjective symptoms of neuropathy and GI involvement.

Aims

The overall aim of this thesis was to explore potential shared mechanisms linking cardiovascular autonomic dysfunction, GI features, and peripheral neuropathic manifestations in POTS. Through clinical studies performed in a well-described POTS cohort with comparisons to matched healthy controls, this work aims to contribute to a more integrated and multidisciplinary understanding of POTS as a systemic disorder.

The aims of the individual studies were as follows:

Paper I

To describe socioeconomic factors and lifestyle habits in patients with POTS, with focus on dietary patterns and nutritional parameters, and to examine their associations with GI symptoms.

Paper II

To characterize GI symptoms in POTS using validated questionnaires and to investigate associations between GI symptoms and cardiovascular autonomic parameters. Secondary aims were to assess the prevalence of autoantibodies associated with systemic rheumatic autoimmune diseases and to evaluate markers of mast cell activation, and to explore their potential association with GI symptoms.

Paper III

To examine circulating levels of gut hormones involved in GI motility, secretion, blood flow, and glucose homeostasis, as well as long-term glycemic control assessed by HbA1c in patients with POTS, and to investigate their associations with GI symptoms and hemodynamic parameters.

Paper IV

To evaluate subjective symptoms and objective measures of both small- and large fiber neuropathy in POTS, and to examine associations between objective neuropathic findings, subjective neuropathic symptoms, and GI manifestations.

Materials and Methods

Cohorts and study population

SYSTEMA cohort and head up tilt test

The papers included in this thesis are based on material from The Syncope Study of Unselected Population in Malmö (SYSTEMA), conducted between 2008 and 2021. Patients with unclear syncope or severe orthostatic intolerance were referred to the Syncope Unit at the Skåne University Hospital in Malmö, Sweden, after routine clinical investigations such as continuous 24-h electrocardiogram (ECG), echocardiography, or brain imaging. Patients with a well-defined underlying etiology, severe physical disability or dementia were excluded (85).

All included patients underwent a head-up tilt test, to provoke orthostatic symptoms and to assess hemodynamic responses. The protocol involved 10 minutes supine rest followed by passive tilt table elevation to 60°–70° for 20 minutes (30). Continuous cardiovascular monitoring was performed with ECG, and photoplethysmographic BP measurement (Nexfin, BMEYE, Amsterdam, The Netherlands or Finapres Nova, Finapres Medical Systems, PH Enschede, The Netherlands). BP and HR were recorded at supine position, and after 1, 3, and 10 minutes of tilt, as well as the maximum HR during testing. Orthostatic HR increase was expressed as Δ HR. POTS was defined as symptoms of orthostatic intolerance lasting for ≥ 3 months, with a sustained Δ HR ≥ 30 bpm, or HR ≥ 120 bpm during tilt, without orthostatic hypotension (30).

In total, 3040 patients were included in the SYSTEMA cohort, after data revision from the original 3081 enrolled (86). Among them, 329 (10.8%) met diagnostic criteria for POTS (87).

The POTS sub-study and active standing test

Between 2017 and 2021, patients diagnosed with POTS in the SYSTEMA cohort were invited to participate in additional studies focusing on POTS. Moreover, some patients with POTS who were followed clinically at the Syncope Unit (previously enrolled via the SYSTEMA cohort) were also included. However, not all patients with POTS from 2008 to 2021 were approached. In total, 93 patients with POTS, as well as more than 80 healthy controls, were included in the POTS sub-study. The

recruitment took place at the Clinical Research Unit of the Department of Internal Medicine, Skåne University Hospital, Malmö.

Most patients and all controls underwent active standing test (88). BP and HR were measured in the supine position and then after 1, 3, and 5 minutes of standing using a validated automated oscillometer device (Omron, Kyoto, Japan). Orthostatic HR increase was calculated as the difference between standing and supine HR, as in the tilt test. Before testing, participants were asked to fast overnight and avoid smoking, and the patients were also instructed to discontinue all cardiovascular medication 48 hours prior to testing. Between 2017 and 2019, blood samples were collected in all participants in the morning immediately before the active standing test and stored at -80°C.

While the head-up tilt test passively tilts the patient to assess hemodynamic responses under controlled, muscle-inactive conditions, the active standing test evaluates the same responses during voluntary standing, where muscle activity increases venous return. Therefore, the orthostatic HR increase may be less exaggerated in active standing tests (89).

Patients

The patients in papers I–IV were recruited from the POTS sub-study. Inclusion criteria to the present study were a definite POTS diagnosis, age 18–70 years, and the ability to understand the Swedish language. Exclusion criteria were severe psychiatric or somatic co-morbidity, inability to understand study information, and severe opioid or alcohol abuse. Additionally, if the patient was living too far away from Malmö, it was considered an exclusion criterion. Therefore, invitation letters were sent via regular mail to 82 of the 93 patients from the POTS sub-study, followed by a telephone call to provide additional information if a valid phone number was available. Patients who agreed to participate received written study information and questionnaires by mail to complete before the scheduled appointment.

Of the 82 invited patients, 39 patients were excluded for the following reasons: did not respond to invitation letter and/or no telephone number available (n=22), relocation from Skåne (n=1), symptoms of viral infection at the time of appointment (n=2), declined participation (n=8), debilitating symptoms of POTS (n=3), did not meet diagnostic criteria for POTS after re-evaluation (n=3). After exclusion, 43 patients were included in the study.

Paper III was partly based on data from 42 patients included in the original POTS sub-study. Their previously collected fasting blood samples were thawed and re-analyzed to assess metabolic and hormonal parameters. Seventeen of these 42 patients were also participating in the studies presented in papers I–IV, while the remaining 25 contributed only biobank samples. Figure 3 illustrates the recruitment process.

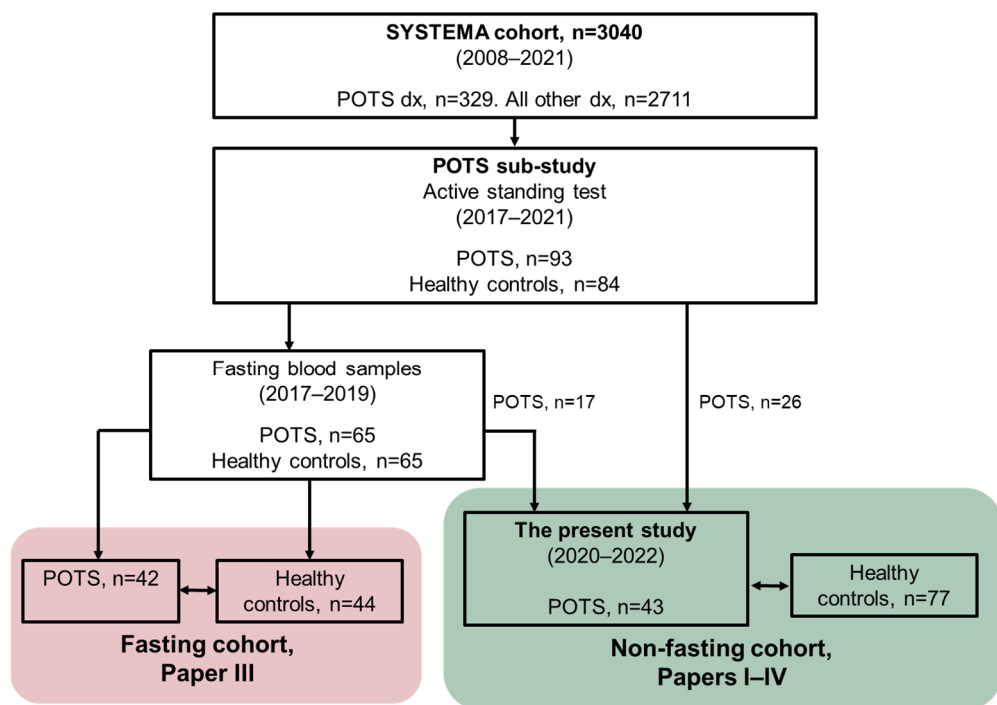


Figure 3. Flowchart on study population recruitment.

SYSTEMA: Syncope study of unselected population in Malmö; POTS dx: postural orthostatic tachycardia syndrome diagnosis; GI: gastrointestinal.

Controls

For Paper I–IV, healthy control participants were recruited through personal invitations and advertisements among hospital staff and medical students at Skåne University Hospital, Malmö. The controls were required to have no chronic illness or significant GI symptoms. Use of multivitamins and hormonal contraceptives was accepted, as well as temporary use of medications, such as seasonal allergy medicines. In total, 77 healthy controls were recruited and examined, with minor protocol variations to suit the aims of each paper.

For Paper III, data and bio-banked blood samples from 44 controls examined within the POTS sub-study were utilized. These participants were recruited through personal invitations (e.g. healthy medical students and hospital staff) and from ongoing population-based epidemiological studies in Malmö. Controls had no history of syncope, orthostatic intolerance, cancer, cardiovascular, or endocrine disease. None of the controls showed a significant HR increase during active standing test (88).

Study design

The study was cross-sectional and conducted in two study sessions. During the first visit, the participants handed in completed questionnaires, and blood samples were collected and stored at -20 °C or -80°C. Weight and height were recorded. POTS patients but not controls underwent a routine clinical examination including cardiac, pulmonary, abdominal, and neurological status. However, sensory neurological examinations were performed in controls as well. A second study visit was scheduled within a few weeks for POTS patients and a subset of the controls, where an additional comprehensive examination of large and small sensory nerve fibers, including skin punch biopsy, was completed. Participants were non-fasting, and the patients continued their regular medications before both sessions. The recruitment and examinations took place at the Department of Gastroenterology and the Department of Hand Surgery, Skåne University Hospital, Malmö, between October 1, 2020, and May 31, 2023. Since the study was conducted during the COVID-19 pandemic, none of the participants were allowed to have any symptoms indicative of bacterial or viral infection during any of the study visits. Due to the same reason, all external recruitment of patients and controls was temporarily suspended; consequently, in-house hospital staff and students were asked to serve as controls during that period.

For Paper III, fasting serum and plasma stored and frozen at -80°C were analyzed from randomly selected POTS patients and age- and sex-matched healthy controls, originally recruited to the POTS sub-study. Corresponding hemodynamic data from the same occasion were also included.

Data obtained from questionnaires, biochemical analyses, hemodynamic recordings, clinical and histopathological examinations were compared between patients with POTS and controls, and correlations between selected parameters were analyzed, according to the specific research questions.

Questionnaires

Participants were asked to complete a study questionnaire, from which several parameters and instruments were used in more than one of the included papers, as described below. Additional questionnaire data specific to each paper is presented in the respective paper-specific sections.

Clinical study questionnaire

Participants in Papers I–IV completed a clinical questionnaire covering sociodemographic factors (education, marital status, occupation) and lifestyle habits (smoking, alcohol use, physical activity), where the background variables were categorized either as binary or measured on an ordinal scale. In addition, medical history (pregnancies and childbirth, previous and current illnesses, surgeries, family history, and current pharmacological medications) was recorded in free text format.

The Visual Analogue Scale for Irritable Bowel Syndrome

The visual analogue scale for irritable bowel syndrome (VAS-IBS) is a validated instrument assessing GI symptoms common in IBS (90). It includes seven core items: abdominal pain, diarrhea, constipation, bloating and flatulence, vomiting and nausea, psychological well-being, and the intestinal symptoms' influence on daily life. The respondent rates the degree of symptoms for the past 2 weeks on a 0–100 mm scale, where 100 mm indicates very severe symptoms. In this study, the scales were inverted from the original version (90). Two additional “yes/no” items cover urgency and sensation of incomplete evacuation of stools. The VAS-IBS was used in Papers I–III.

The Irritable Bowel Syndrome Severity Scoring System

The irritable bowel syndrome severity scoring system (IBS-SSS) is a validated scoring system used in IBS research, comprising four items on abdominal pain, abdominal distension, satisfaction with bowel habits, and the impact of bowel habits on daily life, each rated on a 0–100 mm scale, where 100 mm means very severe symptoms. In addition, one item records the number of days of abdominal pain during the past 10 days. Scores are summed to yield a total IBS-SSS score (0–500), where 75–175 suggests mild IBS, 176–300 suggest moderate IBS, and >300 suggest severe IBS (91). An additional ten-item extraintestinal scale assesses nausea, early satiety, headache, back pain, lethargy, excess wind, heartburn, urinary symptoms, thigh pain, and bodily pain, 0–100 mm, with higher scores indicating greater severity of symptoms (91, 92). In this study, total IBS-SSS scores were used in Papers II–IV, and extraintestinal scores were used in Paper II.

Statistics

Statistical analyses were performed using SPSS© software, versions 25–29 for Windows (IBM, New York, USA). Continuous variables were tested for normality using the Kolmogorov-Smirnov and/or Shapiro-Wilk tests, and by visual inspection

of histograms. In the POTS group, the distribution of symptomatic parameters tended to be normally distributed. However, none of the symptom-related variables in the control group were normally distributed, and the overall sample size was relatively small. Therefore, non-parametric tests were used for comparisons between groups (Mann-Whitney U-test, Fisher's exact test) and for correlation analyses (Spearman's rank order test). Data are presented as median and interquartile range (IQR), or as numbers and percentages. A p-value <0.05 was considered statistically significant for comparisons between groups, and for correlation analyses in Paper II. In Papers I and III, a p-value <0.01 was considered statistically significant for the correlations analyses, to adjust for multiple testing. Adjustments for confounders were performed in some cases, see Paper-specific sections.

Paper I: Specific methods

Forty-three POTS patients and 61 healthy controls were included in this study.

Questionnaires including food diary

Data from the clinical study questionnaire and the VAS-IBS were analyzed. Participants were also asked to complete a 4-day food diary, recording all ingested foods and beverages along with any associated GI symptoms. Each ingested item should be as thoroughly described as possible, including the timing of consumption. The food intake was categorized into meat, fish, vegetables/legumes, fruits/berries, dairy products, cereals, candies, soda, juice, protein drinks, and energy- or fluid replacement drinks. The frequency of fast food and calorie-containing beverage consumption was noted, as well as whether participants reported regular or irregular eating habits.

Laboratory methods

Serum levels of the micronutrients cobalamins, folic acid, iron, total iron-binding capacity, ferritin, sodium, potassium, magnesium, phosphorus, albumin, and 25-hydroxyvitamin D (25-OH vitamin D), as well as high-sensitive C-reactive protein (CRP), were analyzed according to clinical routines at the Department of Laboratory Medicine, Skåne University Hospital, Malmö (93).

Statistics

A generalized linear model was used to examine associations between continuous variables, with adjustments for age and gender. Variables that differed significantly between patients with POTS and controls – including nutritional and inflammatory parameters, body weight, and body mass index (BMI) – were analyzed as dependent variables, with cohort group or GI symptom scores as predictors. In complementary analyses, GI symptom scores were used as dependent variables and nutritional intake variables as predictors.

For categorical variables, logistic regression was applied to adjust for age and gender, using cohort group as the dependent variable and sociodemographic and lifestyle factors as independent variables. Due to the low number of participants, tests for trend were applied for ordinal variables, except for marital status. Results are presented as β coefficients or odds ratios (ORs), with 95% confidence interval (CI).

Paper II: Specific methods

Forty-three patients with POTS and 74 healthy controls were included in this study.

Questionnaires including prevalence of gastrointestinal symptoms

Data from the VAS-IBS and IBS-SSS instruments were analyzed in POTS and controls. In addition, participants completed a modified questionnaire previously used to assess prevalence of gastroparesis symptoms in diabetes (94). This instrument contains 14 common gastroparesis-related symptoms, where respondents indicate the presence or absence of each symptom. The symptoms covered were loss of appetite, dysphagia, meal-related cough, early satiety, nausea, vomiting, weight loss, abdominal fullness, bloating, regurgitation, constipation, diarrhea with gas, evacuation incontinence, and postprandial perspiration.

GI symptoms were evaluated in relation to the hemodynamic parameters that were recorded during the previously performed diagnostic head-up tilt and active standing tests, see section “Cohorts and study population”.

Laboratory analyses

The presence of IgG autoantibodies commonly targeted in classic systemic autoimmune rheumatic diseases was evaluated using a custom-designed multiplex solid-phase microarray platform (Thermo Fisher Scientific Immunodiagnosics, Uppsala, Sweden) (95). Thirty-three antigens (including systemic, nuclear,

cytoplasmic, and post-translationally modified), as well as paired native control antigens, were arranged separately on glass slides with one slide per participant. The inclusion of paired native and modified peptide antigens provides internal specificity control and allows for discrimination between true modification-specific immune responses – typically seen in rheumatoid arthritis – and non-specific reactivity. Serum from the participants were added to the slides. After incubation and wash-off, potential antibodies to the antigens were bound. Then, a fluorescent secondary IgG-directed antibody was added to the slide. Finally, the fluorescent intensity was measured with a laser scanner, resulting in a value that corresponds to the anti-body levels to each antigen in all participants. The cut-off level for each individual antigen was based on the 98th percentile value among 400 healthy controls (95). A list of antigens used in the assay can be found in Supplementary table 1 and includes 20 antigen targets commonly incorporated in classical antinuclear antibodies (ANA) analyses (96). This analysis was performed at Department of Medicine, Division of Rheumatology at Karolinska Institute, Stockholm.

Total IgE and tryptase were analyzed in serum according to clinical routines at the Department of Clinical Immunology, Skåne University Hospital, Lund. Reference values for healthy volunteers are given for tryptase (97) and total IgE (98), and thus, these parameters were not analyzed in the control group.

Statistics

Subgroup analyses were performed within the POTS cohort to explore whether commonly reported comorbid conditions influenced GI symptoms or hemodynamic responses. For each comorbidity, patients were dichotomized based on self-reported presence or absence of the condition. Separate subgroup comparisons were conducted for the following comorbidities: HSD/EDS, IBS, organic GI disease, and allergic disease (defined as asthma, allergic disorders, and/or use of anti-allergic medication). Hemodynamic parameters obtained from the diagnostic head-up tilt test and GI symptom scores were compared between patients with and without each comorbidity.

Sensitivity analyses were performed excluding patients with concomitant organic GI disorders (n=7). A generalized linear model was used to calculate associations adjusted for psychological wellbeing, with continuous symptom-related parameters as dependent variables, and Δ HRs from the head up tilt test as predictors, and results presented as β coefficient with 95% CI.

Paper III: Specific methods

In this paper, two partially overlapping groups of patients from the POTS sub-study were studied (Fig 3). Fasting samples from 2017–2019 were analyzed and linked to hemodynamic data from the active standing test from the same occasion in 42 POTS patients and 44 controls. Non-fasting blood samples were analyzed for this specific study in 43 patients and 52 controls.

Laboratory methods

HbA1c (mmol/mol) was analyzed in non-fasting samples, according to clinical routines at the Department of Laboratory Medicine, Skåne University Hospital, Malmö, Sweden (99). Hemoglobin (g/L) and erythrocyte mean corpuscular volume (fL) were measured in the same cohort to exclude hemoglobinopathies that could affect HbA1c levels (100, 101). Morning plasma cortisol (nmol/L) was measured in fasting samples according to clinical routines at the same department (102).

Levels of C-peptide, total glucose-dependent insulintropic polypeptide (GIP), total glucagon-like peptide-1 (GLP-1), glucagon, insulin, leptin, and total peptide YY (PYY) were measured using the Mesoscale Discovery® (MSD) U-PLEX® Human Diabetes Combo 1 Multiplex Assay (K15274K, Maryland, USA) in serum (fasting blood samples) and ethylenediaminetetraacetic acid (EDTA) plasma (non-fasting blood samples). Each hormone was captured by specific antibodies, which were coupled to a U-PLEX™ linker in the assay plate. To prepare the plate, 50 µL of each antibody solution was added to the wells. In addition, aprotinin and a dipeptidyl peptidase-4 inhibitor (Merck, Darmstadt, Germany) was added to the plate to prevent protein- and hormone breakdown. After washing the assay plate, 50 µL of calibrator, and serum/plasma (diluted 1:2) were added to each well. After incubation, the plate was washed again, and 50 µL of SULFO-TAG™ detection antibody was added. After a new incubation and washing procedure, 150 µL of MSD GOLD™ Read Buffer B was added to each well, to create an electrochemiluminescence reaction. The plates were then read using an MSD® instrument, where the intensity of emitted light by the reaction is directly proportional to the amount of each hormone in the samples. The hormone levels were analyzed in relation to calibrators, with defined concentrations of each hormone, to act as reference points and ensure accuracy of the method.

Statistics

Multiple linear regression was performed to calculate associations between plasma leptin as dependent variable, and the presence of POTS diagnosis as predictor, adjusting for gender and BMI. Results are expressed as β coefficients and 95% CI.

Paper IV: Specific methods

Forty-three POTS patients and 54 controls were included in the study.

Neuropathy Symptom Score

In addition to the IBS-SSS, a modified version of the validated neuropathy symptom score (NSS) was used in the questionnaires to assess subjective symptoms of peripheral neuropathy in the hands and feet (103). The symptoms evaluated included numbness, warmth/cold sensation, paresthesia, burning sensation, radiating pain, aching pain, and nocturnal exacerbation caused by contact with bed sheets. Participants rated each symptom as 0=never, 1=sometimes, 2=often, and 3=nocturnal. Scores for all items regarding hands and feet were summed up to yield a composite total score, with a maximum of 42 points. If the respondent answered that symptoms occurred both often (2 points) and nocturnally (3 points), only the higher score was used in the composite total.

Clinical examinations including routine neurological sensory testing

Patients with POTS underwent a clinical examination including routine neurological assessment (104), and all participants were examined with standard neurological sensory tests. Examination of sensory nerve fibers and mechanoreceptors was performed distally at the feet and legs with light touch and by 10 g monofilament (Bailey Instruments, Manchester, UK). To assess vibration perception thresholds (VPT), a neurothesiometer (Scientific Laboratory Supplies, Nottingham, UK) was used with the instrument directed at the bony prominence of the medial malleolus and the tip of the great toe bilaterally. The vibration frequency of the device was preset to 50–60 Hertz (Hz), and the amplitude of the vibratory signal was gradually increased until the participant sensed the vibration. Three measurements were performed on each site, and a mean value was calculated. Phalen's and Tinel's tests were performed in all participants to exclude carpal tunnel syndrome (105).

Multi-Frequency Vibrometry

During the second session, VPTs were examined in 40 POTS patients using the VibroSense Meter® II device (VibroSense Dynamics AB, Malmö, Sweden). Measurements were performed with seven frequencies (4, 8, 16, 32, 64, 125, and 250 Hz) on the head of the first metatarsal bone of the right foot, and with three frequencies (32, 125, and 250 Hz) on the index and little finger of the right hand. The results were expressed as Z-scores, based on data obtained from previous studies with healthy age- and sex-matched controls (106). Unlike assessment with

neurothesiometry, multi-frequency vibrometry (MFV) measures VPTs for several different vibrating frequencies, which allows for a broader evaluation of large A β sensory nerve fibers, different mechanoreceptors and different innervation areas (107).

Skin biopsy procedure and evaluation of intraepidermal nerve fiber density

Skin punch biopsies (3 mm, circular) were obtained from the distal right leg 10 cm proximal to the lateral malleolus in 39 POTS patients and 13 controls after local anesthesia (Carbocain® 10 mg/mL). Biopsy samples were fixed in a 4% buffered formaldehyde solution for at least 24 hours before dehydration and paraffin embedding. Thereafter, the paraffin-embedded blocks were sectioned at 5 μ m and immunohistochemically stained with a rabbit polyclonal protein gene product 9.5 antibody (Cell Marque, Rocklin, USA, dilution 1:3000). The IENFD, expressed as the number of fibers per mm of epidermal length, was manually assessed in each specimen, as previously validated and described in a large normal material (108). The observer was blinded to the characteristics of the participants when assessing IENFD. The inter- and intra-observer reliability was good in a previous study by the same assessor, using the same methods as here (109).

Statistics

Patients with POTS were divided into two equally sized groups according to the total NSS-symptom scores, with the median total NSS used as cutoff to define low and high neuropathy symptom groups. The same procedure was performed with the median total IBS-SSS value in POTS, to define low and high GI symptom groups. VPTs from neurothesiometry and MFV, as well as IENFD were compared between patients with low/high neuropathy symptoms, and between low/high GI symptoms.

Ethics

The Swedish Ethical Review Authority approved the studies presented in Papers I–IV, permission numbers 2020–02432 (approval date 26/08/2020) and 2021–00049 (approval date 11/02/2021). The SYSTEMA project and the POTS sub-study were originally approved by the Regional Ethical Committee in Lund, Sweden, with approval numbers 82/2008 and 2017/295, respectively. All subjects gave their written and informed consent before participating in the studies, and all were informed about their right to withdraw their consent at any time after enrolment.

All information was considered sensitive, and therefore password-protected and accessible only to the primary investigators of the research team. The data were transferred to coded datasets, including blood samples and histopathological specimens. The code keys were kept separated from the data and could only be linked to the participants by the primary investigators of the research team.

Results

Characteristics of the POTS cohort

Forty-three patients with POTS were included in the study, and data from these patients are presented in Papers I–IV. The time from symptom onset to diagnosis by tilt test was 3 (1–10) years, and the time from diagnosis to the current study inclusion was 3 (1–7) years. The median age was 30.6 (26.0–41.0) years and the BMI was 24.2 (21.3–27.0) kg/m². Most patients were female (93%). Sociodemographic factors and lifestyle habits are described in Table 1.

Table 1. Sociodemographic factors and lifestyle habits in POTS (n=43)

Values are presented as number (percentages).

Education		Marital status	
Primary school	3 (7.0%)	Living alone	20 (46.5%)
Secondary school	14 (32.6%)	Married/living together	23 (53.5%)
>1 year of university studies	9 (20.9%)	Smoking	
University exam	17 (39.5%)	Never smoked	32 (74.4%)
Occupation		Quit smoking	5 (10.9%)
Full time work or studies	14 (32.6%)	Sometimes	1 (2.2%)
Part-time work 50–99%	6 (14.0%)	Regularly	5 (11.6%)
Sick leave	19 (44%)	Physical activity/week	
Retired	1 (2.3%)	No time at all	8 (18.6%)
Unemployment	3 (7.0%)	<30 minutes	6 (14.0%)
Weekly alcohol intake (standard glasses)		30–60 minutes	10 (23.3%)
<1	38 (88.4%)	60–90 minutes	11 (25.6%)
1–4	4 (9.3%)	90–120 minutes	2 (4.7%)
5–9	1 (2.2%)	>120 minutes	6 (14.0%)

Physical activity was defined as activities that lead to shortness of breath.

Many patients with POTS reported being on sick leave. It is not known whether patients who were working part-time also were on part-time sick leave. Most patients were non-smokers, and the reported alcohol intake was very low. Few patients reported regular intense physical activity.

The most common self-reported comorbidities in POTS patients were HSD/EDS (n=12, 28%), IBS (n=12, 28%), asthma (n=9, 21%), migraine (n=7, 16%), thyroid disease (n=6, 14%), neuropsychiatric disorders (n=6, 14%), endometriosis (n=5, 11.6%) and myalgic encephalomyelitis (n=5, 11.6%). Seven patients (16%) reported organic GI disorder, including celiac disease (n=2), inflammatory bowel disease (n=2), microscopic colitis (n=2), and persistent *Helicobacter pylori* infection due to antibiotic resistance (n=1).

Pharmacological treatment was common. The most frequently reported drug classes were anti-hypotensive agents (e. g. norepinephrine prodrugs, α -1 agonists), heart rate-lowering agents (β -blockers, I(f)-channel inhibitors), anti-allergy medications (e.g. histamine H1 antagonists), and central nervous system stimulants. The median number of reported medications per patient was 4 (IQR 2–6; range 0–11; unpublished data). A list of medications is reported in Supplementary Table 2.

The results of the GI- and extraintestinal symptoms measured on the VAS-IBS and IBS-SSS instruments are presented in Table 2 and Fig 4. These variables were compared with those of healthy controls with slight modifications in all papers, and all symptom scores reported by POTS patients were significantly more severe than those reported by controls ($p < 0.001$).

Upon clinical examination, cardiac and pulmonary findings were normal, and resting BP was within normal limits. Many patients complained of diffuse pain during abdominal examinations, however, no abnormal abdominal findings were detected.

The characteristics of the additional 42 patients who were included for fasting hormonal analyses are presented under the specific section of Paper III.

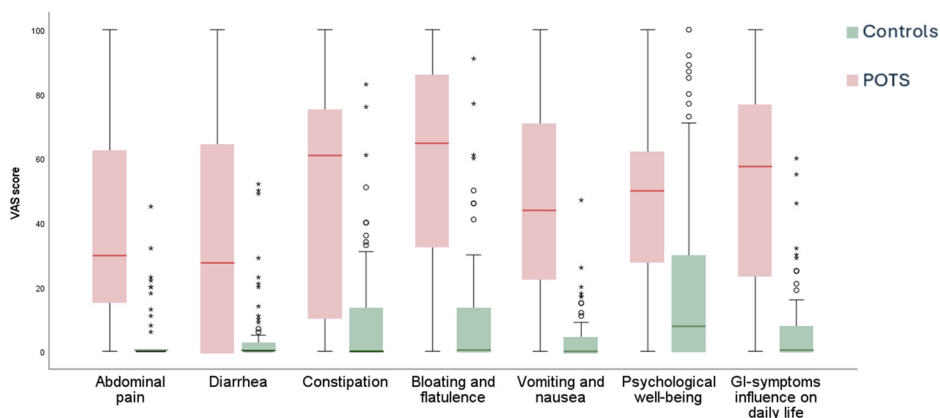


Figure 4. Gastrointestinal symptoms in POTS (n=43) and healthy controls (n=74).

Symptoms were measured in millimeters on the VAS-IBS scale. VAS-IBS: Visual analogue scale for irritable bowel syndrome.

Table 2. Gastrointestinal and extraintestinal symptoms in POTS (n=43).

Values are presented as median (interquartile range) or number (percentages).

VAS-IBS		IBS-SSS Extraintestinal symptoms	
Abdominal pain	30 (16–62)	Early satiety	39 (5–60)
Diarrhea	28 (0–64)	Headache	71 (33–85)
Constipation	61 (11–74)	Back pain	50 (15–77)
Bloating and flatulence	65 (25–88)	Lethargy	92 (81–100)
Vomiting and nausea	44 (23–70)	Excess wind	60 (29–82)
Psychological well-being	50 (28–62)	Heart burn	23 (3–60)
Symptoms' influence on daily life	58 (23–76)	Urinary symptoms	44 (8–84)
Urgency	17 (40.5%) ^a	Thigh pain	42 (2–58)
Incomplete evacuation	30 (69.8%)	Bodily pain	76 (29–96)
Total IBS-SSS	213 (135–319)^b	Total extraintestinal score	277 (178–338)

^a1 missing value

^b3 missing values

Paper I

Patients with POTS were slightly younger than the 61 included controls ($p=0.030$) and the prevalence of female sex was higher among patients (93% vs. 74%; $p=0.019$). Therefore, adjustments for age and sex were performed if it was considered relevant in the following analyses. BMI was comparable between patients and controls after adjustments ($p=0.128$).

Sociodemographic and lifestyle factors including food diaries

After adjustments for age and sex, patients had lower educational level (OR 0.386 [0.216–0.689]; p for trends=0.001, see Errata), were more often on sick leave (OR 1.368 [1.108–1.688]; p for trends=0.004), and had less physical activity than controls (OR 0.691 [0.525–0.910]; p for trends=0.009). In addition, the patients consumed significantly less alcohol than controls (OR 0.116 [0.043–0.312]; p for trends <0.001).

Twenty-four (55.8%) of the patients and only 20 (32.8%) controls returned the 4-day food diaries. The patients reported more frequent intake of various drinks including soda, energy drinks, protein drinks, and juices compared with controls (17 vs. 7 times; $p=0.032$). Other than that, the overall nutrient intake was in general similar between patients and controls.

Micronutrients, inflammatory parameters and symptoms

High-sensitive CRP was significantly higher in patients, and albumin levels were significantly lower. However, both variables were generally within their respective reference ranges. Sodium levels were significantly higher, and potassium levels were significantly lower in patients, although the absolute differences were small. After adjustments for age and gender, the difference in potassium levels between patients and controls disappeared (β : -0.084; 95% CI: -0.188 to 0.021; $p=0.117$). All other micronutrient levels were comparable between patients and controls, also after adjustments for age and gender (Table 3).

Table 3. Serum levels of CRP and nutrient parameters.

Values are presented as median (interquartile range). Comparisons were performed with Mann-Whitney U-test before adjusting for confounders.

	POTS (n=43) ^a	Controls (n=61) ^b	P-value
High-sensitive CRP	1.35 (0.62–2.98)	0.68 (0.35–1.38)	0.006
Albumin	41 (40–44)	44 (41–45)	0.014
Cobalamin	300 (244–363)	294 (231–362)	0.549
Folic acid	18 (12–27)	19 (15–24)	0.503
25-OH Vitamin D	68 (54–80)	63 (49–80)	0.292
Iron	17 (12–22)	18 (14–22)	0.719
Total iron binding capacity	69 (60–76)	67 (61–73)	0.692
Ferritin	31 (14–50)	34 (15–74)	0.574
Sodium	142 (141–143)	141 (139–142)	0.003
Potassium	3.9 (3.8–4.0)	4.0 (3.8–4.2)	0.029
Magnesium	0.80 (0.76–0.84)	0.78 (0.75–0.83)	0.169
Phosphorus	1.1 (1.0–1.3)	1.2 (1.1–1.3)	0.103

CRP: C-reactive protein.

^a2 missing values

^b10 missing values

In all participants (i.e. POTS and controls), CRP levels were not associated with GI symptoms after adjustments for age and gender. Albumin levels correlated inversely with abdominal pain (β : -0.004; 95% CI: -0.052 to -0.010); $p=0.004$) and with intestinal symptoms' influence on daily life (β : -0.020; 95% CI: -0.038 to -0.003); $p=0.025$). Sodium levels correlated inversely with abdominal pain (β : 0.020; 95% CI: 0.005–0.035; $p=0.011$), vomiting and nausea (β : 0.021; 95% CI: 0.007–0.035; $p=0.003$), and intestinal symptoms' influence on daily life (β : 0.014; 95% CI: 0.002–0.027; $p=0.026$).

Paper II

The median age was comparable between patients with POTS and 74 included controls ($p=0.068$), but the prevalence of female sex was higher in the POTS cohort (93% vs. 78.4%; $p=0.041$). BMI was comparable between POTS and controls ($p=0.413$).

Mapping of gastrointestinal symptoms in patients with POTS

The most prevalent GI symptom was nausea, reported by 79.1% of the patients, followed by abdominal fullness (69.8%) and bloating (66.7%). The most severe symptoms were bloating and flatulence, and constipation (Table 2, Fig 4).

There was a significant association between lower levels of psychological well-being and all reported GI symptoms ($p < 0.001$).

In patients with self-reported HSD/EDS, constipation was more pronounced than in those without HSD/EDS (74 [43–92] vs. 51 [7–72]; $p = 0.030$). Regarding hemodynamic tilt data, the $\Delta HR_{\max}$ was higher in the non-HSD/EDS group (47 [38–54] vs. 32 [30–45]; $p = 0.005$).

Diarrhea was more pronounced in patients with self-reported IBS compared with those without IBS (57 [21–80] vs. 10 [0–64]; $p = 0.011$). Otherwise, there were no significant differences in the degree of GI symptoms, or hemodynamic data between groups. No differences were seen regarding GI symptoms and hemodynamic data when comparing patients with and without self-reported organic GI disorder, or when comparing patients with and without self-reported asthma/allergy, or on anti-allergy medication ($n = 15$).

There was an inverse correlation between $\Delta HR_{\max}$ and abdominal pain ($r = -0.406$, $p = 0.007$). This association remained significant after adjustment for psychological well-being (β : -0.915; 95% CI: -1.478 to -0.351; $p = 0.002$). After adjustment, an inverse relationship was also observed between $\Delta HR_{10\min}$ and total IBS-SSS (β : -4.748; 95% CI: -9.172 to -0.324; $p = 0.036$). When the seven patients with organic GI disorders were excluded from the analysis, the same pattern became even more apparent, with inverse correlations between ΔHR and abdominal pain, constipation, intestinal symptoms' influence on daily life, and total IBS-SSS, respectively. These results remained after adjusting for psychological well-being. Similar analyses using data from the more recent active standing tests yielded the same pattern of results.

Autoantibodies and immunological factors in POTS

A microarray for systemic autoimmunity was performed in 34 patients and 39 controls, randomly selected from the study cohort, with no significant differences in age or gender distribution between groups. The prevalence of individuals positive for any autoantibody in the array was similar between groups (29.4% in POTS vs. 33.3% in controls; $p = 0.803$). ANA-related autoantibodies were detected in ten patients with POTS and in eleven healthy controls (29.4 % vs. 28.2 %). In most cases, autoantibody positivity was restricted to a single tested ANA-associated antigen. Anti-topoisomerase 1 (Anti-Scl-70) antibodies, which are associated with systemic sclerosis, were detected in four patients in the absence of broader ANA

reactivity, but in none of the controls. Apart from this finding, no differences in autoantibody expression were observed between patients and controls (Fig 5). The presence of any autoantibody was not associated with GI symptoms.

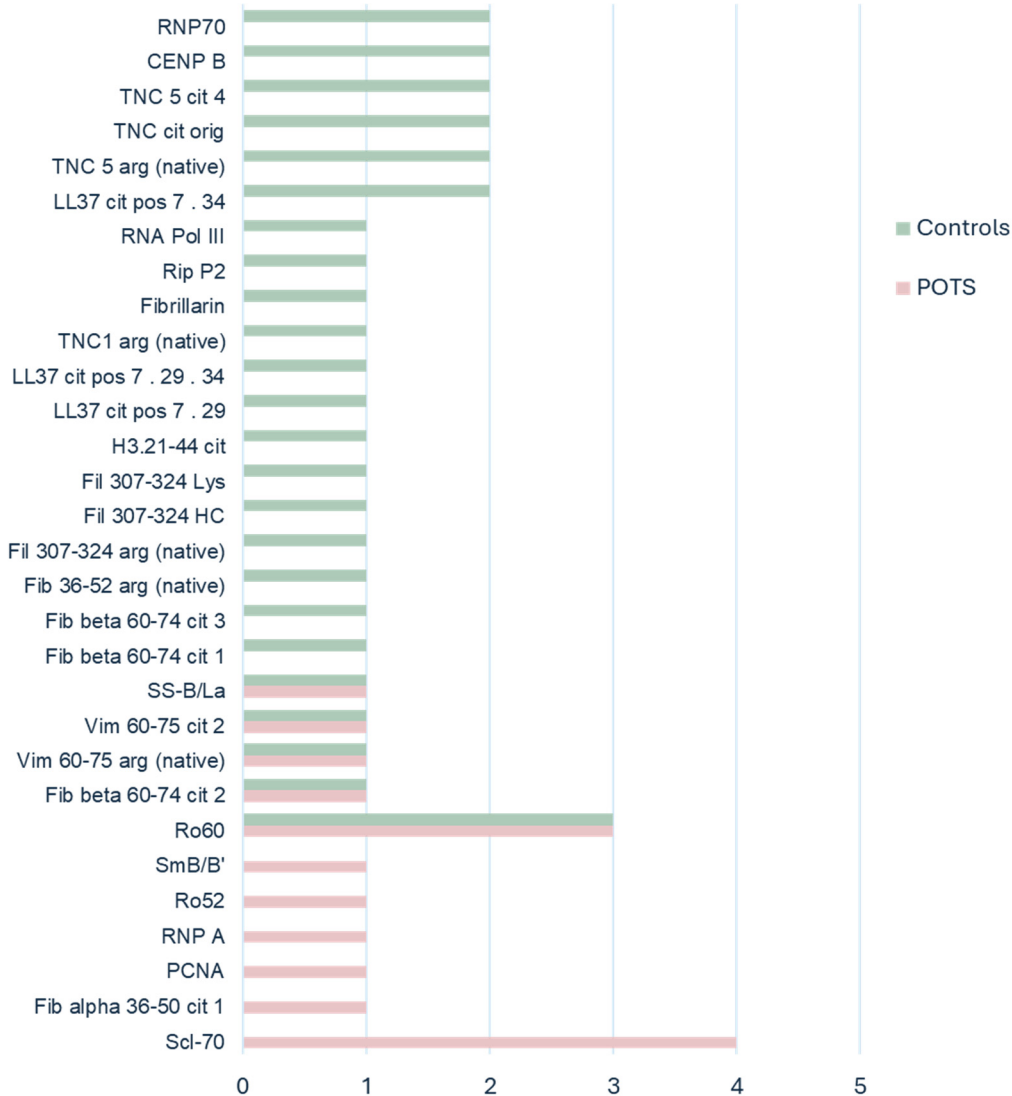


Figure 5. Autoantibody positivity by antigen in POTS and healthy controls. On the x-axis the number of positive subjects. Explanations of antigens can be found in Supplementary table 1.

Forty-one patients were screened for tryptase and 40 for total IgE levels. One patient had slightly elevated tryptase concentration (12.4 µg/L, reference upper limit 11.3 µg/L). Total IgE was above the upper normal limit (129 kU/L) in five patients. One of these patients reported asthma, whereas the others did not report asthma, atopic disease, or use of anti-allergy medications.

Paper III

The results are reported separately for the *fasting cohort*, i.e. randomly selected patients and controls recruited to the POTS sub-study, and the *non-fasting cohort*, with the same patient cohort and healthy controls as in Papers I, II and IV (Fig 3).

Fasting cohort

Forty-two patients and 44 age- and sex matched controls were included. BMI was equal in the two groups, however, data on BMI was only reported by 19 patients (45%) and eight controls (18%). The level of serum insulin was significantly higher in patients compared to controls (14.8 [11.0–19.2] vs. 11.9 [9.1–15.7] µUI/mL; $p=0.029$). C-peptide was also higher in POTS, although the significance level was not reached ($p=0.054$). Leptin levels were comparable between groups, as were morning cortisol levels. There were strong positive correlations between levels of C-peptide/insulin, and systolic/diastolic BP during rest as well as during active standing from the same occasion in patients but not in controls (Fig 6). There were no correlations between any of the gut hormones and Δ HR in POTS, and no correlations between cortisol levels and hemodynamic parameters.

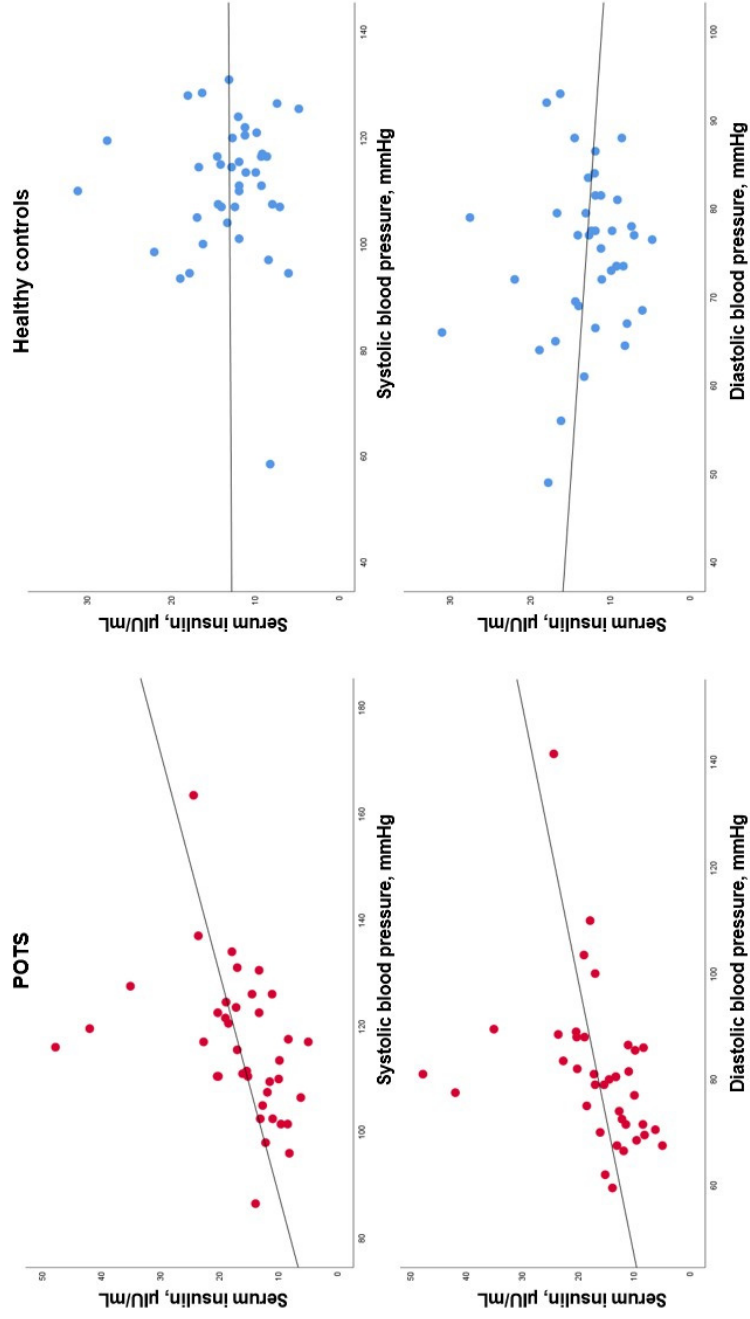


Figure 6. Correlations between serum insulin and blood pressure in POTS and healthy controls. Fasting serum insulin ($\mu\text{IU/mL}$) on the y-axis. Systolic/diastolic blood pressure (mmHg) after 3 minutes of standing on the x-axis. Correlations in POTS (left panel) and in healthy controls (right panel).

Non-fasting cohort

Forty-three patients with POTS and 52 controls were included, with no significant differences between groups regarding age, gender distribution, or BMI. Examinations and blood samplings for both patients and controls were conducted approximately during the same time span of the day. There were no significant differences between groups in levels of HbA1c, hemoglobin, erythrocyte mean corpuscular volume, C-peptide, insulin, glucagon, GIP, GLP-1, or PYY. Leptin levels were significantly higher in patients compared with controls (16 645 [8 879–47 871] vs. 11 041 [5 143–20 463] pg/mL; $p=0.003$), but after adjustments for gender and BMI, the association between POTS and leptin was no longer significant ($\beta=-5\,574$; 95% CI: -12 313–1 165; $p=0.104$).

There were no observed correlations between any of the analyzed non-fasting gut hormones and GI symptoms.

Paper IV

Forty-three patients and 54 controls were included for evaluation of symptoms and basal sensory neurological examinations. The controls were slightly older than the patients (38 [30–46] vs. 31 [26–41] years; $p=0.010$). Smoking habits were equal between groups, but alcohol consumption was significantly lower among POTS.

Neuropathy symptoms and clinical examinations

Four (7.4%) of the 54 control participants and 36 (83.7%) of the 43 patients reported one or more variables on the NSS to occur often or nocturnally in hands or feet. The symptoms that patients reported most frequently were “Abnormal sensation to heat and cold” and “Sensation of pins and needles”. Significant differences in total NSS were found between patients and controls where the median composite score for patients was 14.5 (6.0–21.3) compared to 0.0 (0.0–2.0) in the control group ($p<0.001$).

Clinical routine neurological examinations, including tendon reflex tests and pinprick test with monofilament, were generally normal in patients. In a few sporadic cases, mild generalized weakness was observed, leading to slightly reduced motor function. However, the clinical findings did not raise suspicion of any neurological disorder in those cases. VPTs, tested by neurothesiometer, were normal in POTS, and generally comparable between patients and controls. Phalen’s and Tinel’s tests were positive in a handful of patients and controls. One patient fulfilled criteria for carpal tunnel syndrome and one control ended up with a diagnosis of

ulnar nerve entrapment, both participants re-evaluated at the Department of Hand Surgery, Skåne University Hospital, Malmö.

Multi-frequency vibrometry

Z-scores for vibratory potential thresholds measured by MFV were pathological (Z-score >1.65) in three patients in the feet, and in five patients regarding index and/or little finger.

Intraepidermal nerve fiber density

Thirty-nine (90.4%) patients and 13 (24.1%) controls underwent skin punch biopsy. There were no significant differences between groups regarding age, gender distribution and BMI. Median IENFD was comparable between patients and controls (2.26 [1.62–3.08] fibers/mm vs. 1.63 [0.73–2.68] fibers/mm; $p=0.108$) (Fig 7). In general, skin biopsies were well tolerated. A mild and local skin infection occurred in one patient who consequently received antibiotic therapy with no residual complications.

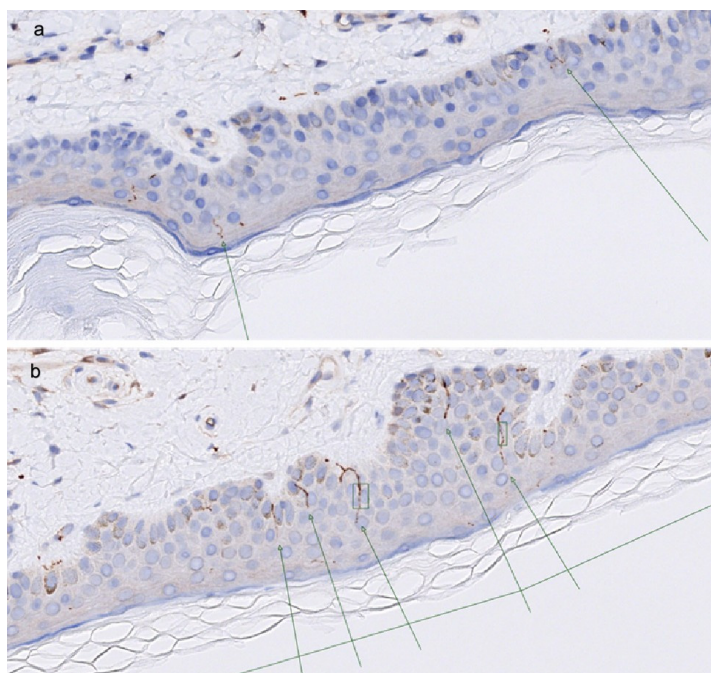


Figure 7. Intraepidermal nerve fibers.

Intraepidermal nerve fibers in 5 μ m skin sections on a 50X magnification in a) a patient with low IENFD and b) a patient with high IENFD. IENFD: intraepidermal nerve fiber density.

Nerve function measures in relation to symptom severity

Patients with NSS-scores >14.5 (the median value) were classified as “High NSS group”, as opposed to the “Low NSS group”. There were no differences in routine sensory testing between high vs low NSS groups. IENFD were comparable. MFV Z-scores were higher in the “High NSS group”, although the median values were within normal ranges (Table 4). The total IBS-SSS score was 297 (185–376) in the “High NSS-group”, and 165 (133–250) in the “Low NSS-group”, $p=0.018$ (unpublished data).

When the same procedure was performed with the median total IBS-SSS value, the same results were seen. The NSS was significantly higher in the “high IBS-SSS group” than in the “low IBS-SSS group” (18.0 [14.3–22.8] vs. 11.0 [4.0–15.0]; $p=0.002$) (Fig 8).

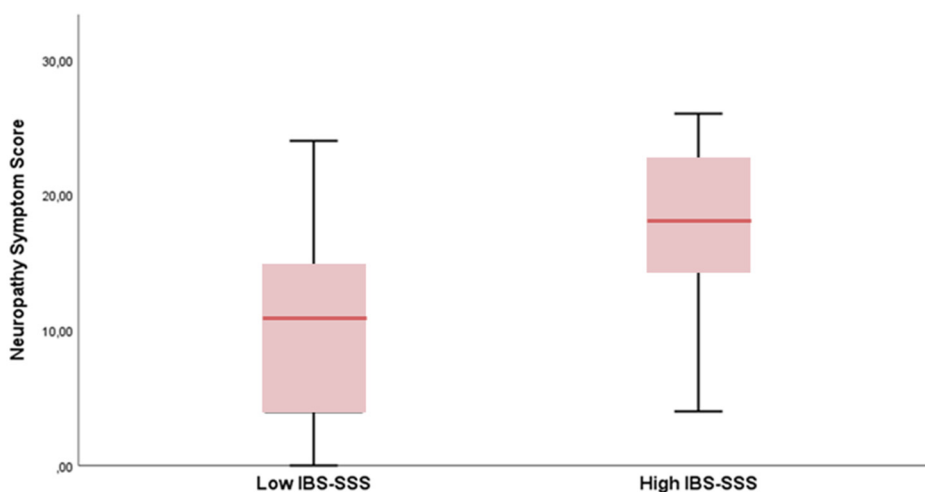


Figure 8. Comparison of neuropathy symptom scores in patients with POTS with high or low scores of IBS-SSS.

NSS 11.0 (4.0–15.0) vs. 18.0 (14.3–22.8); $p=0.002$. NSS: Neuropathy symptom score; IBS-SSS: Irritable bowel syndrome severity scoring system.

Table 4. Measures of nerve function in relation to self-reported neuropathy symptoms in POTS.

Comparison of objective signs of peripheral neuropathy in patients with POTS with low and high burden of self-reported neuropathy symptoms measured with NSS. Values are presented as number (percentages) or median (interquartile range). Comparative analyses were performed with Fisher's Exact test or Mann-Whitney U-test. A p-value <0.05 was considered statistically significant.

	Low NSS (<14.5), n=21	High NSS (>14.5), n=21	P-value
10 g Monofilament			
Right foot, normal (%)	19 (95.0%) ^a	19 (95.0%) ^a	0.756
Left foot, normal (%)	20 (100%) ^a	19 (95.0%) ^a	0.500
Neurothesiometer (V)			
Right side			
Medial malleolus	7.0 (5.5–8.7)	7.5 (5.6–9.3) ^a	0.593
Big toe	4.8 (3.9–6.2)	5.3 (4.1–7.8) ^a	0.411
Left side			
Medial malleolus	5.7 (4.5–7.8)	7.4 (5.8–9.2) ^a	0.105
Big toe	4.7 (3.3–6.7)	5.6 (4.1–7.7) ^a	0.235
IENFD (fibers/mm)	2.40 (1.10–3.11) ^a	2.26 (1.66–2.85) ^a	0.930
MFV (average Z-score)			
Hand			
Index finger	0.1 (-0.5–0.6) ^b	0.7 (-0.2–1.3) ^b	0.129
Little finger	0.0 (-0.5–0.8) ^b	0.9 (0.3–1.3) ^a	0.006
Foot	-0.3 (-0.6–0.4) ^b	0.6 (-0.1–1.2) ^b	0.011

NSS: Neuropathy Symptom Score; IENFD: Intraepidermal Nerve Fiber Density. MFV: Multi-frequency vibrometry; V=volts.

^a1 missing value.

^b2 missing values.

Discussion

POTS is a chronic, debilitating disorder associated with substantial impairment in quality of life. Previous studies have proposed multiple systemic pathophysiological mechanisms, although with varying levels of evidence. This thesis explored clinical features and potential mechanisms linking cardiovascular autonomic dysfunction, GI symptoms, and peripheral neuropathic signs and symptoms in POTS, with comparisons to matched healthy control participants.

Summary of principal findings

According to the four studies included in this thesis, the findings support the concept that POTS is associated with multisystem involvement and not only isolated cardiovascular autonomic dysfunction. However, no single pathophysiological mechanism could fully explain the observed symptom complexity after consideration of results from cardiovascular autonomic testing, GI symptom assessment, metabolic and gut-hormonal analyses, inflammatory markers, and peripheral nerve examinations.

The patients were highly symptomatic, with all assessed symptoms being significantly more severe than in healthy controls. Although the primary focus was on GI and neuropathic symptoms, results from the extraintestinal domain of the IBS-SSS questionnaire revealed a more widespread symptom burden, as described in Paper II. In addition, patients reported significantly lower psychological well-being than controls, which was strongly associated with the severity of all GI symptoms and nearly all extraintestinal symptoms. However, within the scope of present studies, no conclusions could be drawn regarding whether reduced psychological well-being contributes to increased symptom severity or represents a consequence of chronic symptoms.

Objective findings of nutritional deficiency, inflammation, hormonal abnormalities, autoimmunity, or peripheral neuropathy were scarce or absent, when compared with healthy controls. The only pathophysiological mechanistic finding distinguishing patients from controls was indirect evidence of SNS activation, as reported in Paper III.

Contrary to an expected linear relationship between ANS and ENS dysfunction, GI symptom burden was inversely correlated with the magnitude of orthostatic tachycardia. This finding, which was reported in Paper II, may reflect the influence of comorbid conditions rather than a direct association between cardiovascular autonomic dysfunction and GI symptoms.

Finally, subjective symptom severity tended to co-occur across different organ systems. Patients who reported more severe GI symptoms also reported worse peripheral neuropathic symptom burden, despite minimal or absent objective evidence of peripheral nerve pathology compared with healthy controls, as demonstrated in Paper IV. Together, these findings indicate that symptom burden in POTS is high, but not directly proportional to the severity of measurable autonomic or peripheral nerve dysfunction.

General discussion

Clinical phenotype and symptom burden

The studied cohort was dominated by young female patients, consistent with the known epidemiology of POTS (6, 23, 110). Although severe GI involvement requiring enteral or parenteral nutritional support has been described in selected cases (25), most previous studies report normal BMI in patients with POTS (2, 18, 22, 41, 42, 44, 51, 75, 81, 111-116), in agreement with the findings of the present thesis. Reported comorbidities were also in line with previous cohorts, including migraine, HSD/EDS, allergic conditions, and IBS (6, 28, 29, 117). The median number of regularly used medications was four, with some patients reporting up to eleven concurrent treatments. This is notable given the young age of the cohort and the limited evidence base for pharmacological treatment of POTS (118-120). This likely reflects symptom burden, comorbidities, and fragmented management.

Lifestyle factors differed in some respects between patients and controls. Smoking prevalence was low and comparable between POTS and controls, whereas alcohol consumption was significantly lower in POTS. Although not formally captured in the published data, patients frequently reported symptom exacerbation following alcohol intake, which made alcohol intake undesirable. Physical activity levels were also lower in POTS, consistent with previous studies, which have demonstrated exercise intolerance, reduced peak oxygen uptake, and reduced physical fitness (36, 37, 42, 121).

Dietary habits have been sparsely studied in POTS. A small recent study did not find major differences compared with controls (122). In Paper I, data from food diaries revealed increased intake of fluids, consistent with recommended medical

advice in POTS (31), but no other substantial differences in dietary patterns between POTS and controls. This contrasts with IBS where poor dietary habits are common and dietary intervention may improve GI symptoms (60, 62). Electrolytes, micronutrients, and serum albumin levels were within normal ranges, arguing against malnutrition and/or malabsorption. Thus, nutritional deficiencies seem unlikely to be the primary cause of symptoms or neuropathic features in POTS.

Socioeconomic consequences were substantial, as in previous studies on the subject (33, 35). Only a small proportion of patients worked or studied full-time (14%), while a large proportion were on sick leave (44%). These findings emphasize the considerable functional impact of the disorder.

The symptom burden was high, as reported in Paper II and IV. GI symptoms were markedly more severe in POTS than in controls, with nausea being the most frequently reported symptom. This is consistent with its classification as an orthostatic symptom (6, 29, 54, 55). The most severe symptoms, however, were bloating and flatulence, and constipation, which are symptoms rather regarded as non-orthostatic and also reported in previous studies (6, 24, 25, 123). The median total IBS-SSS score corresponded to moderately severe IBS (91). Notably, a comorbid diagnosis of IBS or organic GI disease did not significantly influence the GI symptom burden in POTS. This raises the question whether GI symptoms in POTS represent a distinct clinical entity or largely overlap with IBS. Patients with comorbid HSD/EDS reported more severe constipation than those without, in line with known GI involvement in this condition (124). Extraintestinal symptoms were pronounced, notably lethargy, bodily pain, and headache, as were peripheral neuropathic symptoms. The reported symptom pattern was similar to that described in a previous large survey-based study on POTS (6).

Overall, the patients included and presented in all four of the papers resemble other well-characterized POTS cohorts. The patients are highly symptomatic across multiple organ systems, with substantial consequences on socioeconomic and lifestyle factors. However, the observed nutritional and anthropometric parameters remain normal, and these observations argue against nutritional deficiency as a primary explanation for neuropathic features in POTS but highlight the complex and wide symptom burden.

Gastrointestinal symptoms and orthostatic physiology

The GI symptoms assessed in this thesis were analyzed in relation to the magnitude of HR increase recorded during the diagnostic head-up tilt test, and the subsequent active standing test. Based on the assumption of shared involvement of ANS and ENS in POTS, a positive association between orthostatic tachycardia and chronic GI symptom burden was expected. Contrary to this hypothesis, a greater HR increase during orthostatic testing was associated with *less* severe chronic,

predominantly non-orthostatic GI symptoms. This association persisted, and in some analyses strengthened, after exclusion of patients with organic GI disorders, as demonstrated in Paper II.

This inverse relationship was unexpected and has not been extensively explored in the POTS literature. Adolescents with orthostatic intolerance reported similar symptom burden regardless of the magnitude of HR increase upon tilt test (125). In contrast, a recent study, based on the SYSTEMA cohort, reported a positive association between HR increase and a newly developed composite score that included GI symptoms alongside multiple other orthostatic complaints (53). These findings underscore that associations between orthostatic tachycardia and symptom burden may depend on how symptoms are defined and aggregated.

Heart rate variability (HRV), another marker of autonomic regulation, has previously been studied in POTS, as well as in disorders of gut-brain interaction such as IBS. Reduced HRV, generally interpreted as reduced cardiovagal tone, has been associated with GI symptom severity, subtype, and stress in IBS, although findings are inconsistent across studies and methodologies (126-132). In POTS, reduced HRV has been demonstrated both supine (133), and during orthostatic challenge (134, 135), but associations with individual symptoms have not been systematically examined. HRV was, however, not examined in the present thesis.

The presence of comorbid HSD/EDS partially influenced the observed inverse relationship, with a tendency of less pronounced HR increase during orthostatic testing and slightly higher GI symptom burden. This observation may reflect the heterogeneity of POTS and suggests that in some patients, GI symptoms may be driven more strongly by comorbid conditions than by cardiovascular autonomic dysfunction per se.

Altogether, orthostatic tachycardia and chronic GI symptoms may represent parallel but partially independent processes in POTS. Severe non-orthostatic GI symptoms appear to dominate the clinical picture in patients closer to the diagnostic threshold for POTS. These findings challenge the assumption that the magnitude of orthostatic tachycardia reflects the global burden of autonomic dysfunction in POTS.

Metabolic findings, insulin, and sympathetic activation

Disturbances in glucose homeostasis and insulin resistance have been connected to the development of neuropathy, even in the absence of overt diabetes (136). Subtle signs of insulin resistance have previously been described in POTS (44, 137), as well as in conditions that often co-occur with POTS such as IBS and migraine (138-141). In line with these observations, fasting insulin levels were modestly higher in patients with POTS compared with controls, as reported in Paper III. In contrast, the non-fasting insulin levels did not differ between groups, underscoring the importance of cautious interpretation. Unfortunately, it was not possible to analyze

fasting plasma glucose from the blood samples stored in the freezer, but HbA1c was normal and comparable between POTS and controls. To our knowledge, this is the first study to report HbA1c levels in POTS.

Elevated fasting insulin levels in the context of normal HbA1c could generally be interpreted as a marker of insulin resistance (142). Insulin is a potent hormone with central roles in glucose- and lipid metabolism, with widespread effects all over the human body (143, 144). Hyperinsulinemia is associated with the metabolic syndrome, which is characterized by dyslipidemia, visceral adiposity, and hypertension (145). In the present study, however, BP values were within normal range, and although there were substantial missing data on BMI, it was in general normal and leptin levels did not differ between POTS and controls. These factors argue against metabolic syndrome as the primary explanation for the observed hyperinsulinemia in POTS.

Notably, fasting insulin and C-peptide levels correlated strongly with both supine and orthostatic BP levels in POTS, but not in controls. Given the absence of metabolic syndrome features, this association may instead reflect SNS activation, even at rest. SNS activation is known to induce insulin resistance through stimulation of hepatic glucose production and inhibition of peripheral glucose uptake (146). Hyperadrenergic states represent one of the central pathophysiological mechanisms in POTS (23, 32, 38).

Because cortisol is another stress-related hormone capable of increasing blood glucose and insulin-levels, and also associated with elevated BP (147, 148), fasting cortisol was assessed. Previous studies have demonstrated largely preserved function of the adrenal cortical function in POTS (114), although increased morning cortisol levels have been reported (113, 149). In the present study, morning cortisol levels were within normal limits and showed no association with insulin, C-peptide, or BP in POTS. Thus, cortisol is unlikely the driver of the observed metabolic findings in the present study.

Insulin and C-peptide levels did not correlate with GI symptom severity in the non-fasting cohort, and symptom questionnaires were not completed at the time of fasting blood sampling. Consequently, the present data do not allow firm conclusions regarding an association between possible SNS activation and GI symptoms.

Taken together, these results suggest that modest fasting hyperinsulinemia in POTS may reflect chronic sympathetic activation rather than underlying metabolic disease. Elevated insulin levels may therefore serve as an indirect marker of autonomic imbalance in POTS, detectable even in the supine resting state. Similar mechanisms could potentially be relevant in associated conditions such as IBS or migraine, although this hypothesis was not addressed within the scope of the present thesis.

Gut hormones, hemodynamics, and gastrointestinal symptoms

Gut hormones have been only sparsely investigated in POTS. A previous small study demonstrated increased levels of GIP and PYY in patients with POTS following an oral glucose tolerance test, where elevated GIP levels during subsequent tilt testing correlated significantly with decreased stroke volume in POTS but not in controls. This finding suggested a potential pathogenetic role for gut hormones in postprandial cardiovascular dysregulation in POTS (44). Worsening of orthostatic symptoms has also recently been observed in a patient with POTS treated with a combined GLP-1 and GIP receptor agonist used for weight reduction (150).

In contrast to those findings, no differences were observed in fasting or non-fasting circulating levels of GIP, GLP-1, or PYY between patients with POTS and controls, as reported in Paper III. Moreover, circulating gut hormone levels were not associated with hemodynamic measures or GI symptom severity. These results suggest that baseline or random systemic levels of gut hormones are not altered in POTS.

Gut hormones are secreted by entero-endocrine cells throughout the GI tract in response to ingested food and nutrient exposure. Once released, they exert local effects on motility, secretion, permeability, and visceral sensitivity. They also signal via enteric, autonomic, and finally central neural pathways (70, 151). GIP and GLP-1 exert a strong stimulating effect on pancreatic β -cells to release insulin in response to ingested intraluminal nutrients (152).

In disorders of gut-brain interaction such as IBS, altered density and distribution of entero-endocrine cells have been demonstrated, possibly explained by interaction with diet and gut microbiota. However, circulating hormone levels are often normal or inconsistent across studies (70, 153-157). This discrepancy is likely explained by the rapid enzymatic degradation of gut hormones. In addition, gut hormones have a predominantly paracrine mode of action, which makes fasting or random plasma measurements insensitive to local alterations (152, 158, 159). This limits the interpretability of fasting or random measurements of gut hormones.

Therefore, the absence of systemic gut hormone abnormalities in POTS in this thesis doesn't exclude altered local entero-endocrine signaling, including postprandial responses. Importantly, the modest fasting hyperinsulinemia observed in POTS is unlikely driven by the effects of GIP or GLP-1, as these incretin hormones are released in response to luminal content rather than fasting glucose levels in blood (160). Instead, sympathetic activation with stimulation of hepatic gluconeogenesis and thereby direct stimulation of pancreatic insulin secretion provides a more plausible explanation.

Autoimmunity

Autoimmune mechanisms have been proposed to contribute to the pathophysiology in POTS and immune-modulating therapies have been suggested in selected patients. However, current evidence is still limited and largely case-based (32, 49, 161). Broadly, the POTS literature has focused on two distinct immunological hypotheses: 1) an increased prevalence of established systemic autoimmune or autoinflammatory diseases interpreted as autoimmunity in general, and 2) the presence of autoantibodies directed against autonomic receptors or other structures involved in hemodynamic regulation, with direct effects on autonomic functions. To date, no causative antibody has been found (49, 162).

Several systemic autoimmune diseases have been described to occur more often in POTS than in the general population, including celiac disease, Sjögren's syndrome, systemic lupus erythematosus, antiphospholipid syndrome, rheumatoid arthritis, and thyroid disease (6, 27, 49). However, the quality of these data is limited by retrospective designs, self-reported diagnoses, lack of matched control material, and risk of selection bias (26).

Markers of classical systemic autoimmunity were assessed using a microarray targeting autoantibodies associated with systemic autoimmune diseases, as presented in Paper II. These conditions were selected due to their known associations with autonomic dysfunction and GI involvement (46, 163). Overall, the prevalence of any autoantibody positivity did not differ between POTS and controls, and the frequency of ANA reactivity was comparable between groups. This finding is contrasting to a previous retrospective study where ANA was positive in 25% of POTS patients versus the estimated 16% in the general population (27). In the present thesis, the presence of antibodies in POTS was not associated with GI symptom burden. A strength of the present study is that antibodies were tested broadly and in unselected patients, with comparisons to matched controls, which differs methodologically from previous studies that were performed retrospectively (26).

Anti-Scl-70 antibodies were detected in four patients with POTS but in none of the controls. This finding is noteworthy and has never been shown before. Anti-Scl-70 is strongly associated with systemic sclerosis, and linked to internal organ involvement, including GI dysmotility (164, 165). Given that POTS is a diagnosis of exclusion, the presence of anti-Scl-70 antibodies raise the possibility that these individuals may represent early or atypical systemic sclerosis rather than POTS with autoimmune features. Although patients with POTS share some general symptom patterns with systemic sclerosis, clinical features specific to this disorder were not investigated in the present POTS cohort (166). From a clinical perspective, this finding emphasizes the importance of carefully considering alternative diagnoses when systemic autoimmune markers are present.

Apart from these isolated cases, the results do not support the notion that classical IgG-mediated systemic immunity is a defining feature of confirmed POTS. The inclusion of well-matched controls and unselected testing in the POTS cohort strengthens this conclusion. Immune dysregulation may still play a role in subsets of patients, particularly through mechanisms not captured by conventional autoantibody screening. However, the present findings argue against routine screening for systemic autoimmune diseases in unselected patients with POTS.

Mast cells and allergies

In classical allergic reactions, mast cells are acutely activated and release mediators such as tryptase, which typically peaks 1–2 hours after activation before gradually returning to baseline. Persistently elevated baseline tryptase levels are usually seen in clonal mast cell disorders such as mastocytosis and may also be present in hereditary α -tryptasemia (50, 167-170). In contrast, mast cell activation syndrome (MCAS) is characterized by episodic and transient elevations of mast cell mediators and symptom improvement following treatment with mast cell-stabilizing therapy (171).

MCAS has been proposed as a contributor to GI symptoms in POTS, based on reports of elevated mast cell metabolites in urine and blood (50, 51). Local alterations in mast cell distribution, density, and function have also been a proposed mechanism in IBS (68, 172). Conversely, patients with primary mast cell disorders may report pronounced autonomic symptoms despite relatively mild objective autonomic dysfunction (173).

Baseline serum tryptase levels in this thesis were largely within normal range. Only one patient exhibited mildly elevated levels, and three additional patients had values in the upper normal range. Due to the small number of individuals in this subgroup, further analysis of GI symptoms was not performed. These findings are consistent with a recent large retrospective study of 250 patients with POTS, in which only a small minority exhibited tryptase levels in the upper normal range or slightly elevated. The estimated prevalence of hereditary α -tryptasemia was comparable to that of the general population (168).

Because of the link between mast cell activity and allergic disorders, co-occurring allergic conditions were also examined in POTS. In total, 44% of patients reported allergic comorbidities, use of anti-allergic medication, and/or exhibited elevated IgE-levels in blood. This proportion is likely somewhat higher than that reported in the age-matched general population in Sweden (174, 175). Importantly, GI symptom burden did not differ between patients with and without allergic features, as shown in Paper II.

A limitation is that baseline tryptase measurement may fail to capture the dynamic nature of MCAS, which requires paired sampling of mast cell mediators during

symptomatic episodes and at baseline (171). Nevertheless, given the high and persistent symptom burden in this POTS cohort, one might expect at least a tendency toward elevated baseline tryptase levels if MCAS were a dominant mechanism. The absence of such a pattern suggests that primary mast cell disorders or inherited tryptase abnormalities are unlikely to represent common drivers of symptoms in an unselected POTS cohort. However, there is still a possibility that localized or episodic mast cell involvement may exist in selected patients.

Peripheral neuropathy – subjective symptoms versus objective findings

Neuropathic symptoms were common and often severe in POTS, with abnormal sensations of heat and cold being the most frequently reported complaints. These are symptoms typically attributed to SFN (176). Patients also reported numbness and dull pain in the hands and feet, which are symptoms more often associated with dysfunction of moderately myelinated A β nerve fibers (107, 176). These findings are consistent with previously reported symptom patterns in POTS (6). Neurological examinations, including deep tendon reflexes, were largely normal, in line with earlier reports (76, 83). A small number of patients showed non-specific motor abnormalities, most likely reflecting general muscle weakness or deconditioning rather than neuropathy.

Objective assessment of large-fiber function using neurothesiometry demonstrated normal age-adjusted VPTs in all patients, comparable with controls. However, when using MFV, minor deviations in VPTs were observed in a subset of patients, particularly among those who reported higher NSS burden. While this may suggest subtle sensory variations, the absolute differences were small, and largely within normal limits. This may thus represent chance findings or multiple testing rather than clinical meaningful neuropathy.

Assessment of small-fiber pathology using IENFD did not reveal differences between POTS and controls. This contrasts with several previous studies reporting reduced IENFD in subsets of POTS patients (75-79, 82). However, only one of these included matched healthy controls and simultaneous symptom assessment (75). In alignment with the negative findings in this thesis, Singer et al. found no evidence of SFN by IENFD (177), and corneal confocal microscopy likewise failed to demonstrate SFN in a small symptomatic POTS cohort (178). Some prior studies have suggested functional impairment of autonomic nerve fibers such as abnormal sudomotor function, with or without structural loss of nerve fibers (81-84, 177). SFN is not specific to POTS and occurs in a range of systemic metabolic, endocrine, autoimmune, inflammatory, and infectious disorders (179). Symptoms and routine clinical signs indicative of SFN have recently been reported in subsets of patients with IBS, and reduced sudomotor function has been shown in underweight patients with anorexia nervosa, highlighting the complexity of the interpretation of SFN-related findings (180, 181).

Patients with a high NSS burden also reported greater GI symptom burden than those with fewer NSS complaints. However, there were no differences between these groups in routine sensory nerve assessments or IENFD. Although GI symptom burden correlated with self-reported NSS burden, no associations were found between GI symptoms and objective measures of peripheral neuropathy. Overall, no robust relationship was identified between subjective neuropathic symptoms and objective measures of large- or small-fiber pathology, arguing against peripheral neuropathy as a unifying mechanism underlying both GI and neuropathic symptoms in POTS.

A major strength of the present study is the comprehensive evaluation of peripheral neuropathy, combining symptom assessment with clinical examination and detailed investigation of both large and small nerve fibers, allowing direct comparison between subjective and objective findings. However, a limitation worth discussing is the use of 5 μ m biopsy sections. Since many other studies on the subject have demonstrated at least subtle signs of SFN, it is possible that this size is too small to find reduced IENFD in POTS. Biopsy cuts of 50 μ m are recommended as gold standard for supporting the diagnosis of SFN affecting A δ - or C-fibers and/or autonomic nerve fibers (182). However, the assessment of 50 μ m skin biopsies is time-consuming and impractical for routine clinical application (73). Importantly, thinner sections (5–10 μ m) have previously yielded reliable IENFD assessments in diabetic neuropathy and SFN cohorts (107, 109, 183). In any case, it is reasonable to ask whether subtle structural changes, potentially missed by thinner sections, could reasonably explain the full symptom burden observed in POTS.

The lack of clear association between subjective neuropathic symptoms and objective neuropathic findings, together with the tendency for symptom clustering across organ systems, argues against structural peripheral neuropathy as a unifying pathophysiological mechanism in POTS. Instead, these findings support a role for functional and/or central mechanisms in symptom generation and perception, consistent with recent evidence showing a mismatch between self-reported symptoms and objective measures of dysautonomia in POTS (184).

Understanding gastrointestinal and neuropathic features in POTS

In summary, the findings of this thesis support the concept that POTS is associated with a high symptom burden, but do not support POTS as a generalized systemic peripheral neuropathic disorder. The observations from the initial double case study that served as the conceptual inspiration for the thesis work-up could not be replicated in the four complementary studies presented (7).

Importantly, these findings do not exclude the possibility that some of the mechanisms tested and presented throughout the thesis may be relevant in selected

subsets of patients. Rather, the findings suggest that such mechanisms are unlikely to represent typical universal features in an unselected POTS cohort.

The tendency for widespread symptom severity, together with a lack of concordance between symptom burden and objective measurable findings, may point toward functional and/or centrally mediated mechanisms influencing symptom severity. Central sensitization, a phenomenon described in IBS and other comorbidities common in POTS, may provide one explanatory framework (56, 185). Somatic hypervigilance has also been reported in POTS and may further amplify symptom perception (24, 186). Throughout this work, similarities between POTS and IBS were noted, and parallels have therefore been used as a framework for the discussion. These parallels include shared GI symptom profiles, overlapping comorbidities, glucose-insulin features, similarities in ANS regulation, mast cell biology, and reports of small fiber dysfunction in both conditions.

Furthermore, genetic studies in IBS and other disorders with increased pain perception suggest that polymorphisms in catecholaminergic receptor pathways may modulate symptom severity (187, 188). Reduced activity of presynaptic α 2-adrenergic receptors with consequent increased sympathetic activity has also been shown in IBS, which also may contribute to the symptom severity (189). These autonomic receptor pathways have also been associated with measurable structural alterations in neuroimaging studies. Of particular interest are areas involved in pain modulation, arousal and affective processing (189, 190). In addition, psychosocial factors seem to interact with these altered catecholaminergic pathways and structural findings, where early adverse life events seem to be of special interest (187-189). These childhood risk factors are more common in IBS than in controls, and particularly emotional abuse seem to be associated with development of IBS (191).

Interestingly, similar alterations in brain areas associated with visceral sensation, emotion, and stress regulation have been reported in small neuroimaging studies of POTS, although it remains unclear whether these findings represent predisposing vulnerabilities or secondary adaptations to chronic symptoms. Early adverse life events are currently emerging as possible risk factors for symptom severity in POTS as well (190, 192-194). In both IBS and POTS, psychological well-being is closely linked to symptom severity, as also observed in the present thesis. This supports a model in which autonomic imbalance, central sensitization, and psychosocial factors interact to shape the clinical phenotype in both POTS and IBS.

Strengths and limitations

While methodological considerations have been addressed within the relevant sections above, several overarching strengths and limitations warrant emphasis. All four studies were cross-sectional and compared patients with POTS to largely matched healthy controls. The inclusion of a control group strengthens interpretability, particularly in the light of several negative findings. However, a cross-sectional design captures only a snapshot and does not allow conclusions regarding temporal relationships, disease progression, or causality.

The main patient cohort was relatively small ($n=43$) and selected from a tertiary referral center. There is a risk of selection bias due to the study design focus on neuropathic features. Invited patients who experienced more sensory symptoms may have been more willing to participate in this study due to this overarching study question, thus limiting generalizability. At the same time, the demographic characteristics, comorbidity patterns, and symptom profiles were broadly consistent with other well-described POTS cohorts, supporting external validity.

Healthy controls were recruited among hospital staff and students and may represent a particularly healthy subset of the general population. This may potentially increase the between-group contrasts for self-reported symptoms and lifestyle factors. In addition, some sub-analyses were based on smaller sample sizes (e.g. dietary diaries in 24 patients and IENFD measurements in 13 controls), which reduces statistical power and increases the risk of type II error (i.e. failing to detect true group differences).

Most comorbidities were not used as exclusion criteria, reflecting real-world clinical POTS populations. However, this increases heterogeneity and makes it difficult to separate findings specific to POTS from those driven by comorbid conditions. Patients were generally not asked to discontinue medications, to preserve feasibility and safety of the inclusion procedure. Use of medications may have influenced symptom reporting and some physiological measures, including the metabolic factors (195, 196). Notably, participants in the fasting cohort in Paper III largely abstained from medication.

Finally, the study design included broad phenotyping using questionnaires and validated GI instruments, which facilitate the basic understanding of POTS and enable comparisons with disorders of gut-brain interaction such as IBS. Ideally, symptoms questionnaires would have been collected closer in time to the diagnostic tilt test. However, data correlations with later performed active standing tests were similar to those obtained by the tilt tests, which supported the robustness of the observed associations.

Conclusions

POTS is associated with a high symptom burden in multiple organ systems, with substantial impact on socioeconomic participation and lifestyle factors. This thesis focused primarily on GI and neuropathic symptoms, however, extraintestinal symptoms were also common and severe, underscoring the multisystem nature of the disorder.

An inverse relationship between the magnitude of HR increase during orthostatic testing and chronic, predominantly non-orthostatic, GI symptoms were observed. This finding challenges the assumption of a linear positive relationship between cardiovascular autonomic dysfunction and enteric symptom severity. Instead, comorbid conditions may play a more prominent role in GI symptom expression.

The most consistent physiological finding was indirect evidence of sympathetic activation. A strong association between fasting insulin and C-peptide levels and BP was present in POTS but not in healthy controls, supporting autonomic imbalance as one contributor to the clinical phenotype.

In contrast, objective evidence of nutritional deficiency, chronic mast cell disorders, classical systemic autoimmunity, metabolic disturbances, baseline gut-hormonal abnormalities, or generalized peripheral neuropathy was scarce and did not meaningfully differ from matched healthy controls. These findings indicate that symptom burden in POTS is not readily explained by measurable structural pathology in an unselected cohort.

Overall, the results support a model in which autonomic imbalance interacts with central and functional mechanisms to shape symptoms perception and severity in POTS.

Future perspectives

This thesis provides a snapshot of POTS and highlights several challenges faced by patients and caregivers. However, no novel pathophysiological mechanisms or clinically relevant findings were identified. Instead, the results mainly emphasize functional aspects of POTS.

To improve understanding of disease development and potential long-term complications of POTS – including possible associations with metabolic syndrome, overt diabetes, or autoimmune disorders – longitudinal studies with comparisons to matched population-based controls are needed. The SYSTEMA register in Malmö, established in 2008, may represent a valuable resource for future investigations.

Although this thesis aimed to capture a broad clinical picture of POTS, certain observations fell outside the predefined study's aims and were therefore not formally analyzed. These included clinical impressions of hypervigilance and highly engaged caregivers. Such observations raise hypotheses regarding behavioral or learned patterns of symptom perception, which would be interesting to further explore. Qualitative research approaches may contribute to a deeper understanding of behavioral dimensions of POTS, including perspectives of both patients and caregivers. While qualitative studies are emerging in the field, they are still limited (197-201).

No evidence of classical autoimmunity was seen in the POTS cohort included in this thesis. However, recent studies have reported antibodies or functional activity directed against G protein-coupled receptors in the autonomic nervous system, including α - and β -adrenergic and muscarinic receptors, in subsets of patients with POTS. Activity at these receptors has been associated with GI or orthostatic symptoms in some studies (47, 202). This emerging field deserves attention, particularly in studies that may integrate autonomic phenotyping, neuroimaging, and factors associated with vulnerability, such as early adverse life events. Such approaches may also be relevant to research in IBS and other disorders of gut-brain interaction.

Despite the use of pharmacological agents, patients included in this thesis did not report satisfactory symptom relief. Although commonly used medications in POTS appear to influence various physiological parameters, large placebo-controlled trials evaluating major symptom relief remain scarce (203-205). This represents an important area for future research. In parallel, non-pharmacological strategies such

as use of compression garments, increased fluid and salt intake, exercise training, yoga, and deep-breathing practices are currently under investigation in POTS (118, 121, 206-210). These largely low-risk lifestyle modifications may be integrated into clinical care. Behavioral therapies are currently insufficiently studied but have shown promising preliminary results (211, 212) and warrant further research.

Throughout this thesis, similarities between POTS and IBS were noted. Management strategies for IBS, refined over several decades, emphasize a strong physician-patient relationship grounded in person-centered care, including confident diagnosis, clear explanation, reassurance, and patient education. Lifestyle advice, behavioral interventions, and pharmacological treatments are then tailored to individual needs (213, 214). A similar framework may be applicable to POTS and merits further investigation, particularly regarding its feasibility in primary care settings.

Populärvetenskaplig sammanfattning

Posturalt ortostatiskt takykardisyndrom (POTS) är ett tillstånd där hjärtat slår onormalt snabbt vid uppresning från liggande till stående. Förutom hjärtrusning drabbas patienterna av trötthet, yrsel och illamående vid uppresning. Vid POTS ses inget tydligt blodtrycksfall vid uppresning, vilket skiljer det från tillståndet ortostatism, som är en vanlig orsak till svimning bland äldre människor. Innan diagnosen POTS ställs behöver symptomen ha funnits i minst tre månader och andra medicinska orsaker som kan ge likartad symptombild, såsom hormonella sjukdomar eller blodbrist, ska vara uteslutna.

POTS är inte ett nytt tillstånd men det fick sitt nuvarande namn på 1990-talet efter att man sett den typiska bilden hos amerikanska veteraner. Under senare år har intresset för POTS ökat kraftigt, bland annat på grund av likheter med långvariga symptom efter COVID-19. Trots detta är kunskapen om varför POTS uppstår fortfarande begränsad. Det man däremot vet är att sjukdomen är förknippad med störningar i det autonoma nervsystemet, som styr bland annat puls, hjärtats pumpförmåga och blodkärlens funktion utan att vi själva egentligen är medvetna om det.

Den typiska patienten med POTS är en ung kvinna som ofta fått vänta länge på diagnos. Många har även andra besvär, bland annat migrän, fibromyalgi, överrörlighet i leder och mag- och tarmbesvär, vilka ofta påminner om irritabel tarm (IBS). Symptombilden är alltså ofta bred och varierande, vilket kan göra det svårt att förstå vad som ligger bakom besvären. En stor del av patienterna rapporterar att symptomen utlöstes av en eller flera händelser, till exempel infektioner, operationer eller graviditeter. För en del patienter ebbar symptomen ut med åren, men för många tenderar de att bli kroniska eller återkommande.

Denna avhandling utgår från en tidigare pilotstudie som gjordes i Malmö under 2018. Två patienter med väldokumenterad motorikstörning med nervpåverkan i mag- och tarmkanalen undersöktes med avsikt att finna nervpåverkan även i andra delar av nervsystemet utanför själva hjärnan. Båda patienterna hade avvikande resultat på uppresningstesterna, och vid biopsitagning från huden i underbenen sågs ovanligt få tunna nervfibrer jämfört med friska individer. Detta väckte frågan om det finns gemensamma mekanismer som kan förklara både hjärt- och kärlsymptom, mag- och tarmbesvär och nervsymptom vid POTS.

Det övergripande syftet med avhandlingsarbetet var därför att kartlägga olika gemensamma nämnare som kan knyta ihop nedsatt funktion i det autonoma nervsystemet med störningar i mag- och tarmfunktion samt tecken på störningar i känselnerv i huden. Avhandlingen består av fyra delarbeten där 43 patienter med POTS studerades och resultaten jämfördes med friska kontrollpersoner. I de fyra delstudierna undersöktes livsstil, kost, mag- och tarmsymptom, hormonella och metabola faktorer, immunologiska markörer samt tecken på nervpåverkan.

Resultaten visar att patienter med POTS har en mycket hög symptomnivå jämfört med friska kontroller, särskilt från mag- och tarmkanalen och nervsystemet. Det fanns en stark koppling mellan symtomens svårighetsgrad och patienternas självskattade psykologiska mående. De patienter som upplevde svårast mag-och tarmsymptom hade även de svåraste nervsymptomen. Patienterna uppvisade även inskränkningar i livet i övrigt, med lägre utbildningsnivå, lägre sysselsättningsgrad, hög grad av sjukskrivning, nedsatt fysisk aktivitet och ett betydligt lägre alkoholintag än friska kontrollpersoner. Samtidigt kunde få tydliga mätbara objektiva avvikelser påvisas. Det fanns inga tecken på näringsbrist, uttalad inflammation, autoimmuna sjukdomar, mastcellsjukdom eller avvikande nivåer av hormoner som styr mag- och tarmkanalens funktion. Några tecken på generell nervskada kunde inte heller visas efter jämförelse med friska kontrollpersoner.

Det enda objektiva undersökningsfyndet som skiljde sig tydligt från kontrollpersonerna var lätt förhöjda insulinnivåer vid fasta hos patienter med POTS, som dessutom var kopplade till stegrade blodtrycksnivåer. Detta fynd kan tala för en överaktivitet i den sympatiska delen av det autonoma nervsystemet, vilket ibland brukar benämnas som "fight or flight". Långtidsblodsocker var däremot normalt i patientgruppen, trots de ökade insulinnivåerna.

Avhandlingen visar också att graden av hjärtrusning vid uppresning inte speglar hur uttalade de långvariga mag-tarmbesvären är. Tvärtom hade vissa patienter med mindre uttalad pulsökning svårare mag-tarmsymptom, vilket möjligtvis beror på annan samsjuklighet.

Sammanfattningsvis visar denna avhandling att POTS är ett tillstånd med stor symptomtyngd och påverkan på vardagsliv, arbete och livskvalitet. Däremot visar resultaten ingen tydlig koppling mellan patienternas upplevda symptom och objektiva mätbara fynd som skulle kunna tyda på, eller ligga bakom, störningar i nervsystemet. Det är möjligt att centrala mekanismer i hjärnan kan spela en viktig roll i hur symptomen uppstår och upplevs. Framtida forskning bör därför fokusera både på vad som föregår POTS-diagnosen, kanske med hjälp av mer djupgående intervjuer, och vad som händer med patienterna på lång sikt. Även forskning på behandling mot symptom vid POTS är viktig, eftersom dagens behandling mot POTS främst bygger på expertråd och inte placebokontrollerade behandlingsstudier.

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References

1. Low PA, Opfer-Gehrking TL, Textor SC, Benarroch EE, Shen WK, Schondorf R, et al. Postural tachycardia syndrome (POTS). *Neurology*. 1995;45(4 Suppl 5):S19-25.
2. Björnson M, Wijnblad K, Törnberg A, Svensson-Raskh A, Svensson A, Ståhlberg M, et al. Prevalence and Clinical Impact of Postural Orthostatic Tachycardia Syndrome in Highly Symptomatic Long COVID. *Circ Arrhythm Electrophysiol*. 2025;18(10):e013629.
3. World Health Organization. 8D89.2 Postural orthostatic tachycardia syndrome. Available from: <https://icd.who.int/browse/2025-01/mms/en#1533647472>. [Accessed December 15, 2025].
4. Dysautonomia International. New ICD-10 Code for POTS. Available from: <https://www.dysautonomiainternational.org/pdf/ICDProviderLetter.pdf>. [Accessed December 15, 2025].
5. Olshansky B, Cannom D, Fedorowski A, Stewart J, Gibbons C, Sutton R, et al. Postural Orthostatic Tachycardia Syndrome (POTS): A critical assessment. *Prog Cardiovasc Dis*. 2020;63(3):263-70.
6. Shaw BH, Stiles LE, Bourne K, Green EA, Shibao CA, Okamoto LE, et al. The face of postural tachycardia syndrome - insights from a large cross-sectional online community-based survey. *J Intern Med*. 2019;286(4):438-48.
7. Ohlsson B, Dahlin LB, Englund E, Veress B. Autonomic and peripheral neuropathy with reduced intraepidermal nerve fiber density can be observed in patients with gastrointestinal dysmotility. *Clin Case Rep*. 2020;8(1):142-8.
8. Martin JH. *Neuroanatomy: Text and Atlas*. 5th ed. New York: McGraw Hill; 2021.
9. Townsend KL. One Nervous System: Critical Links Between Central and Peripheral Nervous System Health and Implications for Obesity and Diabetes. *Diabetes*. 2024;73(12):1967-75.
10. Benarroch EE. *Physiology and Pathophysiology of the Autonomic Nervous System*. Continuum (Minneapolis). 2020;26(1):12-24.
11. Sanvictores T, Jozsa F, Tadi P. *Neuroanatomy, Autonomic Nervous System Visceral Afferent Fibers and Pain*. StatPearls. Treasure Island (FL): StatPearls Publishing. Copyright © 2025, StatPearls Publishing LLC.; 2025.

12. Waxenbaum JA, Reddy V, Varacallo MA. Anatomy, Autonomic Nervous System. StatPearls. Treasure Island (FL): StatPearls Publishing. Copyright © 2025, StatPearls Publishing LLC.; 2025.
13. Squire LDB, Bloom FE, du Lac S, Ghosh A, Spitzer NC. Fundamental Neuroscience. 4th ed: Academic Press (Elsevier); 2012.
14. Hall JE, Hall ME. Guyton and Hall Textbook of Medical Physiology. 14th ed. Philadelphia: Elsevier; 2021.
15. Gibbons CH. Basics of autonomic nervous system function. *Handb Clin Neurol*. 2019;160:407-18.
16. Koloski NA, Jones M, Kalantar J, Weltman M, Zaguirre J, Talley NJ. The brain--gut pathway in functional gastrointestinal disorders is bidirectional: a 12-year prospective population-based study. *Gut*. 2012;61(9):1284-90.
17. Bazira PJ. An overview of the nervous system. *Surgery (Oxford)*. 2024;42(8):525-35.
18. Raj SR, Biaggioni I, Yamhure PC, Black BK, Paranjape SY, Byrne DW, et al. Renin-aldosterone paradox and perturbed blood volume regulation underlying postural tachycardia syndrome. *Circulation*. 2005;111(13):1574-82.
19. Jacob G, Ertl AC, Shannon JR, Furlan R, Robertson RM, Robertson D. Effect of standing on neurohumoral responses and plasma volume in healthy subjects. *J Appl Physiol* (1985). 1998;84(3):914-21.
20. Gordan R, Gwathmey JK, Xie LH. Autonomic and endocrine control of cardiovascular function. *World J Cardiol*. 2015;7(4):204-14.
21. Arnold AC, Ng J, Raj SR. Postural tachycardia syndrome - Diagnosis, physiology, and prognosis. *Auton Neurosci*. 2018;215:3-11.
22. Hamrefors V, Spahic JM, Nilsson D, Senneby M, Sutton R, Melander O, et al. Syndromes of orthostatic intolerance and syncope in young adults. *Open Heart*. 2017;4(1):e000585.
23. Vernino S, Bourne KM, Stiles LE, Grubb BP, Fedorowski A, Stewart JM, et al. Postural orthostatic tachycardia syndrome (POTS): State of the science and clinical care from a 2019 National Institutes of Health Expert Consensus Meeting - Part 1. *Auton Neurosci*. 2021;235:102828.
24. DiBaise JK, Harris LA, Goodman B. Postural Tachycardia Syndrome (POTS) and the GI Tract: A Primer for the Gastroenterologist. *Am J Gastroenterol*. 2018;113(10):1458-67.
25. Mehr SE, Barbul A, Shibao CA. Gastrointestinal symptoms in postural tachycardia syndrome: a systematic review. *Clin Auton Res*. 2018;28(4):411-21.
26. Mannan H, Pain CM. Sex adjusted standardized prevalence ratios for celiac disease and other autoimmune diseases in patients with postural orthostatic tachycardia

- syndrome (POTS): A systematic review and meta-analysis. *Heliyon*. 2023;9(2):e12982.
27. Blitshteyn S. Autoimmune markers and autoimmune disorders in patients with postural tachycardia syndrome (POTS). *Lupus*. 2015;24(13):1364-9.
 28. Liao Y, Zhang QY, Li HX, Wang YL, Liu P, Du JB. [Co-morbidity of vasovagal syncope and postural tachycardia syndrome with allergic diseases in children]. *Beijing Da Xue Xue Bao Yi Xue Ban*. 2017;49(5):783-8.
 29. Chelimsky G, Chelimsky T. The gastrointestinal symptoms present in patients with postural tachycardia syndrome: A review of the literature and overview of treatment. *Auton Neurosci*. 2018;215:70-7.
 30. Brignole M, Moya A, de Lange FJ, Deharo JC, Elliott PM, Fanciulli A, et al. 2018 ESC Guidelines for the diagnosis and management of syncope. *Eur Heart J*. 2018;39(21):1883-948.
 31. Raj SR, Fedorowski A, Sheldon RS. Diagnosis and management of postural orthostatic tachycardia syndrome. *Cmaj*. 2022;194(10):E378-e85.
 32. Fedorowski A. Postural orthostatic tachycardia syndrome: clinical presentation, aetiology and management. *J Intern Med*. 2019;285(4):352-66.
 33. Bourne KM, Chew DS, Stiles LE, Shaw BH, Shibao CA, Okamoto LE, et al. Postural orthostatic tachycardia syndrome is associated with significant employment and economic loss. *J Intern Med*. 2021;290(1):203-12.
 34. Raj SR, Bourne KM, Stiles LE, Miglis MG, Cortez MM, Miller AJ, et al. Postural orthostatic tachycardia syndrome (POTS): Priorities for POTS care and research from a 2019 National Institutes of Health Expert Consensus Meeting - Part 2. *Auton Neurosci*. 2021;235:102836.
 35. Boris JR, Shadiack EC, 3rd, McCormick EM, MacMullen L, George-Sankoh I, Falk MJ. Long-Term Postural Orthostatic Tachycardia Syndrome Outcomes Survey: Educational, Economic, and Social Impact. *J Am Heart Assoc*. 2025;14(21):e042365.
 36. Benarroch EE. Postural tachycardia syndrome: a heterogeneous and multifactorial disorder. *Mayo Clin Proc*. 2012;87(12):1214-25.
 37. Fu Q, Levine BD. Exercise in the postural orthostatic tachycardia syndrome. *Auton Neurosci*. 2015;188:86-9.
 38. Garland EM, Raj SR, Black BK, Harris PA, Robertson D. The hemodynamic and neurohumoral phenotype of postural tachycardia syndrome. *Neurology*. 2007;69(8):790-8.
 39. Cervi AL, Lukewich MK, Lomax AE. Neural regulation of gastrointestinal inflammation: role of the sympathetic nervous system. *Auton Neurosci*. 2014;182:83-8.

40. Jacob G, Costa F, Shannon JR, Robertson RM, Wathen M, Stein M, et al. The neuropathic postural tachycardia syndrome. *N Engl J Med*. 2000;343(14):1008-14.
41. Loavenbruck A, Iturrino J, Singer W, Sletten DM, Low PA, Zinsmeister AR, et al. Disturbances of gastrointestinal transit and autonomic functions in postural orthostatic tachycardia syndrome. *Neurogastroenterol Motil*. 2015;27(1):92-8.
42. Fu Q, Vangundy TB, Galbreath MM, Shibata S, Jain M, Hastings JL, et al. Cardiac origins of the postural orthostatic tachycardia syndrome. *J Am Coll Cardiol*. 2010;55(25):2858-68.
43. Mar PL, Raj SR. Postural Orthostatic Tachycardia Syndrome: Mechanisms and New Therapies. *Annu Rev Med*. 2020;71:235-48.
44. Breier NC, Paranjape SY, Scudder S, Mehr SE, Diedrich A, Flynn CR, et al. Worsening Postural Tachycardia Syndrome Is Associated With Increased Glucose-Dependent Insulinotropic Polypeptide Secretion. *Hypertension*. 2022;79(5):e89-e99.
45. Mustafa HI, Raj SR, Diedrich A, Black BK, Paranjape SY, Dupont WD, et al. Altered systemic hemodynamic and baroreflex response to angiotensin II in postural tachycardia syndrome. *Circ Arrhythm Electrophysiol*. 2012;5(1):173-80.
46. Roth B, Schiro DB, Ohlsson B. Diseases which cause generalized peripheral neuropathy: a systematic review. *Scand J Gastroenterol*. 2021;56(9):1000-10.
47. Kharraziha I, Axelsson J, Ricci F, Di Martino G, Persson M, Sutton R, et al. Serum Activity Against G Protein-Coupled Receptors and Severity of Orthostatic Symptoms in Postural Orthostatic Tachycardia Syndrome. *J Am Heart Assoc*. 2020;9(15):e015989.
48. Sandroni P, Low PA. Other autonomic neuropathies associated with ganglionic antibody. *Auton Neurosci*. 2009;146(1-2):13-7.
49. Vernino S, Stiles LE. Autoimmunity in postural orthostatic tachycardia syndrome: Current understanding. *Auton Neurosci*. 2018;215:78-82.
50. Kohno R, Cannom DS, Olshansky B, Xi SC, Krishnappa D, Adkisson WO, et al. Mast Cell Activation Disorder and Postural Orthostatic Tachycardia Syndrome: A Clinical Association. *J Am Heart Assoc*. 2021;10(17):e021002.
51. Shibao C, Arzubiaga C, Roberts LJ, 2nd, Raj S, Black B, Harris P, et al. Hyperadrenergic postural tachycardia syndrome in mast cell activation disorders. *Hypertension*. 2005;45(3):385-90.
52. Tu Y, Abell TL, Raj SR, Mar PL. Mechanisms and management of gastrointestinal symptoms in postural orthostatic tachycardia syndrome. *Neurogastroenterol Motil*. 2020;32(12):e14031.
53. 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*. 2023;293(1):91-9.

54. Sullivan SD, Hanauer J, Rowe PC, Barron DF, Darbari A, Oliva-Hemker M. Gastrointestinal symptoms associated with orthostatic intolerance. *J Pediatr Gastroenterol Nutr.* 2005;40(4):425-8.
55. Fortunato JE, Wagoner AL, Harbinson RL, D'Agostino RB, Jr., Shaltout HA, Diz DI. Effect of fludrocortisone acetate on chronic unexplained nausea and abdominal pain in children with orthostatic intolerance. *J Pediatr Gastroenterol Nutr.* 2014;59(1):39-43.
56. Ford AC, Sperber AD, Corsetti M, Camilleri M. Irritable bowel syndrome. *Lancet.* 2020;396(10263):1675-88.
57. Camilleri M. Gastrointestinal motility disorders in neurologic disease. *J Clin Invest.* 2021;131(4).
58. Huang RJ, Chun CL, Friday K, Triadafilopoulos G. Manometric abnormalities in the postural orthostatic tachycardia syndrome: a case series. *Dig Dis Sci.* 2013;58(11):3207-11.
59. Park KJ, Singer W, Sletten DM, Low PA, Bharucha AE. Gastric emptying in postural tachycardia syndrome: a preliminary report. *Clin Auton Res.* 2013;23(4):163-7.
60. Nilholm C, Larsson E, Roth B, Gustafsson R, Ohlsson B. Irregular Dietary Habits with a High Intake of Cereals and Sweets Are Associated with More Severe Gastrointestinal Symptoms in IBS Patients. *Nutrients.* 2019;11(6).
61. Nilholm C, Larsson E, Sonestedt E, Roth B, Ohlsson B. Assessment of a 4-Week Starch- and Sucrose-Reduced Diet and Its Effects on Gastrointestinal Symptoms and Inflammatory Parameters among Patients with Irritable Bowel Syndrome. *Nutrients.* 2021;13(2).
62. Roth B, Larsson E, Ohlsson B. Poor intake of vitamins and minerals is associated with symptoms among patients with irritable bowel syndrome. *J Gastroenterol Hepatol.* 2022;37(7):1253-62.
63. Baum P, Toyka KV, Blüher M, Kosacka J, Nowicki M. Inflammatory Mechanisms in the Pathophysiology of Diabetic Peripheral Neuropathy (DN)-New Aspects. *Int J Mol Sci.* 2021;22(19).
64. Toru S, Yokota T, Inaba A, Yamawaki M, Yamada M, Mizusawa H, et al. Autonomic dysfunction and orthostatic hypotension caused by vitamin B12 deficiency. *J Neurol Neurosurg Psychiatry.* 1999;66(6):804-5.
65. Jarjour IT, Jarjour LK. Low iron storage and mild anemia in postural tachycardia syndrome in adolescents. *Clin Auton Res.* 2013;23(4):175-9.
66. Öner T, Guven B, Tavli V, Mese T, Yilmazer MM, Demirpence S. Postural orthostatic tachycardia syndrome (POTS) and vitamin B12 deficiency in adolescents. *Pediatrics.* 2014;133(1):e138-42.
67. Hayes PA, Fraher MH, Quigley EM. Irritable bowel syndrome: the role of food in pathogenesis and management. *Gastroenterol Hepatol (N Y).* 2014;10(3):164-74.

68. Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology*. 2004;126(3):693-702.
69. Gunning WT, 3rd, Stepkowski SM, Kramer PM, Karabin BL, Grubb BP. Inflammatory Biomarkers in Postural Orthostatic Tachycardia Syndrome with Elevated G-Protein-Coupled Receptor Autoantibodies. *J Clin Med*. 2021;10(4).
70. El-Salhy M, Seim I, Chopin L, Gundersen D, Hatlebakk JG, Hausken T. Irritable bowel syndrome: the role of gut neuroendocrine peptides. *Front Biosci (Elite Ed)*. 2012;4(8):2783-800.
71. El-Salhy M, Mazzawi T, Gundersen D, Hatlebakk JG, Hausken T. The role of peptide YY in gastrointestinal diseases and disorders (review). *Int J Mol Med*. 2013;31(2):275-82.
72. El-Salhy M, Gilja OH, Gundersen D, Hatlebakk JG, Hausken T. Interaction between ingested nutrients and gut endocrine cells in patients with irritable bowel syndrome (review). *Int J Mol Med*. 2014;34(2):363-71.
73. Raasing LRM, Vogels OJM, Veltkamp M, van Swol CFP, Grutters JC. Current View of Diagnosing Small Fiber Neuropathy. *J Neuromuscul Dis*. 2021;8(2):185-207.
74. Tufvesson H, Hamrefors V, Ohlsson B. Mechanisms behind diffuse idiopathic peripheral neuropathy in humans – a systematic review. *Scandinavian Journal of Gastroenterology*. 2023;58(6):572-82.
75. Gibbons CH, Bonyhay I, Benson A, Wang N, Freeman R. Structural and functional small fiber abnormalities in the neuropathic postural tachycardia syndrome. *PLoS One*. 2013;8(12):e84716.
76. Haensch CA, Tosch M, Katona I, Weis J, Isenmann S. Small-fiber neuropathy with cardiac denervation in postural tachycardia syndrome. *Muscle Nerve*. 2014;50(6):956-61.
77. Billig SCI, Schauer mann JC, Rolke R, Katona I, Schulz JB, Maier A. Quantitative sensory testing predicts histological small fiber neuropathy in postural tachycardia syndrome. *Neurol Clin Pract*. 2020;10(5):428-34.
78. Moak JP, Ramwell CB, Gordish-Dressman H, Sule SD, Bettini E. Small fiber neuropathy in children, adolescents, and young adults with chronic orthostatic intolerance and postural orthostatic tachycardia syndrome: A retrospective study. *Auton Neurosci*. 2024;253:103163.
79. Miglis MG, Seliger J, Shaik R, Gibbons CH. A case series of cutaneous phosphorylated α -synuclein in Long-COVID POTS. *Clin Auton Res*. 2022;32(3):209-12.
80. Buchmann SJ, Penzlin AI, Kubasch ML, Illigens BM, Siepmann T. Assessment of sudomotor function. *Clin Auton Res*. 2019;29(1):41-53.

81. Peltier AC, Garland E, Raj SR, Sato K, Black B, Song Y, et al. Distal sudomotor findings in postural tachycardia syndrome. *Clin Auton Res.* 2010;20(2):93-9.
82. Zhang R, Mayuga K, Shields R, Cantrell C, Wilson R. Skin Biopsy and Quantitative Sudomotor Axon Reflex Testing in Patients With Postural Orthostatic Tachycardia Syndrome. *Cureus.* 2022;14(11):e31021.
83. Thieben MJ, Sandroni P, Sletten DM, Benrud-Larson LM, Fealey RD, Vernino S, et al. Postural orthostatic tachycardia syndrome: the Mayo clinic experience. *Mayo Clin Proc.* 2007;82(3):308-13.
84. Al-Shekhlee A, Lindenberg JR, Hachwi RN, Chelimsky TC. The value of autonomic testing in postural tachycardia syndrome. *Clin Auton Res.* 2005;15(3):219-22.
85. Fedorowski A, Burri P, Struck J, Juul-Möller S, Melander O. Novel cardiovascular biomarkers in unexplained syncopal attacks: the SYSTEMA cohort. *J Intern Med.* 2013;273(4):359-67.
86. Johansson M, Gagaouзова BS, van Rossum IA, Thijs RD, Hamrefors V, van Dijk JG, et al. Aetiology and haemodynamic patterns of orthostatic hypotension in a tertiary syncope unit. *Europace.* 2025;27(5).
87. Sheldon RS, Grubb BP, 2nd, Olshansky B, Shen WK, Calkins H, Brignole M, et al. 2015 heart rhythm society expert consensus statement on the diagnosis and treatment of postural tachycardia syndrome, inappropriate sinus tachycardia, and vasovagal syncope. *Heart Rhythm.* 2015;12(6):e41-63.
88. Johansson M, Ricci F, Schulte J, Persson M, Melander O, Sutton R, et al. Circulating levels of growth hormone in postural orthostatic tachycardia syndrome. *Sci Rep.* 2021;11(1):8575.
89. Plash WB, Diedrich A, Biaggioni I, Garland EM, Paranjape SY, Black BK, et al. Diagnosing postural tachycardia syndrome: comparison of tilt testing compared with standing haemodynamics. *Clin Sci (Lond).* 2013;124(2):109-14.
90. Bengtsson M, Ohlsson B, Ulander K. Development and psychometric testing of the Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS). *BMC Gastroenterol.* 2007;7:16.
91. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharmacol Ther.* 1997;11(2):395-402.
92. Ohlsson B. Extraintestinal manifestations in irritable bowel syndrome: A systematic review. *Therap Adv Gastroenterol.* 2022;15:17562848221114558.
93. Laboratoriemedicin Region Skåne. Analysportalen. <http://analysportalen-labmedicin.skane.se/2022> [Accessed September 27, 2022].
94. Faraj J, Melander O, Sundkvist G, Olsson R, Thorsson O, Ekberg O, et al. Oesophageal dysmotility, delayed gastric emptying and gastrointestinal symptoms in patients with diabetes mellitus. *Diabet Med.* 2007;24(11):1235-9.

95. Hansson M, Mathsson L, Schleder T, Israelsson L, Matsson P, Nogueira L, et al. Validation of a multiplex chip-based assay for the detection of autoantibodies against citrullinated peptides. *Arthritis Res Ther*. 2012;14(5):R201.
96. Agmon-Levin N, Damoiseaux J, Kallenberg C, Sack U, Witte T, Herold M, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *Ann Rheum Dis*. 2014;73(1):17-23.
97. Laboratoriemedicin Region Skåne. Tryptas. Available from: <http://analysportalen-labmedicin.skane.se/pics/Labmedicin/analysportalen/KIT/Tryptas.pdf>. [Accessed October 6, 2023].
98. Laboratoriemedicin Region Skåne. Immunoglobulin E (Total IgE). Available from: [http://analysportalen-labmedicin.skane.se/pics/Labmedicin/analysportalen/KIT/Immunoglobulin%20IgE%20\(total%20IgE\).pdf](http://analysportalen-labmedicin.skane.se/pics/Labmedicin/analysportalen/KIT/Immunoglobulin%20IgE%20(total%20IgE).pdf). [Accessed October 6, 2023].
99. Laboratoriemedicin Region Skåne. B-HbA1c (IFCC). Available from: [http://analysportalen-labmedicin.skane.se/pics/Labmedicin/Verksamhetsomr%C3%A5den/Klinisk%20kemi/Analyser/Skane/B-HbA1c%20\(IFCC\).pdf](http://analysportalen-labmedicin.skane.se/pics/Labmedicin/Verksamhetsomr%C3%A5den/Klinisk%20kemi/Analyser/Skane/B-HbA1c%20(IFCC).pdf). [Accessed August 27, 2025].
100. Laboratoriemedicin Region Skåne. B-Hemoglobin (Hb), B-Erythrocyter, Erc-MCV, Erc-MCH, Erc-MCHC, B-EVF, Sysmex XN-10 samt XN-20. Available from: [http://analysportalen-labmedicin.skane.se/pics/Labmedicin/Verksamhetsomr%C3%A5den/Klinisk%20kemi/Analyser/Skane/B-Hemoglobin%20\(Hb\),%20B-Erythrocyter,%20Erc-MCV,%20Erc-MCH,%20Erc-MCHC,%20B-EVF,%20Sysmex%20XN-10%20samt.pdf](http://analysportalen-labmedicin.skane.se/pics/Labmedicin/Verksamhetsomr%C3%A5den/Klinisk%20kemi/Analyser/Skane/B-Hemoglobin%20(Hb),%20B-Erythrocyter,%20Erc-MCV,%20Erc-MCH,%20Erc-MCHC,%20B-EVF,%20Sysmex%20XN-10%20samt.pdf). [Accessed August 27, 2025].
101. Weykamp C. HbA1c: a review of analytical and clinical aspects. *Ann Lab Med*. 2013;33(6):393-400.
102. Laboratoriemedicin Region Skåne. P- Kortisol. Available from: <http://analysportalen-labmedicin.skane.se/pics/Labmedicin/Verksamhetsomr%C3%A5den/Klinisk%20kemi/Analyser/Skane/P-Kortisol%20p%C3%A5%20Cobas%20Pro.pdf>. [Accessed August 27, 2025].
103. Thrainsdottir S, Malik RA, Dahlin LB, Wiksell P, Eriksson KF, Rosén I, et al. Endoneurial capillary abnormalities presage deterioration of glucose tolerance and accompany peripheral neuropathy in man. *Diabetes*. 2003;52(10):2615-22.
104. Karling P et al. Kliniska färdigheter : mötet mellan patient och läkare. Studentlitteratur AB; 2021. 406 p.
105. Cannon J. Performing Tinel's test and Phalen's test. *Nursing*. 2009;39(8):16.
106. Ekman L, Lindholm E, Brogren E, Dahlin LB. Normative values of the vibration perception thresholds at finger pulps and metatarsal heads in healthy adults. *PLoS One*. 2021;16(4):e0249461.

107. Ekman L. Assessment of peripheral neuropathy [Doctoral Thesis]. Lund, Sweden: Lund University; 2024.
108. Ekman L, Dahlin LB, Englund E. Assessment of intraepidermal nerve fiber densities in 5 μ m sections from arm and leg - a search for normative age-related values. *Free Neuropathol.* 2024;5:24.
109. Ekman L, Pourhamidi K, Englund E, Lagali N, Rolandsson O, Dahlin LB. Temporal trend of small nerve fibre degeneration in people with and without type 2 diabetes mellitus. *Diabet Med.* 2022;39(3):e14691.
110. Bourne KM, Hall J, Stiles LE, Sheldon RS, Shibao CA, Okamoto LE, et al. Symptom Presentation and Access to Medical Care in Patients With Postural Orthostatic Tachycardia Syndrome: Role of Sex. *CJC Open.* 2021;3(12 Suppl):S44-s52.
111. Anderson JW, Lambert EA, Sari CI, Dawood T, Esler MD, Vaddadi G, et al. Cognitive function, health-related quality of life, and symptoms of depression and anxiety sensitivity are impaired in patients with the postural orthostatic tachycardia syndrome (POTS). *Front Physiol.* 2014;5:230.
112. de Oliveira MCS, Távora-Mehta MZP, Mehta N, Magajevski AS, Concato L, Ortiz MR, et al. Distinct Hemodynamic Responses That Culminate With Postural Orthostatic Tachycardia Syndrome. *Am J Cardiol.* 2023;197:3-12.
113. Khan HF, Ambreen S, Raziq H, Hayat A. Comparison of cortisol levels in patients with vasovagal syncope and postural tachycardia syndrome. *Pak J Med Sci.* 2022;38(1):185-9.
114. Laurin JKH, Oyewunmi OA, Garland EM, Gamboa A, Nwazue VC, Paranjape SY, et al. Adrenal gland response to adrenocorticotrophic hormone is intact in patients with postural orthostatic tachycardia syndrome. *Auton Neurosci.* 2023;248:103105.
115. Leys F, Verginer M, Kirchler E, Marino L, Goebel G, Campese N, et al. Postural orthostatic tachycardia syndrome is the most frequent cardiovascular autonomic disorder following COVID-19 infection or vaccination. *J Neurol.* 2025;272(12):783.
116. Okamoto LE, Urechie V, Rigo S, Abner JJ, Giesecke M, Muldowney JAS, et al. Hyperadrenergic Postural Tachycardia Syndrome: Clinical Biomarkers and Response to Guanfacine. *Hypertension.* 2024;81(11):2237-47.
117. Miller AJ, Stiles LE, Sheehan T, Bascom R, Levy HP, Francomano CA, et al. Prevalence of hypermobile Ehlers-Danlos syndrome in postural orthostatic tachycardia syndrome. *Auton Neurosci.* 2020;224:102637.
118. Wells R, Elliott AD, Mahajan R, Page A, Iodice V, Sanders P, et al. Efficacy of Therapies for Postural Tachycardia Syndrome: A Systematic Review and Meta-analysis. *Mayo Clin Proc.* 2018;93(8):1043-53.
119. Kwok CS, Lee S, Hall M, Qureshi AI, Lip GYH, Loke YK, et al. The evidence for treatments for postural orthostatic tachycardia syndrome: a systematic review of randomized trials. *Trends Cardiovasc Med.* 2025;35(8):517-27.

120. Lyonga Ngonge A, Nyange C, Ghali JK. Novel pharmacotherapeutic options for the treatment of postural orthostatic tachycardia syndrome. *Expert Opin Pharmacother.* 2024;25(2):181-8.
121. Galbreath MM, Shibata S, VanGundy TB, Okazaki K, Fu Q, Levine BD. Effects of exercise training on arterial-cardiac baroreflex function in POTS. *Clin Auton Res.* 2011;21(2):73-80.
122. Ishimwe JA, Breier N, Saleem M, Kastner PD, Kirabo A, Shibao CA. The Gut Microbiota and Short-Chain Fatty Acids Profile in Postural Orthostatic Tachycardia Syndrome. *Front Physiol.* 2022;13:879012.
123. Wang LB, Culbertson CJ, Deb A, Morgenshtern K, Huang H, Hohler AD. Gastrointestinal dysfunction in postural tachycardia syndrome. *J Neurol Sci.* 2015;359(1-2):193-6.
124. Tai FWD, Palsson OS, Lam CY, Whitehead WE, Sperber AD, Tornblom H, et al. Functional gastrointestinal disorders are increased in joint hypermobility-related disorders with concomitant postural orthostatic tachycardia syndrome. *Neurogastroenterol Motil.* 2020;32(12):e13975.
125. Boris JR, Huang J, Bernadzikowski T. Orthostatic heart rate does not predict symptomatic burden in pediatric patients with chronic orthostatic intolerance. *Clin Auton Res.* 2020;30(1):19-28.
126. Aggarwal A, Cutts TF, Abell TL, Cardoso S, FAMILONI B, Bremer J, et al. Predominant symptoms in irritable bowel syndrome correlate with specific autonomic nervous system abnormalities. *Gastroenterology.* 1994;106(4):945-50.
127. Heitkemper M, Jarrett M, Cain KC, Burr R, Levy RL, Feld A, et al. Autonomic nervous system function in women with irritable bowel syndrome. *Dig Dis Sci.* 2001;46(6):1276-84.
128. Polster A, Friberg P, Gunterberg V, Öhman L, Le Nevé B, Törnblom H, et al. Heart rate variability characteristics of patients with irritable bowel syndrome and associations with symptoms. *Neurogastroenterol Motil.* 2018;30(7):e13320.
129. Ali MK, Chen JDZ. Roles of Heart Rate Variability in Assessing Autonomic Nervous System in Functional Gastrointestinal Disorders: A Systematic Review. *Diagnostics (Basel).* 2023;13(2).
130. Mazur M, Furgała A, Jabłoński K, Mach T, Thor P. Autonomic nervous system activity in constipation-predominant irritable bowel syndrome patients. *Med Sci Monit.* 2012;18(8):Cr493-9.
131. Chang L. The role of stress on physiologic responses and clinical symptoms in irritable bowel syndrome. *Gastroenterology.* 2011;140(3):761-5.
132. Cheng P, Shih W, Alberto M, Presson AP, Licudine A, Mayer EA, et al. Autonomic response to a visceral stressor is dysregulated in irritable bowel syndrome and correlates with duration of disease. *Neurogastroenterol Motil.* 2013;25(10):e650-9.

133. Inbaraj G, Udupa K, Vasuki PP, Nalini A, Sathyaprabha TN. Resting heart rate variability as a diagnostic marker of cardiovascular dysautonomia in postural tachycardia syndrome. *J Basic Clin Physiol Pharmacol*. 2023;34(1):103-9.
134. Crnošija L, Krbot Skorić M, Adamec I, Lovrić M, Junaković A, Mišmaš A, et al. Hemodynamic profile and heart rate variability in hyperadrenergic versus non-hyperadrenergic postural orthostatic tachycardia syndrome. *Clin Neurophysiol*. 2016;127(2):1639-44.
135. Swai J, Hu Z, Zhao X, Rugambwa T, Ming G. Heart rate and heart rate variability comparison between postural orthostatic tachycardia syndrome versus healthy participants; a systematic review and meta-analysis. *BMC Cardiovasc Disord*. 2019;19(1):320.
136. Sumner CJ, Sheth S, Griffin JW, Cornblath DR, Polydefkis M. The spectrum of neuropathy in diabetes and impaired glucose tolerance. *Neurology*. 2003;60(1):108-11.
137. Chopoorian AH, Wahba A, Celedonio J, Nwazue V, Smith EC, Garland EM, et al. Impaired Endothelial Function in Patients With Postural Tachycardia Syndrome. *Hypertension*. 2021;77(3):1001-9.
138. Gulcan E, Taser F, Toker A, Korkmaz U, Alcelik A. Increased frequency of prediabetes in patients with irritable bowel syndrome. *Am J Med Sci*. 2009;338(2):116-9.
139. Eriksson EM, Andrén KI, Eriksson HT, Kurlberg GK. Irritable bowel syndrome subtypes differ in body awareness, psychological symptoms and biochemical stress markers. *World J Gastroenterol*. 2008;14(31):4889-96.
140. Sun WX, Chen TY, Song MM, Gao YJ, Xu SY. Energy metabolism disorders in migraine: triggers, pathways, and therapeutic repurposing. *Front Neurol*. 2025;16:1561000.
141. Dean YE, Loayza Pintado JJ, Rouzan SS, Nale LL, Abbas A, Aboushaira A, et al. The Relationship Between Irritable Bowel Syndrome and Metabolic Syndrome: A Systematic Review and Meta-Analysis of 49,662 Individuals. *Endocrinol Diabetes Metab*. 2025;8(2):e70041.
142. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*. 1999;22(9):1462-70.
143. Norton L, Shannon C, Gastaldelli A, DeFronzo RA. Insulin: The master regulator of glucose metabolism. *Metabolism*. 2022;129:155142.
144. Mancusi C, Izzo R, di Gioia G, Losi MA, Barbato E, Morisco C. Insulin Resistance the Hinge Between Hypertension and Type 2 Diabetes. *High Blood Press Cardiovasc Prev*. 2020;27(6):515-26.

145. da Silva AA, do Carmo JM, Li X, Wang Z, Mouton AJ, Hall JE. Role of Hyperinsulinemia and Insulin Resistance in Hypertension: Metabolic Syndrome Revisited. *Can J Cardiol.* 2020;36(5):671-82.
146. Jensen J, Ruge T, Lai YC, Svensson MK, Eriksson JW. Effects of adrenaline on whole-body glucose metabolism and insulin-mediated regulation of glycogen synthase and PKB phosphorylation in human skeletal muscle. *Metabolism.* 2011;60(2):215-26.
147. Rizza RA, Mandarino LJ, Gerich JE. Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor defect of insulin action. *J Clin Endocrinol Metab.* 1982;54(1):131-8.
148. Coupal KE, Heeney ND, Hockin BCD, Ronsley R, Armstrong K, Sanatani S, et al. Pubertal Hormonal Changes and the Autonomic Nervous System: Potential Role in Pediatric Orthostatic Intolerance. *Front Neurosci.* 2019;13:1197.
149. Lin J, Zhao H, Shen J, Jiao F. Salivary Cortisol Levels Predict Therapeutic Response to a Sleep-Promoting Method in Children with Postural Tachycardia Syndrome. *J Pediatr.* 2017;191:91-5.e1.
150. Hedge ET, Grappe SR, Vernino S, Almandoz JP, Levine BD. Exacerbation of Postural Orthostatic Tachycardia Syndrome With Tirzepatide Prescribed for Weight Loss. *JACC Case Rep.* 2025;30(32):105430.
151. Karhunen LJ, Juvonen KR, Huotari A, Purhonen AK, Herzig KH. Effect of protein, fat, carbohydrate and fibre on gastrointestinal peptide release in humans. *Regul Pept.* 2008;149(1-3):70-8.
152. Drucker DJ. The role of gut hormones in glucose homeostasis. *J Clin Invest.* 2007;117(1):24-32.
153. Saidi K, Nilholm C, Roth B, Ohlsson B. A carbohydrate-restricted diet for patients with irritable bowel syndrome lowers serum C-peptide, insulin, and leptin without any correlation with symptom reduction. *Nutr Res.* 2021;86:23-36.
154. El-Salhy M, Hatlebakk JG, Gilja OH, Hausken T. Densities of rectal peptide YY and somatostatin cells as biomarkers for the diagnosis of irritable bowel syndrome. *Peptides.* 2015;67:12-9.
155. El-Salhy M, Hatlebakk JG, Hausken T. Diet in Irritable Bowel Syndrome (IBS): Interaction with Gut Microbiota and Gut Hormones. *Nutrients.* 2019;11(8).
156. Khan U, Brønstad I, Hillestad EMR, Steinsvik EK, Hausken T, Berentsen B, et al. Increase in circulating GLP-1 following low FODMAP diet in irritable bowel syndrome patients. *Front Nutr.* 2025;12:1615671.
157. Li ZY, Zhang N, Wen S, Zhang J, Sun XL, Fan XM, et al. Decreased glucagon-like peptide-1 correlates with abdominal pain in patients with constipation-predominant irritable bowel syndrome. *Clin Res Hepatol Gastroenterol.* 2017;41(4):459-65.

158. Kuhre RE, Wewer Albrechtsen NJ, Hartmann B, Deacon CF, Holst JJ. Measurement of the incretin hormones: glucagon-like peptide-1 and glucose-dependent insulinotropic peptide. *J Diabetes Complications*. 2015;29(3):445-50.
159. Albrechtsen NJW, Rehfeld JF. On premises and principles for measurement of gastrointestinal peptide hormones. *Peptides*. 2021;141:170545.
160. Holst JJ. The incretin system in healthy humans: The role of GIP and GLP-1. *Metabolism*. 2019;96:46-55.
161. Blitshteyn S, Funez-dePagnier G, Szombathy A, Hutchinson M. Immunotherapies for postural orthostatic tachycardia syndrome, other common autonomic disorders, and Long COVID: current state and future direction. *Front Cell Infect Microbiol*. 2025;15:1647203.
162. Lukáčová M, Mitro P, Lazúrová Z, Hijová E, Bertková I, Valová K. Autoimmune Antibodies in Orthostatic Intolerance Syndromes. *Physiol Res*. 2025;74(2):255-62.
163. Paroli M, Sirinian MI. Pathogenic Crosstalk Between the Peripheral and Central Nervous System in Rheumatic Diseases: Emerging Evidence and Clinical Implications. *Int J Mol Sci*. 2025;26(13).
164. Stochmal A, Czuwara J, Trojanowska M, Rudnicka L. Antinuclear Antibodies in Systemic Sclerosis: an Update. *Clin Rev Allergy Immunol*. 2020;58(1):40-51.
165. Mehra S, Walker J, Patterson K, Fritzler MJ. Autoantibodies in systemic sclerosis. *Autoimmun Rev*. 2013;12(3):340-54.
166. Adler BL, Kuchinad K, Jeong SJ, Stiles LE, Shah AA, Chung T, et al. Colour changes in the feet: a sign of autonomic symptoms in systemic sclerosis. *Rheumatology (Oxford)*. 2025;64(5):3080-4.
167. CADTH Health Technology Review. Retesting Intervals for Tryptase: Rapid Review. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; 2024.
168. Huang J, Imam K, Criado JR, Luskin KT, Liu Y, Puglisi LH, et al. Hereditary alpha-tryptasemia in patients with postural orthostatic tachycardia syndrome. *J Allergy Clin Immunol Pract*. 2024;12(2):528-9.e1.
169. Abbas M, Goldin J. Type I Hypersensitivity Reaction. StatPearls. Treasure Island (FL): StatPearls Publishing. Copyright © 2025, StatPearls Publishing LLC.; 2025.
170. Amin K. The role of mast cells in allergic inflammation. *Respir Med*. 2012;106(1):9-14.
171. Valent P, Hoermann G, Bonadonna P, Hartmann K, Sperr WR, Broesby-Olsen S, et al. The Normal Range of Baseline Tryptase Should Be 1 to 15 ng/mL and Covers Healthy Individuals With HαT. *J Allergy Clin Immunol Pract*. 2023;11(10):3010-20.

172. Molfetta R, Carnevale A, Marangio C, Putro E, Paolini R. Beyond the "Master" Role in Allergy: Insights into Intestinal Mast Cell Plasticity and Gastrointestinal Diseases. *Biomedicines*. 2025;13(2).
173. Stino AM, Nelson J, Gutsell O, Eldokla A, Liu J, Akin C. Autonomic function testing and symptom severity in patients with suspected mast cell activation disorders. *Auton Neurosci*. 2025;262:103362.
174. Vasileiadou S, Ekerljung L, Bjerg A, Goksör E. Asthma increased in young adults from 2008-2016 despite stable allergic rhinitis and reduced smoking. *PLoS One*. 2021;16(6):e0253322.
175. Goksör E, Wennergren G, Vasileiadou S, Mincheva R, Nwaru B, Lundbäck B, et al. Increased prevalence of allergic rhinitis in young men in Western Sweden. *European Respiratory Journal*. 52(suppl 62):PA3906.
176. Gwathmey KG, Pearson KT. Diagnosis and management of sensory polyneuropathy. *Bmj*. 2019;365:l1108.
177. Singer W, Spies JM, McArthur J, Low J, Griffin JW, Nickander KK, et al. Prospective evaluation of somatic and autonomic small fibers in selected autonomic neuropathies. *Neurology*. 2004;62(4):612-8.
178. Cantrell C, Rilinger R, Stallkamp Tidd SJ, Wilson R. Corneal Confocal Microscopy in Postural Orthostatic Tachycardia Syndrome (POTS) as a Diagnostic Tool for Small Fiber Neuropathy. *Cureus*. 2025;17(4):e82781.
179. Chan AC, Wilder-Smith EP. Small fiber neuropathy: Getting bigger! *Muscle Nerve*. 2016;53(5):671-82.
180. Motaghi P, Adibi I, Adibi P, Ghasemi M. Small fiber neuropathy in irritable bowel syndrome. *Gastroenterol Hepatol Bed Bench*. 2024;17(1):57-63.
181. Jenkins ZM, Castle DJ, Eikelis N, Phillipou A, Lambert GW, Lambert EA. Autonomic nervous system function in women with anorexia nervosa. *Clin Auton Res*. 2022;32(1):29-42.
182. European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society. *J Peripher Nerv Syst*. 2010;15(2):79-92.
183. Koskinen M, Hietaharju A, Kyläniemi M, Peltola J, Rantala I, Udd B, et al. A quantitative method for the assessment of intraepidermal nerve fibers in small-fiber neuropathy. *J Neurol*. 2005;252(7):789-94.
184. Novak P, Systrom DM, Marciano SP, Knief A, Felsenstein D, Giannetti MP, et al. Mismatch between subjective and objective dysautonomia. *Sci Rep*. 2024;14(1):2513.
185. Cook GA, Jr., Sandroni P. Management of headache and chronic pain in POTS. *Auton Neurosci*. 2018;215:37-45.

186. Khurana RK. Experimental induction of panic-like symptoms in patients with postural tachycardia syndrome. *Clin Auton Res*. 2006;16(6):371-7.
187. Diatchenko L, Anderson AD, Slade GD, Fillingim RB, Shabalina SA, Higgins TJ, et al. Three major haplotypes of the beta2 adrenergic receptor define psychological profile, blood pressure, and the risk for development of a common musculoskeletal pain disorder. *Am J Med Genet B Neuropsychiatr Genet*. 2006;141b(5):449-62.
188. Orand A, Gupta A, Shih W, Presson AP, Hammer C, Niesler B, et al. Catecholaminergic Gene Polymorphisms Are Associated with GI Symptoms and Morphological Brain Changes in Irritable Bowel Syndrome. *PLoS One*. 2015;10(8):e0135910.
189. Berman S, Suyenobu B, Naliboff BD, Bueller J, Stains J, Wong H, et al. Evidence for alterations in central noradrenergic signaling in irritable bowel syndrome. *Neuroimage*. 2012;63(4):1854-63.
190. Blankstein U, Chen J, Diamant NE, Davis KD. Altered brain structure in irritable bowel syndrome: potential contributions of pre-existing and disease-driven factors. *Gastroenterology*. 2010;138(5):1783-9.
191. Bradford K, Shih W, Videlock EJ, Presson AP, Naliboff BD, Mayer EA, et al. Association between early adverse life events and irritable bowel syndrome. *Clin Gastroenterol Hepatol*. 2012;10(4):385-90.e1-3.
192. Umeda S, Harrison NA, Gray MA, Mathias CJ, Critchley HD. Structural brain abnormalities in postural tachycardia syndrome: A VBM-DARTEL study. *Front Neurosci*. 2015;9:34.
193. Reiss S, Reiss R, Reiss C. Autonomic Dysfunction and Postural Orthostatic Tachycardia Syndrome Secondary to Abuse. *JACC Case Rep*. 2025;30(38):105773.
194. Dorris BG, Plisco MK. Early Relational Trauma and the Development of Internalizing Symptoms in Females with Postural Orthostatic Tachycardia Syndrome. *J Trauma Dissociation*. 2025;26(5):617-33.
195. Pollare T, Lithell H, Selinus I, Berne C. Sensitivity to insulin during treatment with atenolol and metoprolol: a randomised, double blind study of effects on carbohydrate and lipoprotein metabolism in hypertensive patients. *Bmj*. 1989;298(6681):1152-7.
196. Sawicki PT, Siebenhofer A. Betablocker treatment in diabetes mellitus. *J Intern Med*. 2001;250(1):11-7.
197. Walsh EG, Bonnet K, Schlundt DG, Kelly EC, Rogalski K, Broadbent C, et al. A qualitative exploration of illness-related experiences, emotions, and coping among adults with postural orthostatic tachycardia syndrome (POTS). *J Health Psychol*. 2025;30(12):3553-68.
198. Walsh EG, Birdee GS, Bonnet K, Schlundt DG, Broadbent C, Kelly EC, et al. Patient perspectives on exercise among adults with postural orthostatic tachycardia syndrome: a mixed methods study. *Clin Auton Res*. 2025.

199. Eftekhari H, Pearce G, Staniszewska S, Seers K. Understanding the experiences of people who have Postural Orthostatic Tachycardia Syndrome (POTS) and the health professionals who care for them: A grounded theory study. *Int J Nurs Stud*. 2025;164:104986.
200. Frye WS, King CK, Schaefer MR, Decker J, Kuhn B. "You Look Perfectly Healthy to Me": Living With Postural Orthostatic Tachycardia Syndrome Through Adolescents' and Parents' Eyes. *Clin Pediatr (Phila)*. 2023;62(6):622-32.
201. Waterman S, Opie M, Waterman D, Langdon D. Experiences of living with postural tachycardia syndrome. *Chronic Illn*. 2023;19(1):184-96.
202. Sunami Y, Sugaya K, Takahashi K. G protein-coupled receptors related to autoimmunity in postural orthostatic tachycardia syndrome. *Immunol Med*. 2025;48(2):141-8.
203. Pierson BC, Apilado K, Franzos MA, Allard R, Mancuso JD, Tribble D, et al. Oral medications for the treatment of postural orthostatic tachycardia syndrome; a systematic review of studies before and during the COVID-19 pandemic. *Front Neurol*. 2024;15:1515486.
204. Ross AJ, Ocon AJ, Medow MS, Stewart JM. A double-blind placebo-controlled cross-over study of the vascular effects of midodrine in neuropathic compared with hyperadrenergic postural tachycardia syndrome. *Clin Sci (Lond)*. 2014;126(4):289-96.
205. Taub PR, Zadourian A, Lo HC, Ormiston CK, Golshan S, Hsu JC. Randomized Trial of Ivabradine in Patients With Hyperadrenergic Postural Orthostatic Tachycardia Syndrome. *J Am Coll Cardiol*. 2021;77(7):861-71.
206. Wheatley-Guy CM, Shea MG, Parks JK, Scales R, Goodman BP, Butterfield RJ, et al. Semi-supervised exercise training program more effective for individuals with postural orthostatic tachycardia syndrome in randomized controlled trial. *Clin Auton Res*. 2023;33(6):659-72.
207. Bourne KM, Karalasingham K, Siddiqui T, Mammarella B, Patel A, Exner DV, et al. Abdominal-only Compression Garments Reduce Orthostatic Tachycardia and Improve Symptoms in Patients With Postural Orthostatic Tachycardia Syndrome. *Can J Cardiol*. 2025.
208. Garland EM, Gamboa A, Nwazue VC, Celedonio JE, Paranjape SY, Black BK, et al. Effect of High Dietary Sodium Intake in Patients With Postural Tachycardia Syndrome. *J Am Coll Cardiol*. 2021;77(17):2174-84.
209. Oka T, Lkhagvasuren B. Effects of recumbent isometric yoga on the orthostatic cardiovascular response of patients with myalgic encephalomyelitis/chronic fatigue syndrome. *Biopsychosoc Med*. 2025;19(1):16.
210. Stick M, Leone A, Fischer F, Schulz JB, Maier A. Deep abdominal breathing reduces heart rate and symptoms during orthostatic challenge in patients with postural orthostatic tachycardia syndrome. *Eur J Neurol*. 2024;31(10):e16402.

211. Freedenberg VA, Hinds PS, Friedmann E. Mindfulness-Based Stress Reduction and Group Support Decrease Stress in Adolescents with Cardiac Diagnoses: A Randomized Two-Group Study. *Pediatr Cardiol.* 2017;38(7):1415-25.
212. Ralston TE, Kanzler KE. Symptom Discrimination and Habituation: A Case Study of Behavioral Treatment for Postural Orthostatic Tachycardia Syndrome (POTS). *Behav Med.* 2016;42(1):57-62.
213. Simrén M, Törnblom H, Palsson OS, Whitehead WE. Management of the multiple symptoms of irritable bowel syndrome. *Lancet Gastroenterol Hepatol.* 2017;2(2):112-22.
214. Chey WD, Keefer L, Whelan K, Gibson PR. Behavioral and Diet Therapies in Integrated Care for Patients With Irritable Bowel Syndrome. *Gastroenterology.* 2021;160(1):47-62.

Supplementary tables

Supplementary table 1. Antigens targeted in systemic autoimmune rheumatic diseases

IgG autoantibodies were tested against these antigens commonly targeted in classic systemic autoimmune rheumatic diseases using a custom-designed multiplex solid-phase microarray platform.

Antigen	Protein	ANA/ENA	Peptid sequence
Cit Fiba ₃₆₋₅₀	Fibrinogen α -chain, 36-50	No	GP(cit) VVE (cit)HQ SAC KDSK
Cit Fibβ ₆₀₋₇₄	Fibrinogen β -chain, 60-74	No	(cit)PA PPP ISG GGY (cit)A(cit)
Cit Fibβ ₃₆₋₅₂	Fibrinogen β -chain, 36-52	No	NEE GFF SA(cit) GHR PLD KK
Cit Fil ₃₀₇₋₃₂₄	Filaggrin, 307-324	No	HQC HQE ST(cit) GRS RGR CGR SGS
Acet Fil ₃₀₇₋₃₂₄	Filaggrin, 307-324	No	HQC HQE ST (AC) GRS RGR CGR SGS
Carb Fil ₃₀₇₋₃₂₄	Filaggrin, 307-324	No	HQC HQE ST (HC) GRS RGR CGR SGS
Cit H3 ₁₋₃₀	Histone 3, 1-30	No	A(cit)T KQT A(cit)K STG GKA P(cit)K QLA TKA A(cit)K SAP
Cit H3 ₂₁₋₄₄	Histone 3, 21-44	No	ATK AA(cit)KSA PAT GGV KKP H(cit)Y (cit)PG G GK
H4 ₁₋₁₈	Histone 4, 1-18	No	SGR GKG GKG LGK GGA KRH RKVL
Acet H4 _{1-18 -Ac8, 12,16}	Histone 4, 1-18	No	SGR GKG GK(Ac)G LGK(Ac) GGA K(Ac)RH RKVL
Acet H4 _{1-18 -Ac16}	Histone 4, 1-18	No	SGR GKG GKG LGK GGA K(Ac)RH RKVL
Acet H4 _{1-18 -Ac5}	Histone 4, 1-18	No	SGR GK(Ac)G GKG LGK GGA KRH RKVL
Cit TNC1	Tenascin, 2026-2040	No	VFL RRK NG(cit) ENF YQNW
Cit TNC5	Tenascin, 2176-2200	No	EHS IQF AEM KL(cit) PSN F(cit)N LEG (cit)(cit)KR
Cit Vim ₆₀₋₇₅	Vimentin, 60-75	No	VYA T(cit)S SAV (cit)L(cit) SSVF
CENP B	Centromer protein B	Yes	
dsDNA	Double stranded DNA	Yes	
Fibrillarin	Fibrillarin	Yes	
Jo-1	Histidyle-tRNA synthetase	Yes	

Mi-2	Mi-2 nuclear antigen	Yes	
PCNA	Proliferating Cell Nuclear Antigen	Yes	
PM-Scl100	Also known as Rrp6	Yes	
Rip P0	Phosphorylated P0 protein of the ribosomalstalk	Yes	
Rip P1	Phosphorylated P1 protein of the ribosomalstalk	Yes	
Rip P2	Phosphorylated P2 protein of the ribosomalstalk	Yes	
RNA Pol III	RNA Pol III	Yes	
RNP70	U1-snRNP complex, 70kD protein	Yes	
RNP A	U1-snRNP complex, protein A	Yes	
RNP C	U1-snRNP complex, protein C	Yes	
Ro52	Also known as TRIM21	Yes	
Ro60	Also known as TROVE2	Yes	
Scl-70	70 kD immunoreactive fragment of Topoisomeras I	Yes	
Sm	Smith protein complex	Yes	
SmB/B'	Smith protein complex B protein	Yes	
SS-B/La	Sjögren syndrome type B antigen/ Lupus La antigen	Yes	

Protein sequences are not shown. Some peptide sequences are not shown due to undisclosed information by Thermo Fisher Scientific Immunodiagnostics, Uppsala, Sweden. ANA/ENA: Anti-nuclear antibodies/Extractable nuclear antigen antibodies.

Supplementary Table 2. Pharmacotherapy in POTS and healthy controls.
Self-reported pharmacotherapy in participants in Papers I–IV.

Drug class		POTS n=43	Healthy controls n=77
Cardiovascular and POTS-related drugs	Alpha-1 adrenergic agonists	17	0
	I(f) receptor antagonists	16	0
	Betablockers	13	0
	Central nervous system stimulants	8	0
	Cholinesterase inhibitors	6	0
	Mineral corticoids	3	0
	Short acting nitroglycerine	3	0
	Calcium channel inhibitors	2	0
	Angiotensin receptor antagonists	1	0
Asthma and allergy	Histamine H1 antagonists	13	2
	Inhaled β_2 agonists	7	0
	Leukotriene receptor agonists	5	0
	Inhaled steroids	4	0
	Sodium cromoglicate	3	0
	Nasal steroids	2	1
	Inhaled ipratropiumbromide	1	0
	Oral budesonide	1	0
Hormonal drugs	Combined hormonal contraceptives	8	3
	Progesterone, contraceptives	6	1
	Thyroid hormones	5	0
	GLP-1 agonists	1	0
Pain killers	Acetaminophen	6	1
	Opioids and opioid-like drugs	5	0
	Non-steroidal anti-inflammatory drugs	3	1
	Muscle relaxing agents	2	0
	Gabapentinoids	2	0
	Spasmolytics	1	0
Vitamin and mineral supplementation	B12 (oral or injected)	6	0
	D-vitamins	4	0
	Multivitamins	3	2
	Folic acid	2	0
	Potassium chloride	1	0
	Magnesium	1	0
	Sodium chloride	1	0

Antidepressants and mood stabilizers	Selective serotonin reuptake inhibitors	7	2 (of which one cyclic due to PMDD)
	Serotonin and noradrenaline reuptake inhibitors	3	0
	Noradrenaline reuptake inhibitors	3	0
	Antiepileptic drugs	2	0
	Tricyclic antidepressants	2	0
	Other antidepressants	1	0
Gastrointestinal drugs	Histamine H2 receptor antagonists	7	0
	Proton pump inhibitors	2	1
	5-HT3 receptor antagonists	2	0
	5-HT4 receptor antagonists	2	0
	Laxatives	1	0
Sleeping pills	Melatonin	4	1
	Benzodiazepine-like	2	1
	Others	3	0
Tranquilizers	Non-benzodiazepines	3	0
	Benzodiazepines	1	0
Miscellaneous	Triptans	2	0
	Statins	2	0
	TNF- α antagonist	1	0
	Direct oral anticoagulants	1	0

GLP-1: Glucagon-like peptide-1; PMDD: premenstrual dysphoric disorder; 5-HT: Serotonin; TNF: tumor necrosis factor.

Paper I



Research Article

Sociodemographic Factors, Nutritional Status, and Inflammatory Markers in Patients with Postural Orthostatic Tachycardia Syndrome

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Objectives. Postural orthostatic tachycardia syndrome (POTS) is characterized by cardiovascular autonomic dysfunction of unknown etiology with high a prevalence of gastrointestinal symptoms. The aim of the present study was to examine sociodemographic, lifestyle, and nutritional factors as well as inflammatory markers in patients with POTS. **Materials and Methods.** Forty-three patients with POTS and 61 healthy controls completed questionnaires about sociodemographic factors, lifestyle habits, and gastrointestinal symptoms. Blood samples were analyzed for serum levels of cobalamins, folic acid, iron, total iron-binding capacity (TIBC), ferritin, sodium, potassium, magnesium, phosphorus, albumin, high-sensitive C-reactive protein (CRP), and 25-hydroxyvitamin D (25-OH vitamin D). **Results.** POTS patients were predominantly women with a lower education level and were more often working part-time, on sick leave, and living alone compared with healthy controls. They reported lower alcohol intake and physical activity levels than controls. The nutrient intake was in general similar in both groups, but POTS patients had a higher intake of different drinks and reported more gastrointestinal symptoms than controls. POTS was associated with higher CRP levels (β : 1.370; 95% CI: 0.004–2.737; $p = 0.049$), lower albumin levels (β : -1.443; 95% CI: -2.648–(-0.238); $p = 0.019$), and higher sodium levels (β : 1.392; 95% CI: 0.559–2.225; $p = 0.001$). Abdominal pain ($p = 0.004$) and intestinal symptoms' influence on daily life ($p = 0.025$) were negatively associated with albumin levels. Abdominal pain ($p = 0.011$), vomiting and nausea ($p = 0.003$), and intestinal symptoms' influence on daily life ($p = 0.026$) were associated with higher sodium levels. Serum levels representing iron and vitamin metabolism were equal between groups. **Conclusions.** POTS is associated with poorer sociodemographic status, but malnutrition cannot explain POTS or related gastrointestinal symptoms. Higher CRP and lower albumin levels suggest low-grade inflammation as one possible etiological factor.

1. Introduction

Postural orthostatic tachycardia syndrome (POTS) is characterized by pronounced symptoms of orthostatic intolerance and a heart rate increase > 30 beat per min (bpm) from a recumbent to a standing position or more than

120 bpm in standing, in the absence of orthostatic hypotension [1, 2]. The diagnosis can be confirmed by either an active stand test for 10 min or tilt table testing. The heart rate increase must occur in the absence of orthostatic hypotension [1, 2]. The prevalence of POTS is significantly higher among women in their fertile age compared with men. The

etiology and pathophysiology of this cardiovascular autonomic dysfunction are unknown [3]. It is well described that patients with POTS often have abdominal pain and nausea [4], but also a wide range of other gastrointestinal symptoms have been described [5]. Similar gastrointestinal symptoms of unknown origin in young women have been reported in irritable bowel syndrome (IBS) and endometriosis [6, 7]. IBS is associated with female sex, smoking, and unemployment [6], whereas endometriosis is associated with lower alcohol intake and lower physical activity [7]. These factors have never been examined in POTS, which may be of importance in the care planning of the patients.

Previous studies have shown that IBS patients have poor dietary habits, with a low intake of vegetables and fruits and a high intake of fast food, candy, and soda [8]. The gastrointestinal symptoms are correlated with poor dietary intake, and improvement of dietary habits leads to markedly reduced symptoms [8, 9]. Malnutrition may cause neuropathy, e.g., vitamin B12 deficiency and iron deficiency are well-known etiologies of autonomic neuropathy and orthostatic hypotension [10, 11]. Low levels of B12 have been described in adolescents with POTS [12], but the degree of malnutrition has not been studied in adult POTS patients.

Low-grade inflammation is another hypothesis for gastrointestinal symptoms in IBS [13], and a high prevalence of autoantibodies and abnormal cytokine levels have been described in POTS [14].

While most studies on POTS have focused on regulatory molecular mechanisms, few studies have examined the sociodemographic factors and lifestyle habits. It has been proposed that lifestyle habits and poor nutrient intake could explain the autonomic neuropathy and gastrointestinal symptoms. Accordingly, the aim of the present study was therefore to examine sociodemographic factors, lifestyle habits, nutritional factors, and inflammatory markers in patients with POTS and explore associations between these factors and gastrointestinal symptoms.

2. Materials and Methods

2.1. Study Design. Study participants were recruited for this cross-sectional study at the Department of Gastroenterology, Skåne University Hospital, Malmö, Sweden, between October 2020 and January 2022. Invitations including study information were sent by regular mail to patients with previously confirmed POTS at the Department of Cardiology of the same hospital, as described previously [15]. The patients were contacted by telephone to give further information and book a visit. During the same time frame, healthy controls were recruited among hospital staff and students. All study participants were asked to complete a 4-day food diary at home. During the first study visit, the participants were asked to fill in study questionnaires, and a clinical examination was performed. Blood samples were drawn and kept frozen at -20°C . The blood sample analyses were performed at the Department of Clinical Chemistry. At the time of the examination and blood sampling, none of the participants had any symptoms indicative of a viral or bacterial infection.

2.2. Study Population

2.2.1. Patients. The Syncope Study of Unselected Population in Malmö (SYSTEMA) cohort, with over 3000 patients investigated for syncope and severe orthostatic intolerance at the Skåne University Hospital, was conducted during 2008–2021. Details of the SYSTEMA cohort are described elsewhere [16]. The patients underwent cardiovascular autonomic tests with head-up tilt (HUT) testing with continuous hemodynamic monitoring, as well as other cardiac tests including ambulatory electrocardiogram (ECG) or 24 h ambulatory blood pressure monitoring, when appropriate [16]. The HUT testing protocol included supine rest for 10 min preceding table elevation to $60\text{--}70^{\circ}$ for 20 min [17]. POTS diagnosis was defined as symptoms of orthostatic intolerance lasting for ≥ 6 months associated with a pathological HUT test showing a heart rate (HR) increase > 30 bpm from a supine position or more than 120 bpm in standing, in the absence of orthostatic hypotension [1]. Moreover, all patients had been thoroughly evaluated by a senior expert (AF) to exclude other primary causes of the hemodynamic findings. As part of the clinical follow-up, POTS patients had been given lifestyle advice, including increased water and sodium intake.

From the SYSTEMA cohort, 93 patients with a clinically confirmed POTS diagnosis were included in the POTS sub-study. These patients were further examined at the Clinical Research Unit, Department of Internal Medicine, to reconfirm the POTS diagnosis. If possible, from the clinical status of the included patients, blood pressure and heart rate were measured twice in the supine position by a validated automated oscillometer device (Omron, Kyoto, Japan), and then after 1, 3, 5, and 10 min of standing. An average of two measurements in the supine position was used for group comparisons. For the orthostatic heart rate increase, the difference between standing heart rate after 3 min and supine heart rate was calculated. All cardiovascular pharmacological agents such as beta-blockers, ivabradine, midodrine, and droxidopa were discontinued 72 h prior to the examination. If patients were unable to abstain from medication due to pronounced symptoms, the POTS diagnosis established during previous tilt-testing and characteristic symptoms were considered valid [17].

Patients from the POTS sub-study obtained written information about the present study if they were living within a reasonable proximity to Malmö ($n = 82$) (Supplementary Figure 1). The patients were later contacted by telephone by one of the authors (HT) to give more information about the study. The inclusion criteria were age 18–70 years and the ability to fully understand the study information.

2.2.2. Control Subjects. Healthy control participants were recruited among hospital staff and medical students at Skåne University Hospital through personal invitation and advertisement. The controls were not allowed to have any current chronic or acute illness or significant gastrointestinal symptoms. Intake of multivitamins and hormonal contraceptive medicines was accepted, but otherwise, only temporary use of medications, such as seasonal allergy medicines, was allowed.

2.3. Clinical Examinations. During the first study visit, POTS patients were examined for heart, lung, abdominal, and neurological status. Neurological sensory testing was performed distally at the feet and distal leg with touch and by 10 g monofilament (Bailey Instruments, UK). A neurothesiometer (Horwell, London, UK) examination was performed to assess vibratory thresholds bilaterally at the medial malleolus and the tip of the big toe. A tendon hammer was used to assess the Achilles and patellar reflex. The current weight was obtained, as well as resting blood pressure from the right arm, in a supine position after approximately 5 min of rest. The healthy controls were examined with neurological sensory testing in the same way as the patients.

2.4. Questionnaires

2.4.1. Study Questionnaire. All study participants were asked to complete a questionnaire regarding sociodemographic factors, lifestyle habits, pregnancies and childbirth, previous and current illnesses, family history, current pharmacological treatment, and specific lifestyle modifications in case of gastrointestinal complaints.

2.4.2. Food Diary Questionnaire. The study participants were asked to keep a dietary record of all ingested food and drinks as well as associated gastrointestinal symptoms for 4 days. Each ingested item should be as thoroughly described as possible. Details about how to keep a food diary have been published previously [18]. The food intake during the 4 days was grouped into categories of meat, fish, vegetables/legumes, fruits/berries, dairy products, cereals, candies, soda, juice, protein drinks, and energy drinks including fluid replacement by a senior physician (BO). The number of times they ingested any fast food or calorie-containing liquid was registered, as well as whether they had regular or irregular food habits.

2.4.3. The Visual Analog Scale for Irritable Bowel Syndrome. The visual analog scale for irritable bowel syndrome (VAS-IBS) is a validated questionnaire regarding gastrointestinal symptoms common in IBS [19]. The respondent marks their degree of symptoms on a VAS between 0 and 100 mm, where 100 mm means very severe symptoms. The items covered are abdominal pain, diarrhea, constipation, bloating and flatulence, vomiting and nausea, psychological well-being, and the intestinal symptoms' influence on daily life. The scales are inverted from the original version [19]. Reference values for 52 healthy women are published [20].

2.5. Blood Samples. Serum levels of cobalamins, folic acid, iron, total iron-binding capacity (TIBC), ferritin, sodium, potassium, magnesium, phosphorus, albumin, high-sensitive C-reactive protein (CRP), and 25-hydroxyvitamin D (25-OH vitamin D) were analyzed according to clinical routines at the Department of Clinical Chemistry [21].

2.6. Statistical Analyses. Statistical analyses were performed using the software SPSS®, version 25 for Windows (IBM, New York, USA). Comparisons between groups were performed by the Mann-Whitney *U* test or Fisher's exact test

as appropriate. Spearman's correlation test was used to assess correlations between food intakes, serum levels of nutrients and inflammatory parameters, and symptoms. Since age and sex differed between groups, a generalized linear model was used to calculate associations with continuous variables after adjustments for these confounders, using significantly different nutrient and inflammatory parameters, weight, and body mass index (BMI) as dependent variables, and cohort group or gastrointestinal symptoms as predictors, and gastrointestinal symptoms as dependent variables and nutrient intake as predictors. For adjustment of age and sex in categorical variables, logistic regression was used with groups as dependent variables and sociodemographic and lifestyle habits as independent variables. Due to the low number of participants, *p* for trend was calculated except for marital status. Values are presented as median and interquartile range, numbers and percentages, β -value with a 95% confidence interval (CI), or odds ratio (OR) with a 95% CI. $p < 0.05$ was considered statistically significant. $p < 0.01$ was considered statistically significant in correlation tests due to several calculations.

3. Results

3.1. Basal Characteristics. In total, 61 controls and 43 patients with POTS were included. The time from symptom onset to POTS diagnosis was 3 [1–10] years, and the time from diagnosis to study inclusion was 3 [1–7] years. The patients were slightly younger than the controls ($p = 0.030$), with a higher prevalence of female sex ($p = 0.019$). After the adjustment for age and sex, weight was higher in patients, but BMI did not differ between groups. POTS patients had a lower education level, seldom worked full time, and were more often on sick leave or unemployed than healthy controls. They more often lived alone compared with healthy controls. Both groups had a low prevalence of smokers, whereas the patients drank less alcohol and had less physical activity than the controls (Table 1). The patients reported they felt sick from drinking alcohol.

The most common comorbidities among patients were the Ehlers-Danlos syndrome ($n = 12$; 27.9%), IBS ($n = 12$; 27.9%), asthma ($n = 8$; 18.6%), migraine ($n = 7$; 16.3%), neuropsychiatric disorders (ADHD/ADD/autism spectrum disorders) ($n = 6$; 14.0%), endometriosis ($n = 5$; 11.6%), and myalgic encephalomyelitis ($n = 5$; 11.6%). Sporadic cases of other diseases were observed such as celiac disease and inflammatory bowel disease. In the control group, allergy was found in four participants. The most common drugs in the POTS patients were antihypotensive drugs (midodrine and droxidopa) ($n = 17$), ivabradine ($n = 16$), beta blockers ($n = 13$), histamine H1 blockers ($n = 13$), and combined hormonal contraceptives ($n = 8$). A complete list of drugs used during inclusion is given in Supplementary Table 1. Several patients were taken vitamins such as B12 ($n = 6$), D-vitamin ($n = 4$), multivitamins ($n = 3$), and folic acid ($n = 2$). Sporadic users of antiallergic treatment ($n = 2$), contraceptives ($n = 4$), and multivitamins ($n = 2$) were found among healthy controls.

TABLE 1: Basal characteristics.

	Healthy controls N = 61	POTS N = 43	<i>p</i> value	β or OR (95% CI)	<i>p</i> value
Age (years)	38 (29–44)	31 (26–41)	0.030		
Women (<i>n</i> , %)	45 (73.8)	40 (93.0)	0.019		
Weight (kg)	65.0 (57.5–76.2)	70.0 (60.0–82.6)	0.132	7.031 (2.012–12.050)	0.006*
BMI (kg/m ²)	22.7 (21.4–25.3)	24.2 (21.3–27.0)	0.416	1.330 (–0.382–3.042)	0.128*
Education (<i>n</i> , %)			<0.001		
Primary school (9 years)		3 (7.0)			
Secondary school (3 years)	4 (6.6)	14 (32.6)			
At least 1 year of higher education	10 (16.4)	9 (20.9)			
University	47 (77.0)	17 (39.5)			
<i>p</i> for trends				0.386 (0.216–689)	0.001**
Occupation (<i>n</i> , %)			<0.001		
100%	41 (67.2)	6 (14.0)			
99–51%	9 (14.8)	5 (11.6)			
50%		1 (2.3)			
Sick leave		19 (44.2)			
Retired	1 (1.6)	1 (2.3)			
Unemployment		3 (7.0)			
Studying	10 (16.4)	8 (18.6)			
<i>p</i> for trends				1.368 (1.108–1.688)	0.004**
Marital status (<i>n</i> , %)			0.023		
Living alone	15 (24.6)	20 (46.5)		1.00	
Married/living together	46 (75.4)	23 (53.5)		0.451 (0.184–1.110)	0.083**
Smoking (<i>n</i> , %)			0.361		
Never smoked	42 (68.9)	32 (74.4)			
Quit smoking	12 (19.7)	5 (10.9)			
Sometimes	4 (6.6)	1 (2.2)			
Regularly	3 (4.9)	5 (11.6)			
<i>p</i> for trends				0.975 (0.614–1.548)	0.914**
Weekly alcohol consumption (standard glasses)			<0.001		
<1	19 (31.1)	38 (88.4)			
1–4	32 (52.5)	4 (9.3)			
5–9	8 (13.1)	1 (2.2)			
9–14	2 (3.3)				
<i>p</i> for trends				0.116 (0.043–0.312)	<0.001**
Weekly physical activity (min)			0.025		
No time at all	3 (4.9)	8 (18.6)			
<30	7 (11.5)	6 (14.0)			
30–60	13 (21.3)	10 (23.3)			
60–90	9 (14.8)	11 (25.6)			
90–120	11 (18.0)	2 (4.7)			
>120	18 (29.5)	6 (14.0)			
<i>p</i> for trends				0.691 (0.525–0.910)	0.009**

POTS: postural orthostatic tachycardia syndrome. Values are given as number and percentages or median and interquartile ranges. Differences are calculated by Fisher's exact test and Mann–Whitney *U* test. β Coefficient and 95% confidence interval (CI) (continuous variables) or odds ratio (OR) and 95% CI (categorical variables) are given after adjustment for age and sex by generalized linear model* or logistic regression**. *p* for trend was given for dichotomous variables, except for marital status. *p* < 0.05 was considered statistically significant.

3.2. Nutrient Intake. Only 20 (32.8%) of the controls and 24 (55.8%) of the POTS patients returned diary books, with no differences in age ($p = 0.080$) or sex distribution ($p = 0.118$) between groups. Patients had a lower intake of vegetables and legumes compared with controls, and a tendency to have a lower intake of candies and cakes (Table 2). On the other hand, the intake of sugar-rich soda was higher in patients. There was a more frequent intake of different drinks such as energy drinks, protein drinks, and juice in patients than in controls, although not statistically significant for each drink. However, when calculated together, the higher drink intake in patients reached statistical significance (Table 3). When studying the diary books, several meals were replaced by energy-rich drinks in the POTS group (data not shown).

3.3. Serum Levels of CRP and Nutrient Parameters. Although only three (7.3%) patients and one (2.0%) control had CRP levels above the reference value, the levels of high-sensitive CRP were higher in patients than in controls (Table 4) (Figure 1). CRP levels correlated inversely with the levels of albumin ($rs = -0.470$, $p < 0.001$) and iron ($rs = -0.348$, $p = 0.004$). All participants had albumin levels within reference values, but the levels were lower in patients than in controls (Table 4) (Figure 1), and albumin levels correlated with levels of iron ($rs = 0.268$, $p = 0.010$) and ferritin ($rs = 0.293$, $p = 0.005$).

Sodium levels were higher in patients than in controls, with corresponding lower potassium levels (Table 4) (Figure 1).

Among the controls, three (5.9%) had low iron levels, eight (15.7%) had higher TIBC levels, and eight (15.7%) had low ferritin levels. Among patients, four (9.8%) had low iron levels ($p = 0.119$), seven (17.1%) had high TIBC levels ($p = 0.190$), and six (14.6%) had ferritin levels below reference values ($p = 0.211$). Regarding 25-OH vitamin D, 36 (70.6%) controls and 29 (70.7%) patients had levels below reference values ($p = 0.204$). When adjusting for sex and age, which may affect levels of ferritin and phosphorus, there were still no differences between controls and patients (β : -8.980; 95% CI: -29.859–11.900; $p = 0.399$ and β : 0.052; 95% CI: -0.029–0.132; $p = 0.209$, respectively).

There were no correlations between nutrient intake and nutrient levels in serum (data not shown).

3.4. Gastrointestinal Symptoms. POTS patients had more gastrointestinal symptoms than healthy controls (Table 5). Bread was the only food intake which correlated with symptoms and showed negative correlations with vomiting and nausea ($rs = -0.443$, $p = 0.003$) and psychological well-being ($rs = -0.406$, $p = 0.006$). Dietary habits did not associate with any symptoms after adjustments for the confounders' age and sex (data not shown).

CRP levels correlated with diarrhea ($rs = 0.379$, $p = 0.002$), bloating and flatulence ($rs = 0.397$, $p = 0.001$), and the intestinal symptoms' influence on daily life ($rs = 0.336$, $p = 0.006$). Albumin correlated negatively with abdominal pain ($rs = -0.327$, $p = 0.001$), vomiting and nausea ($rs = -0.276$, $p = 0.008$), and the intestinal symptoms' influence on daily

TABLE 2: Nutrient intake.

	Healthy controls N = 20	POTS N = 24	p value
Meat	4.00 (1.25–7.75)	4.00 (1.25–5.75)	0.794
Fish/seafood	1.00 (0–2.00)	1.00 (0–2.00)	0.712
Vegetables/legumes	6.00 (4.00–8.00)	5.50 (3.00–7.00)	0.042
Fruits/berries	4.00 (2.25–5.00)	3.00 (0.25–6.50)	0.550
Dairy products	6.00 (3.25–9.50)	5.50 (2.5–8.00)	0.531
Cereals	9.00 (7.25–11.75)	7.00 (3.25–9.00)	0.333
Candies	3.00 (3.00–5.00)	2.00 (1.25–4.00)	0.051

POTS: postural orthostatic tachycardia syndrome. Values are given as median and interquartile ranges. Mann-Whitney U test. $p < 0.05$ was considered statistically significant.

life ($r = -0.280$, $p = 0.007$). Sodium levels correlated with abdominal pain ($rs = 0.291$, $p = 0.005$), vomiting and nausea ($rs = 0.304$, $p = 0.003$), and the intestinal symptoms' influence on daily life ($rs = 0.292$, $p = 0.005$). Potassium levels correlated negatively with diarrhea ($rs = -0.316$, $p = 0.002$) and vomiting and nausea ($rs = -0.275$, $p = 0.008$).

3.5. Associations with Serum Levels of CRP and Nutrient Parameters. Since levels of CRP, albumin, sodium, and potassium differed between patients and controls (Figure 1), calculations were performed after adjustments for the confounders; age and sex. POTS was associated with higher CRP levels (β : 1.370; 95% CI: 0.004–2.737; $p = 0.049$), lower albumin levels (β : -1.443; 95% CI: -2.648–(-0.238); $p = 0.019$), and higher sodium levels (β : 1.392; 95% CI: 0.559–2.225; $p = 0.001$). There was no association between POTS and lower potassium levels (β : -0.084; 95% CI: -0.188–0.021; $p = 0.117$).

CRP levels were not associated with any gastrointestinal symptoms after adjustment for confounders (data not shown). Abdominal pain (β : -0.004; 95% CI: -0.052–(-0.010); $p = 0.004$) and intestinal symptoms' influence on daily life (β : -0.020; 95% CI: -0.038–(-0.003); $p = 0.025$) were negatively associated with albumin levels. Abdominal pain (β : 0.020; 95% CI: 0.005–0.035; $p = 0.011$), vomiting and nausea (β : 0.021; 95% CI: 0.007–0.035; $p = 0.003$), and intestinal symptoms' influence on daily life (β : 0.014; 95% CI: 0.002–0.027; $p = 0.026$) were associated with higher sodium levels. Diarrhea (β : -0.002; 95% CI: -0.004–(-0.001); $p = 0.006$) and vomiting and nausea (β : -0.002; 95% CI: -0.004–(-0.001); $p = 0.011$) were negatively associated with potassium levels.

4. Discussion

The main findings of the present study were the associations of POTS with sociodemographic factors, signs of low-grade inflammation, and higher sodium levels compared with healthy controls.

The current findings suggest that POTS patients more often are living alone, less often complete any education, and less often are working full time compared with healthy subjects. Although it may be suspected that the disease

TABLE 3: The number of participants who ingested the food or beverage at any time for 4 days.

	Healthy controls N = 20	POTS N = 24	p value
Regular meals	16 (80.0%)	15 (62.5%)	0.321
Fast food	6 (30.0%)	8 (33.3%)	1.000
Soda			
Sugar-rich	2 (10.0%)	9 (37.5%)	0.044
Sugar-free	3 (15.0%)	3 (12.6%)	1.000
Juice	5 (25.0%)	8 (33.3%)	0.742
Protein drinks	1 (5.0%)	3 (12.6%)	0.614
Energy drinks	0	4 (16.6%)	0.114
All drinks together	7 (35.0%)	17 (70.8%)	0.032

POTS: postural orthostatic tachycardia syndrome. Values are given as number and percentage of participants in each group. One participant could ingest several different drinks. Fisher's exact test. $p < 0.05$ was considered statistically significant.

may contribute to these associations, no causality can be confirmed in this cross-sectional study. The low intake of alcohol and lower degree of physical activity are also suggested to be secondary effects of the disease. The patients said that they felt sick when they drank alcohol and therefore avoided or reduced alcohol intake. Low physical activity may further impair their health and their psychological and physical capacities since physical activity has been shown to reduce the burden of gastrointestinal unhealth and depression [22, 23]. Low physical activity is also a risk factor for physical deconditioning, which may worsen symptoms in POTS [2].

IBS was present in one-third of the POTS patients, and endometriosis was present in 12% of patients. These comorbidities are much more frequent than expected since the prevalence in the society of IBS is 4–11% [24, 25] and the prevalence of endometriosis is 6–10% [26]. Some of the patients may have got diagnosis with IBS due to their gastrointestinal symptoms, since the physician may not have recognized POTS as associated with such symptoms. Self-reported IBS in the general population showed the strongest associations with female sex and smoking, and gastrointestinal symptoms were associated with unemployment and inversely associated with age ≥ 50 years [6]. In POTS patients, a female predominance is at hand, but there were no differences in smoking habits between groups, and the differences in age, education, occupation, marital status, alcohol intake, and physical activity cannot explain the development of gastrointestinal symptoms [6]. In endometriosis, low alcohol intake and low physical activity were observed, but education, occupation, and marital status did not differ between endometriosis patients and controls from the general population [7]. These observations suggest that patients with POTS are more affected by this condition with a greater impact on daily life than what is observed in patients with IBS and endometriosis. This may be due to that POTS patients do not only have gastrointestinal symptoms but also have symptoms of cardiovascular and, possibly, another autonomic dysfunction. The sociodemographic

consequences are most likely secondary to the severity of symptoms and do not have a causal role. There is a delay from symptom onset to diagnosis, and the inability to work full-time or complete higher educational levels remains years after diagnosis.

The higher levels of sodium in POTS than in controls are in accordance with other studies [27], which may indicate hypovolemia and increased intake of salt by patients following common POTS recommendations [28]. POTS often have a reduced intravascular volume [29–31] and high-standing plasma norepinephrine [32]. A radiolabeled serum albumin investigation found a plasma volume deficit of nearly 13% [29]. A reduced plasma volume should stimulate the renin–angiotensin–aldosterone system (RAAS) to promote sodium retention. Although POTS patients have been shown to have lower sodium excretion than healthy controls [33], the regulatory RAAS might be defective in POTS [29]. Elevation in plasma norepinephrine suggests an increase in the neural tone of the sympathetic nervous system, leading to deficient RAAS activity [34]. However, the affected sodium levels may also depend on gastrointestinal disturbances with dysregulated electrolytes as a result.

Intravascular hypovolemia may be exaggerated in the upright position by blood pooling in the abdomen, pelvis, and legs [35]. The decreased return of blood to the heart and the reduced cardiac output lead to less stimulation of carotid and aortic baroreceptors, triggering an increase in sympathetic outflow [36, 37]. The high sympathetic activity renders a hyperadrenergic state with palpitations, tachycardia, near syncope, chest pain, and dyspnea, as well as symptoms from other organs like the gastrointestinal system, the genitourinary system, and psychological effects of high sympathetic activity such as anxiousness [38]. This may start a negative spiral since gastrointestinal symptoms of diarrhea and vomiting may worsen dehydration and electrolyte imbalance with the loss of potassium and albumin. The sympathetic activation continues until the patient is supine, or until the patient increases plasma volume through hydration [38]. In accordance with the hypovolemia theory, POTS patients experienced a reduction in heart rate acceleration and standing plasma norepinephrine upon upright position following a short-term period of elevated dietary sodium intake [27]. The current finding with the replacement of a meal of fast food with liquid intake in POTS may depend on increased thirst as a response to hypovolemia.

Previous studies have suggested POTS to be an autoimmune disease, with high a prevalence of autoantibodies and abnormal cytokine levels [14]. Although within reference values in most patients, CRP levels were associated with POTS but not with gastrointestinal symptoms, in contrast to potassium levels which were associated with gastrointestinal symptoms but not with POTS. A normal CRP value does not exclude presence of low-grade inflammation [39]. For comparison, CRP levels did not differ between controls and IBS patients, which are also considered a low-grade inflammatory disease [18]. The findings of higher CRP and lower albumin levels, in correlation with altered levels of iron, TIBC, and ferritin, support the hypothesis that low-grade inflammation is common in POTS [40]. If the patients

TABLE 4: Serum levels of CRP and nutrient parameters.

	Healthy controls N = 61	POTS N = 43	p value
High-sensitive CRP (<5 mg/L)	0.68 (0.35–1.38)	1.35 (0.62–2.98)	0.006
Albumin (36–48 g/L)	44 (41–45)	41 (40–44)	0.014
Cobalamin (156–672 pmol/L)	294 (231–362)	300 (244–363)	0.549
Folic acid (>8 nmol/L)	19 (15–24)	18 (12–27)	0.503
25-OH vitamin D (>75 nmol/L)	63 (49–80)	68 (54–80)	0.292
Iron (9–34 μ mol/L)	18 (14–22)	17 (12–22)	0.719
TIBC (47–80 μ mol/L)	67 (61–73)	69 (60–76)	0.692
Ferritin (men: 22–322 μ g/L) (women: 10–291 μ g/L)	34 (15–74)	31 (14–50)	0.574
Sodium (137–145 mmol/L)	141 (139–142)	142 (141–143)	0.003
Potassium (3.5–4.4 mmol/L)	4.0 (3.8–4.2)	3.9 (3.8–4.0)	0.029
Magnesium (0.70–0.95 mmol/L)	0.78 (0.75–0.83)	0.80 (0.76–0.84)	0.169
Phosphorus (men 18–50 μ mol/L; women $\geq$ 18 μ mol/L; 0.80–1.5 mmol/L)	1.2 (1.1–1.3)	1.1 (1.0–1.3)	0.103

Two missing values in POTS and 10 missing values in controls. CRP: C-reactive protein; POTS: postural orthostatic tachycardia syndrome; TIBC: total iron-binding capacity. Reference values within brackets are taken from the Department of Clinical Chemistry, Skåne University Hospital, Malmö, Sweden [21]. Values are given as median and interquartile ranges. Mann-Whitney *U* test. $p < 0.05$ was considered statistically significant.

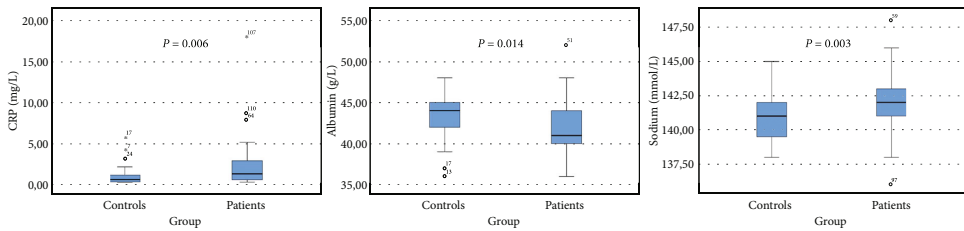


FIGURE 1: Serum levels of C-reactive protein, albumin, and sodium in healthy controls and patients with postural orthostatic tachycardia syndrome. Mann-Whitney *U* test. $p < 0.05$ was considered statistically significant.

TABLE 5: Gastrointestinal symptoms.

	Controls N = 61	POTS N = 43	p value
Abdominal pain (5 (1–15))	0	30 (11–62)	<0.001
Diarrhea (3 (0–10))	0	28 (0–64)	<0.001
Constipation (9 (1–22))	0 (0–13)	53 (7–74)	<0.001
Bloating and flatulence (14 (1–29))	0 (0–10)	65 (14–88)	<0.001
Vomiting and nausea (2 (0–3))	0	44 (21–70)	<0.001
Intestinal symptoms influence on daily life (2 (0–18))	0 (0–7)	50 (22–61)	<0.001
Psychological well-being (4 (0–16))	3 (0–18)	52 (20–76)	<0.001

POTS: postural orthostatic tachycardia syndrome. Gastrointestinal symptoms were assessed by the visual analog scale for irritable bowel syndrome, 0–100 mm, where 0 represents no symptoms and 100 maximal symptoms [19]. Reference values for VAS-IBS from healthy women are shown within brackets [20]. Values are given as median and interquartile ranges. Mann-Whitney *U* test. $p < 0.05$ was considered statistically significant.

were hypovolemic, the true albumin levels could be even lower than observed.

A previous study has found lower levels of B12 in adolescents with POTS [12]. This could not be found in the present study in adults. Furthermore, although POTS patients ingested several energy-rich and sugar-rich drinks, they had overall similar dietary habits to the healthy controls; they had similar serum levels of micronutrients; and their

dietary habits did not associate with any gastrointestinal symptoms. We could thus not find any evidence that malnutrition could explain the pathophysiology behind POTS and related gastrointestinal symptoms, as we have found in IBS patients [9]. However, more patients than controls ingested vitamin tablets, which may have improved the circulating levels of nutrients. The high intake of drinks was based on individual experience and not prescribed by the physician.

The strength of the present study is the comparison between patients and controls from the same region. One limitation is the low-reported frequency of diary books and the differences in age and sex between the two groups. However, these differences were adjusted for in the statistical calculations. Another limitation is that the study was conducted in a tertiary hospital setting and that the included POTS patients may not be representative of findings in milder forms of POTS. On the other hand, the study of well-phenotypic POTS patients may increase the sensitivity for finding differences in the examined factors between POTS and healthy controls.

5. Conclusion

In conclusion, the diagnosis of POTS is associated with sociodemographic factors in the form of less often being married or living together, less often having completed an education, and an impaired ability to work full time. POTS patients drink less alcohol and demonstrate low physical activity but have adequate dietary habits. Malnutrition may not explain the neuropathy in POTS. Low-grade inflammation may be involved in the pathophysiology of POTS and its related symptoms, as well as dysregulation of sodium levels and hypovolemia.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

The present study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Review Board at Uppsala University, Dnr 2020-02432 and 2021-00049. The Regional Ethical Committee in Lund, Sweden, approved the SYSTEMA project (82/2008) and the POTS-substudy (2017/295).

Consent

All study participants provided informed written consent prior to inclusion.

Conflicts of Interest

The authors have no conflict of interest to declare.

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Supplementary Materials

Supplementary 1. Supplementary Figure 1: flow chart of the recruitment of study participants. SYSTEMA: the Syncope Study of Unselected Population in Malmö; POTS: postural orthostatic tachycardia syndrome.

Supplementary 2. Supplementary Table 1: medication treatment in the group of patients with postural orthostatic tachycardia syndrome.

References

- [1] M. Brignole, A. Moya, F. J. de Lange et al., "2018 ESC guidelines for the diagnosis and management of syncope," *European Heart Journal*, vol. 39, no. 21, pp. 1883–1948, 2018.
- [2] A. Fedorowski, "Postural orthostatic tachycardia syndrome: clinical presentation, aetiology and management," *Journal of Internal Medicine*, vol. 285, no. 4, pp. 352–366, 2019.
- [3] S. Vernino, K. M. Bourne, L. E. Stiles et al., "Postural orthostatic tachycardia syndrome (POTS): State of the science and clinical care from a 2019 National Institutes of Health Expert Consensus Meeting - Part 1," *Autonomic Neuroscience*, vol. 235, article 102828, 2021.
- [4] Y. Tu, T. L. Abell, S. R. Raj, and P. L. Mar, "Mechanisms and management of gastrointestinal symptoms in postural orthostatic tachycardia syndrome," *Neurogastroenterology & Motility*, vol. 32, no. 12, article e14031, 2020.
- [5] J. K. DiBaise, L. A. Harris, and B. Goodman, "Postural tachycardia syndrome (POTS) and the GI tract: a primer for the gastroenterologist," *The American Journal of Gastroenterology*, vol. 113, no. 10, pp. 1458–1467, 2018.
- [6] D. Nilsson and B. Ohlsson, "Gastrointestinal symptoms and irritable bowel syndrome are associated with female sex and smoking in the general population and with unemployment in men," *Frontiers in Medicine*, vol. 8, article 646658, 2021.
- [7] M. Ek, B. Roth, P. M. Nilsson, and B. Ohlsson, "Characteristics of endometriosis: a case-cohort study showing elevated IgG titers against the TSH receptor (TRAb) and mental comorbidity," *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, vol. 231, pp. 8–14, 2018.
- [8] C. Nilholm, E. Larsson, B. Roth, R. Gustafsson, and B. Ohlsson, "Irregular dietary habits with a high intake of cereals and sweets are associated with more severe gastrointestinal symptoms in IBS patients," *Nutrients*, vol. 11, no. 6, p. 1279, 2019.
- [9] B. Roth, E. Larsson, and B. Ohlsson, "Poor intake of vitamins and minerals is associated with symptoms among patients with irritable bowel syndrome," *Journal of Gastroenterology and Hepatology*, vol. 37, no. 7, pp. 1253–1262, 2022.
- [10] S. Toru, T. Yokota, A. Inaba et al., "Autonomic dysfunction and orthostatic hypotension caused by vitamin B12 deficiency," *Journal of Neurology, Neurosurgery, and Psychiatry*, vol. 66, no. 6, pp. 804–805, 1999.
- [11] P. Baum, K. V. Toyka, M. Blüher, J. Kosacka, and M. Nowicki, "Inflammatory mechanisms in the pathophysiology of diabetic peripheral neuropathy (DN)-new aspects," *International Journal of Molecular Sciences*, vol. 22, no. 19, p. 10835, 2021.
- [12] T. Öner, B. Guven, V. Tavli, T. Mese, M. Muhtar Yilmazer, and S. Demirpence, "Postural orthostatic tachycardia syndrome (POTS) and vitamin B12 deficiency in adolescents," *Pediatrics*, vol. 133, no. 1, pp. e138–e142, 2014.
- [13] P. A. Hayes, M. H. Fraher, and E. M. M. Quigley, "Irritable bowel syndrome: the role of food in pathogenesis and management," *Gastroenterology & Hepatology*, vol. 10, pp. 164–174, 2014.
- [14] W. T. Gunning, S. M. Stepkowski, P. M. Kramer, B. L. Karabin, and B. P. Grubb, "Inflammatory biomarkers in postural

- orthostatic tachycardia syndrome with elevated G-protein-coupled receptor autoantibodies," *Journal of Clinical Medicine*, vol. 10, no. 4, p. 623, 2021.
- [15] M. Johansson, F. Ricci, J. Schulte et al., "Circulating levels of growth hormone in postural orthostatic tachycardia syndrome," *Scientific Reports*, vol. 11, no. 1, p. 8575, 2021.
- [16] A. Fedorowski, P. Burri, J. Struck, S. Juul-Möller, and O. Melander, "Novel cardiovascular biomarkers in unexplained syncopal attacks: the SYSTEMA cohort," *Journal of Internal Medicine*, vol. 273, no. 4, pp. 359–367, 2013.
- [17] J. M. Spahic, V. Hamrefors, M. Johansson et al., "Malmö POTS symptom score: assessing symptom burden in postural orthostatic tachycardia syndrome," *Journal of Internal Medicine*, vol. 293, no. 1, pp. 91–99, 2023.
- [18] C. Nilholm, E. Larsson, E. Sonestedt, B. Roth, and B. Ohlsson, "Assessment of a 4-week starch- and sucrose-reduced diet and its effects on gastrointestinal symptoms and inflammatory parameters among patients with irritable bowel syndrome," *Nutrients*, vol. 13, no. 2, p. 416, 2021.
- [19] M. Bengtsson, B. Ohlsson, and K. Ulander, "Development and psychometric testing of the visual analogue scale for irritable bowel syndrome (VAS-IBS)," *BMC Gastroenterology*, vol. 7, no. 1, p. 16, 2007.
- [20] M. Bengtsson, O. Hammar, T. Mandl, and B. Ohlsson, "Evaluation of gastrointestinal symptoms in different patient groups using the visual analogue scale for irritable bowel syndrome (VAS-IBS)," *BMC Gastroenterology*, vol. 11, no. 1, p. 122, 2011.
- [21] Medical Service, "Lab Medicine Skåne Analysportalen," AnalysPortalen-Labmedicin Skåne, 2022, <https://www.skane.se/>.
- [22] H. Jo, J. Lee, S. Lee, H. Lee, Y. S. Ahn, and S. B. Koh, "The longitudinal effect of leisure time physical activity on reduced depressive symptoms: the ARIRANG study," *Journal of Affective Disorders*, vol. 282, pp. 1220–1225, 2021.
- [23] R. Sámano, F. Esparza-Juárez, G. Chico-Barba, E. González-Medina, B. Sánchez-Jiménez, and M. Hernández-Trejo, "Association of diet, body mass index, and lifestyle on the gastrointestinal health risk in a sample of adults," *International Journal of Environmental Research and Public Health*, vol. 19, no. 17, article 10569, 2022.
- [24] A. D. Sperber, D. Dumitrascu, S. Fukudo et al., "The global prevalence of IBS in adults remains elusive due to the heterogeneity of studies: a Rome foundation working team literature review," *Gut*, vol. 66, no. 6, pp. 1075–1082, 2017.
- [25] O. S. Palsson, W. Whitehead, H. Törnblom, A. D. Sperber, and M. Simren, "Prevalence of Rome IV functional bowel disorders among adults in the United States, Canada, and the United Kingdom," *Gastroenterology*, vol. 158, no. 5, pp. 1262–1273.e3, 2020.
- [26] L. C. Guidice and L. C. Kao, "Endometriosis," *Lancet*, vol. 364, no. 9447, pp. 1789–1799, 2004.
- [27] E. M. Garland, A. Gamboa, V. C. Nwazue et al., "Effect of high dietary sodium intake in patients with postural tachycardia syndrome," *Journal of the American College of Cardiology*, vol. 77, no. 17, pp. 2174–2184, 2021.
- [28] S. Raj, A. Fedorowski, and R. S. Sheldon, "Diagnosis and management of postural orthostatic tachycardia syndrome," *Canadian Medical Association Journal*, vol. 194, no. 10, pp. E378–E385, 2022.
- [29] S. R. Raj, I. Biaggioni, P. C. Yamhure et al., "Renin-aldosterone paradox and perturbed blood volume regulation underlying postural tachycardia syndrome," *Circulation*, vol. 111, no. 13, pp. 1574–1582, 2005.
- [30] S. R. Raj and D. Robertson, "Blood volume perturbations in the postural tachycardia syndrome," *The American Journal of the Medical Sciences*, vol. 334, no. 1, pp. 57–60, 2007.
- [31] H. I. Mustafa, E. M. Garland, I. Biaggioni et al., "Abnormalities of angiotensin regulation in postural tachycardia syndrome," *Heart Rhythm*, vol. 8, no. 3, pp. 422–428, 2011.
- [32] E. M. Garland, S. R. Raj, B. K. Black, P. A. Harris, and D. Robertson, "The hemodynamic and neurohumoral phenotype of postural tachycardia syndrome," *Neurology*, vol. 69, no. 8, pp. 790–798, 2007.
- [33] Q. Zhang, Y. Liao, C. Tang, J. Du, and H. Jin, "Twenty-four-hour urinary sodium excretion and postural orthostatic tachycardia syndrome," *The Journal of Pediatrics*, vol. 161, no. 2, pp. 281–284, 2012.
- [34] E. M. Garland, J. E. Celedonio, and S. R. Raj, "Postural tachycardia syndrome: beyond orthostatic intolerance," *Current Neurology and Neuroscience Reports*, vol. 15, no. 9, p. 60, 2015.
- [35] G. Jacob, I. Biaggioni, R. Mosqueda-Garcia, R. M. Robertson, and D. Robertson, "Relation of blood volume and blood pressure in orthostatic intolerance," *The American Journal of the Medical Sciences*, vol. 315, no. 2, pp. 95–100, 1998.
- [36] D. S. Kimmerly and J. K. Schoemaker, "Hypovolemia and neurovascular control during orthostatic stress," *American Journal of Physiology. Heart and Circulatory Physiology*, vol. 282, no. 2, pp. H645–H655, 2002.
- [37] N. Charkoudian, E. A. Martin, F. A. Dinennen, J. H. Eisenach, N. M. Dietz, and M. J. Joyner, "Influence of increased central venous pressure on baroreflex control of sympathetic activity in humans," *American Journal of Physiology-Heart and Circulatory Physiology*, vol. 287, no. 4, pp. H1658–H1662, 2004.
- [38] H. Snapper and W. P. Cheshire, "Oral and intravenous hydration in the treatment of orthostatic hypotension and postural tachycardia syndrome," *Autonomic Neuroscience*, vol. 238, article 102951, 2022.
- [39] A. Olsson, S. Gustavsen, K. Gisselø Lauridsen et al., "Neutrophil-to-lymphocyte ratio and CRP as biomarkers in multiple sclerosis: a systematic review," *Acta Neurologica Scandinavica*, vol. 143, no. 6, pp. 577–586, 2021.
- [40] M. Yamamoto, H. Adachi, M. Enomoto et al., "Lower albumin levels are associated with frailty measures, trace elements, and an inflammation marker in a cross-sectional study in Tanushimaru," *Environmental Health and Preventive Medicine*, vol. 26, no. 1, p. 25, 2021.

Paper II





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Gastrointestinal symptoms in patients with postural orthostatic tachycardia syndrome in relation to hemodynamic findings and immunological factors

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Gastrointestinal (GI) symptoms are common in postural orthostatic tachycardia syndrome (POTS). We aimed to explore the prevalence and severity of GI symptoms in POTS, and to investigate immunological factors, hemodynamic findings, and their possible association with GI symptoms in POTS. Forty-three patients (93% female, median age 30.6 (26.0–41.0) years), previously diagnosed with POTS and 74 healthy controls (78% female, median age 35.6 (28.8–41.7) years) were included. The participants completed a questionnaire including prevalence of GI symptoms, the irritable bowel syndrome severity scoring system (IBS-SSS), and visual analog scale for IBS (VAS-IBS). All POTS patients were previously examined by tilt test (2010–2021) and the vast majority with more recent active standing test (2017–2021), which included monitoring of heart rate (HR). Δ HR was calculated as difference between supine and upright position. Continuous variables from IBS-SSS and VAS-IBS were correlated to Δ HR. A microarray containing several autoantigens commonly targeted in systemic autoimmune disorders was used to assess prevalent autoantibodies in POTS and controls. Total IgE and S-tryptase were analyzed. GI symptoms were more prevalent and severe in POTS than in controls; nausea being the most prevalent (79.1% vs 4.9%, $p < 0.001$) and bloating and flatulence being the most severe (median 65 (25–88) vs 0 (0–14), $p < 0.001$). The median total IBS-SSS was 213 (135–319) in POTS vs 13 (0–54) in controls ($p < 0.001$). Total IBS-SSS was associated with low psychological wellbeing ($r = 0.539$, $p < 0.001$) in POTS. Δ HR_{max} correlated inversely with abdominal pain ($r = -0.406$, $p = 0.007$). After adjustments for psychological wellbeing, total IBS-SSS still associated inversely with Δ HR_{10min} (β : 4.748; 95% CI: -9.172 to -0.324; $p = 0.036$). Similar results were seen with active standing test. The prevalence of autoantibodies did not differ between POTS and controls (29.4% vs 33.3%, $p = 0.803$). There was no association between GI symptoms and autoantibody status. Total IgE and tryptase were elevated in a few cases. This study confirms the high prevalence of GI symptoms in POTS. More pronounced tachycardia upon tilt table testing

seems to be inversely correlated with severity of chronic GI symptoms in POTS. This study did not support the hypothesis that POTS is associated with immunological factors.

KEYWORDS

postural orthostatic tachycardia (POTS), gastrointestinal tract, tilt table test, autoimmunity, ehlers-danlos syndrome, irritable bowel syndrome, mast cells

1 Introduction

Postural orthostatic tachycardia syndrome (POTS) is a cardiovascular autonomic disorder, characterized by a heart rate (HR) increase >30 beats per minute (bpm) or a HR of >120 bpm within 10 min upon standing, and no significant reduction in blood pressure (BP) (Brignole et al., 2018; Vernino et al., 2021). To fulfill the diagnostic criteria, the patient must have chronic symptoms (>3 months) of orthostatic intolerance, i.e., palpitations, blurred vision, fatigue, and lightheadedness upon standing that improve when the patient returns to supine position. Other causes of tachycardia and orthostatic intolerance, such as anemia, should be ruled out (Vernino et al., 2021; Raj et al., 2022). As diagnostic tool, the head up tilt test can be performed to measure HR and BP responses from supine to standing (Brignole et al., 2018). The more easily performed active standing test can be used as well, with the same diagnostic criteria (Brignole et al., 2018; Fedorowski, 2019). The epidemiology of POTS is not fully explored, but previous studies estimate a prevalence ranging from 0.2% to 1.0% in the U.S. population, with females over-represented with a 4:1 ratio, especially in younger ages (Fedorowski, 2019). POTS frequently presents with chronic comorbidities or conditions that are unrelated to orthostatic intolerance, such as migraine, fibromyalgia, chronic fatigue, and allergic disorders (Liao et al., 2017; Chelimsky and Chelimsky, 2018). Hypermobile spectrum disorders (HSD) including hypermobile Ehlers-Danlos syndrome (hEDS) are also more common in POTS (Miller et al., 2020; Tai et al., 2020). Autoimmunity has been proposed as a possible etiological mechanism in POTS, with high prevalence of diverse antibodies in previous reports (Fedorowski, 2019). Moreover, increased prevalence of co-morbid systemic autoimmune rheumatic disorders have been reported in POTS (Mannan and Pain, 2023), but causative autoantibodies have not been found so far (Vernino and Stiles, 2018). Mast cell activation disorder has been proposed to be associated with POTS with atypical symptom presentation, such as predominant gastrointestinal (GI) symptomatology (Kohn et al., 2021).

GI symptoms are very frequent in POTS, well described in several studies (Chelimsky and Chelimsky, 2018; DiBaise et al., 2018; Mehr et al., 2018; Tu et al., 2020). Some GI symptoms may be triggered by orthostatic challenge, and are thereby relieved in recumbent position, whereas most other GI complaints are non-orthostatic (Chelimsky and Chelimsky, 2018; Tu et al., 2020). The enteric nervous system is a part of the autonomous nervous system (Gibbons, 2019), receiving input from parasympathetic and sympathetic nerves, as well as input from the central nervous system (Koloski et al., 2012; Mehr et al., 2018). Disturbances of the enteric nervous system can cause GI disorders such as gastroparesis, and symptoms like pain and altered bowel

habits (Camilleri, 2021). Since POTS is associated with disturbances of the cardiovascular autonomic nervous system (Vernino et al., 2021), our hypothesis was that more exaggerated pulse increase on tilt testing is associated with more severe GI symptoms, as a surrogate marker of autonomic and enteric nervous dysfunction.

Consequently, the first aim of this study was to explore GI symptoms in a POTS cohort compared with healthy controls using validated GI symptom questionnaires, and to investigate whether GI symptoms correlated with pulse increase during tilt test as a surrogate of disease severity. The second aim was to explore the prevalence of common autoantibodies found in systemic autoimmune rheumatic disorders, as well as markers of allergy and mast cell activation in POTS, compared with controls, and to investigate whether these immunological factors are associated with GI symptoms.

2 Materials and methods

2.1 Study design

Study participants were recruited at the Department of Gastroenterology, Skåne University Hospital, Malmö, Sweden between October 2020 and January 2022. Invitations including study information were sent by regular mail to patients with previously confirmed POTS by tilt testing during 2010–2021 at the Department of Cardiology at the same hospital. One of the authors (HT) contacted the patients by telephone, to give further information and book a visit. During the same time frame, healthy controls were recruited among hospital staff and students. The study participants were asked to fill in study questionnaires during the study visit where a clinical examination and blood sampling were performed (Tufvesson et al., 2023). All blood samples were drawn in a non-fasting state, and the patients took their regular medications prior to the study visit.

2.2 Study population

2.2.1 The POTS cohort

2.2.1.1 Patients

The Syncope Study of Unselected Population in Malmö (SYSTEMA) cohort, with over 3,000 patients investigated for syncope and severe orthostatic intolerance at the Skåne University Hospital in Malmö, Sweden, was conducted between 2008 and 2021. Details of the SYSTEMA cohort are described elsewhere (Fedorowski et al., 2013). From the SYSTEMA cohort, 93 patients with a clinically confirmed POTS were included in a

POTS sub-study cohort with additional tests, at the Clinical Research Unit, Department of Internal Medicine, during 2017–2020 (Spahic et al., 2023).

2.2.1.2 Cardiovascular autonomic testing

During inclusion in the SYSTEMA cohort 2008–2021, the patients underwent cardiovascular autonomic tests with tilt testing and continuous hemodynamic monitoring (Johansson et al., 2021). Moreover, all patients had been thoroughly evaluated by a senior expert (AF) to exclude other primary causes of the hemodynamic findings. The tilt testing protocol included supine rest for 10 min preceding table elevation to 60°–70° for 20 min. Data on BP and HR derived from 30 s stable periods were collected at supine position, and after 1, 3, and 10 min upon the tilt test. Also, the maximum HR during tilt test was noted (Spahic et al., 2023). POTS diagnosis was defined as symptoms of orthostatic intolerance lasting for ≥ 3 months associated with a pathological tilt test showing a HR increase >30 bpm from supine position or a HR > 120 bpm in standing, with no evidence of orthostatic hypotension (Brignole et al., 2018). For each patient, a Δ HR was calculated from the supine position and the maximum HR and the HR at 1, 3 and 10 min of up-right position, to measure the magnitude of chronotropic response during the tilt test.

Since some of the tilt tests were performed up to 10 years ago, we also included more recent data from active standing tests that were performed between 2017 and 2021 in the majority of the POTS-patients that were included in the current study. Δ HR was calculated from supine position and for 1, 3, and 5 min after standing as well as maximal HR. When re-tested with active standing test, the patients were asked to discontinue cardiovascular pharmacological agents 48 h prior to examination (Spahic et al., 2023).

2.2.2 Study participants

2.2.2.1 Patients

Eligible patients from the POTS sub-study ($n = 82$) obtained written information about the present study if they were living within a reasonable proximity to Malmö, Sweden. If the patients were interested in participating in the study, they were asked to contact one of the authors (HT), who later contacted them by telephone to give further information. The inclusion criteria were age 18–70 years and ability to fully understand the study information.

2.2.2.2 Control subjects

Healthy control participants were recruited among hospital staff and medical students at Skåne University Hospital, Malmö, through personal invitation and advertisement. The controls were not allowed to have any current chronic or acute illness or significant GI symptoms. Intake of multivitamins and hormonal contraceptive medicines was accepted, as well as temporary use of medications, such as seasonal allergy medicines and pain killers.

2.3 Clinical examinations

During the study visit, POTS patients were examined with heart-, lung-, abdominal-, and neurological status. The current weight and height were obtained, as well as resting BP from the

right arm, in a supine position after approximately 5 min of rest. The healthy controls were examined with height, weight, resting BP, and HR.

2.4 Questionnaires

2.4.1 Study questionnaire

All study participants were asked to complete a questionnaire regarding sociodemographic factors, lifestyle habits, pregnancies and childbirth, previous and current illnesses, family history, current pharmacological treatment, and specific lifestyle modifications in case of GI complaints.

2.4.2 Gastroparesis questionnaire

The gastroparesis questionnaire has been used previously in studies regarding symptoms in diabetic gastroparesis (Faraj et al., 2007). This modified questionnaire contains 14 items common in gastroparesis, where the respondent marks whether the symptom is prevalent or not. The symptoms covered are loss of appetite, dysphagia, meal-related cough, early satiety, nausea, vomiting, weight loss, abdominal fullness, bloating, regurgitation, constipation, diarrhea with gas, evacuation incontinence, and postprandial perspiration. Symptoms that are seen specifically in diabetic gastroparesis including postprandial glycemia pitfalls and symptomatic postprandial hypoglycemia were excluded in the present study.

2.4.3 The visual analog scale for irritable bowel syndrome (VAS-IBS)

The VAS-IBS is a validated questionnaire regarding GI symptoms common in IBS (Bengtsson et al., 2007). The respondent marks their degree of symptoms for the past 2 weeks on a visual analog scale (VAS) between 0 and 100 mm, where 100 mm means very severe symptoms. The items covered are abdominal pain, diarrhea, constipation, bloating and flatulence, vomiting and nausea, psychological wellbeing, and the intestinal symptoms' influence on daily life. The scales are inverted from the original version (Bengtsson et al., 2007).

2.4.4 The irritable bowel syndrome severity scoring system (IBS-SSS)

The IBS-SSS questionnaire is a validated scoring system used in IBS, consisting of four items regarding abdominal pain, abdominal distension, satisfaction with bowel habit, and the impact of bowel habits on daily life, measured in mm on a 0–100 mm VAS, where 100 mm means very severe symptoms. In addition, one question asks the number of days of abdominal pain in the last 10 days. Scores from these questions are added, producing total IBS-SSS, with a maximum score of 500 (Francis et al., 1997). Scores between 75 and 175 suggests mild IBS, 175–300 suggest moderate IBS, and >300 suggest severe IBS. Additionally, there are ten extraintestinal items (nausea, early satiety, headache, back pain, lethargy, excess wind, heartburn, urinary symptoms, thigh pain, and bodily pain) measured on a VAS, that together produce an extraintestinal score, with a maximum score of 500. More severe symptoms are associated with higher scores (Francis et al., 1997; Ohlsson, 2022). In the current study, total IBS-SSS scores and extraintestinal scores were used.

2.5 Laboratory analyses

2.5.1 Microarray

The presence of autoantibodies was evaluated using a custom-designed multiplex solid-phase microarray platform (Thermo Fisher Scientific Immunodiagnosics, Uppsala, Sweden) containing 33 different peptide and protein autoantigens targeted in systemic autoimmune rheumatic diseases, as well as control antigens (Hansson et al., 2012). The list of antigens used in the assay can be found in [Supplementary Table S1](#). The cut-off level for each individual antigen is based on the 98th percentile value among 400 healthy controls.

2.5.2 Total IgE and tryptase

Total IgE and tryptase were analyzed in serum according to clinical routines at the Department of Clinical Chemistry, Skåne University Hospital, Malmö. Reference values for healthy volunteers are given for tryptase (Lab Medicine, 2023b) and total IgE (Lab Medicine, 2023a).

2.6 Statistical analyses

Comparisons between groups were performed by Mann-Whitney *U* test or Fisher's exact test as appropriate. Spearman's correlation test was used to assess correlations between Δ HR parameters and symptoms, and between psychological wellbeing and symptoms. Generalized linear model was performed to calculate associations after adjustment for psychological wellbeing, using continuous parameters of the VAS-IBS, total IBS-SSS, total extra-intestinal IBS-SSS and all extraintestinal symptoms of the IBS-SSS as dependent variables, and Δ HR at 1, 3, and 10 min as well as Δ HR_{max} as predictors. Sensitivity analyses were performed excluding patients with concomitant organic GI disorders. Values are presented as median and interquartile range, numbers and percentages, or β -value with 95% confidence interval (CI). $p < 0.05$ was considered statistically significant. Statistical analyses were performed using software SPSS®, version 28 for Windows (IBM, New York, United States).

2.7 Ethics

The present study was performed in accordance with the Declaration of Helsinki, and it was approved by the Ethics Review Board at Uppsala University, Dnr 2020–02432 and 2021–00049. The Regional Ethical Committee in Lund, Sweden, approved the SYSTEMA project (82/2008) and the POTS sub-study (2017/295).

3 Results

3.1 Clinical characteristics

In total, 43 of 82 contacted patients with POTS and 74 healthy control subjects were recruited to the present study. The median age was 30.6 (26.0–41.0) years in POTS and 35.6 (28.8–41.7) years in controls ($p = 0.068$), and the median body mass index was 24.2 (21.3–27.0) kg/m² in POTS and 22.8 (21.4–25.3) kg/m² in controls ($p = 0.413$). A vast majority, 93.0%, were female in the POTS group, compared with 78.4% in controls ($p = 0.041$). At clinical

TABLE 1 Head up tilt test-data 2011–2021.

	POTS N = 43
Systolic BP supine	127 (117–136)
Diastolic BP supine	78 (65–86)
HR supine	85 (71–91)
Systolic BP 1 min	137 (126–144)
Diastolic BP 1 min	96 (85–105)
HR 1 min	107 (98–122)
Δ HR 1 min	28 (21–33)
Missing data	7
Systolic BP 3 min	130 (122–140)
Diastolic BP 3 min	93 (83–104)
HR 3 min	114 (102–127)
Δ HR 3 min	29 (25–41)
Systolic BP 10 min	127 (118–140)
Diastolic BP 10 min	91 (84–103)
HR 10 min	117 (102–126)
Δ HR 10 min	34 (28–40)
Missing data	10
Maximum HR	124 (114–139)
Δ HR _{max}	44 (36–53)

Values presented as median and interquartile range. BP, blood pressure; HR, heart rate.

examination, there were no major pathological findings in the POTS group regarding heart-, lung-, and abdominal status.

The median time from definite POTS diagnosis by tilt test to the current study examination was 3.0 (1.0–7.0) years. The most common self-reported comorbidities in POTS were hypermobile spectrum disorders/Ehlers-Danlos syndrome (HSD/EDS) ($n = 12$, 28%), IBS ($n = 12$, 28%), asthma ($n = 9$, 21%), migraine ($n = 7$, 16%), and neuropsychiatric disorders ($n = 6$, 14%). Ten patients (23%) reported comorbid autoimmune disorders (psoriasis, psoriasis arthritis, lichen sclerosus, autoimmune thyroid disorders, and celiac disease), and another five patients (12%) reported other inflammatory disorders (inflammatory bowel disease, microscopic colitis, and rosacea) ([Supplementary Table S2](#)). Four of the patients who reported HSD/EDS also reported IBS. Seven patients (16%) reported organic GI disorder, including celiac disease ($n = 2$), inflammatory bowel disease ($n = 2$), microscopic colitis ($n = 2$), and persistent *helicobacter pylori* infection due to antibiotic resistance ($n = 1$). Two of the patients with organic GI disorder also reported IBS. Pharmacological treatment was abundant in the POTS group, especially cardiovascular and anti-allergy drugs. Notably histamine H1-blockers were taken by 13 patients ([Supplementary Table S3](#)). In total, 15 patients reported asthma, allergy, or were treated with anti-allergy drugs.

The tilt testing variables including HR and BP are presented in [Table 1](#). In total, 38 (88%) patients performed an active standing test in the POTS sub-study, and variables are presented in [Table 2](#). Nine

TABLE 2 Active standing test 2017–2019.

	POTS N = 38
Systolic BP supine	116 (104–123)
Diastolic BP supine	69 (65–79)
HR supine	72 (64–81)
Systolic BP 1 min	118 (109–132)
Diastolic BP 1 min	81 (77–92)
HR 1 min	99 (89–110)
Δ HR 1 min	26 (16–34)
Missing data	1
Systolic BP 3 min	116 (106–126)
Diastolic BP 3 min	80 (75–86)
HR 3 min	104 (88–111)
Δ HR 3 min	27 (23–35)
Systolic BP 5 min	116 (108–126)
Diastolic BP 5 min	79 (73–87)
HR 5 min	95 (83–114)
Δ HR 5 min	28 (18–37)
Missing data	2

Values presented as median and interquartile range. BP, blood pressure; HR, heart rate.

of these patients did not manage to discontinue all of the cardiovascular medications 48 h before the examination.

3.2 Gastrointestinal symptom scoring systems

All items on the gastroparesis score were significantly more prevalent in POTS than in controls ($p < 0.01$), and both specific and overall GI symptoms were more severe in POTS than in controls ($p < 0.001$) (Table 3; Figure 1). Nausea was the most prevalent symptom reported in POTS (79.1%), followed by abdominal fullness (69.8%) and bloating (66.7%) (Table 3). The most severe symptoms were bloating and flatulence (65 (25–88)) and constipation (61 (11–74)) (Table 3; Figure 1).

The total IBS-SSS score was 213 (135–319) in POTS compared with 13 (0–54) in controls ($p < 0.001$). All extra-intestinal symptoms were aggravated in the POTS group, and the total extraintestinal symptom score was 277 (178–338) in POTS compared with 29 (16–58) in controls ($p < 0.001$) (Table 3).

There was no significant difference between patients with and without self-reported HSD/EDS regarding the prevalence of GI symptoms (data not shown). Constipation (74 (43–92) vs 51 (7–72), $p = 0.03$) and bodily pain (92 (68–99) vs 67 (16–84), $p = 0.034$) were more exaggerated in HSD/EDS compared with the non-HSD/EDS group (Table 4). Abdominal pain tended to be more pronounced in HSD/EDS, but the significance level was not reached (58 (24–76) vs 30 (16–50), $p = 0.051$). The HR_{max} was higher in the non-HSD/EDS group (130 (116–147) vs 119 (103–130), $p = 0.035$),

as well as the Δ HR_{max} (47 (38–54) vs. 32 (30–45), $p = 0.005$) (Table 4).

Postprandial perspiration was more prevalent in patients with self-reported IBS compared with those without IBS (66.7% vs 29.0%, $p = 0.037$). Diarrhea was more pronounced in IBS (57 (21–80) vs 10 (0–64), $p = 0.011$), but otherwise, no significant differences were found regarding the degree of GI symptoms (Table 5). There were no significant differences between patients with and without IBS regarding tilt data (data not shown).

No significant differences were found between patients with and without organic GI disorder regarding GI symptoms and tilt data (Supplementary Table S4).

No significant differences were found between patients with and without asthma/allergy/anti-allergy drugs ($n = 15$) regarding GI symptoms and tilt data (Supplementary Table S5).

3.3 Correlations between psychological wellbeing and gastrointestinal symptoms in POTS

There was a significant positive correlation between lower levels of psychological wellbeing and abdominal pain ($r = 0.495$, $p < 0.001$), diarrhea ($r = 0.403$, $p = 0.007$), constipation ($r = 0.332$, $p = 0.030$), bloating and flatulence ($r = 0.352$, $p = 0.020$), vomiting and nausea ($r = 0.490$, $p < 0.001$), intestinal symptoms' influence on daily life ($r = 0.587$, $p < 0.001$), total IBS-SSS ($r = 0.539$, $p < 0.001$), early satiety ($r = 0.535$, $p < 0.001$), back pain ($r = 0.321$, $p = 0.036$), excess wind ($r = 0.497$, $p < 0.001$), heart burn ($r = 0.431$, $p = 0.004$), bodily pain ($r = 0.336$, $p = 0.027$), and total extraintestinal symptoms ($r = 0.450$, $p = 0.002$).

3.4 Correlations between hemodynamic response and gastrointestinal symptoms in POTS

The Δ HR_{max} correlated inversely with abdominal pain ($r = -0.406$, $p = 0.007$). There were no significant correlations between Δ HR and extraintestinal symptoms (Table 6). When the seven patients with organic GI disorders were excluded from the analyses, inverse correlations were seen between Δ HR_{10min} and abdominal pain, constipation, and total IBS-SSS, respectively, and between Δ HR_{max} and abdominal pain, intestinal symptoms' influence on daily life, and total IBS-SSS, respectively (Table 6).

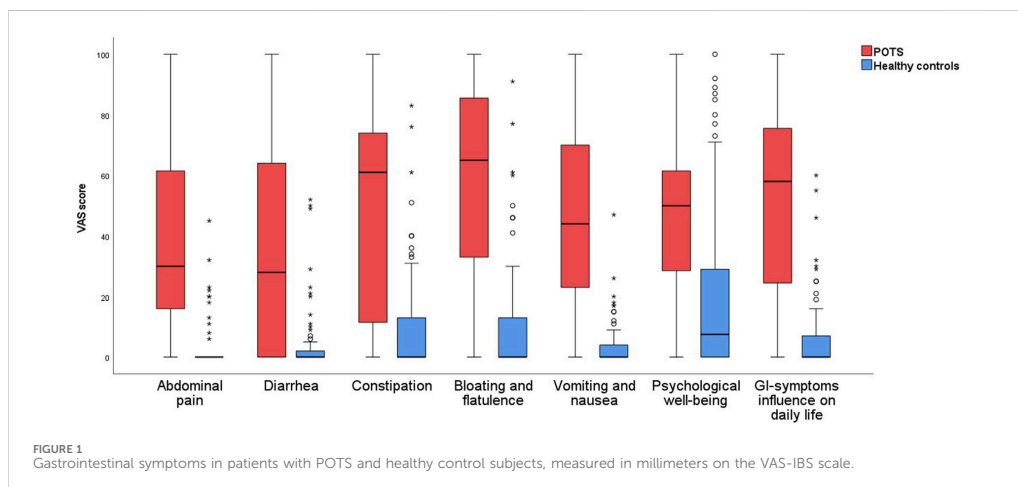
After adjustment for psychological wellbeing, inverse associations were found between Δ HR_{10min} and abdominal pain (β : -1.137; 95% CI: -2.157 to -0.117; $p = 0.030$) and total IBS-SSS (β : -4.748; 95% CI: -9.172 to -0.324; $p = 0.036$). An inverse association was also seen between Δ HR_{max} and abdominal pain (β : -0.915; 95% CI: -1.478 to -0.351; $p = 0.002$). Furthermore, adjustments for psychological wellbeing did not affect the results regarding correlations between hemodynamic data and GI symptoms when the seven patients with organic GI disorders were excluded from the analyses (data not shown).

No significant correlations were observed between Δ HR from the active standing test and symptoms. After exclusion of six patients

TABLE 3 Gastrointestinal and autonomic symptoms in POTS and healthy controls.

	Healthy controls N = 74	POTS N = 43
Gastroparesis score		
Loss of appetite	1 (1.6%)	20 (46.5%)
Dysphagia	0 (0%)	16 (37.2%)
Meal-related cough	1 (1.6%)	14 (32.6%)
Early satiety	2 (3.3%)	23 (53.5%)
Nausea	3 (4.9%)	34 (79.1%)
Vomiting	0 (0%)	6 (14.3%)
Weight loss	0 (0%)	13 (31.0%)
Abdominal fullness	7 (11.5%)	30 (69.8%)
Bloating	4 (6.6%)	28 (66.7%)
Regurgitation	2 (3.3%)	23 (53.5%)
Constipation	10 (16.4%)	28 (65.1%)
Diarrhea with gas	5 (8.2%)	23 (53.5%)
Evacuation incontinence	1 (1.6%)	10 (23.3%)
Postprandial perspiration	0 (0%)	17 (39.5%)
VAS-IBS		
Abdominal pain	0 (0–0)	30 (16–62)
Diarrhea	0 (0–2)	28 (0–64)
Constipation	0 (0–13)	61 (11–74)
Bloating and flatulence	0 (0–14)	65 (25–88)
Vomiting and nausea	0 (0–4)	44 (23–70)
Psychological wellbeing	8 (0–30)	50 (28–62)
Symptoms'influence on daily life	0 (0–7)	58 (23–76)
Urgency	4 (5.6%)	17 (40.5%)
Incomplete evacuation	20 (27.4%)	30 (69.8%)
IBS-SSS		
Total IBS-SSS	13 (0–54)	213 (135–319)
Nausea	0 (0–4)	44 (23–70)
Early satiety	0 (0–0)	39 (5–60)
Headache	9 (0–23)	71 (33–85)
Back pain	6 (0–19)	50 (15–77)
Lethargy	16 (2–32)	92 (81–100)
Excess wind	0 (0–14)	60 (29–82)
Heart burn	0 (0–5)	23 (3–60)
Urinary symptoms	0 (0–4)	44 (8–84)
Thigh pain	0 (0–0)	42 (2–58)
Bodily pain	0 (0–12)	76 (29–96)
Total extraintestinal symptom score	29 (16–58)	277 (178–338)

Gastrointestinal symptom prevalence was assessed by the gastroparesis score. Gastrointestinal symptom severity was assessed by IBS-SSS, Irritable Bowel Syndrome-Severity Scoring System and VAS-IBS, visual analog scale for irritable bowel syndrome, 0–100 mm, where 0 represents no symptoms and 100 maximal symptoms. Values presented as number (percent) or median (interquartile range). Comparative analyses were performed with Fisher's Exact test or Mann Whitney-U, test. The *p*-value was <0.001 between patients and controls in all symptoms, except the gastroparesis score vomiting, that was *p* = 0.004. A few missing values were noted.



with organic GI disorder, inverse correlations between GI symptoms and Δ HR was observed, including intestinal symptoms' influence on daily life, diarrhea, bloating and flatulence, and total IBS-SSS (Table 7).

3.5 Autoantibodies and immunological factors

Microarray for systemic autoimmunity were performed in 34 patients with POTS and in 39 controls, all randomly selected. The most prevalent autoantibodies were Ro-60 ($n = 6$) and Scl-70 ($n = 4$). Ro-60 was equally distributed between POTS and controls, whereas Scl-70 was only found in the POTS cohort. Otherwise, the prevalence of subjects positive for any autoantibody was equal between groups (29.4% in POTS vs 33.3% in controls, $p = 0.803$). No statistically significant differences were found between patients or controls with or without expression of autoantibodies regarding the severity of GI symptoms or tilt test (data not shown).

Baseline serum total tryptase levels were measured in 41 of the 43 patients with POTS. One patient had a slightly elevated tryptase level (12.4 $\mu\text{g/L}$), according to the upper limit for normal value (11.3 $\mu\text{g/L}$) (Lab Medicine, 2023b). Another three patients had tryptase levels $>8.0 \mu\text{g/L}$, which is suggested as the lower level for the most common genetic trait, hereditary α -tryptasemia, causing elevated tryptase and symptoms of mast cell activation (Valent et al., 2023). Serum total IgE levels were measured in 40 patients with POTS. Five patients had total IgE levels above the upper limit for normal value (129 kU/L) (Lab Medicine, 2023a). One of these patients reported asthma, whereas the other four patients neither reported any atopic disease, nor any anti-allergy drugs. Total IgE was normal in all four patients with tryptase levels $>8.0 \mu\text{g/L}$.

4 Discussion

The main finding of the present study was that patients with POTS have a high burden of GI- and extraintestinal symptoms, compared with healthy controls. The most prevalent symptoms were nausea, abdominal fullness, and bloating. The most severe GI symptoms were bloating and flatulence, and constipation. GI symptoms were associated with low psychological wellbeing. Importantly, the magnitude of HR acceleration on the initial tilt test was inversely correlated with GI symptoms, i.e., a pronounced HR acceleration at diagnosis was associated with less severe chronic GI symptoms. Finally, there was no overexpression of circulating autoantibodies commonly seen in systemic autoimmune rheumatic diseases, or elevated baseline tryptase levels among POTS patients, and there was no association between autoantibodies and GI symptoms.

To our knowledge, this is the first study where specific GI symptoms have been studied in a POTS cohort in terms of prevalence and the degree of severity on validated GI symptom scales. Additionally, this is the first study that has investigated how the hemodynamic response, as well as signs of autoimmunity, correlated with specific GI- and extraintestinal symptoms.

The results are in line with previous studies examining GI symptoms in POTS (Huang et al., 2013; Wang et al., 2015; Mehr et al., 2018; Tai et al., 2020). In a review by Mehr et al. (2018), pooled data from six original articles showed that the most common, non-cardiovascular symptoms in POTS were nausea and abdominal pain. Conceptually, there is an important difference between orthostatic and non-orthostatic GI symptoms in POTS (Chelimsky and Chelimsky, 2018). Orthostatic GI symptoms, such as nausea and vomiting, can be relieved by recumbent position and cardiovascular treatment strategies (Sullivan et al., 2005; Fortunato et al., 2014), whereas non-orthostatic GI symptoms require other management strategies (Chelimsky and Chelimsky, 2018). Multisystem comorbid

TABLE 4 GI symptoms and tilt-data in POTS with and without HSD/EDS.

	HSD/EDS N = 12	Non-HSD/EDS N = 31	p-value
VAS-IBS			
Abdominal pain	58 (24–76)	30 (16–50)	0.051
Diarrhea	51 (0–71)	23 (0–64)	0.603
Constipation	74 (43–92)	51 (7–72)	0.030
Bloating and flatulence	64 (32–87)	70 (15–88)	0.797
Vomiting and nausea	48 (28–70)	40 (12–73)	0.655
Psychological wellbeing	60 (37–76)	50 (22–60)	0.155
Symptoms' influence on daily life	68 (23–84)	50 (23–74)	0.408
Urgency	5 (41.7%)	12 (40%) 1 missing	1.000
Incomplete evacuation	8 (66.7%)	22 (71%)	1.000
IBS-SSS			
Total IBS-SSS	297 (143–387)	193 (126–296) 3 missing	0.275
Nausea	48 (28–70)	40 (12–73)	0.655
Early satiety	51 (7–75)	25 (2–54)	0.321
Headache	65 (38–79)	77 (25–93)	0.309
Back pain	56 (36–93)	50 (8–77)	0.378
Lethargy	92 (86–99)	94 (78–100)	0.913
Excess wind	73 (40–82)	50 (27–87)	0.735
Heart burn	56 (24–71)	21 (2–54)	0.139
Urinary symptoms	52 (25–81)	44 (8–85)	0.924
Thigh pain	46 (1–54)	19 (2–60)	0.892
Bodily pain	92 (68–99)	67 (16–84)	0.034
Total extraintestinal score	322 (244–354)	248 (154–316)	0.176
Tilt-data			
HR supine	80 (69–97)	85 (72–90)	0.807
HR 1 min	111 (93–129)	107 (99–120)	0.697
Δ HR 1 min	30.5 (24.5–32.3)	24.5 (19.8–33.3)	0.548
Missing data	5	2	
HR 3 min	107 (95–126)	115 (104–127)	0.310
Δ HR 3 min	37.5 (22.8–35.3)	30 (26–41)	0.233
HR 10 min	117 (94–128)	117 (102–126)	0.906
Δ HR 10 min	30.5 (21–41.5)	34 (30–39)	0.410
Missing data	2	8	
Max HR	119 (103–130)	130 (116–147)	0.035
Δ HR max	32 (30–45)	47 (38–54)	0.005

Values presented as median (interquartile range), or number (percent). Comparative analyses were performed with Mann Whitney-U, test or Fisher's Exact test. VAS-IBS, Visual Analog Scale for Irritable Bowel Syndrome; IBS-SSS, Irritable Bowel Syndrome-Severity Scoring System. HSD/EDS, hypermobile spectrum disorder/Ehlers-Danlos syndrome. HUT, Head up tilt; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate. There were 3 missing values regarding Total-IBS-SSS, and 1 regarding urgency in the non-HSD/EDS, group.

TABLE 5 GI symptoms in POTS with and without IBS.

	IBS N = 12	Non-IBS N = 31	<i>p</i> -value
Gastroparesis score			
Loss of appetite	7 (58.3%)	13 (41.9%)	0.497
Dysphagia	3 (25%)	13 (41.9%)	0.484
Meal-related cough	3 (25%)	11 (35.5%)	0.720
Early satiety	9 (75%)	14 (45.2%)	0.099
Nausea	11 (91.7%)	23 (74.2%)	0.405
Vomiting	2 (18.2%) 1 missing	4 (12.9%)	0.644
Weight loss	2 (18.2%) 1 missing	11 (35.5%)	0.453
Abdominal fullness	9 (75%)	21 (67.7%)	0.727
Bloating	9 (75%)	19 (63.3%) 1 missing	0.719
Regurgitation	7 (58.3%)	16 (51.6%)	0.745
Constipation	7 (58.3%)	21 (67.7%)	0.723
Diarrhea with gas	9 (75%)	14 (45.2%)	0.099
Evacuation incontinence	2 (16.7%)	8 (25.8%)	0.698
Postprandial perspiration	8 (66.7%)	9 (29%)	0.037
VAS-IBS			
Abdominal pain	38 (30–63)	28 (8–53)	0.162
Diarrhea	57 (21–80)	10 (0–64)	0.011
Constipation	38 (12–79)	65 (11–74)	0.714
Bloating and flatulence	81 (54–95)	62 (14–80)	0.098
Vomiting and nausea	48 (27–75)	40 (10–70)	0.465
Psychological wellbeing	57 (39–60)	47 (28–63)	0.448
Symptoms' influence on daily life	64 (51–84)	44 (28–63)	0.129
Urgency	7 (63.6%) 1 missing	10 (32.3%)	0.086
Incomplete evacuation	9 (75%)	21 (67.7%)	0.727
IBS-SSS			
Total IBS-SSS	250 (188–392) 1 missing	173 (91–313) 2 missing	0.093
Nausea	48 (27–75)	40 (10–70)	0.465
Early satiety	32 (19–62)	40 (0–60)	0.497
Headache	71 (61–87)	75 (25–85)	0.694
Back pain	45 (17–80)	50 (9–77)	0.807
Lethargy	91 (88–100)	92 (78–98)	0.512
Excess wind	74 (40–82)	50 (20–83)	0.350
Heart burn	22 (10–57)	25 (2–73)	0.654
Urinary symptoms	75 (25–100)	38 (7–83)	0.071
Thigh pain	15 (2–59)	44 (2–58)	0.967
Bodily pain	81 (24–97)	76 (29–93)	0.684
Total extraintestinal score	286 (201–354)	277 (154–316)	0.481

Values presented as median (interquartile range), or number (percent). Comparative analyses were performed with Mann Whitney-U, test or Fisher's Exact test. VAS-IBS, Visual Analog Scale for Irritable Bowel Syndrome; IBS-SSS, Irritable Bowel Syndrome-Severity Scoring System.

TABLE 6 Spearman’s rank correlation between heart rate acceleration during tilt test and gastrointestinal/extra-intestinal symptoms in POTS.

	$\Delta\text{HR}_{1\text{ min}}$	$\Delta\text{HR}_{3\text{ min}}$	$\Delta\text{HR}_{10\text{ min}}$	$\Delta\text{HR}_{\text{max}}$
Abdominal pain				
r	−0.055	−0.179	−0.339 (−0.439)	−0.406 (−0.453)
p-value	0.752	0.250	0.054 (0.022)	0.007 (0.006)
Diarréa				
r	−0.108	0.091	−0.162	−0.181
p-value	0.529	0.561	0.368	0.245
Constipation				
r	0.045	0.018	−0.263 (−0.380)	−0.157
p-value	0.795	0.909	0.140 (0.050)	0.314
Bloating and flatulence				
r	−0.002	0.135	−0.189	−0.082
p-value	0.992	0.388	0.293	0.601
Vomiting and nausea				
r	0.177	0.029	0.005	−0.082
p-value	0.301	0.852	0.980	0.602
Psychological wellbeing				
r	0.155	0.040	−0.025	−0.027
p-value	0.368	0.797	0.892	0.864
Symptoms’ influence on daily life				
r	0.008	0.008	−0.189	−0.293 (−0.367)
p-value	0.962	0.957	0.292	0.066 (0.028)
Total-IBS-SSS				
r	−0.075	−0.106	−0.348 (−0.540)	−0.293 (−0.458)
p-value	0.669	0.515	0.051 (0.004)	0.066 (0.007)
Nausea				
r		0.029	0.005	−0.082
p-value		0.852	0.980	0.602
Early satiety				
r	0.232	0.064	−0.113	−0.084
p-value	0.172	0.658	0.530	0.593
Headache				
r	−0.135	−0.027	−0.259	−0.046
p-value	0.433	0.863	0.145	0.772
Backpain				
r	−0.079	−0.135	−0.258	−0.138
p-value	0.649	0.388	0.147	0.379
Lethargy				
r	−0.013	−0.066	−0.138	−0.125
p-value	0.939	0.676	0.443	0.423

(Continued on following page)

TABLE 6 (Continued) Spearman's rank correlation between heart rate acceleration during tilt test and gastrointestinal/extra-intestinal symptoms in POTS.

	$\Delta\text{HR}_{1\text{ min}}$	$\Delta\text{HR}_{3\text{ min}}$	$\Delta\text{HR}_{10\text{ min}}$	$\Delta\text{HR}_{\text{max}}$
Excess wind				
<i>r</i>	0.110	0.149	-0.054	-0.061
<i>p</i> -value	0.521	0.341	0.767	0.699
Heart burn				
<i>r</i>	0.070	0.108	-0.153	-0.177
<i>p</i> -value	0.683	0.492	0.394	0.256
Urinary symptoms				
<i>r</i>	0.006	0.055	-0.163	-0.083
<i>p</i> -value	0.973	0.726	0.366	0.597
Thigh pain				
<i>r</i>	0.062	0.030	-0.224	0.008
<i>p</i> -value	0.720	0.848	0.210	0.959
Bodily pain				
<i>r</i>	0.156	-0.039	-0.013	-0.111
<i>p</i> -value	0.363	0.805	0.942	0.479
Total extraintestinal score				
<i>r</i>	0.094	0.024	-0.200	-0.146
<i>p</i> -value	0.586	0.877	0.264	0.349

Correlation analyses between heart rate acceleration during tilt test and continuous GI symptoms performed with Spearman's rank correlation test. In brackets *r*- and *p*-value in the sensitivity analysis without the patients with organic GI-disorder ($n = 7$). HR, heart rate; IBS-SSS, Irritable Bowel Syndrome-Severity Scoring System.

conditions are common in POTS, including functional GI disorders (FGID) (DiBaise et al., 2018). However, there is no evidence that POTS itself contribute mechanistically to these conditions, according to a previous study in children (Chelimsky et al., 2015). In the present study, the most severe GI symptoms were bloating and flatulence and constipation. These items are probably non-orthostatic, rather representing comorbidities. Twenty-eight percent of the POTS group reported a diagnosis of IBS, and the median total IBS-SSS score in POTS was 213, corresponding to moderate IBS (Francis et al., 1997). Moreover, 28% reported HSD/EDS and previous research has shown that HSD/EDS is associated with FGIDs, with and without POTS (Beckers et al., 2017; Fikree et al., 2017; Tai et al., 2020). In addition, seven patients reported a concomitant organic GI disorder. Hence, comorbid functional and organic GI disorders may explain the magnitude of GI symptoms in POTS in this study.

Almost all GI symptoms correlated with low psychological wellbeing in the present study. It is well known that low psychological wellbeing is associated with IBS and other FGIDs, where bilateral communication between the brain and the gut results in visceral hypersensitivity (Koloski et al., 2012; Ford et al., 2020). In POTS, previous studies have shown mild to moderate depression and anxiety levels, as well as positive associations between the degree of depression and self-rated orthostatic symptom burden (Raj et al., 2018). Somatic hypervigilance has been described in POTS (Khurana, 2006; DiBaise et al., 2018), suggesting central visceral

sensitization (DiBaise et al., 2018). Previous studies regarding FGIDs have shown that lower psychological wellbeing is associated with less effective central pain modulation (Marcuzzi et al., 2019), including an increased membrane excitability, a facilitated synaptic strength, and decreased inhibitory influence on the dorsal horn neurons, all contributing to an abnormal responsiveness to noxious and non-noxious stimuli (Latremoliere and Woolf, 2009; Ossipov et al., 2014). According to a previous study, IBS patients with lowered pain thresholds upon testing also have higher circulating levels of cortisol and corticotrophin, as well as higher blood pressure, indicating a disturbed hypothalamic-pituitary-adrenal axis, possibly due to higher stress levels (Chang et al., 2009; Zhou et al., 2010). It is tempting to believe that these mechanisms contribute to central sensitization and hypervigilance in POTS as well. However, in the setting of the present study, we cannot draw any conclusions whether GI symptoms in POTS are caused by lowered psychological wellbeing with increased hypersensitivity, or *vice versa*.

To further explore the nature of GI symptoms in POTS, correlation tests were performed between symptoms and hemodynamic parameters. Surprisingly, there was a significant inverse correlation between maximum orthostatic HR acceleration at diagnostic tilt test and abdominal pain. The inverse correlation between HR acceleration and various chronic GI symptoms became even more distinct when the patients with organic GI disorders were excluded from the analyses. The same tendency between hemodynamic data and GI symptoms was seen

TABLE 7 Spearman's rank correlation between heart rate acceleration and gastrointestinal/extraintestinal symptoms in POTS in the active standing tests.

	$\Delta HR_{1 \text{ min}}$	$\Delta HR_{3 \text{ min}}$	$\Delta HR_{5 \text{ min}}$
Abdominal pain			
r	−0.091	0.013	−0.219
p-value	0.591	0.936	0.200
Diarréa			
r	−0.210 (−0.357)	−0.070	−0.113
p-value	0.212 (0.049)	0.674	0.511
Constipation			
r	0.054	−0.068	−0.218
p-value	0.752	0.684	0.202
Bloating and flatulence			
r	−0.069	0.031	−0.236 (−0.355)
p-value	0.686	0.854	0.166 (0.050)
Vomiting and nausea			
r	−0.041	0.187	0.018
p-value	0.811	0.260	0.915
Psychological wellbeing			
R	−0.052	0.081	−0.121
p-value	0.758	0.627	0.481
Symptoms' influence on daily life			
R	−0.017 (−0.642)	0.043	−0.270 (−0.412)
p-value	0.921 (0.033)	0.797	0.111 (0.021)
Total-IBS-SSS			
R	0.018	0.063	−0.299 (−0.466)
p-value	0.919	0.718	0.091 (0.012)

N = 38. Correlation analyses between heart rate acceleration during tilt test and continuous gastrointestinal symptoms. In brackets significant r- and p-value when re-analyzing the group without the patients with organic gastrointestinal disorder (n = 6). HR, heart rate; IBS-SSS, Irritable Bowel Syndrome-Severity Scoring System.

when analyzing the active standing test performed a few years after the tilt test, closer to the recruitment period of the present study.

There are some possible theoretical explanations to the findings regarding the inverse correlation between orthostatic HR acceleration and GI symptoms among POTS patients. First, there may be a possible treatment bias. Patients who present with a pronounced HR acceleration may receive more aggressive treatment or more active follow-up. Active advice on higher salt- and fluid intake, as well as prescription of some cardiovascular drugs, often result in improvement of the typical orthostatic GI symptoms (Sullivan et al., 2005; Fortunato et al., 2014). A second explanation to the finding is that POTS represents a heterogenous syndrome rather than a disease, where multiple mechanisms can affect the phenotypic outcome (Mehr et al., 2018; Fedorowski, 2019). Some of the most important pathophysiological mechanisms behind POTS are (I) a hyperadrenergic state, (II) partial autonomic neuropathy, (III) a persistent predilection for hypovolemia leading to reflex orthostatic tachycardia, and (IV) physical

deconditioning (Benarroch, 2012; Tu et al., 2020; Raj et al., 2022). These mechanisms interact; thus, one patient can exhibit characteristics from two or more of these pathophysiological mechanisms (Tu et al., 2020). Signs of autonomic neuropathy and distal peripheral neuropathy are seen in approximately 50% of the POTS population (Low et al., 2009). GI motility disorders are well described in POTS (Huang et al., 2013; Park et al., 2013; Loavenbruck et al., 2015), where up to 18% have delayed gastric emptying, and up to 48% have rapid gastric emptying (Loavenbruck et al., 2015). These disturbances may be considered manifestations of neuropathy. In a study by Gibbons et al. (2013), the HR increase at tilt test was less pronounced in POTS patients with signs of neuropathy, compared with non-neuropathic POTS. More exaggerated GI symptoms have been reported in neuropathic POTS (Al-Shekhlee et al., 2005). Thus, neuropathic POTS may explain why a subset of patients in the present study exhibit more GI symptoms, but less pronounced HR acceleration. Lastly, more pronounced GI symptoms and less exaggerated orthostatic pulse

acceleration were measured in the 28% with self-reported HSD/EDS in the present study. The mechanistic association between POTS and HSD/EDS is not entirely clear (Tai et al., 2020), but common mechanisms behind HSD/EDS and POTS may partly explain the inverse correlations between GI symptoms and HR acceleration in the present study.

Autoantibody microarray assay did not show any difference in expression of autoantibodies commonly found in systemic autoimmune rheumatic disorders, when comparing POTS with controls. Previous studies have shown that comorbid autoimmune disorders in POTS are prevalent (Vernino and Stiles, 2018; Vernino et al., 2021), as in our study, where approximately one-third of participants self-reported such disorders. However, contrasting our study, Blitshteyn (2015) observed in a retrospective study that the prevalence of antinuclear antibodies (ANA) and anti-phospholipid antibodies (aPL) were higher in POTS compared with the normal population. The results were compared with known cut-off values for normal population, and not with comparable controls (Blitshteyn, 2015). Further, others reported ganglionic acetylcholine receptor (AChR) autoantibodies and G-protein coupled receptor autoantibodies being more expressed in POTS, but the pathological significance remains uncertain (Vernino and Stiles, 2018). In the present study, no association was seen between expression of autoantibodies and any GI symptoms, suggesting that systemic autoimmunity is not a major driver of GI symptoms in POTS.

Allergic comorbidity and medications were common in the present study, and total IgE was elevated in a subset of patients. However, baseline total serum tryptase was only slightly elevated in one patient. GI symptoms were not more pronounced in patients with self-reported allergic disorders or on anti-allergic medications. Thus, the present study could not confirm the results by Kohno et al. (2021), who were suggesting that mast cell activation is a major driver of atypical POTS symptoms such as GI complaints.

The strength of this study is use of different validated scoring systems for assessment of GI symptom burden. The patients were thoroughly examined at a tertiary cardiovascular center with a syncope unit. The tilt data was present in all patients, and active standing test was performed in 88% of the patients at a later time point, which had the same correlation tendency to GI symptoms as the tilt test did, reflecting robustness of the findings. In addition, all patients were examined clinically at inclusion.

One limitation to the study may be a selection bias since POTS patients were recruited from a highly specialized center, and thus, might have more exaggerated symptoms than the POTS population in general. Furthermore, 82 patients with POTS were invited to the study, but only 43 ended up at inclusion. This is a limitation that has some explanations. Firstly, we wanted to examine the patients physically, and many patients declined to participate in the study due to long distance to the study center. In addition, some of the patients were hindered by severe orthostatic symptoms. Lastly, the study was conducted during the covid pandemic, and some patients were excluded due to covid symptoms. Another limitation was that a control group recruited from hospital staff might be healthier with less symptoms than controls recruited more randomly. All data on

comorbidities were self-reported, which is especially problematic when it comes to HSD/EDS, as these diagnostic terms are used inconsistently among patients according to previous studies (Tai et al., 2020). Both HSD and EDS present with musculoskeletal symptoms and joint hypermobility, but whereas EDS is a rare disorder of the connective tissue with various systemic manifestations, HSD is more common, affecting approximately 3% of the population, but without systemic manifestations (Miller et al., 2020). However, the prevalence rates regarding self-reported HSD/EDS were fairly in line with previous studies (Miller et al., 2020; Tai et al., 2020). The patients were not asked whether GI symptoms worsened upon standing, which would have been interesting, to discriminate between orthostatic and non-orthostatic GI symptoms. Another limitation was that the tilt tests were performed some years ago in several patients, although the diagnosis was confirmed by active standing test during enrolment. Regarding measurement of baseline tryptase, the study was not designed to diagnose mast cell activation syndrome, where tryptase must be measured both at baseline, and upon acute symptoms. The patients were not fasting at blood sampling, and two patients were on oral mast cell stabilizing therapy. Finally, pharmacological treatment was abundant among POTS patients. Several of the reported agents, including opioids and cholinesterase inhibitors, are known to cause adverse GI symptoms (Kanjwal et al., 2011; Drewes et al., 2016).

A recent Indian study (Inbaraj et al., 2023) has investigated heart rate variability (HRV) in POTS, based on the assumption that HRV is the most reliable marker of cardiovascular autonomic dysfunction, showing affected HRV parameters in POTS compared with controls (Inbaraj et al., 2023). Based on the results from that study, future studies would be to further investigate the relationship between GI symptoms and parameters of HRV in POTS.

5 Conclusion

Gastrointestinal symptoms are common in POTS and are associated with low psychological wellbeing. More pronounced tachycardia during tilt testing or active standing test is associated with less severe GI complaints in POTS. This finding may reflect different contributing pathophysiological mechanisms and comorbidities in POTS. Finally, this study did not find any evidence supporting that POTS is associated with conventional biomarkers of autoimmunity.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Review Board at Uppsala University, Sweden and The Regional Ethical Committee in Lund, Sweden. The studies were conducted in

accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HT: Data curation, Formal Analysis, Funding acquisition, Investigation, Writing–original draft. VH: Data curation, Writing–review and editing. AF: Conceptualization, Data curation, Methodology, Writing–review and editing. MH: Methodology, Writing–original draft, Writing–review and editing. BO: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2024.1342351/full#supplementary-material>

References

- Al-Shekhlee, A., Lindenberg, J. R., Hachwi, R. N., and Chelimsy, T. C. (2005). The value of autonomic testing in postural tachycardia syndrome. *Clin. Auton. Res.* 15, 219–222. doi:10.1007/s10286-005-0282-7
- Beckers, A. B., Keszthelyi, D., Fikree, A., Vork, L., Masclee, A., Farmer, A. D., et al. (2017). Gastrointestinal disorders in joint hypermobility syndrome/Ehlers-Danlos syndrome hypermobility type: a review for the gastroenterologist. *Neurogastroenterol. Motil.* 29. doi:10.1111/nmo.13013
- Benarroch, E. E. (2012). Postural tachycardia syndrome: a heterogeneous and multifactorial disorder. *Mayo Clin. Proc.* 87, 1214–1225. doi:10.1016/j.mayocp.2012.08.013
- Bengtsson, M., Ohlsson, B., and Ulander, K. (2007). Development and psychometric testing of the visual analogue scale for irritable bowel syndrome (VAS-IBS). *BMC Gastroenterol.* 7, 16. doi:10.1186/1471-230x-7-16
- Blitshteyn, S. (2015). Autoimmune markers and autoimmune disorders in patients with postural tachycardia syndrome (POTS). *Lupus* 24, 1364–1369. doi:10.1177/0961203315587566
- Brignole, M., Moya, A., de Lange, F. J., Deharo, J.-C., Elliott, P. M., Fanciulli, A., et al. (2018). 2018 ESC Guidelines for the diagnosis and management of syncope. *Eur. Heart J.* 39, 1883–1948. doi:10.1093/eurheartj/ehy037
- Camilleri, M. (2021). Gastrointestinal motility disorders in neurologic disease. *J. Clin. Invest.* 131, e143771. doi:10.1172/jci143771
- Chang, L., Sundaresh, S., Elliott, J., Anton, P. A., Baldi, P., Licudine, A., et al. (2009). Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis in irritable bowel syndrome. *Neurogastroenterol. Motil.* 21, 149–159. doi:10.1111/j.1365-2982.2008.01171.x
- Chelimsy, G., and Chelimsy, T. (2018). The gastrointestinal symptoms present in patients with postural tachycardia syndrome: a review of the literature and overview of treatment. *Auton. Neurosci.* 215, 70–77. doi:10.1016/j.autneu.2018.09.003
- Chelimsy, G., Kovacic, K., Nugent, M., Mueller, A., Simpson, P., and Chelimsy, T. C. (2015). Comorbid conditions do not differ in children and young adults with functional disorders with or without postural tachycardia syndrome. *J. Pediatr.* 167, 120–124. doi:10.1016/j.jpeds.2015.03.039
- DiBaise, J. K., Harris, L. A., and Goodman, B. (2018). Postural tachycardia syndrome (POTS) and the GI tract: a primer for the gastroenterologist. *Am. J. Gastroenterol.* 113, 1458–1467. doi:10.1038/s41395-018-0215-4
- Drewes, A. M., Munkholm, P., Simrén, M., Breivik, H., Kongsgaard, U. E., Hatlebakk, J. G., et al. (2016). Definition, diagnosis and treatment strategies for opioid-induced bowel dysfunction-Recommendations of the Nordic Working Group. *Scand. J. Pain* 11, 111–122. doi:10.1016/j.sjpain.2015.12.005
- Faraj, J., Melander, O., Sundkvist, G., Olsson, R., Thorsson, O., Ekberg, O., et al. (2007). Oesophageal dysmotility, delayed gastric emptying and gastrointestinal symptoms in patients with diabetes mellitus. *Diabet. Med.* 24, 1235–1239. doi:10.1111/j.1464-5491.2007.02236.x
- Fedorowski, A. (2019). Postural orthostatic tachycardia syndrome: clinical presentation, aetiology and management. *J. Intern. Med.* 285, 352–366. doi:10.1111/joim.12852
- Fedorowski, A., Burri, P., Struck, J., Juul-Möller, S., and Melander, O. (2013). Novel cardiovascular biomarkers in unexplained syncopal attacks: the SYSTEMA cohort. *J. Intern. Med.* 273, 359–367. doi:10.1111/joim.12043
- Fikree, A., Aziz, Q., and Sifrim, D. (2017). Mechanisms underlying reflux symptoms and dysphagia in patients with joint hypermobility syndrome, with and without postural tachycardia syndrome. *Neurogastroenterol. Motil.* 29, 29. doi:10.1111/nmo.13029
- Ford, A. C., Sperber, A. D., Corsetti, M., and Camilleri, M. (2020). Irritable bowel syndrome. *Lancet* 396, 1675–1688. doi:10.1016/S0140-6736(20)31548-8
- Fortunato, J. E., Wagoner, A. L., Harbinson, R. L., D'Agostino, R. B., Shaltout, H. A., and Diz, D. I. (2014). Effect of fludrocortisone acetate on chronic unexplained nausea and abdominal pain in children with orthostatic intolerance. *J. Pediatr. Gastroenterol. Nutr.* 59, 39–43. doi:10.1097/MPG.0000000000000305
- Francis, C. Y., Morris, J., and Whorwell, P. J. (1997). The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment. Pharmacol. Ther.* 11, 395–402. doi:10.1046/j.1365-2036.1997.142318000.x
- Gibbons, C. H. (2019). Basics of autonomic nervous system function. *Handb. Clin. Neurol.* 160, 407–418. doi:10.1016/B978-0-444-64032-1.00027-8

- Gibbons, C. H., Bonyhay, L., Benson, A., Wang, N., and Freeman, R. (2013). Structural and functional small fiber abnormalities in the neuropathic postural tachycardia syndrome. *PLoS One* 8, e84716. doi:10.1371/journal.pone.0084716
- Hansson, M., Mathsson, L., Schleider, T., Israelsson, L., Mattsson, P., Nogueira, L., et al. (2012). Validation of a multiplex chip-based assay for the detection of autoantibodies against citrullinated peptides. *Arthritis Res. Ther.* 14, R201. doi:10.1186/ar4039
- Huang, R. J., Chun, C. L., Friday, K., and Triadafilopoulos, G. (2013). Manometric abnormalities in the postural orthostatic tachycardia syndrome: a case series. *Dig. Dis. Sci.* 58, 3207–3211. doi:10.1007/s10620-013-2865-9
- Inbaraj, G., Udupa, K., Vasuki, P. P., Nalini, A., and Sathyaprabha, T. N. (2023). Resting heart rate variability as a diagnostic marker of cardiovascular dysautonomia in postural tachycardia syndrome. *J. Basic Clin. Physiol. Pharmacol.* 34, 103–109. doi:10.1515/jbcpp-2022-0069
- Johansson, M., Ricci, F., Schulte, J., Persson, M., Melander, O., Sutton, R., et al. (2021). Circulating levels of growth hormone in postural orthostatic tachycardia syndrome. *Sci. Rep.* 11, 8575. doi:10.1038/s41598-021-87983-5
- Kanjwal, K., Karabin, B., Sheikh, M., Elmer, L., Kanjwal, Y., Saeed, B., et al. (2011). Pyridostigmine in the treatment of postural orthostatic tachycardia: a single-center experience. *Pacing Clin. Electrophysiol.* 34, 750–755. doi:10.1111/j.1540-8159.2011.03047.x
- Khurana, R. K. (2006). Experimental induction of panic-like symptoms in patients with postural tachycardia syndrome. *Clin. Auton. Res.* 16, 371–377. doi:10.1007/s10286-006-0365-0
- Kohn, R., Cannon, D. S., Olshansky, B., Xi, S. C., Krishnapa, D., Adkisson, W. O., et al. (2021). Mast cell activation disorder and postural orthostatic tachycardia syndrome: a clinical association. *J. Am. Heart Assoc.* 10, e021002. doi:10.1161/jaha.121.021002
- Koloski, N. A., Jones, M., Kalantar, J., Weltman, M., Zaguirre, J., and Talley, N. J. (2012). The brain-gut pathway in functional gastrointestinal disorders is bidirectional: a 12-year prospective population-based study. *Gut* 61, 1284–1290. doi:10.1136/gutjnl-2011-300474
- Lab Medicine (2023a). Region Skåne, analysportalen: immunglobulin E (total IgE). Available at: [http://analysportalen-labmedicin.skane.se/pics/Labmedicin/analysportalen/KIT/Immunglobulin%20IgE%20\(total%20IgE\).pdf](http://analysportalen-labmedicin.skane.se/pics/Labmedicin/analysportalen/KIT/Immunglobulin%20IgE%20(total%20IgE).pdf) (Accessed October 6, 2023).
- Lab Medicine (2023b). Region Skåne, analysportalen: tryptas. Available at: <http://analysportalen-labmedicin.skane.se/pics/Labmedicin/analysportalen/KIT/Tryptas.pdf> (Accessed October 6, 2023).
- Latremoliere, A., and Woolf, C. J. (2009). Central sensitization: a generator of pain hypersensitivity by central neural plasticity. *J. Pain* 10, 895–926. doi:10.1016/j.jpain.2009.06.012
- Liao, Y., Zhang, Q. Y., Li, H. X., Wang, Y. L., Liu, P., and Du, J. B. (2017). Comorbidity of vasovagal syncope and postural tachycardia syndrome with allergic diseases in children. *Beijing Da Xue Xue Bao Yi Xue Ban.* 49, 783–788. doi:10.3969/j.issn.1671-167X.2017.05.007
- Loavenbruck, A., Iturrino, J., Singer, W., Sletten, D. M., Low, P. A., Zinsmeister, A. R., et al. (2015). Disturbances of gastrointestinal transit and autonomic functions in postural orthostatic tachycardia syndrome. *Neurogastroenterol. Motil.* 27, 92–98. doi:10.1111/nmo.12480
- Low, P. A., Sandroni, P., Joyner, M., and Shen, W. K. (2009). Postural tachycardia syndrome (POTS). *J. Cardiovasc. Electrophysiol.* 20, 352–358. doi:10.1111/j.1540-8167.2008.01407.x
- Mannan, H., and Pain, C. M. (2023). Sex adjusted standardized prevalence ratios for celiac disease and other autoimmune diseases in patients with postural orthostatic tachycardia syndrome (POTS): a systematic review and meta-analysis. *Heliyon* 9, e12982. doi:10.1016/j.heliyon.2023.e12982
- Marcuzzi, A., Chakiath, R. J., Siddall, P. J., Kellow, J. E., Hush, J. M., Jones, M. P., et al. (2019). Conditioned pain modulation (cpm) is reduced in irritable bowel syndrome: a systematic review and meta-analysis of cpm and the role of psychological factors. *J. Clin. Gastroenterol.* 53, 399–408. doi:10.1097/MCG.0000000000001181
- Mehr, S. E., Barbul, A., and Shibao, C. A. (2018). Gastrointestinal symptoms in postural tachycardia syndrome: a systematic review. *Clin. Auton. Res.* 28, 411–421. doi:10.1007/s10286-018-0519-x
- Miller, A. J., Stiles, L. E., Sheehan, T., Bascom, R., Levy, H. P., Francomano, C. A., et al. (2020). Prevalence of hypermobile Ehlers-Danlos syndrome in postural orthostatic tachycardia syndrome. *Auton. Neurosci.* 224, 102637. doi:10.1016/j.autneu.2020.102637
- Ohlsson, B. (2022). Extraintestinal manifestations in irritable bowel syndrome: a systematic review. *Ther. Adv. Gastroenterol.* 15, 17562848221114558. doi:10.1177/17562848221114558
- Ossipov, M. H., Morimura, K., and Porreca, F. (2014). Descending pain modulation and chronification of pain. *Curr. Opin. Support Palliat. Care* 8, 143–151. doi:10.1097/SPC.0000000000000055
- Park, K. J., Singer, W., Sletten, D. M., Low, P. A., and Bharucha, A. E. (2013). Gastric emptying in postural tachycardia syndrome: a preliminary report. *Clin. Auton. Res.* 23, 163–167. doi:10.1007/s10286-013-0193-y
- Raj, S. R., Fedorowski, A., and Sheldon, R. S. (2022). Diagnosis and management of postural orthostatic tachycardia syndrome. *Cmaj* 194, E378–E385. doi:10.1503/cmaj.211373
- Raj, V., Opie, M., and Arnold, A. C. (2018). Cognitive and psychological issues in postural tachycardia syndrome. *Auton. Neurosci.* 215, 46–55. doi:10.1016/j.autneu.2018.03.004
- Spahic, J. M., Hamrefors, V., Johansson, M., Ricci, F., Melander, O., Sutton, R., et al. (2023). Malmö POTS symptom score: assessing symptom burden in postural orthostatic tachycardia syndrome. *J. Intern. Med.* 293, 91–99. doi:10.1111/joim.13566
- Sullivan, S. D., Hanauer, J., Rowe, P. C., Barron, D. F., Darbari, A., and Oliva-Hemker, M. (2005). Gastrointestinal symptoms associated with orthostatic intolerance. *J. Pediatr. Gastroenterol. Nutr.* 40, 425–428. doi:10.1097/01.mpg.0000157914.40088.31
- Tai, F. W. D., Palsson, O. S., Lam, C. Y., Whitehead, W. E., Sperber, A. D., Tornblom, H., et al. (2020). Functional gastrointestinal disorders are increased in joint hypermobility-related disorders with concomitant postural orthostatic tachycardia syndrome. *Neurogastroenterol. Motil.* 32, e13975. doi:10.1111/nmo.13975
- Tu, Y., Abell, T. L., Raj, S. R., and Mar, P. L. (2020). Mechanisms and management of gastrointestinal symptoms in postural orthostatic tachycardia syndrome. *Neurogastroenterol. Motil.* 32, e14031. doi:10.1111/nmo.14031
- Tufvesson, H., Hamrefors, V., Roth, B., Fedorowski, A., and Ohlsson, B. (2023). Sociodemographic factors, nutritional status, and inflammatory markers in patients with postural orthostatic tachycardia syndrome. *Acta Neurol. Scand.* 2023, 1–9. doi:10.1155/2023/3992718
- Valent, P., Hoermann, G., Bonadonna, P., Hartmann, K., Sperr, W. R., Broesby-Olsen, S., et al. (2023). The normal range of baseline tryptase should be 1 to 15 ng/mL and covers healthy individuals with HaT. *J. Allergy Clin. Immunol. Pract.* 11, 3010–3020. doi:10.1016/j.jaip.2023.08.008
- Vernino, S., Bourne, K. M., Stiles, L. E., Grubb, B. P., Fedorowski, A., Stewart, J. M., et al. (2021). Postural orthostatic tachycardia syndrome (POTS): state of the science and clinical care from a 2019 national institutes of health expert consensus meeting - Part I. *Auton. Neurosci.* 235, 102828. doi:10.1016/j.autneu.2021.102828
- Vernino, S., and Stiles, L. E. (2018). Autoimmunity in postural orthostatic tachycardia syndrome: current understanding. *Auton. Neurosci.* 215, 78–82. doi:10.1016/j.autneu.2018.04.005
- Wang, L. B., Culbertson, C. J., Deb, A., Morgenshtern, K., Huang, H., and Hohler, A. D. (2015). Gastrointestinal dysfunction in postural tachycardia syndrome. *J. Neurol. Sci.* 359, 193–196. doi:10.1016/j.jns.2015.10.052
- Zhou, Q., Fillingim, R. B., Riley, J. L., 3rd, Malarkey, W. B., and Verne, N. G. (2010). Central and peripheral hypersensitivity in the irritable bowel syndrome. *Pain* 148, 454–461. doi:10.1016/j.pain.2009.12.005

Paper IV



RESEARCH ARTICLE

Symptoms and objective signs of peripheral sensory neuropathy in POTS and correlations to gastrointestinal symptoms

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Abstract

Aim

Postural orthostatic tachycardia syndrome (POTS) is a disorder with cardiovascular autonomic dysfunction where multiple and variable symptoms are common, including those from the peripheral and enteric nervous systems. We aimed to investigate subjective and objective signs of small and large fiber neuropathy in a Swedish POTS cohort compared with healthy controls. Secondly, we wanted to examine potential associations between gastrointestinal symptoms and neuropathy signs in POTS.

Methods

Forty-three patients with POTS (93% female) and 54 healthy controls (76% female) were included in the study. All participants completed a questionnaire including modified neuropathy symptoms score (NSS) and irritable bowel syndrome severity scoring system (IBS-SSS) for gastrointestinal evaluation. Small nerve fibers were investigated by assessing the intraepidermal nerve fiber density (IENFD). Large nerve fiber function was examined through pinprick and vibration perception thresholds (VPTs), using neurothesiometry and multi-frequency vibrometry (MFV). The patients were classified as "High NSS" and "High IBS-SSS" if their total NSS vs. IBS-SSS were above median levels in POTS.

Results

Peripheral sensory and gastrointestinal symptoms were more prevalent and severe in POTS than in controls. Median VPTs were normal and IENFDs were comparable

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government.se/information-material/2009/09/public-access-to-information-and-secrecy-act/) but data can be made available for researches after a special Review that includes approval of the research Project by both an ethics committee and the authorities' data safety committees. Data access queries are referred to the Swedish Ethical Review Authority (<https://etikproving-smyndigheten.se/>) and KVB, the department responsible for patient data disclosures at Skåne University Hospital, Malmö, Sweden (<https://vardgivare.skane.se/kompetens-utveckling/forskning-inom-region-skane/utlamanande-av-patientdata-samradkvb/>). Data that cannot compromise patient confidentiality has been added as a supplementary Excel file.

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between POTS and controls (2.26 [1.62–3.08] vs. 1.63 [0.73–2.68] fibers/mm; $p=0.108$). The patients with "high NSS" had slightly higher VPTs measured by MFV, although within normal ranges, compared to patients with "low NSS". The patients within the "high IBS-SSS" group had higher NSS (18.0 [14.3–22.8] vs. 11.0 [4.0–15.0]; $p=0.002$) compared to patients with low total IBS-SSS.

Conclusion

Symptoms of peripheral and enteric neuropathy are common in POTS but no solid evidence was found regarding functional or morphological signs of small or large fiber neuropathy. Neuropathic and gastrointestinal symptoms were closely associated within POTS.

1. Introduction

Postural orthostatic tachycardia syndrome (POTS) is a disorder characterized by debilitating orthostatic symptoms and pulse acceleration upon standing with no significant drop in blood pressure [1]. The mechanisms behind POTS are probably multifactorial and still not fully elucidated [2]. One mechanistic subtype is the neuropathic POTS, where the suggested pathophysiology involves underlying small nerve fiber neuropathy (SFN), characterized by degeneration and/or dysfunction of thinly myelinated A δ -fibers and unmyelinated C-fibers [3]. Both the somatic and the autonomic peripheral nervous systems, including the enteric nerves, can be affected by SFN [4,5], but the accessibility to peripheral nerves is superior to enteric nerves [5]. Patients with POTS may experience symptoms consistent with peripheral sensory small and large nerve fiber neuropathy [6]. Previous studies have reported objective signs of SFN in some POTS patients, where assessments of intraepidermal nerve fiber density (IENFD) in skin biopsies have shown reduced nerve fiber density in up to 56% of the POTS population [5,7–10]. Moreover, post-ganglionic sudomotor dysfunction, measured by quantitative sudomotor axon reflex test (QSART), reflecting partial sympathetic denervation is common in various autonomic neuropathies [11] and is present in some POTS patients [9,12–14]. We have previously shown that gastrointestinal symptoms were common and moderately severe in POTS, resembling the symptomatology found in irritable bowel syndrome (IBS), as well as in gastroparesis [15]. Additionally, we have published a case report where two patients with known enteric neuropathy also presented with symptoms of peripheral neuropathy, and reduced IENFD. One of the patients also fulfilled criteria for POTS [5].

Regarding peripheral neuropathic involvement, few studies have compared patients with POTS to healthy matched controls [7]. Thereto, of the studies that have shown reduced IENFD in POTS, three were retrospective [8–10]. Thus, no symptom evaluation was performed within temporal proximity to the biopsy sampling. In the study by Gibbons et al [7], POTS patients with objective signs of post-ganglionic sudomotor dysfunction reported more gastrointestinal symptoms, suggesting a more widespread neuropathy. However, symptoms of peripheral sensory neuropathy were

not assessed in that study. To our knowledge, the only previous study that has examined large nerve fiber function in POTS was conducted retrospectively [10].

Therefore, we aimed to assess symptoms and objective signs of peripheral small and large fiber neuropathy in POTS to determine any correlations between the severity of symptoms and the extent of objective neuropathy. Additionally, since patients with POTS also experience symptoms that may originate from the enteric nervous system, we aimed to examine whether gastrointestinal symptoms were associated with subjective or objective signs of peripheral neuropathy, reflecting a more widespread neuropathy.

2. Materials & methods

2.1. Ethical statement

The present study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Review Board at Uppsala University, permission no. 2020–02432 (approval date 26/08/2020) and 2021–00049 (approval date 11/02/2021). Written informed consent was obtained from all study participants.

2.2. Study design

In the present cross-sectional study, participants were recruited at the Department of Gastroenterology and the Department of Hand Surgery, Skåne University Hospital, Malmö, Sweden, between the 1:st of October 2020 and 31:st of May 2023. Invitations, including study information, were sent by regular mail to patients with a previously described and confirmed diagnosis of POTS at the Department of Cardiology at Skåne University Hospital, Malmö, Sweden [16]. One of the authors (HT) contacted the participants by telephone, to give further information and book a visit. At the same time, healthy control participants were recruited among hospital staff and students. The study was conducted in two study sessions. During the first study visit, the participants were asked to fill in study questionnaires, and a clinical examination was performed. Large sensory nerve fiber function [17] was examined with routine clinical tests commonly used for neuropathy screening: 10 g monofilament test for pinprick sense, and neurothesiometry for vibrotactile sense [18]. A second study visit was then booked within a couple of weeks, where an additional, more comprehensive, examination of large sensory nerve fibers was performed with multi-frequency vibrometry (MFV), and two skin biopsies were obtained from the distal leg to determine IENFD and small nerve fiber neuropathy.

2.3. Study population

2.3.1. Patients with POTS. Study participants (n=43) were recruited from the Syncope Study of Unselected Population in Malmö (SYSTEMA) cohort. This cohort involves over 3000 patients investigated for syncope and severe orthostatic intolerance at the Skåne University Hospital in Malmö, Sweden, and was conducted between 2008 and 2021 [19]. All patients of the cohort underwent cardiovascular autonomic tests with head-up tilt testing with continuous hemodynamic monitoring, as well as other cardiac tests, including ambulatory electrocardiogram or 24-hour ambulatory blood pressure monitoring, when appropriate. The head-up tilt testing protocol included supine rest for 10 min preceding table elevation to 60–70° for 20 min [20]. The diagnosis of POTS was defined as symptoms of orthostatic intolerance lasting for ≥ 3 months associated with a pathological head-up tilt test showing a heart rate (HR) increase of ≥ 30 beats per minute (bpm) or HR ≥ 120 bpm, with no significant drop in blood pressure [21]. All patients fulfilled the diagnostic criteria for POTS. The recruitment process from this cohort has been described in detail previously [15], and is summarized in a flow chart in supplementary figure 1. Inclusion criteria were ages between 18–70 years and the ability to fully understand the study information.

2.3.2. Healthy controls. Healthy control participants (n=54) were recruited among hospital staff, medical students, and their relatives at Skåne University Hospital, Malmö, through personal invitation and advertisement. The controls were

not allowed to have any current chronic or acute illness. Intake of multivitamins and hormonal contraceptive medicines was accepted, but otherwise, only temporary use of medications, such as seasonal allergy medicines, was allowed.

2.4. Questionnaires

All study participants were asked to complete a questionnaire, including sociodemographic factors, lifestyle habits, previous and current illnesses, family history, and current pharmacological treatment. Furthermore, two validated symptom scores, described below, were used to assess subjective symptoms of peripheral neuropathy and gastrointestinal symptoms.

2.4.1. Neuropathy Symptom Score (NSS). A modified version of the validated Neuropathy Symptom Score (NSS) was used to assess subjective signs of peripheral neuropathy in the hands and feet [22]. The symptoms assessed are numbness, warmth/cold sensation, paresthesia, burning sensation, radiating pain, aching pain, and nocturnal exacerbation by sheets. The participants scored the symptoms as 0 = never, 1 = sometimes, 2 = often, and 3 = nocturnal. Scores for all questions regarding both hands and feet were added to produce a composite total score (maximum 42 points). If the respondent answered that symptoms occurred both often (2 points) and nocturnally (3 points), the highest point was used in the composite score.

2.4.2. The irritable bowel syndrome severity scoring system (IBS-SSS). While most of the patients in the present study did not have an official IBS diagnosis, the comorbidity with IBS is high in POTS [6,15]. We have previously used this tool for assessment of gastrointestinal symptoms in this very POTS cohort, revealing a wide span of gastrointestinal symptom severity [15]. The IBS-SSS questionnaire is a validated scoring system used in IBS, consisting of four items regarding abdominal pain, abdominal distension, satisfaction with bowel habits, and the impact of bowel habits on daily life. The items are rated on a visual analog scale from zero to 100 millimeters, where 100 mm indicates very severe symptoms. In addition, one question asks the number of days of abdominal pain in the last 10 days. Scores from these questions are added together, producing a total IBS-SSS, with a maximum score of 500. Scores between 75 and 174 suggest mild IBS, 175–299 suggest moderate IBS, and ≥ 300 suggest severe IBS [23].

2.5. Clinical examinations

Patients with POTS were clinically examined with heart-, lung-, abdominal- and routine neurological status [24]. In all participants, neurological sensory testing for large sensory nerve fibers and mechanoreceptors was performed distally at the feet and leg with touch, by 10 g monofilament (Bailey Instruments, Manchester, UK), and by neurothesiometry. Examination with Horwell Neurothesiometer (Scientific Laboratory Supplies, Nottingham, UK) was performed with the participants in a supine position, to assess vibration perception thresholds (VPTs) at the bony prominence of the medial malleolus and the tip of the big toe bilaterally. The vibrating frequency was preset to 50–60 Hz on this device and the vibratory signal was increased slowly until the participant sensed the vibration, measured in volts (V). Three measurements were performed at each site, and a mean value was calculated. Phalen's and Tinel's tests were performed, as well as anamnestic symptoms of median nerve affection were asked for, to exclude carpal tunnel syndrome in all participants [25]. The current weight and height were obtained. All clinical examinations were performed by an internal medicine specialist (HT).

2.6. Multi-frequency vibrometry

Vibration perception thresholds were further examined in patients with POTS using the VibroSense Meter® II device (VibroSense Dynamics AB, Malmö, Sweden) on the index and little finger of the right hand as well as on the head of the first metatarsal bone of the right foot. A full program, i.e., seven frequencies (4, 8, 16, 32, 64, 125, and 250 Hz), was used for examining VPTs in the foot. For VPT assessment in the index and little finger, a screening procedure was applied, consisting of only three frequencies (32, 125, and 250 Hz). A detailed description of the method has been presented

previously [26]. The results were expressed as Z-scores, based on data obtained from previous studies with healthy age- and sex-matched controls [26]. Thus, none of the healthy controls recruited for the present study were examined through MFV.

2.7. Skin biopsy procedure and evaluation of intraepidermal nerve fiber density

A skin punch biopsy was obtained from the distal right leg, 10 cm proximal to the lateral malleolus, using a 3 mm disposable circular needle during local anesthesia (0.5 ml Carbocain® 10 mg/ml) (LD). The skin defect was closed with one Ethilon 4–0 suture (Ethicon®, Raritan, USA) and covered by a small dressing allowing free activities. Biopsy samples were immediately fixed in a 4% buffered formaldehyde solution for at least 24 hours before dehydration and paraffin embedding.

Thereafter, the paraffin-embedded blocks were sectioned at 5 µm and immunohistochemically stained with a rabbit polyclonal Protein Gene Product (PGP) 9.5 antibody (Cell Marque, Rocklin, USA, dilution 1:3000). The IENFD, expressed as the number of fibers per millimeter of epidermal length, was manually assessed in each specimen by one of the authors (LE). Nerve fibers were counted as previously validated, and described in a large normal material [27]. The observer was blinded to the characteristics of the participants when assessing IENFD. Inter- and intra-observer reliability was assessed with good results in a previous study by the same author (LE) using the same methods as here [28]. For choice of methods, please see Discussion.

2.8. Statistical analyses

Statistical analyses were performed in SPSS, version 28. Data are presented as median and interquartile range (IQR) or numbers and percentages (N, %). Data on VPTs assessed with MFV are presented as average Z-scores for each measuring point (index finger, little finger, first metatarsal head). Differences between patients and controls were compared using the non-parametric Mann-Whitney *U* test. Fisher's exact test was used for dichotomous variables. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Basic characteristics

A total of 43 patients (age 31 [26–41] years) and 54 controls (age 38 [30–46] years; $p = 0.010$) were included in the study. There was a higher proportion of female sex ($n = 40$; 93.0%) in the patient group in comparison to the control group ($n = 41$; 75.6%; $p = 0.029$). There was no difference in BMI between patients (24.2 [21.3–27.0] kg/m²) and controls (22.7 [20.8–25.3] kg/m²; $p = 0.294$). Most of the patients (74.4%) and the controls (72.2%) had never smoked ($p = 0.528$). The vast majority (88.4%) of the patients consumed less than one standard glass of alcohol per week, which was significantly fewer than in the control group (31.5% < 1 glass; $p < 0.001$).

Besides POTS, the most common self-reported comorbidities among patients were Ehlers-Danlos syndrome ($n = 12$; 27.9%), IBS ($n = 12$; 27.9%), asthma ($n = 8$; 18.6%), migraine ($n = 7$; 16.3%), neuropsychiatric disorders ($n = 6$; 14.0%), endometriosis ($n = 5$; 11.6%), and myalgic encephalomyelitis ($n = 5$; 11.6%). In the control group, seasonal allergies were found in four (7.4%) participants. The most common drugs in the POTS patients were antihypertensive drugs ($n = 17$; 39.5%), ivabradine ($n = 16$; 37.2%), beta-blockers ($n = 13$; 30.2%), histamine H1 blockers ($n = 13$; 30.2%), and combined hormonal contraceptives ($n = 8$; 18.6%). A full list of current drug treatments in this POTS cohort has previously been published [15]. Sporadic users of anti-allergy treatment, contraceptives, and multivitamins were found among healthy controls.

3.2. Questionnaires

3.2.1. Neuropathy Symptom Score (NSS). Four (7.4%) of the 54 control participants and 36 (83.7%) of the 43 patients with POTS reported one or more variables on the NSS to occur often or nocturnally in hands or feet. Significant

differences in total NSS were found between patients and controls where the median composite score for patients was 14.5 (6.0–21.3) compared to 0.0 (0.0–2.0) in the control group ($p < 0.001$; Table 1).

The most frequently reported symptoms in POTS patients were “Abnormal sensation to heat or cold”, reported to occur often or nocturnally by 25 (59.5%) and 28 (66.7%) patients in hands and feet, respectively, and “Sensation of pins and needles”, reported to occur often or nocturnally by 20 (47.6%) and 19 (45.2%) patients in hands and feet, respectively. All items on the NSS questionnaire were reported to occur more or less often in the POTS cohort. There was no difference in the distribution of neuropathic symptoms between hands and feet in POTS (S1 Table).

3.2.2. The irritable bowel syndrome severity scoring system (IBS-SSS). The median total IBS-SSS score was 213 (135–319) in POTS compared to 11 (0–47) in healthy controls ($p < 0.001$, Table 1).

3.3. Clinical examinations

Clinical routine neurological status was generally normal and without any objective signs of severe neurological disorder in POTS. Abnormal signs of motor neuropathy were observed in sporadic cases: walking test ($n = 2$; 4.7%), walking on toes ($n = 3$; 7.0%), walking on heels ($n = 5$; 11.6%), finger strength ($n = 2$; 4.7%), shoulder abduction ($n = 2$; 4.8%), and leg raises ($n = 6$; 14.0%). In all these cases, general weakness was observed clinically, however, the clinical examination did not raise any suspicion of neurological disorders in any of these cases. Five of the POTS patients were using wheelchairs due to orthostatic intolerance or general muscle weakness (clinical observation). Brachial, patellar, and Achilles tendon

Table 1. Clinical characteristics and subjective and objective signs of sensory nerve fiber neuropathy in control participants and patients with POTS.

	Controls N = 54	POTS N = 43	P-value
Age (years)	38 (30–46)	31 (26–41)	0.010
Female (%)	41 (75.6%)	40 (93.0%)	0.029
Monofilament, normal (%)	46 (90.2%) ^d	39 (95.1%) ^b	0.455
Neurothesiometer (V)			
Right big toe	5.8 (4.8–7.7) ^a	4.9 (4.0–7.1) ^a	0.065
Left big toe	5.8 (4.2–7.9) ^a	5.1 (3.3–6.7) ^a	0.085
Right lateral malleolous	7.3 (5.8–9.5) ^a	7.3 (5.7–9.2) ^a	0.810
Left lateral malleolous	7.7 (6.2–9.7) ^a	6.5 (4.7–8.5) ^a	0.016
IBS-SSS total	11.0 (0.0–46.8)	212.5 (134.5–318.8) ^c	<0.001
NSS total	0.0 (0.0–2.0)	14.5 (6.0–21–3) ^a	<0.001
Multi-frequency vibrometry (average Z-score)			
Hand			
Index finger	n/a	0.3 (–0.4–1.1) ^d	
Little finger	n/a	0.6 (–0.3–1.1) ^d	
Foot			
	n/a	0.1 (–0.4–0.7) ^d	
IENFD (fibers/mm)	1.63 (0.73–2.68) ^a	2.26 (1.62–3.08) ^d	0.108

Values presented as number (percent) or median (interquartile range). Comparative analyses were performed with Fisher’s Exact test or Mann Whitney-U test. A p -value < 0.05 was considered statistically significant. IBS-SSS: Irritable Bowel Syndrome – Severity Scoring System [23]; NSS: Neuropathy Symptom Score [22]; IENFD: Intraepidermal Nerve Fiber Density. n/a = not applicable

^a1 missing value.

^b2 missing values.

^c3 missing values.

^d4 missing values.

^e13 control participants were examined.

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reflexes were tested in all patients. In a handful of patients, reflexes were difficult to elicit in one or two reflex sites. However, there were no patients who had absent reflexes in all of the tested sites.

No differences were found between patients and controls regarding pinprick sensation with monofilament testing (Table 1). The VPTs measured by neurothesiometer were lower in the left medial malleolus in patients compared with controls, although within the normal range (Table 1). Four (9.3%) of the patients experienced unilaterally mildly decreased or increased sensitivity in the arms, and five (11.6%) experienced unilaterally mildly decreased or increased sensitivity in the legs upon touching. None of these patients had abnormal results from neurothesiometer or monofilament testing.

Phalen's test was positive in three patients and three controls, whereas Tinel's test was positive in seven of the patients and three controls. One POTS patient fulfilled the criteria for carpal tunnel syndrome after re-evaluation at the Department of Hand Surgery and the other two of the patients with positive Phalen's test were already operated on for carpal tunnel syndrome with no effect on symptoms. All of the POTS patients with positive Phalen's test reported various neuropathic symptoms graded with the NSS as often in both hands and feet. None of the control participants with positive Phalen's test had any subjective symptoms of neuropathy measured on the NSS. One control participant had clinical signs of ulnar nerve entrapment, which was confirmed after re-evaluation at the Department of Hand Surgery.

3.4. Multi-frequency vibrometry

Average Z-scores for VPTs assessed through MFV are presented for patients in Table 1. Pathological measures (average Z-score >1.65) were found in the feet of three patients, as well as in the index and/or little fingers of five patients. Among the patients with a vibrometry Z-score >1.65 in the index and/or little fingers, only one showed a positive Tinel's test. None of these patients presented with a positive Phalen's test.

3.5. Intraepidermal nerve fiber density

Thirty-nine (90.4%) of the patients with POTS and 13 (24.1%) of the healthy control participants underwent skin punch biopsy. In general, skin biopsies were well tolerated in both POTS and controls. A mild and local skin infection occurred in one patient who consequently received antibiotic therapy with no residual complications. Regarding the subgroup of POTS patients and healthy controls who underwent skin biopsy, there were no significant differences between groups in baseline characteristics, including age, sex, BMI, or smoking habits (S2 Table). Alcohol consumption was higher in controls compared to POTS patients. There were no significant differences in sensory thresholds measured with monofilament or neurothesiometry between groups (S2 Table). Median IENFD was comparable between POTS and controls (2.26 [1.62–3.08] fibers/mm vs. 1.63 [0.73–2.68] fibers/mm, respectively; $p=0.108$) (Fig 1). We could not identify any obvious subgroup within POTS with low IENFD compared with healthy controls (Supplementary data file).

3.6. Measures of nerve function and IENFD in relation to self-reported neuropathy symptoms

Patients with POTS were divided into two groups according to their symptom status regarding peripheral neuropathy symptoms, based on the median value of the NSS in the whole group. Patients with NSS below 14.5 were included in the "Low NSS" group, and patients with NSS above 14.5 were included in the "High NSS" group. There were no differences in monofilament perception, neurothesiometer values, or IENFD between the two groups. Vibrometry Z-scores were higher in the "High NSS" group when measured in the little finger and foot, although the median values were still within normal ranges (Table 2). Six (31.6%) of the patients with an average vibrometry Z-score >1.65 in hands/feet were included in the "high NSS" group, whereas only one (5.3%) patient was included in the "low NSS" group ($p=0.090$).

3.7. Measures of nerve function and IENFD in relation to self-reported gastrointestinal symptoms

Patients with POTS were divided into two groups according to gastrointestinal symptoms, based on the median value of the total IBS-SSS score. Patients with a total IBS-SSS score below 212.5 were included in the "Low IBS-SSS" group, and

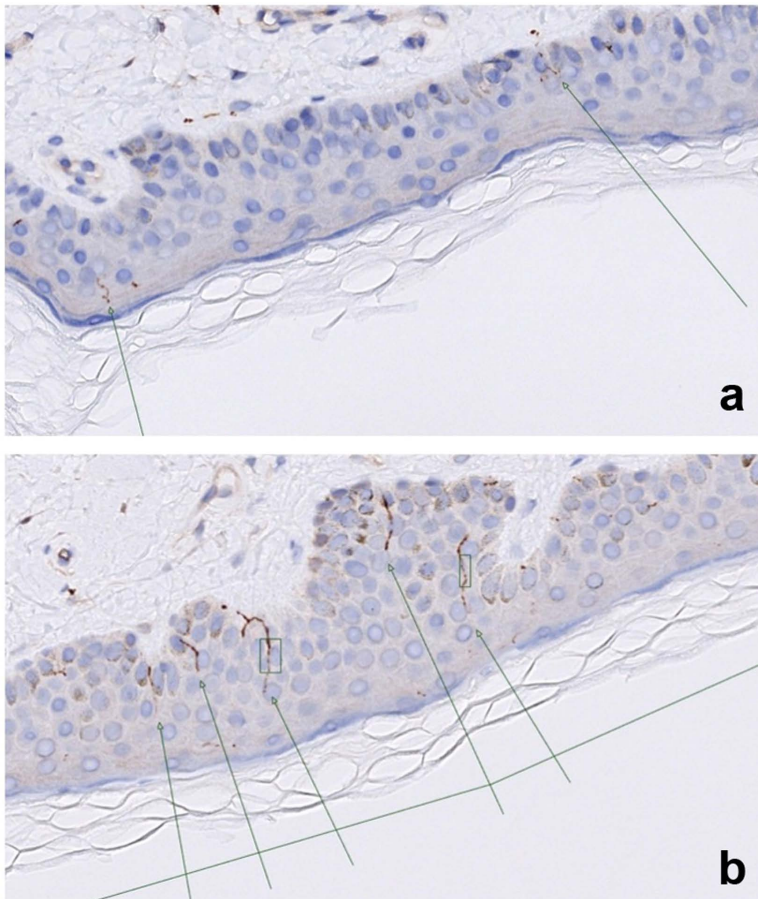


Fig 1. Intraepidermal nerve fibers. Intraepidermal nerve fibers in 5 μ m skin section on a 50X magnification in a) a patient with low IENFD and b) high IENFD. Nerve fibers are indicated by arrows. IENFD: Intraepidermal nerve fiber density.

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patients with total IBS-SSS score above 212.5 were included in the “High IBS-SSS” group. There were no differences in monofilament perception, neurothesiometer values, or IENFD between the two groups. Median vibrometry Z-scores were higher in the “High IBS-SSS” group when measured in the little finger and foot, however within normal ranges (Table 3).

4. Discussion

The main finding of the present study was the absence of solid evidence for peripheral small or large fiber neuropathy in POTS when compared to healthy controls, despite a high burden of neuropathic symptoms. In addition, a high

Table 2. Measures of nerve function in relation to self-reported neuropathy symptoms in POTS.

	Low NSS (<14.5) N = 21	High NSS (>14.5) N = 21	P-value
Monofilament Normal (%)			
Right foot	19 (95.0%) ^a	19 (95.0%) ^a	0.756
Left foot	20 (100%) ^a	19 (95.0%) ^a	0.500
Neurothesiometer (V)			
Right side			
Medial malleolus	7.0 (5.5–8.7)	7.5 (5.6–9.3) ^a	0.593
Big toe	4.8 (3.9–6.2)	5.3 (4.1–7.8) ^a	0.411
Left side			
Medial malleolus	5.7 (4.5–7.8)	7.4 (5.8–9.2) ^a	0.105
Big toe	4.7 (3.3–6.7)	5.6 (4.1–7.7) ^a	0.235
IENFD (fibers/mm)	2.40 (1.10–3.11) ^a	2.26 (1.66–2.85) ^a	0.930
Multi-frequency vibrometry (average Z-score)			
Hand			
Index finger	0.1 (–0.5–0.6) ^b	0.7 (–0.2–1.3) ^b	0.129
Little finger	0.0 (–0.5–0.8) ^b	0.9 (0.3–1.3) ^a	0.006
Foot	–0.3 (–0.6–0.4) ^b	0.6 (–0.1–1.2) ^b	0.011

Comparison of objective signs of peripheral neuropathy in patients with POTS with low and high burden of self-reported neuropathy symptoms measured with NSS [22]. Values are presented as number (percent) or median (interquartile range). Comparative analyses were performed with Fisher's Exact test or Mann Whitney-U test. A p-value <0.05 was considered statistically significant. NSS: Neuropathy Symptom Score; IENFD: Intraepidermal Nerve Fiber Density.

^a1 missing value.

^b2 missing values.

<https://doi.org/10.1371/journal.pone.0327549.t002>

burden of gastrointestinal symptoms in POTS was not associated with neuropathy, based on objective measures. However, patients with a high burden of symptoms indicative of peripheral neuropathy had high scores on the IBS-SSS scale.

Questionnaires revealed that the neuropathy symptom burden is high in POTS, both in terms of occurrence and severity. Gastrointestinal symptoms in this POTS cohort are frequent and moderately severe, a finding published previously [15]. Regarding peripheral neuropathy assessed with NSS, the most commonly reported symptoms were abnormal sensations of heat and cold, which are symptoms we expected to find since heat and cold sensations are transmitted by small nerve fibers [17]. The patients also reported numbness and sensations of dull pain in both hands and feet, which are associated with dysfunction of moderately myelinated A β nerve fibers [17,29]. Our findings correspond to results from a large community-based cross-sectional survey in POTS patients, where coldness, tingling, and numbness in hands and feet were reported by 58–84% of the patients [6].

Neurological clinical status was fairly normal in patients with POTS in the present study, which is in line with previous studies on POTS where signs of neuropathy have been assessed [8,13]. A handful of patients had general muscle weakness, and thus, some unspecific neurological motor abnormalities were seen. Deconditioning and muscle weakness are common in POTS [1], probably due to orthostatic intolerance, which may explain these findings. We have previously shown that weekly physical activity is low in this POTS cohort [30].

Vibrometry Z-scores were abnormal in the hands and/or feet of seven POTS patients. When comparing the patients with high vs. low NSS, the patients with higher NSS had slightly higher Z-scores, measured on the little finger and the

Table 3. Measures of nerve function in relation to self-reported gastrointestinal symptoms in POTS.

	Low IBS-SSS N = 20	High IBS-SSS N = 20	P-value
Monofilament Normal (%)			
Right foot	20 (100%)	17 (94.4%) ^b	0.474
Left foot	20 (100%)	17 (94.4%) ^b	0.474
Neurothesiometer (V)			
Right side			
Medial malleolus	7.3 (5.4–9.2)	7.2 (5.7–8.7) ^a	0.593
Big toe	4.8 (3.6–6.8)	5.0 (4.3–6.8) ^a	0.411
Left side			
Medial malleolus	6.5 (4.7–8.3)	6.3 (5.2–7.8) ^a	0.105
Big toe	4.9 (3.3–6.7)	5.2 (4.0–6.5) ^a	0.235
IENFD (fibers/mm)	2.24 (1.21–3.16) ^d	2.26 (1.76–2.94)	0.942
Multi-frequency vibrometry (average Z-score)			
Hand			
Index finger	0.1 (–0.6–1.1) ^c	0.3 (–0.4–1.0) ^a	0.130
Little finger	0.4 (–0.7–0.8) ^c	0.7 (–0.1–1.3) ^a	0.005
Foot	–0.2 (–0.6–0.4) ^c	0.6 (–0.3–1.6) ^a	0.010
Total NSS score	11.0 (4.0–15.0)	18.0 (14.3–22.8)	0.002

Comparison of objective signs of peripheral neuropathy in patients with POTS with low and high burden of self-reported gastrointestinal symptoms measured with IBS-SSS [23]. Values are presented as number (percent) or median (interquartile range). Comparative analyses were performed with Fisher's Exact test or Mann Whitney-U test. A p-value < 0.05 was considered statistically significant. NSS: Neuropathy Symptom Score [22]; IBS-SSS: Irritable Bowel Syndrome-Severity Scoring System; IENFD: Intraepidermal Nerve Fiber Density.

^a1 missing value.

^b2 missing values.

^c3 missing values.

^d4 missing values.

Total NSS was significantly higher in the "High IBS-SSS" group than in the "Low IBS-SSS" group (Table 3, Fig 2).

<https://doi.org/10.1371/journal.pone.0327549.t003>

foot, than patients with fewer symptoms. Moreover, most of the patients with abnormal Z-scores reported high NSS. On the contrary, when measuring VPTs with neurothesiometry, all patients with POTS showed values within the normal age-adjusted range, and even somewhat lower values compared to the healthy control participants. No difference was seen regarding VPTs measured with neurothesiometry between patients with high vs. low NSS. This finding may suggest that MFV is a more sensitive instrument than the neurothesiometer. Unlike assessment with neurothesiometry, MFV measures VPTs for several different vibrating frequencies, which allows for a broader evaluation of large Aβ sensory nerve fibers, different mechanoreceptors, and different innervation areas [29]. The method is also less user-dependent than neurothesiometry [31]. Nevertheless, the observed differences were very small and remained within normal ranges in most patients. While these findings could indicate an early sign of neuropathy, it might also be coincidental and a multiple testing problem, and thus, we should be cautious in drawing any firm conclusions, given the very low values and small differences. In all, we did not find any solid associations between subjective symptoms of neuropathy obtained by questionnaires and clinical findings. This observation corresponds to the results from a recently published large study where no associations could be found between subjectively reported symptoms and objectively measured signs of dysautonomia in POTS [32].

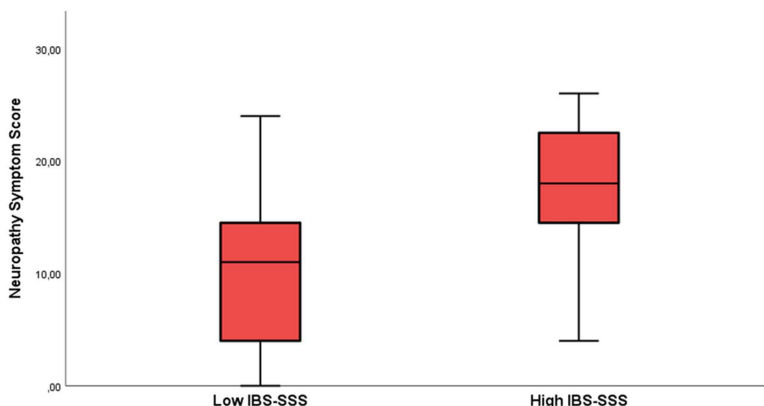


Fig 2. Comparison of neuropathy symptom scores in POTS patients with high or low IBS-SSS. NSS 11.0 (4.0–15.0) vs. 18.0 (14.3–22.8); $p = 0.002$. IBS-SSS: Irritable Bowel Syndrome-Severity Scoring System [23]; NSS: Neuropathy Symptom Score [22].

<https://doi.org/10.1371/journal.pone.0327549.g002>

It is estimated that approximately 50% of the POTS population have a neuropathic mechanism behind their symptoms [2], although no diagnostic criteria for neuropathic POTS exist [9]. This hypothesis has gained support from different assessment methods, including evidence of reduced IENFD on skin biopsies [7–10], findings of decreased norepinephrine spillover in the legs compared to the arms [3], supersensitivity of norepinephrine infusion in distal veins resembling the reaction in denervated veins [33], and impaired post-ganglionic sudomotor function upon measurement with QSART [9,12–14]. Theoretically, degeneration of small autonomic nerve fibers peripherally may lead to impaired vasoconstriction, causing blood pooling in the legs. To compensate for the decreased venous return, the body responds by increasing the heart rate [2]. In the present study, there was no significant difference in IENFD between POTS and controls. These findings are in contrast to previous studies on POTS, where reduced IENFD was found in 24–56% of the examined biopsy specimens [7–10,34,35]. In alignment with our findings, Singer et al. [36] did not find reduced IENFD in a small study of eight POTS patients. However, in three of the skin biopsy specimens, morphological abnormalities were found on the intraepidermal nerve endings [36]. The same study showed impaired post-ganglionic sudomotor function measured with QSART in POTS distally in the legs. This finding supports the thesis that autonomic nerve fiber dysfunction may be involved in the pathogenesis, rather than visible structural abnormalities [36].

The lack of solid association between subjective and objective neuropathic findings in POTS may be hypothetically explained by central sensitization, which has been described in IBS and other functional disorders of the gut-brain axis [37], as well as in other comorbidities commonly seen in POTS [38]. Additionally, somatic hypervigilance has been described in POTS [39,40]. Hence, central mechanisms may theoretically be the unifying link between self-reported symptoms of neuropathy and gastrointestinal symptoms in the present study.

The strength of the present study is that we have used a well-defined, quite large POTS cohort and a well-matched control material. Although the aim of the present study was not to perform a comprehensive testing battery of all types of peripheral somatic nerve fibers, we have utilized more easily accessible methods to scan for possible neuropathies that can explain the patients' symptoms. We have explored symptoms and objective signs of both small and large fiber neuropathy with different modalities. All patients were examined clinically by the same physician (HT) to exclude other causes of neuropathy, and all participants filled in questionnaires regarding neuropathic and gastrointestinal symptoms in close

temporal proximity to neuropathy testing. All MFV testings were performed by the same person (LE), all skin biopsies were obtained by the same surgeon (LD), and finally, all biopsies were examined by the same assessor (LE).

Regarding limitations of the study, the use of 5 μ m thick sections of the skin biopsies is a limitation [41]. It is possible that this size is too small to find reduced IENFD in POTS. Skin punch biopsies with the assessment of IENFD in biopsy cuts of 50 μ m are recommended for supporting the diagnosis of SFN affecting A δ - or C-fibers and/or autonomous nerve fibers [41]. However, the assessment of 50 μ m skin biopsies is time-consuming and not applicable to clinical routine [4]. We have recently demonstrated that 5 μ m biopsy sections can be used for the assessment of IENFD in diabetic subjects with reliable results [28]. A similar, clinically oriented method for assessment of IENFD has been used in Finland with 10 μ m skin biopsy cuts, also with reliable results [42]. Further limitations include that we did not have access to data on heart rate variability and other variables reflecting cardiac autonomic reflex tests [9,43]. It is possible that a subgroup with abnormal cardiac autonomic reflex tests would present with objective signs of neuropathy, including reduced IENFD, according to previous studies [9]. However, in the present cohort, no obvious subgroup with low IENFD was identified. Furthermore, aspects of cardiovascular function in POTS are already well described, hence the focus of the present study to examine peripheral nerves and associations between peripheral neuropathy and gastrointestinal symptoms in POTS. Another potential limitation was that the sample size was too small to explore associations between various symptoms and objective findings within the POTS cohort. We did have a rather small control group for comparisons regarding IENFD, and it is difficult to find healthy controls who are willing to go through an invasive procedure such as a skin punch biopsy. Yet, all control participants were well-matched with the POTS patients, and the results from our study are comparable to normative IENFD values obtained from the same laboratory and the same hospital [27]. There is also a risk of selection bias since the patients were recruited from a highly specialized syncope center, and the patients who agreed to participate in the study might have suffered more from symptoms indicative of peripheral neuropathy than the general POTS population. Other limitations include that the patients continued to take their regular medications during the study, and finally, the many co-morbidities that patients reported.

For future perspectives, it would have been interesting to add measurements of peripheral small nerve fiber function, e.g., with QSART test, to explore potential associations to symptoms of peripheral neuropathy. However, QSART is not available at our hospital, requires both highly trained staff and sensitive equipment, and is thus most often used within specialized centers [11].

5. Conclusion

Symptoms indicative of peripheral small and large nerve fiber neuropathy are common in POTS and are associated with a high burden of gastrointestinal symptoms. However, no solid evidence of peripheral small or large fiber neuropathy was found in POTS when compared to healthy controls in this cross-sectional study, based on objective measures. Future studies are needed to further evaluate the potential presence of small fiber neuropathy in POTS and other conditions of autonomic neuropathy.

Supporting information

S1 Fig. Flow chart demonstrating the recruitment process of POTS patients.

(TIF)

S1 Table. Neuropathy Symptom Score categories, and severity of symptoms within healthy control participants and patients with POTS.

(DOCX)

S2 Table. Clinical characteristics and subjective and objective signs of sensory nerve fiber neuropathy in control participants and patients with POTS who performed skin punch biopsy.

(DOCX)

S1 File. Data on symptoms and objective signs of peripheral neuropathy. (XLSX)

Author contributions

Conceptualization: Hanna Tufvesson, Linnéa Ekman, Lars B. Dahlin, Bodil Ohlsson.

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References

1. Fedorowski A. Postural orthostatic tachycardia syndrome: clinical presentation, aetiology and management. *J Intern Med.* 2019;285(4):352–66. <https://doi.org/10.1111/joim.12852> PMID: 30372565
2. Benarroch EE. Postural tachycardia syndrome: a heterogeneous and multifactorial disorder. *Mayo Clin Proc.* 2012;87(12):1214–25. <https://doi.org/10.1016/j.mayocp.2012.08.013> PMID: 23122672
3. Jacob G, Costa F, Shannon JR, Robertson RM, Wathen M, Stein M, et al. The neuropathic postural tachycardia syndrome. *N Engl J Med.* 2000;343(14):1008–14. <https://doi.org/10.1056/NEJM200010053431404> PMID: 11018167
4. Raasing LRM, Vogels OJM, Veltkamp M, van Swol CFP, Grutters JC. Current view of diagnosing small fiber neuropathy. *J Neuromuscul Dis.* 2021;8(2):185–207. <https://doi.org/10.3233/JND-200490> PMID: 33337383
5. Ohlsson B, Dahlin LB, Englund E, Veress B. Autonomic and peripheral neuropathy with reduced intraepidermal nerve fiber density can be observed in patients with gastrointestinal dysmotility. *Clin Case Rep.* 2019;8(1):142–8. <https://doi.org/10.1002/ccr3.2575> PMID: 31998505
6. Shaw BH, Stiles LE, Bourne K, Green EA, Shibao CA, Okamoto LE, et al. The face of postural tachycardia syndrome - insights from a large cross-sectional online community-based survey. *J Intern Med.* 2019;286(4):438–48. <https://doi.org/10.1111/joim.12895> PMID: 30861229
7. Gibbons CH, Bonyhay I, Benson A, Wang N, Freeman R. Structural and functional small fiber abnormalities in the neuropathic postural tachycardia syndrome. *PLoS One.* 2013;8(12):e84716. <https://doi.org/10.1371/journal.pone.0084716> PMID: 24386408
8. Haensch C-A, Tosch M, Katona I, Weis J, Isenmann S. Small-fiber neuropathy with cardiac denervation in postural tachycardia syndrome. *Muscle Nerve.* 2014;50(6):956–61. <https://doi.org/10.1002/mus.24245> PMID: 24647968
9. Zhang R, Mayuga K, Shields R, Cantrell C, Wilson R. Skin biopsy and quantitative sudomotor axon reflex testing in patients with postural orthostatic tachycardia syndrome. *Cureus.* 2022;14(11):e31021. <https://doi.org/10.7759/cureus.31021> PMID: 36349067
10. Billig SCI, Schauermaier JC, Rolke R, Katona I, Schulz BJ, Maier A. Quantitative sensory testing predicts histological small fiber neuropathy in postural tachycardia syndrome. *Neurol Clin Pract.* 2020;10(5):428–34.
11. Buchmann SJ, Penzlin AI, Kubasch ML, Illigens BM, Siepmann T. Assessment of sudomotor function. *Clin Auton Res.* 2019;29(1):41–53.
12. Peltier AC, Garland E, Raj SR, Sato K, Black B, Song Y, et al. Distal sudomotor findings in postural tachycardia syndrome. *Clin Auton Res.* 2010;20(2):93–9. <https://doi.org/10.1007/s10286-009-0045-y> PMID: 20035362
13. Thieben MJ, Sandroni P, Sletten DM, Benrud-Larson LM, Fealey RD, Vernino S, et al. Postural orthostatic tachycardia syndrome: the Mayo clinic experience. *Mayo Clin Proc.* 2007;82(3):308–13. <https://doi.org/10.4065/82.3.308> PMID: 17352367
14. Al-Shekhlee A, Lindenberg JR, Hachwi RN, Chelimsky TC. The value of autonomic testing in postural tachycardia syndrome. *Clin Auton Res.* 2005;15(3):219–22.

15. Tufvesson H, Hamrefors V, Fedorowski A, Hansson M, Ohlsson B. Gastrointestinal symptoms in patients with postural orthostatic tachycardia syndrome in relation to hemodynamic findings and immunological factors. *Front Physiol.* 2024;15. <https://doi.org/10.3389/fphys.2024.1342351>
16. Johansson M, Ricci F, Schulte J, Persson M, Melander O, Sutton R, et al. Circulating levels of growth hormone in postural orthostatic tachycardia syndrome. *Sci Rep.* 2021;11(1):8575. <https://doi.org/10.1038/s41598-021-87983-5> PMID: [33883597](https://pubmed.ncbi.nlm.nih.gov/33883597/)
17. Gwathmey KG, Pearson KT. Diagnosis and management of sensory polyneuropathy. *BMJ.* 2019;365:l1108. <https://doi.org/10.1136/bmj.l1108> PMID: [31068323](https://pubmed.ncbi.nlm.nih.gov/31068323/)
18. Dubey VN, Dave JM, Beavis J, Coppini DV. Predicting diabetic neuropathy risk level using artificial neural network and clinical parameters of subjects with diabetes. *J Diabetes Sci Technol.* 2022;16(2):275–81. <https://doi.org/10.1177/1932296820965583> PMID: [33090005](https://pubmed.ncbi.nlm.nih.gov/33090005/)
19. Fedorowski A, Burri P, Struck J, Juul-Möller S, Melander O. Novel cardiovascular biomarkers in unexplained syncopal attacks: the SYSTEMA cohort. *J Intern Med.* 2013;273(4):359–67. <https://doi.org/10.1111/joim.12043> PMID: [23510366](https://pubmed.ncbi.nlm.nih.gov/23510366/)
20. 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.* 2023;293(1):91–9. <https://doi.org/10.1111/joim.13566> PMID: [36111700](https://pubmed.ncbi.nlm.nih.gov/36111700/)
21. Brignole M, Moya A, de Lange FJ, Deharo J-C, Elliott PM, Fanciulli A, et al. 2018 ESC Guidelines for the diagnosis and management of syncope. *Eur Heart J.* 2018;39(21):1883–948. <https://doi.org/10.1093/eurheartj/ehy037> PMID: [29562304](https://pubmed.ncbi.nlm.nih.gov/29562304/)
22. Thrainsdottir S, Malik RA, Dahlin LB, Wiksell P, Eriksson KF, Rosén I. Endoneurial capillary abnormalities presage deterioration of glucose tolerance and accompany peripheral neuropathy in man. *Diabetes.* 2003;52(10):2615–22.
23. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharmacol Ther.* 1997;11(2):395–402. <https://doi.org/10.1046/j.1365-2036.1997.142318000.x> PMID: [9146781](https://pubmed.ncbi.nlm.nih.gov/9146781/)
24. Malm J, Liedholm LJ, Lindgren A. Nervsystemet. In: Karling P, ed. *Kliniska färdigheter: mötet mellan patient och läkare*. 4th ed. Lund: Studentlitteratur AB; 2021: 211–32.
25. Cannon J. Performing Tinetti's test and Phalen's test. *Nursing.* 2009;39(8):16. <https://doi.org/10.1097/01.NURSE.0000358565.84950.96> PMID: [19633493](https://pubmed.ncbi.nlm.nih.gov/19633493/)
26. Ekman L, Lindholm E, Brogren E, Dahlin LB. Normative values of the vibration perception thresholds at finger pulps and metatarsal heads in healthy adults. *PLoS One.* 2021;16(4):e0249461. <https://doi.org/10.1371/journal.pone.0249461> PMID: [33822804](https://pubmed.ncbi.nlm.nih.gov/33822804/)
27. Ekman L, Dahlin LB, Englund E. Assessment of intraepidermal nerve fiber densities in 5 µm sections from arm and leg - a search for normative age-related values. *Free Neuropathol.* 2024;5:24. <https://doi.org/10.17879/freeneuropathology-2024-5815> PMID: [39416858](https://pubmed.ncbi.nlm.nih.gov/39416858/)
28. Ekman L, Pourhamidi K, Englund E, Lagali N, Rolandsson O, Dahlin LB. Temporal trend of small nerve fibre degeneration in people with and without type 2 diabetes mellitus. *Diabet Med.* 2022;39(3):e14691. <https://doi.org/10.1111/dme.14691> PMID: [34536243](https://pubmed.ncbi.nlm.nih.gov/34536243/)
29. Ekman L. Assessment of peripheral neuropathy. Lund, Sweden: Lund University; 2024. https://lucris.lub.lu.se/ws/portalfiles/portal/200063739/Avhandling_Linnea_Ekman_LUCRIS.pdf
30. Tufvesson H, Hamrefors V, Roth B, Fedorowski A, Ohlsson B. Sociodemographic factors, nutritional status, and inflammatory markers in patients with postural orthostatic tachycardia syndrome. *Acta Neurologica Scandinavica.* 2023;2023:1–9. <https://doi.org/10.1155/2023/3992718>
31. Ekman L, Dahlin LB, Andersson GS, Lindholm E. Diagnostic contribution of multi-frequency vibrometry to detection of peripheral neuropathy in type 1 diabetes mellitus compared with nerve conduction studies. *PLoS One.* 2024;19(1):e0296661. <https://doi.org/10.1371/journal.pone.0296661> PMID: [38198463](https://pubmed.ncbi.nlm.nih.gov/38198463/)
32. Novak P, Systrom DM, Marciano SP, Knief A, Felsenstein D, Giannetti MP, et al. Mismatch between subjective and objective dysautonomia. *Sci Rep.* 2024;14(1):2513. <https://doi.org/10.1038/s41598-024-52368-x> PMID: [38291116](https://pubmed.ncbi.nlm.nih.gov/38291116/)
33. Streeten DH. Pathogenesis of hyperadrenergic orthostatic hypotension. Evidence of disordered venous innervation exclusively in the lower limbs. *J Clin Invest.* 1990;86(5):1582–8. <https://doi.org/10.1172/JCI114878> PMID: [2243132](https://pubmed.ncbi.nlm.nih.gov/2243132/)
34. Moak JP, Ramwell CB, Gordish-Dressman H, Sule SD, Bettini E. Small fiber neuropathy in children, adolescents, and young adults with chronic orthostatic intolerance and postural orthostatic tachycardia syndrome: A retrospective study. *Auton Neurosci.* 2024;253:103163. <https://doi.org/10.1016/j.autneu.2024.103163> PMID: [38537312](https://pubmed.ncbi.nlm.nih.gov/38537312/)
35. Miglis MG, Seliger J, Shaik R, Gibbons CH. A case series of cutaneous phosphorylated α-synuclein in Long-COVID POTS. *Clin Auton Res.* 2022;32(3):209–12. <https://doi.org/10.1007/s10286-022-00867-0> PMID: [35570247](https://pubmed.ncbi.nlm.nih.gov/35570247/)
36. Singer W, Spies JM, McArthur J, Low J, Griffin JW, Nickander KK, et al. Prospective evaluation of somatic and autonomic small fibers in selected autonomic neuropathies. *Neurology.* 2004;62(4):612–8. <https://doi.org/10.1212/01.wnl.0000110313.39239.82> PMID: [14981179](https://pubmed.ncbi.nlm.nih.gov/14981179/)
37. Ford AC, Sperber AD, Corsetti M, Camilleri M. Irritable bowel syndrome. *Lancet.* 2020;396(10263):1675–88. [https://doi.org/10.1016/S0140-6736\(20\)31548-8](https://doi.org/10.1016/S0140-6736(20)31548-8) PMID: [33049223](https://pubmed.ncbi.nlm.nih.gov/33049223/)
38. Cook GA Jr, Sandroni P. Management of headache and chronic pain in POTS. *Auton Neurosci.* 2018;215:37–45. <https://doi.org/10.1016/j.autneu.2018.06.004> PMID: [29929839](https://pubmed.ncbi.nlm.nih.gov/29929839/)
39. Khurana RK. Experimental induction of panic-like symptoms in patients with postural tachycardia syndrome. *Clin Auton Res.* 2006;16(6):371–7. <https://doi.org/10.1007/s10286-006-0365-0> PMID: [16915526](https://pubmed.ncbi.nlm.nih.gov/16915526/)
40. DiBaise JK, Harris LA, Goodman B. Postural Tachycardia Syndrome (POTS) and the GI tract: a primer for the gastroenterologist. *Am J Gastroenterol.* 2018;113(10):1458–67. <https://doi.org/10.1038/s41395-018-0215-4> PMID: [30072778](https://pubmed.ncbi.nlm.nih.gov/30072778/)

41. Joint Task Force of the EFNS and the PNS. European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society. *J Peripher Nerv Syst.* 2010;15(2):79–92. <https://doi.org/10.1111/j.1529-8027.2010.00269.x> PMID: [20626771](#)
42. Koskinen M, Hietaharju A, Kyläniemi M, Peltola J, Rantala I, Udd B, et al. A quantitative method for the assessment of intraepidermal nerve fibers in small-fiber neuropathy. *J Neurol.* 2005;252(7):789–94. <https://doi.org/10.1007/s00415-005-0743-x> PMID: [15789134](#)
43. Inbaraj G, Udupa K, Vasuki PP, Nalini A, Sathyaprabha TN. Resting heart rate variability as a diagnostic marker of cardiovascular dysautonomia in postural tachycardia syndrome. *J Basic Clin Physiol Pharmacol.* 2023;34(1):103–9.



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