Cardiovascular autonomic dysfunction in syncope related syndromes

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Syncope is a common condition, yet much remains unknown about the pathophysiology of the most common causes. Establishing a syncope diagnosis can be challenging and unexplained syncope is associated with increased mortality.

This thesis examined the pathophysiological mechanisms involved in vasovagal syncope and orthostatic hypotension with focus on cardiovascular biomarkers, and investigated predictors of outcome of syncope evaluation with cardiovascular autonomic testing in a specialized syncope unit.

PARISA TORABI earned her medical degree from Lund University in 2012 and completed specialist training in Clinical physiology at Skåne University Hospital in Malmö in 2022.

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UNIVERSITY

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Abstract:

Background: Autonomic nervous system (ANS) dysfunction may lead to orthostatic intolerance (OI) and syncope. Vasovagal syncope (VVS) is the most common cause of syncope whereas orthostatic hypotension (OH) is the most common cause of OI in elderly persons. Still, much remains unknown about the pathophysiology of VVS and OH. Cardiovascular autonomic testing (CAT) including head-up tilt (HUT) can be used to reveal ANS dysfunction as syncope aetiology and study pathophysiology.

Patients: The SYSTEMA study (n=3029) included patients with OI and unexplained syncope investigated by CAT in the syncope unit at Skåne University Hospital Malmö.

Aims and methods: Paper I included 161 patients with VVS during drug-free HUT and the impact of cardiovascular neurohormones on susceptibility to VVS was studied. Supine and orthostatic plasma concentrations of epinephrine, norepinephrine and pro-hormone peptides of vasopressin, endothelin, atrial natriuretic peptide and adrenomedullin were analysed in relation to time from tilt-up to onset of VVS. Paper II included 584 patients with classical and delayed OH and clinical characteristics and neurohormones (as in paper I) were compared in the two forms. Paper III included 1928 syncope patients and the influence of age at first syncope on clinical characteristics and final HUT diagnosis was assessed. Paper IV included 2663 syncope patients and the predictive value of history and clinical characteristics for unexplained syncope after CAT was investigated.

Results and conclusions: Paper I: Susceptibility to VVS was inversely related to age, higher supine blood pressure and adrenomedullin concentration, whereas greater orthostatic increase in epinephrine and vasopressin predicted shorter time to syncope. Paper II: Compared with delayed OH, classical OH patients were older, had pacemaker-treated arrhythmia, lower glomerular filtration rate, pathologic Valsalva test and Parkinson's disease. Classical OH was associated with increased vasopressin and epinephrine during orthostasis, but blunted increase in norepinephrine. Classical OH is associated with more severe abnormalities of neurohormonal and autonomic regulation and is a more advanced form of OH. Paper III: The first-ever syncope incidence had a bimodal lifetime pattern with peaks at 15 and 70 years. The majority of patients aged ≥60 years had only recent syncope and a higher probability of OH and carotid sinus syndrome. In contrast, older patients with life-long syncope duration had a higher probability of VVS and complex syncope (>1 possible cause of syncope). This study highlights the value of a thorough history in the evaluation of elderly syncope patients. Paper IV: A syncope diagnosis was provided by CAT in 79% of patients. Predictors of negative CAT were older age at first syncope, syncope without prodrome and cardiovascular comorbidities. These are known risk factors for cardiac syncope and patients with inconclusive CAT require further evaluation.

Key words: syncope, head-up tilt, cardiovascular autonomic testing

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To Johan and Oscar

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List of papers

This thesis is based on the following original papers, referred to in the text by their Roman numerals. They are appended at the end of the thesis.

I. Torabi P, Ricci F, Hamrefors V, Melander O, Sutton R, Benditt DG, Fedorowski A. Impact of cardiovascular neurohormones on onset of vasovagal syncope induced by head-up tilt. Journal of the American Heart Association. 2019;8(12):e012559

II. Torabi P, Ricci F, Hamrefors V, Sutton R, Fedorowski A. Classical and delayed orthostatic hypotension in patients with unexplained syncope and severe orthostatic intolerance. Frontiers in Cardiovascular Medicine. 2020;7:21.

III. Torabi P, Rivasi G, Hamrefors V, Ungar A, Sutton R, Brignole M, Fedorowski A. Early and late-onset syncope: insight into mechanisms. European Heart Journal. 2022;43(22), 2116–2123.

IV. Torabi P, Hamrefors V, Sutton R, Brignole M, Fedorowski A. Definitive aetiology of unexplained syncope after cardiovascular autonomic tests in a tertiary syncope unit. Manuscript submitted.

Abstract

Background: Autonomic nervous system (ANS) dysfunction may lead to orthostatic intolerance (OI) and syncope. Vasovagal syncope (VVS) is the most common cause of syncope whereas orthostatic hypotension (OH) is the most common cause of OI in elderly persons. Still, much remains unknown about the pathophysiology of VVS and OH. Cardiovascular autonomic testing (CAT) including head-up tilt (HUT) can be used to reveal ANS dysfunction as syncope aetiology and study pathophysiology.

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Populärvetenskaplig sammanfattning

Bakgrund

Yrsel och svimning är vanliga besvär och ca 40% av befolkningen drabbas av ett svimningsanfall någon gång i livet. Orsaken är oftast den vasovagala reflexen, ”svimningsreflexen”, vilket är ett ofarligt tillstånd. Reflexen kan triggas av långvarigt stillastående, värme, smärta eller obehag och leder till att blodtryck och puls sjunker snabbt, hjärnan får syrebrist och personen förlorar medvetandet. Direkt före svimning kan den drabbade kallsvettas och känna yrsel, illamående eller hjärtklappning. Reflexen går snabbt över när personen faller till golvet, blodtryck och puls återställs och personen återfår medvetandet. Ortostatisk hypotoni (blodtrycksfall i stående) kan orsaka yrsel men också svimning och drabbar främst äldre personer. Tillståndet kan bero på kroniska sjukdomar, biverkningar av läkemedel eller rubbningar i det autonoma (icke-viljestyrda) nervsystemet.

Artiklarna i avhandlingen är baserade på SYSTEMA-studien som inkluderade ca 3000 patienter med yrsel- och svimningsbesvär. Patienterna undersöktes med tippbrädetest på Svimningsenheten på Skånes universitetssjukhus i Malmö. Syftet med undersökningen är att provocera fram patientens symptom samtidigt som blodtryck, puls och syresättning i hjärnan mäts. När patienten tippas till stående, lätt bakåtlutad, samlas ca en liter blod i blodkärlen i nedre delen av kroppen pga. tyngdkraften. När man själv står upp pressar musklerna i benen tillbaka blodet till hjärtat men på tippbrädan är musklerna avslappnade. Har patienten benägenhet att svimma klarar kroppen inte att kompensera för detta och patienten svimmar.

För många patienter är det tillräckligt att få en diagnos och veta att svimningarna inte är farliga. Men ett fåtal patienter svimmar ofta och har stora besvär i vardagen. Äldre multisjuka patienter kan vara hårt drabbade av blodtrycksfall i stående. För dessa patienter saknas i många fall effektiv behandling. Tippbrädetestet gör det möjligt att studera sjukdomsmekanismen vid de olika typerna av svimning. Detta kan i framtiden leda till enklare sätt att ställa rätt diagnos och nya effektiva behandlingar.

Syfte och metod

I första delarbetet ingick 161 patienter med diagnosen vasovagal svimning efter tippbrädetest. Syftet var att undersöka om blodkoncentrationen av hormoner och signalsubstanser som har effekt på hjärta och blodtrycksreglering (adrenalin, noradrenalin, vasopressin, endotelin, natriuretisk förmakspeptid och adrenomedullin) hade ett samband med hur snabbt patienten svimmade under tippbrädetestet.

I andra delarbetet ingick 584 patienter med ortostatisk hypotoni. Syftet var att jämföra två former av sjukdomen, den klassiska varianten som inträffar inom 3 minuter efter stående och den sena typen som börjar efter 3 minuters stående. Vi jämförde symptom, tidigare sjukdomar, signalsubstanser och hormoner (som i första delarbetet).

I tredje delarbetet ingick 1928 patienter med oklar svimning. Syftet var att kartlägga om åldern för patientens allra första svimning hade ett samband med diagnos efter tippbrädetestet.

I fjärde delarbetet ingick 2663 patienter med oklar svimning. Syftet var att undersöka om det utifrån sjukhistoria och andra kliniska faktorer gick att förutsäga vilka som trots tippbrädetest inte fick en säker svimningsdiagnos.

Resultat och slutsatser
I första delarbetet observerade vi att högre ålder, blodtryck samt koncentration av adrenomedullin hade ett samband med minskad känslighet för vasovagal svimning. En större ökning av adrenalin och vasopressin i stående hade ett samband med ökad känslighet för vasovagal svimning. Dessa fynd bidrar till ökad kunskap om sjukdomsmekanismen vid vasovagal svimning.

I andra delarbetet fann vi att jämfört med sen ortostatisk hypotoni var patienter med den klassiska varianten äldre, fler behandlades med pacemaker, hade sämre njurfunktion, Parkinsons sjukdom och tecken på störd funktion i autonoma nervsystemet. Vid klassisk ortostatisk hypotoni uppmättes högre koncentrationer av vasopressin och adrenalin, men utebliven ökning av noradrenalin. Klassisk ortostatisk hypotoni visar tecken på allvarligare störningar i reglering av blodtrycket och autonoma nervsystemet och kan betraktas som en svårare form av sjukdomen.

I tredje delarbetet observerade vi att den allra första svimningen oftast inträffade i ungdomsåren eller vid hög ålder. Majoriteten av de äldre patienterna hade nyligen börjat svimma och hade högre sannolikhet att få diagnoserna ortostatisk hypotoni och karotissinussyndrom (en typ av reflexsvimning). Däremot hade äldre patienter som börjat svimma i ungdomen högre sannolikhet för vasovagal reflex och komplex svimning (fler än en möjlig diagnos till svimningen). Delarbete 3 betonar värdet av en detaljerad sjukhistoria i bedömningen av äldre patienter med svimning.

I delarbete 4 fann vi att tippbrädetestet ledde till diagnos hos 79% av patienterna med svimning. Faktorer som hade ett samband med utebliven diagnos efter undersökningen var hög ålder vid första svimningen, svimning utan förkänningar och förekomst av hjärt- och kärlsjukdomar. Dessa är kända riskfaktorer för svimning orsakad av hjärtsjukdomar, som kan vara livshotande, och därför måste patienter utan diagnos efter tippbrädetestet utredas vidare.

Abbreviations

ADM	adrenomedullin
ANOVA	analysis of variance test
ANS	autonomic nervous system
CAT	cardiovascular autonomic testing
cOH	classical orthostatic hypotension
CSH	carotid sinus hypersensitivity
CSM	carotid sinus massage
CSS	carotid sinus syndrome
CT-proAVP	C-terminal-pro-arginine-vasopressin
CT-proET-1	C-terminal-pro-endothelin-1
DBP	diastolic blood pressure
dOH	delayed orthostatic hypotension
ECG	electrocardiogram
GFR	glomerular filtration rate
HR	heart rate
HUT	head-up tilt
ILR	implantable loop recorder
iOH	immediate orthostatic hypotension
MR-proADM	mid-regional fragment of pro-adrenomedullin
MR-proANP	mid-regional fragment of pro-atrial-natriuretic-peptide
OH	orthostatic hypotension
OI	orthostatic intolerance
POTS	postural orthostatic tachycardia syndrome
PPS	psychogenic pseudosyncope
RAAS	renin-angiotensin-aldosterone system
SBP	systolic blood pressure
VVS	vasovagal syncope

Introduction

The autonomic nervous system and blood pressure regulation

The autonomic nervous system (ANS) consists of the parasympathetic, sympathetic and enteric divisions and innervates most organs in the body. The cardiovascular division of the ANS maintains homeostasis of blood pressure through regulation of vascular resistance, heart rate, cardiac contractility and blood volume through the baroreflex in response to physiological changes such as standing and physical activity (1-3).

Upon standing, up to 1 l of blood is immediately redistributed from the thorax to the capacitance vessels of splanchnic and pelvic circulation and legs, resulting in decreased venous return and cardiac output. In health, this is counteracted by the baroreflex, which continuously and instantaneously regulates blood pressure. High-pressure arterial baroreceptors respond to vascular wall stretch and are located in the adventitia of the carotid sinuses and aortic arch, low-pressure baroreceptors are located in the heart and great veins. As blood pressure falls, baroreceptors are unloaded and relay signals through the glossopharyngeal and vagus nerves to the nucleus tractus solitarii in the medulla oblongata, where cardiovascular reflexes are integrated and coordinated (Figure 1) (1-6).

The efferent pathways of the ANS consist of preganglionic and postganglionic neurons that transmit impulses from the central nervous system to the target organ. The preganglionic neurons of the sympathetic ANS are located in the thoracolumbar regions of the spinal cord. Their axons synapse with the postganglionic neurons located in para- and prevertebral sympathetic ganglion chains that run next to the vertebral column. The preganglionic neurotransmitter is acetylcholine and the postganglionic is norepinephrine for all target organs, with a few exceptions (sweat glands, kidneys and adrenal medulla) (1, 2, 7). The adrenal medulla consists of modified ganglion cells that secrete the neurotransmitters epinephrine and norepinephrine (in the proportions 80% and 20% respectively) directly into the bloodstream (2, 7, 8).

The preganglionic neurons of the parasympathetic division are located in the brainstem and sacral region of the spinal cord and synapse with postganglionic neurons that are

located close to or within target organs (2). Axons of preganglionic neurons located in the brainstem travel via cranial nerves III, VII, IX and X (vagus nerves). The vagus nerves carry approximately 75% of all parasympathetic axons and innervate abdominal and thoracic viscera (2). Acetylcholine is the neurotransmitter in both preganglionic and postganglionic neurons (1).

Thus, the initial decrease in cardiac output and blood pressure leads to decreased vagal outflow and increased sympathetic activity, resulting in increased heart rate, cardiac contractility and peripheral vascular resistance to restore blood pressure (1, 2, 6, 9). The most important factor in maintaining blood pressure is the sympathetic increase in peripheral vascular resistance as an increase in heart rate is usually insufficient to maintain cardiac output (6).

Emotional responses originating in the cerebral cortex and limbic system can modulate ANS activity through pathways in the hypothalamus and brainstem and syncope triggered by emotional upset is one example of this (1, 2).

During prolonged orthostatic stress, increased hydrostatic pressure will lead to transcapillary fluid filtration into the interstitial space and reduce plasma volume by 10-20% (1, 10). The renin-angiotensin-aldosterone system regulates blood pressure and blood volume during prolonged orthostasis. This system can be activated by the baroreflex, decreased sodium chloride concentration or reduced blood flow through the kidney (8, 11). The kidney is mainly under sympathetic autonomic control (12). Renin is secreted by the kidney and converts angiotensinogen into angiotensin I, which in turn is converted to angiotensin II by angiotensin converting enzyme (produced in lung capillaries). Angiotensin II has several important actions, including vasoconstriction of arterioles to increase systemic blood pressure, increased sodium reabsorption and consequently water reabsorption in the kidneys, aldosterone secretion by the adrenal cortex, leading to sodium reabsorption and potassium excretion and vasopressin secretion from the hypothalamus (8).

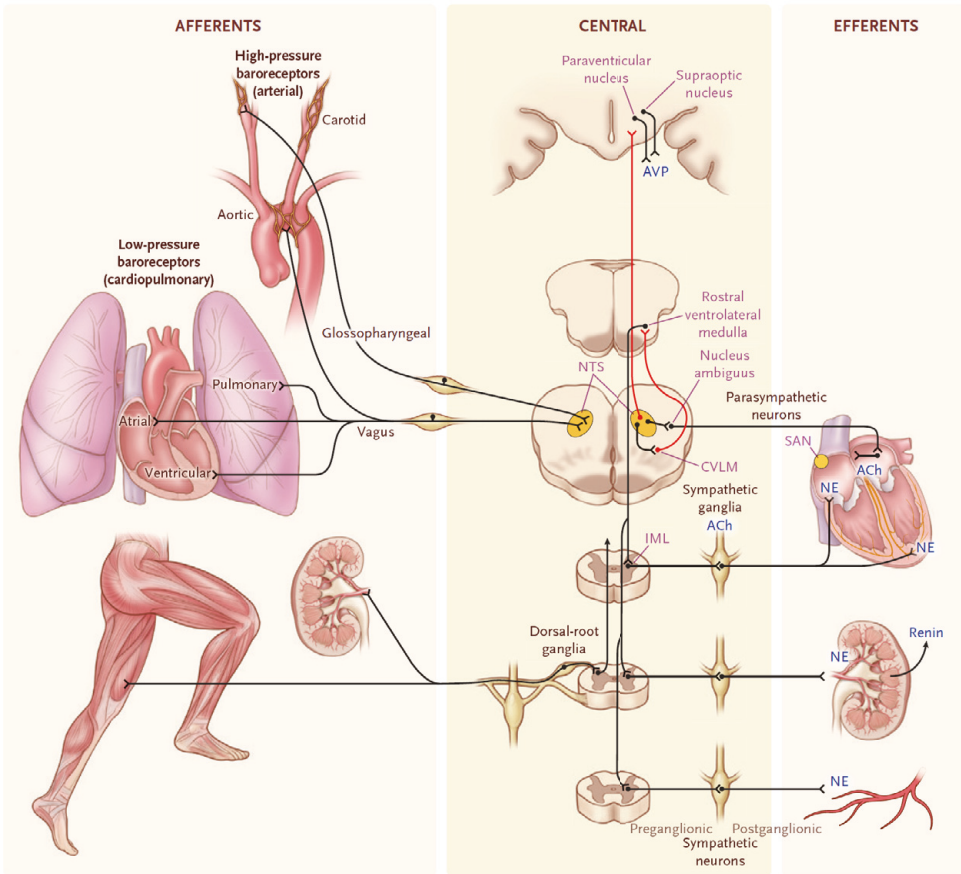

Figure 1. Arterial baroreceptors in the carotid artery and aortic arch and cardiopulmonary baroreceptors in the heart and pulmonary vessels relay signals through the glossopharyngeal and vagus nerves to the nucleus tractus solitarii (NTS) in the medulla oblongata, which signals the caudal ventrolateral medulla (CVLM), which in turn activates the rostral ventrolateral medulla, the origin of sympathetic tone. Simultaneously, the NTS signals the nucleus ambiguous, the origin of parasympathetic tone. The NTS also has projections to the supraoptic and paraventricular nuclei in the hypothalamus, regulating vasopressin (AVP) release from the pituitary gland. Afferent neurons in the kidneys and ergoreceptors in skeletal muscle modulate the baroreflex. A fall in blood pressure leads to decreased vagal outflow and increased sympathetic activity, resulting in increased heart rate, cardiac contractility and peripheral vascular resistance, and release of renin and vasopressin, to restore blood pressure. Abbreviations: ACh=acetylcholine, NE= norepinephrine, SAN=sinoatrial node. Reproduced with permission from (13), Copyright Massachusetts Medical Society.

Changes in cardiovascular autonomic function with normal ageing

Healthy older subjects show a blunted heart rate response to orthostasis, caused by age-related reductions in baroreceptor sensitivity, cardiac β-adrenergic receptor density and postsynaptic β-adrenergic signalling (14-17). To compensate, sympathetic activity and plasma levels of norepinephrine and epinephrine are increased during orthostasis compared with younger individuals (15, 18). During physical activity, increases in heart rate and cardiac contractility are diminished (19). With ageing, vascular α-adrenergic desensitization occurs, which can lead to vasodilation (18). The age-related attenuation of autonomic cardiovascular responses reduces the ability to react to orthostatic stress and can increase the susceptibility to OH and syncope.

Autonomic nervous system dysfunction and syncope

Syncope is defined as a transient loss of consciousness caused by global cerebral hypoperfusion, with a rapid onset and short duration, that is spontaneously resolved with complete recovery (20).

Autonomic nervous system dysfunction can cause inadequate compensatory mechanisms during orthostatic stress and lead to orthostatic intolerance (OI) and ultimately syncope. Reflex syncope, including vasovagal syncope (VVS) and carotid sinus syndrome (CSS), orthostatic hypotension (OH) and postural orthostatic tachycardia syndrome (POTS) are conditions caused by cardiovascular autonomic dysfunction (3).

At rest, the brain receives 15-20% of cardiac output. Syncope occurs when the perfusion of the brain is compromised for 6-10 seconds. Most often this is caused by a fall in systemic blood pressure. Arterial blood pressure is the product of cardiac output (heart rate and stroke volume) and systemic vascular resistance and a decrease in either factor may lead to critically low blood pressure and syncope (21).
Approximately 40% of the population will have one syncopal episode in their lifetime (20, 22-24) and 5% at least 5 episodes (25). Although about 50% of syncope patients never seek medical care (26), syncope accounts for up to 2% emergency department visits (27) and approximately 50% are admitted (20). Establishing a syncope diagnosis can be challenging, especially in elderly patients (28-30). Following hospitalization, up to one third of patients remain undiagnosed (31), with consequently increased morbidity and mortality (32).

Vasovagal syncope

Vasovagal syncope is the most common cause of syncope, with the highest incidence in young persons and women are the most often affected (3, 20, 22-24). Vasovagal syncope is not associated with increased cardiovascular morbidity or mortality (26, 33).

Vasovagal syncope often has a typical trigger, such as orthostatic stress, pain, fear, emotional upset, dehydration or being in a warm environment, however the trigger is not always apparent. Further, it is characterized by typical prodromal symptoms that are caused by autonomic activation and brain and retinal hypoperfusion. Commonly reported symptoms include feeling warm, nausea, yawning, hyperventilation, dizziness, pallor, cold sweat, palpitations, difficulty thinking, hearing loss and tunnel vision (34). The duration and timing of prodromes is variable, but usually start 15-30 seconds before loss of consciousness (34). However, it is not uncommon for older patients to present with syncope without prodromes, unexplained falls or have amnesia for the episode (28-30, 35, 36).

Head-up tilt (HUT) studies have revealed part of the mechanism of orthostatic VVS. Venous pooling in splanchnic and muscular veins leads to diminished venous return, which in turn leads to reduced stroke volume, falling cardiac output and systemic arterial blood pressure. To compensate, sympathetic activity increases and leads to higher heart rate. Total peripheral resistance increases compared to supine levels, but not compared to early HUT levels. Cardiac output continues to slowly decrease, and at a certain point the reflex begins, with withdrawal of sympathetic activity and parasympathetic activation leading to bradycardia. Stroke volume and cardiac output decrease rapidly and the blood pressure decrease accelerates sharply, leading to global cerebral hypoperfusion and syncope (37, 38). Arteriolar vasodilatation is not a major contributor to VVS and total peripheral resistance decreases only slightly at syncope (39).

Although the pathophysiology of vasovagal syncope has been widely studied, there are still many unanswered questions; why does venous pooling occur, without adequate compensation? Why is the effort to compensate falling cardiac output abandoned at one point during the reflex? What is the mechanism in VVS triggered by emotional upset, fear or pain?

Carotid sinus syndrome

Carotid sinus syndrome is a form of reflex syncope, occurring with pressure on the carotid area (such as during head turning, shaving or wearing tight collars) or apparently spontaneously, leading to an exaggerated response by the baroreflex resulting in hypotension, bradycardia and syncope. The pathophysiology is not known in detail.

Elderly men are the most often affected, concomitant cardiovascular disease is common, and patients typically have syncope with very short or no prodrome (40). Carotid sinus hypersensitivity is a phenomenon which is presently ill-understood. Carotid sinus massage causes syncope or near-syncope in CSS with asystole of 3 or more seconds and/or blood pressure fall of at least 50mmHg but in carotid sinus hypersensitivity, carotid sinus massage produces this positive response in an asymptomatic subject which is a common finding in elderly men (up to 40%) (20). Carotid sinus syndrome is unusual in patients under the age of 40 years (21).

Orthostatic hypotension

The prevalence of OH increases with older age and comorbidities, from approximately 3% in individuals under the age of 40 years to 30% in those 75 years and older (20). Orthostatic hypotension is associated with increased risk of recurrent falls and associated injuries (41-43) and increased cardiovascular morbidity and mortality (44-47).

The pathophysiological mechanism in OH involves central or peripheral dysfunction in the efferent pathway of the baroreflex, leading to reduced release of norepinephrine and consequent insufficient arteriolar vasoconstriction of skeletal muscle and splanchnic vessels, leading to reduced venous return and hypotension during standing (3, 10, 13, 48, 49). Patients often report symptoms caused by organ hypoperfusion, including dizziness, nausea, visual blurring, shortness of breath, chest pain, fatigue, head and neck pain and cognitive slowing, that are relieved in the recumbent position (13, 45, 48-51), however up to one third of patients with pronounced orthostatic hypotension are asymptomatic (52).

From a pathophysiological viewpoint, OH can be divided into neurogenic or non-neurogenic types. Neurogenic OH is caused by neurodegenerative disorders such as synucleinopathies, where misfolded α-synuclein is accumulated in neurons and glial cells, including Parkinson's disease, multiple-system atrophy, Lewy-body dementia and pure autonomic failure (13). In these disorders, OH is frequently associated with supine hypertension in (53, 54). Orthostatic hypotension can also be secondary to diseases such as diabetes, renal failure and amyloidosis. Non-neurogenic OH aetiologies include drug treatment, heart failure and venous pooling (48, 55). Drug treatment, mainly diuretics and antihypertensive agents, is a common cause of OH in older persons, and acts through volume depletion or by impairing autonomic and vascular responsiveness (48).

Orthostatic hypotension is clinically classified into classical OH (cOH), delayed OH (dOH) and immediate/initial OH (iOH). In cOH, there is a significant sustained blood pressure fall within 3 minutes of standing, however in dOH, the blood pressure

fall occurs first after 3 minutes and is often less pronounced than in cOH (56) because of gradual impairment of compensatory mechanisms during standing (48). The fall in blood pressure may be associated with compensatory heart rate increase, a reaction that may be blunted or absent in more severe cases of autonomic failure (49). In iOH there is a transient large blood pressure fall immediately upon standing, that in some cases may lead to syncope. The blood pressure fall is usually more pronounced during active standing than on HUT, which involves a slower transition to upright position. Immediate OH is caused by a temporary mismatch between the fall in venous return and compensatory vasoconstriction (48) and is not associated with increased morbidity (43). Associated symptoms are not required for the diagnosis of OH (49).

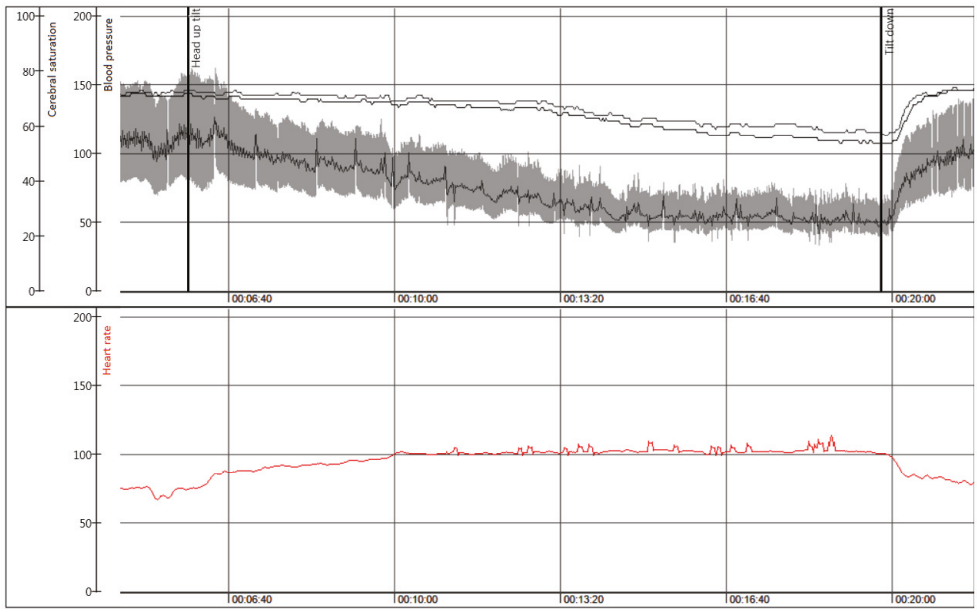

Figure 2. Head-up tilt registration of a 50-year-old woman with classical orthostatic hypotension. Upper panel shows beat-to-beat blood pressure (mmHg) and cerebral oxygen saturation (%) and the lower panel shows heart rate (beats/min). Reprinted from Paper II, Frontiers in Cardiovascular Medicine, under open access CCBY.

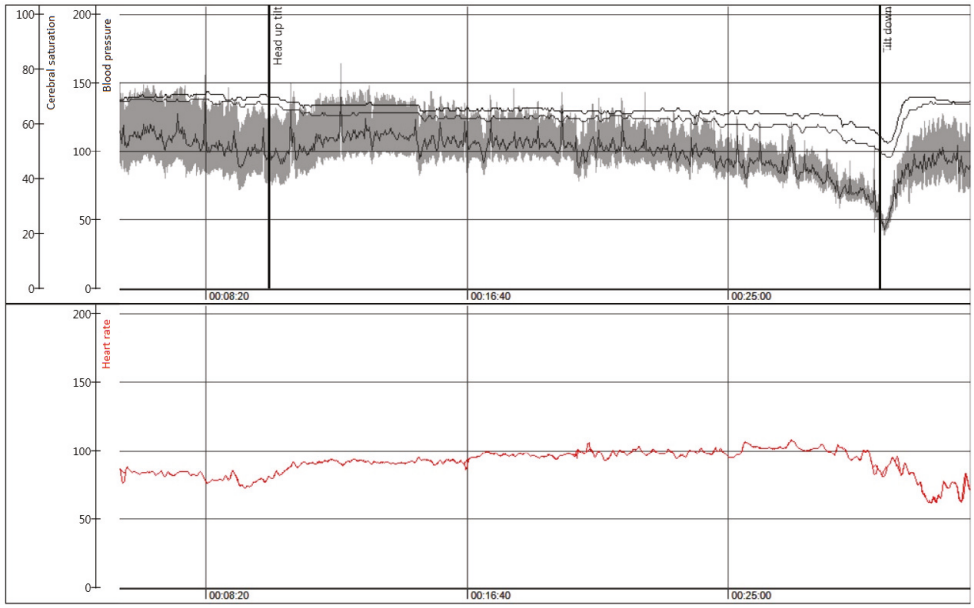

Figure 3. Head-up tilt registration of an 80-year-old man with delayed orthostatic hypotension, leading to vasovagal syncope, an example of complex syncope aetiology. Reprinted from Paper II, Frontiers in Cardiovascular Medicine, under open access CCBY.

Postural orthostatic tachycardia syndrome

Postural orthostatic tachycardia syndrome primarily affects young women. It is characterized by excessive heart rate increase without hypotension upon standing, accompanied by orthostatic symptoms such as dizziness, palpitations, blurred vision, fatigue, chest pain, and persistent symptoms including cognitive impairment, exercise intolerance and gastrointestinal symptoms. The pathophysiology of POTS is heterogenous and not completely understood, and includes immune mediated mechanisms, peripheral sympathetic denervation with excessive venous pooling, excessive sympathetic activity and hypovolemia (20, 57-59). Up to one third of POTS patients have syncope which is similar to the general population and almost always vasovagal in nature, however a majority experience frequent presyncopal episodes (60).

Prevalence of syncope aetiologies

The prevalence of syncope aetiologies differs based on the clinical setting (i.e. general population, emergency department or specialized syncope unit) and age of the patients. Vasovagal syncope is the most common cause in all age groups, with a prevalence of 56-73%, OH is found in 1-10%, cardiac syncope due to structural heart disease

including at channelopathy level in 6-37%, non-syncopal loss of consciousness, mainly psychogenic pseudosyncope in 1-6% and unexplained syncope in 5-20% (20).

The head-up tilt test

The head-up tilt test was introduced into clinical practice for diagnosing VVS in 1986 (61). Initially a passive phase of long duration was used, but eventually shortened to make the test more clinically practical, and pharmacological provocation was used to increase the sensitivity of HUT responses (62). Since its introduction, various HUT protocols have been used, with differences in duration of the passive phase, tilt angle, type of support and pharmacological provocation (62). Presently, the two most widely used protocols, recommended by the European Society of Cardiology syncope guidelines (20) are the Italian protocol (63), which includes a passive phase of 20 minutes and if the passive phase is negative, provocation with sublingual nitroglycerine, and the intravenous isoproterenol protocol (20). The Italian protocol has recently been proposed to be shortened in the passive phase to 10min. with convincing data from a large number of patients (64). HUT can reveal suspected VVS in patients where the diagnosis was not confirmed by initial evaluation. It can also be used to assess OH, POTS and psychogenic pseudosyncope (20).

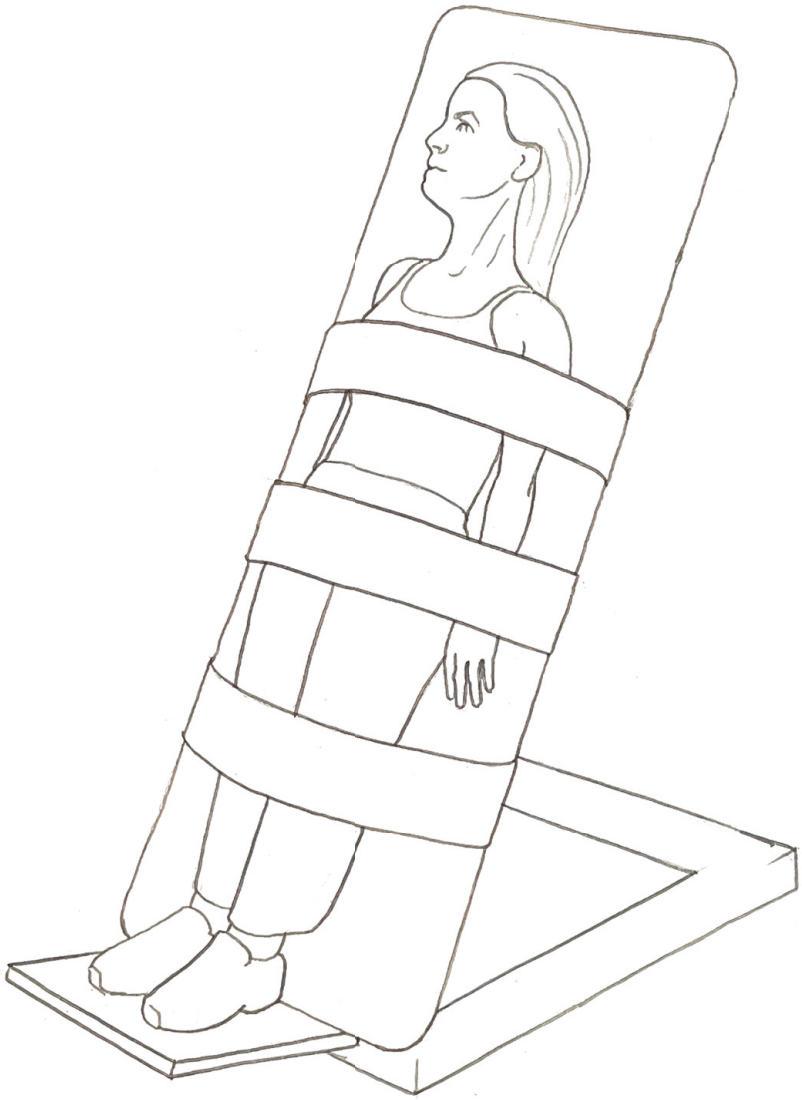

Figure 4. Illustration of head-up tilt, using a tilt angle of 60-70°. Not depicted: ECG and non-invasive continuous blood pressure monitoring. Illustration by Johan Bengtsson.

Cardiovascular biomarkers

A biomarker is a specific quantifiable characteristic of a physiological or pathophysiological process or response to exposure or treatment (65, 66). Some cardiovascular biomarkers, such as cardiac troponins and natriuretic peptides, are used routinely in clinical practice; in diagnostics, risk stratification, prognostication and treatment decisions (65, 67).

Previous studies have shown that supine and standing concentrations of cardiovascular biomarkers (neurohormones) such as catecholamines, natriuretic peptides, endothelin, adrenomedullin and vasopressin are associated with susceptibility to VVS. In OH, the release of norepinephrine from sympathetic postganglionic neurons is reduced, leading to insufficient vasoconstriction and hypotension during standing, which may result in compensatory mechanisms by other neurohormonal systems (10, 68-72).

Various cardiovascular neurohormones presumed to be involved in VVS and autonomic dysfunction have been studied, but it is still unknown whether supine and orthostatic changes in neurohormone levels are responsible for triggering VVS or if they represent a compensatory mechanism. A deeper understanding of neurohormonal pathophysiology may have implications for improved diagnostics, prevention and treatment.

In paper I and II, we analysed C-terminal-pro-arginine-vasopressin (CT-proAVP), C-terminal-pro-endothelin-1 (CT-proET-1), Mid-regional-fragment of pro-atrial-natriuretic-peptide (MR-proANP) and Mid-regional-fragment of pro-adrenomedullin (MR-proADM). The analysed nonactive peptides are surrogate markers of their corresponding biologically active hormones, are generated from the pro-neurohormone molecule in the ratio 1:1 in relation to the active neurohormone and are more stable and suitable for analysis (65).

Epinephrine

Epinephrine is a catecholamine released into the bloodstream by the adrenal medulla in response to sympathetic stimulation and binds to α and β-adrenergic receptors. At low concentrations, it binds to β2-receptors, leading to vasodilation of peripheral arteries, however at higher concentrations the dominant effect is peripheral vasoconstriction through α1-receptors, in addition to positive chronotropic and inotropic effects through β1-receptors (8, 73). Previous studies have shown higher orthostatic concentrations of epinephrine in patients with VVS induced by HUT, compared with HUT-negative individuals (68, 71, 74-77). The orthostatic concentration of epinephrine was higher in patients that developed VVS during the

drug-free phase of HUT compared with nitroglycerine provocation, indicating that higher epinephrine concentration is associated with increased VVS susceptibility (78).

Norepinephrine

Norepinephrine is released from sympathetic postganglionic neurons and from the adrenal medulla and binds to α1, α2 and β1-receptors (73). In healthy individuals, plasma norepinephrine concentration doubles during the first 5 minutes of standing, however OH is associated with impaired release of norepinephrine (79-83). Patients with VVS had comparable levels of norepinephrine prior to syncope compared with HUT-negative controls (74).

C-terminal-pro-arginine-vasopressin

Vasopressin is synthesized in the hypothalamus and released by the posterior pituitary gland in response to hyperosmolarity, reduced blood volume and arterial blood pressure. It leads to water reabsorption in the renal collecting tubes and vasoconstriction (8, 84). In healthy normovolaemic individuals, vasopressin is not critical for maintaining blood pressure (84, 85). However, patients with autonomic dysfunction were more dependent on vasopressin for blood pressure homeostasis, as inhibition of V1 receptors (responsible for vasoconstriction) caused a greater fall in blood pressure in these patients compared with controls (86). Vasopressin release was reduced in patients with OH and diabetic neuropathy (87) and the attenuated response in autonomic dysfunction could be caused by a defective baroreflex arc (88, 89). Patients with HUT-induced VVS had higher orthostatic vasopressin concentrations compared with HUT-negative controls (68, 90).

C-terminal-pro-endothelin-1

Endothelin-1 is a peptide hormone mainly produced by vascular endothelial cells and is a potent vasoconstrictor (8, 91). It regulates vascular tone and renal haemodynamics and is involved in the pathophysiology of cardiovascular diseases such as hypertension and coronary artery disease (92). Contradictory results regarding endothelin-1 concentration in VVS and OH have been reported, with both elevated and reduced levels (70, 93-95).

Mid-regional-fragment of pro-atrial-natriuretic-peptide

Atrial natriuretic peptide is produced by atrial cardiomyocytes in response to atrial distention, sympathetic stimulation, hypernatremia and increased levels of angiotensin

II and endothelin. It regulates blood pressure and volume by natriuretic and diuretic effects on the kidney and vasodilatory effects on vasculature (8, 65, 96). Atrial natriuretic peptide levels decreased during prolonged orthostasis (97). Reduced levels have been reported in reflex syncope (70), and the supine concentration was higher in cardioinhibitory VVS compared with vasodepressor VVS (98).

Mid-regional-fragment of pro-adrenomedullin

Adrenomedullin (ADM) is an ubiquitous peptide hormone and the highest concentrations are found in the adrenal medulla, atria of the heart and lungs (99). It is secreted in response to systemic volume overload and has vasodilatory and natriuretic effects. The vasodilatory effect is mainly due to paracrine signalling in endothelial and vascular smooth muscle cells (100). Adrenomedullin prevents vascular leakage and oedema by maintaining the barrier function of the endothelium (101). Patients with VVS had lower supine levels of ADM compared with HUT-negative controls and lower concentrations predicted cardioinhibitory VVS (69).

Aims

The general aim of this thesis was to investigate the pathophysiological mechanisms involved in vasovagal syncope and orthostatic hypotension with focus on cardiovascular biomarkers, in addition to how history and clinical characteristics are associated with the outcome of syncope evaluation in a specialized syncope unit.

The specific aims of the papers included in this thesis are as follows:

I. to study supine and early orthostatic levels of neurohormones involved in regulation of circulatory homeostasis in relation to the time to onset of head-up tilt-induced vasovagal syncope

II. to compare classical and delayed orthostatic hypotension in terms of clinical characteristics, haemodynamic values and cardiovascular neurohormones

III. to assess the influence of age at first syncope on clinical characteristics and final head-up tilt diagnosis

IV. to investigate the predictive value of history and clinical characteristics for unexplained syncope after cardiovascular autonomic testing and characterize the group with negative results

Methods

Study population: The SYSTEMA cohort

The SYSTEMA (Syncope Study of Unselected Population in Malmö) cohort included patients with severe orthostatic intolerance and unexplained syncope that were investigated in the tertiary syncope unit at Skåne University Hospital in Malmö from August 2008 to May 2021. Unexplained syncope was defined as syncope without an established diagnosis after initial evaluation according to the European Society of Cardiology syncope guidelines (20). Patients were referred from primary care and hospitals in southern Sweden and prior to referral, additional tests were carried out at the discretion of the referring physician, including Holter ECG, echocardiography, exercise test, myocardial perfusion scintigraphy or coronary angiography, brain imaging and electroencephalography. Patients with confirmed non-syncopal loss of consciousness and cardiac syncope were not investigated in the syncope unit.

Cardiovascular autonomic testing

Patients were instructed to fast for 2 hours before the test, but were allowed to take their regular medications and drink water. They completed a questionnaire on medical history and characteristics of syncope-related symptoms. Cardiovascular autonomic testing included Valsalva manoeuvre, carotid sinus massage (CSM) in supine and standing positions (in patients aged >40 years), active standing and HUT according to the Italian protocol (63), i.e. HUT for 20 minutes or until syncope occurred, and if this phase was negative, the addition of 400 μg of sublingual nitroglycerine for another 15 minutes. Electrocardiogram and beat-to-beat blood pressure were continuously monitored using a validated non-invasive photoplethysmographic method (102, 103).

In a subset of patients (during years 2008-2014), blood samples were collected for the analysis of cardiovascular biomarkers. An intravenous line was placed in the antecubital fossa 10 minutes before the test and blood samples were collected in the supine position and after 3 minutes of HUT. Studies (104) have shown that after 3 minutes of orthostasis, the displacement of central blood volume to the lower body, heart rate and total peripheral resistance reach a steady state, indicating that the cardiovascular

neurohormonal responses are fully developed and therefore this time point was chosen for blood sampling. The regional ethical review board in Lund, Sweden approved the study protocol (reference no 82/2008). All study participants gave written informed consent.

Diagnostic criteria

Vasovagal syncope	a typical pattern of sudden-onset hypotension and bradycardia leading to syncope with reproduction of the patient's symptoms (20).
Classical orthostatic hypotension	a sustained decrease in SBP ≥20 mmHg or DBP ≥10 mmHg during the first 3 minutes of being upright (20).
Delayed orthostatic hypotension	a sustained decrease in SBP/DBP ≥20/10mmHg occurring first after 3 minutes of being upright without bradycardia (105).
Immediate orthostatic hypotension	a rapid (within 15 seconds of standing) but transient decrease in SBP/DBP ≥40/20 mmHg (105).
Carotid sinus syndrome	a fall in SBP ≥50 mmHg and/or ventricular pause of ≥3 seconds on CSM, with reproduction of the patient's symptoms or syncope (20).
Complex syncope	detecting ≥2 concomitant diagnoses (CSS, VVS or OH) after CSM and HUT which could contribute to syncope episodes.
Postural orthostatic tachycardia syndrome	a sustained heart rate increase of ≥30 bpm (≥40 bpm in patients aged 12-19 years) or to >120 bpm within 10 minutes of standing, with no significant fall in blood pressure and a history of orthostatic intolerance for >3 months (57).
Psychogenic pseudosyncope	an apparent loss of consciousness during HUT with no decrease or modest increase in blood pressure and heart rate and characteristic features such as closed eyes and long duration of loss of consciousness (106, 107).
Negative CAT/HUT	a normal haemodynamic response to Valsalva manoeuvre, active standing, CSM and HUT.

Study design

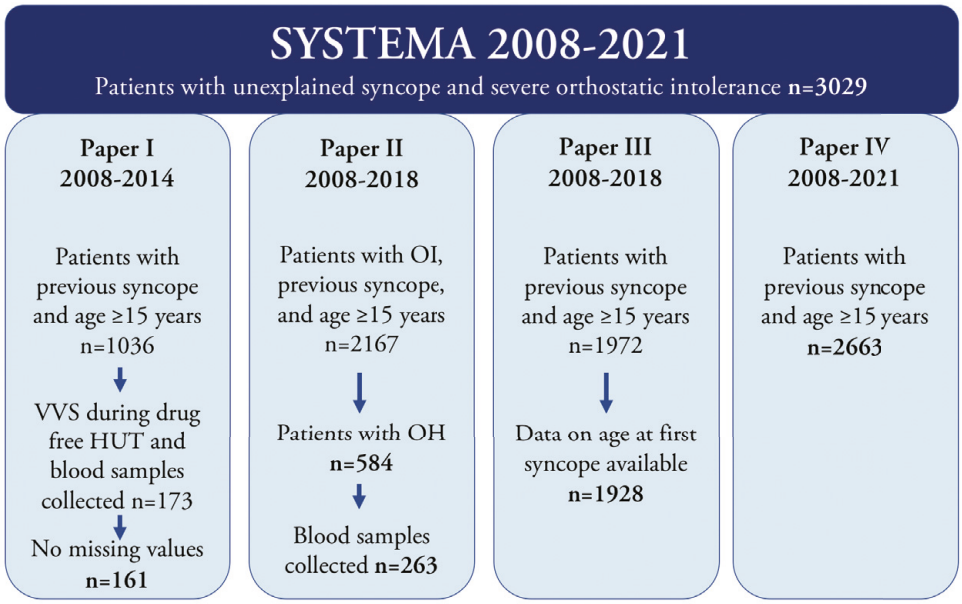

Figure 5. Flowchart of inclusion of patients in papers I-IV. Abbreviations: VVS, vasovagal syncope; HUT, head-up tilt; OI, orthostatic intolerance; OH, orthostatic hypotension.

Paper I

Between 2008 and 2014, 1141 consecutive patients were enrolled in SYSTEMA. Of these, 1036 patients were aged ≥15 years and had unexplained syncope after initial evaluation. Among these, 827 patients had blood samples collected in supine position and after 3 minutes of HUT. Of these, 173 patients developed VVS during the drug-free phase of HUT; 161 patients had no missing values and were included in the analysis. Ten patients developed VVS before 3 minutes of HUT and were excluded from the analyses of orthostatic change in concentrations of cardiovascular biomarkers.

Paper II

Between 2008 and 2018, 2167 consecutive patients aged ≥15 years with unexplained syncope and severe orthostatic intolerance were recruited from SYSTEMA. Of these 548 were diagnosed with orthostatic hypotension (248 with cOH and 336 with dOH) and were included in analysis of clinical characteristics and 263 (111 with cOH and 152 with dOH) had blood samples collected in supine position and after 3 minutes of HUT and were included in analysis of cardiovascular biomarkers.

Paper III

From 2008-2018, 1972 consecutive patients aged ≥15 years with unexplained syncope were recruited from SYSTEMA. Data on age at first syncope was available for 1928 patients and these were included in the analysis.

Paper IV

From 2008-2021, 2663 consecutive patients aged ≥15 years with unexplained syncope were recruited from SYSTEMA and were included in the analysis.

Biomarker analysis

Blood samples were collected in supine position and after 3 minutes of HUT. The concentrations of epinephrine and norepinephrine were measured using high-performance liquid chromatography with fluorescence detection method (108) in the hospital laboratory (Skåne University hospital). For CT-proAVP, CT-proET-1, MR-proANP and MR-proADM, samples were frozen at -80°C and shipped to the manufacturer for analysis, using ThermoScientific BRAHMS assays (BRAHMS GmbH, ThermoFisher Scientific, Hennigsdorf, Germany) (109, 110).

Statistical analysis

The main characteristics of the study population are presented as mean ± standard deviation for normally distributed continuous variables, as median [interquartile range] for non-parametric variables and percentages for categorical variables. All tests were 2-sided and $p<0.05$ was considered significant, unless otherwise stated. Statistical analyses were carried out using IBM SPSS Statistics (SPSS Inc., Chicago, IL, USA) version 25 (Paper I and II), version 27 (paper III) and version 28 (paper IV).

Paper I

A linear regression model adjusted for age and sex was applied; time to syncope was entered as the dependent variable and neurohormone concentration at supine rest (baseline), after 3 minutes of HUT and Δvalue (3 minutes of HUT minus baseline) as the independent variables. Concentrations of neurohormones were non-normally distributed and hence log-transformed and standardized (expressed per 1 standard deviation). The normality assumption of residuals for each linear model was checked by histograms and normal quantile-quantile plots of residuals; the plots showed no

violation of the normality assumption. Log-transformed levels of neurohormones were compared according to tertiles of the time to syncope variable using Kruskal Wallis test.

Paper II

Intergroup differences were analysed using analysis of variance test (ANOVA) for continuous variables and Pearson's chi-squared test for categorical variables. Concentrations of neurohormones were log-transformed and standardized (expressed per 1 standard deviation). A logistic regression model adjusted for age and sex was applied to test intergroup differences in neurohormone levels.

Paper III

To determine the distribution of age at first syncope, a histogram was plotted (Figure 10). The study population was stratified into three age groups; <30, 30–59 and ≥60 years, based on the shape of the age distribution plot. Clinical variables and HUT diagnoses were analysed in relation to age at first syncope and age at investigation. For intergroup comparisons, Pearson's chi-squared test was used for categorical variables and ANOVA and Fisher's least significance difference test were used for continuous variables. To explore the effect of age at first syncope treated as a continuous variable on the final diagnosis in patients aged ≥60 years at examination, a logistic regression model was applied by entering age at first syncope as the independent variable and final HUT diagnosis as the dependent variable. All tests were two-sided and $P<0.05$ was considered significant, except for intergroup comparisons (n=3) where Bonferroni correction was used and the significance level was set at $p=0.017$.

Paper IV

For intergroup comparisons, Pearson's chi-square test was used for categorical variables and ANOVA, Kruskal Wallis test, the Student's T-test and Mann-Whitney U test were used for continuous variables as appropriate. A logistic regression model adjusted for age and sex was applied by entering clinical characteristics as the independent variable and negative CAT as the dependent variable.

Results

Paper I

One hundred and sixty-one patients developed VVS during the drug-free phase of HUT (56% women, age 45±21 years), with a mean time to syncope of 11±7 minutes. The clinical characteristics of the study population are presented in Table 1 and plasma concentrations of neurohormones are shown in Table 2.

Predictors of time to syncope

Older age (β=0.13; SE=0.03, p<0.001), higher supine systolic blood pressure (β=0.06; SE=0.03, p=0.02) and higher supine concentration of MR-proADM (β=2.31; SE=0.77, p=0.003) predicted longer time to syncope, whereas other neurohormones measured in supine position were not associated with time to syncope. After 3 minutes of HUT, higher concentrations of epinephrine (β=–2.13; SE=0.59, p<0.001) and CT-proAVP (β=–1.39; SE=0.68, p=0.043) were predictors of shorter time to syncope, whereas higher MR-proADM (β=1.76; SE=0.82, p=0.035) predicted longer time to syncope. Higher Δepinephrine (i.e. 3-minute HUT minus supine concentration, β=–3.24; SE=0.78, p<0.001) and ΔCT-proAVP (β=–2.07; SE=0.61, p=0.001) were predictors of shorter time to syncope (Table 3). Figure 6 shows the plasma concentration of epinephrine at 3 minutes of HUT in relation to time to syncope.

Figures 7-9 show the plasma concentrations of supine MR-proADM, Δepinephrine, and ΔCT-proAVP grouped according to tertiles of time to syncope. The concentrations were significantly different across tertiles of the time to syncope variable.

Table 1. Clinical characteristics of the study population (n=161).

Characteristic	
Age	45±21
Male sex, n(%)	72 (45)
Body mass index (kg/m2)	24.7±4.1
No. of syncope episodes, median [IQR]	5 [2-15]
Traumatic falls without warning, n(%)	84 (52)
Time to syncope, minutes	11±7
Supine SBP	129±20
Supine DBP	70±9
SBP at 3 min HUT	121±22
DBP at 3 min HUT	73±12
Supine HR	67±11
HR at 3 min HUT	83±17
Use of beta-blockers (n, %)	17 (11)
Use of calcium channel-blockers, n (%)	12 (8)
Use of RAAS antagonists, n (%)	24 (15)
Use of diuretics, n (%)	18 (11)

SBP, systolic blood pressure; DBP, diastolic blood pressure; HUT, head-up tilt; HR, heart rate, RAAS, Renin-angiotensin-aldosterone-system. Data are presented as mean±SD unless otherwise indicated.

Table 2. Plasma concentrations of neurohormones.

Neurohormone	Concentration
Epinephrine (0) (nm/L)	0.12 [0.12]
Epinephrine (3) (nm/L)	0.22 [0.28]
Norepinephrine (0) (nm/L)	1.60 [1.30]
Norepinephrine (3) (nm/L)	2.75 [1.88]
CT-proAVP(0) (pm/L)	6.01 [6.45]
CT-proAVP (3) (pm/L)	6.81 [10.5]
CT-proET-1 (0) (pm/L)	48.0 [19.5]
CT-proET-1 (3) (pm/L)	43.6 [22.2]
MR-proANP (0) (pm/L)	51.4 [44.0]
MR-proANP (3) (pm/L)	51.5 [44.9]
MR-proADM (0) (pm/L)	0.47 [0.21]
MR-proADM (3) (pm/L)	0.42 [0.23]

Plasma concentrations of neurohormones are given as median [interquartile range] for supine (0) and 3 minutes of HUT (3). CT-proAVP, C-terminal-pro-arginine-vasopressin; CT-proET-1, C-terminal-pro-endothelin-1; MR-proANP, mid-regional-fragment of pro atrial-natriuretic-peptide; MR-proADM, mid-regional-fragment of pro-adrenomedullin.

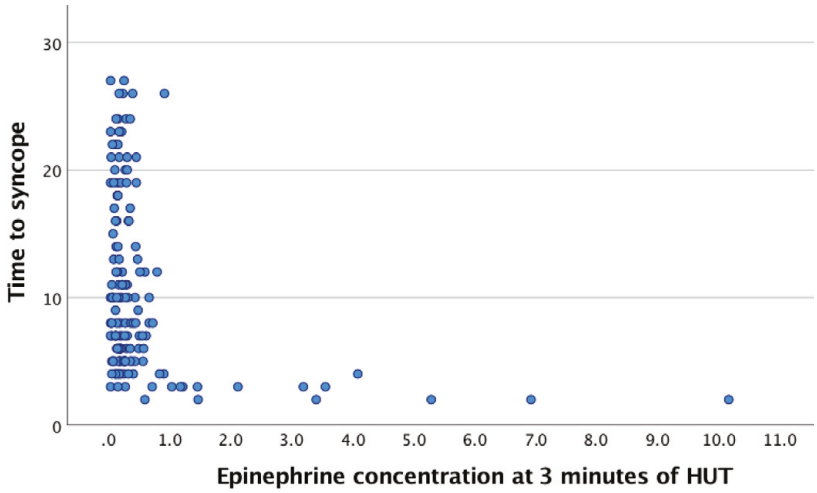

Figure 6. The plasma concentration (nm/L) of epinephrine at 3 minutes of HUT in relation to time to syncope (minutes).

Table 3. Association between neurohormone concentration and time to syncope in a linear regression model adjusted for age and sex reported as β-coefficient and standard error per one standard deviation.

Neurohormone	Supine n=161	P-value	3-min HUT n=151	P-value	Δ value n=151	P-value
Epinephrine	-0.71 (0.59)	0.23	-2.13 (0.59)	<0.001	-3.24 (0.78)	<0.001
Norepinephrine	0.15 (0.65)	0.82	0.97 (0.66)	0.14	2.49 (1.83)	0.18
CT-proAVP	0.46 (0.61)	0.45	-1.39 (0.68)	0.043	-2.07 (0.61)	0.001
CT-proET-1	0.96 (0.66)	0.15	0.70 (0.58)	0.23	0.57 (0.84)	0.50
MR-proANP	0.50 (0.77)	0.52	-0.14 (0.71)	0.84	-0.33 (2.25)	0.88
MR-proADM	2.31 (0.77)	0.003	1.76 (0.82)	0.035	1.83 (1.62)	0.26

CT-proAVP, C-terminal-pro-arginine-vasopressin; CT-proET-1, C-terminal-pro-endothelin-1; HUT, head-up tilt test; MR-proANP, mid-regional-fragment of pro atrial-natriuretic-peptide; MR-proADM, mid-regional-fragment of pro-adrenomedullin.

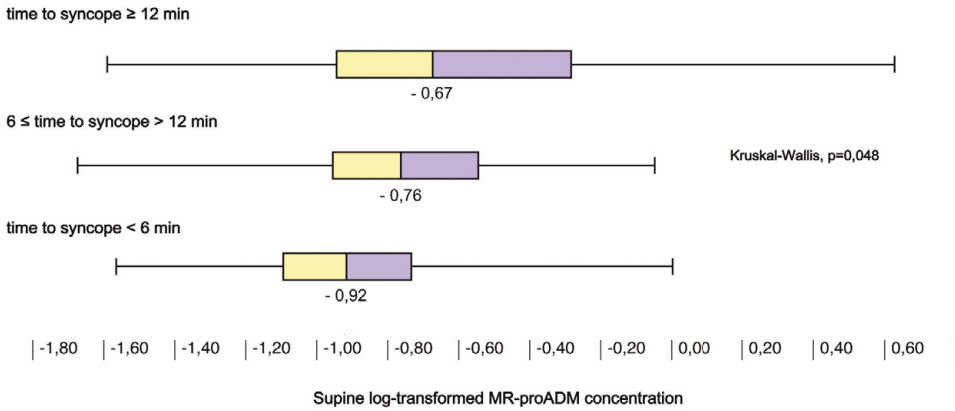

Figure 7. Supine concentration of mid-regional fragment of pro-adrenomedullin (MR-proADM) grouped by tertiles of time to syncope. Higher concentration of MR- proADM was associated with longer time to VVS. Reprinted from Paper I, Journal of the American Heart Association, open access under CC BY-NC-ND

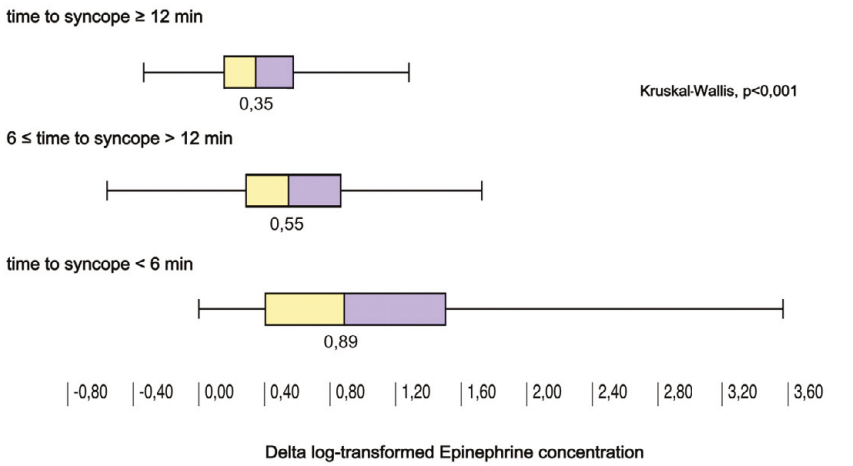

Figure 8. The increase in epinephrine concentration after 3 minutes of HUT grouped by tertiles of time to syncope. Greater increase in epinephrine was associated with shorter time to VVS. Reprinted from Paper I, Journal of the American Heart Association, open access under CC BY-NC-ND.

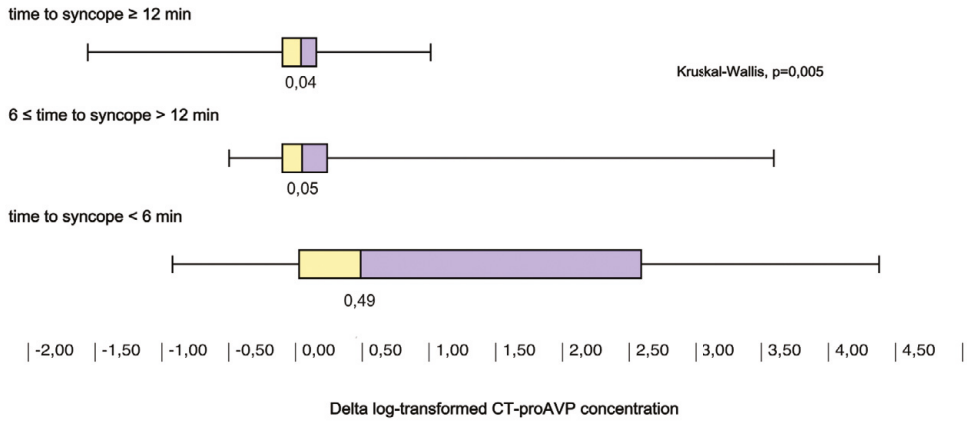

Figure 9. The increase in C-terminal pro–arginine vasopressin (CT-proAVP) concentration after 3 minutes of HUT grouped by tertiles of time to syncope. Greater increase in CT-proAVP was associated with shorter time to VVS. Reprinted from Paper I, Journal of the American Heart Association, open access under CC BY-NC-ND.

Paper II

Clinical characteristics

The clinical characteristics of the study population are presented in Table 4. Compared with dOH, cOH-patients were older (68 vs. 60 years, p<0.001), more likely men (57 vs. 40%, p<0.001) and had a lower proportion of palpitations prior to syncope (16 vs. 29%, p=0.001). Moreover, patients with cOH showed a tendency towards higher supine blood pressure (141 vs. 137 mmHg, p=0.051), had a larger fall in SBP (lowest value 88 vs. 99 mmHg, p<0.001) and DBP (56 vs. 63 mmHg, p<0.001) during HUT and a higher proportion of pathologic Valsalva manoeuvre (43 vs. 18%, p<0.001). The cOH group also had lower estimated glomerular filtration rate (73 vs. 80ml/min/1.73m^2, p=0.003), higher proportions of pacemaker treatment (5 vs. 2%, p=0.04) and Parkinson's disease (5 vs. 1%, p=0.008) compared with dOH. However, cOH and dOH groups did not differ in reported falls, previous syncope, history of atrial fibrillation, heart failure, coronary artery disease or use of antihypertensive drugs.

Neurohormone concentrations

Plasma concentrations of neurohormones are shown in Table 5. Compared to dOH, cOH patients had higher supine and 3 minutes of HUT concentrations of CT-proAVP (p=0.022 and p<0.001 respectively). At 3 minutes of HUT, norepinephrine was higher in dOH patients (p=0.001). Furthermore, Δepinephrine (i.e. 3 minutes of HUT minus

supine concentration; p<0.001) and ΔCT-proAVP (p=0.001) were higher in cOH, whereas Δnorepinephrine was higher in dOH (p=0.045). However, plasma concentrations of CT-proET-1, MR-proANP and MR-proADM were not significantly different between cOH and dOH patients.

Table 4. Clinical characteristics of the study population.

Characteristic	All n=584	Classical OH n=248	Delayed OH n=336	P-value
Age	64±18	68±14	60±20	<0.001
Male sex, n (%)	274 (47)	141 (57)	133 (40)	<0.001
Height (cm)	172±10	173±10	171±10	0.004
Body mass index (kg/m^2)	25±4	25±4	26±5	0.121
Palpitations before syncope, n (%)	110 (23)	31 (16)	79 (29)	0.001
History of orthostatic dizziness, n (%)	439 (76)	183 (74)	256 (77)	0.439
History of syncope, n (%)	527 (90)	220 (89)	307 (91)	0.270
Nr of syncope episodes, median [IQR]	4 [2-10]	4 [2-8]	4 [2-10]	0.160
History of falls, n (%)	319 (55)	137 (56)	182 (55)	0.502
Supine SBP	139±24	141±26	137±22	0.051
Supine DBP	74±12	75±12	73±11	0.155
Supine HR	70±12	69±12	70±12	0.272
Lowest SBP during HUT	95±22	88±22	99±20	<0.001
Lowest DBP during HUT	60±13	56±13	63±12	<0.001
Max HR during HUT	83±16	82±17	85±16	0.069
Pathologic Valsalva manoeuvre, n (%)	135(29)	86(43)	49(18)	<0.001
Estimated GFR (mL/min/1.73m^2)	77±22	73±21	80±22	0.003
Reduced ejection fraction, n (%)	119 (21)	42 (18)	77 (24)	0.068
Atrial fibrillation, n (%)	76 (13)	29 (12)	47 (14)	0.437
Coronary artery disease, n (%)	62(11)	28(11)	34(10)	0.649
Pacemaker therapy, n (%)	20 (3)	13 (5)	7 (2)	0.04
Parkinson's disease, n (%)	16 (3)	12 (5)	4 (1)	0.008
Diabetes, n (%)	59 (10)	25 (10)	34 (10)	0.969
Use of betablockers, n (%)	153 (26)	62 (25)	91 (27)	0.519
Use of calcium channel blockers,n (%)	96 (17)	38 (15)	58 (18)	0.508
Use of RAAS-antagonists, n (%)	95 (16)	44 (18)	51 (15)	0.450
Use of loop-diuretics, n (%)	63 (11)	32 (13)	31 (9)	0.171
Use of alpha-blockers, n (%)	27 (5)	16 (6)	11 (3)	0.075

OH, orthostatic hypotension; IQR, interquartile range; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; HUT, head-up tilt; GFR, glomerular filtration rate; RAAS, renin-angiotensin-aldosterone system. Data are presented as mean ± SD unless indicated otherwise.

Table 5. Supine and orthostatic neurohormone concentrations in classical and delayed OH.

Hormone	All n=263	Classical OH n=111	Delayed OH n=152	P-value*
Epinephrine (0) (nmol/L)	0.15 [0.09-0.24]	0.14 [0.09-0.21]	0.15 [0.09-0.24]	0.954
Epinephrine (3) (nmol/L)	0.22 [0.08-0.35]	0.22 [0.13-0.44]	0.22 [0.13-0.32]	0.128
Norepinephrine (0) (nmol/L)	2.30 [1.60-3.10]	2.20 [1.50-3.10]	2.40 [1.70-3.20]	0.263
Norepinephrine (3) (nmol/L)	3.40 [1.80-4.60]	3.10 [1.90-4.70]	3.60 [2.63-4.56]	0.001
CT-proAVP (0) (pmol/L)	8.18 [4.53-14.4]	9.42 [6.01-16.3]	7.30 [3.94-12.3]	0.022
CT-proAVP (3) (pmol/L)	8.38 [4.57-16.1]	10.5 [6.24-22.5]	6.55 [3.77-12.4]	<0.001
CT-proET-1(0) (pmol/L)	61.8 [50.8-74.2]	63.0 [52.2-74.6]	61.1 [49.9-70.8]	0.406
CT-proET-1 (3) (pmol/L)	56.0 [43.2-71.1]	58.8 [44.5-72.5]	55.0 [41.9-67.4]	0.908
MR-proANP (0) (pmol/L)	109 [65.9-161]	109 [67.3-150]	109 [61.3-168]	0.940
MR-proANP (3) (pmol/L)	109 [61.6-152]	111 [64.6-150]	108 [58.3-173]	0.758
MR-proADM (0) (pmol/L)	0.68 [0.50-0.91]	0.74 [0.50-1.06]	0.63 [0.50-0.85]	0.132
MR-proADM (3) (pmol/L)	0.59 [0.42-0.83]	0.64 [0.44-1.00]	0.56 [0.40-0.81]	0.233

Concentrations given as median [interquartile range] for supine (0) and 3 minutes of HUT (3). CT-proAVP, C-terminal-pro-arginine-vasopressin; CT-proET, C-terminal-pro-endothelin-1; MR-proANP, mid-regional-fragment of pro-atrial-natriuretic-peptide and MR-proADM, mid-regional-fragment of pro-adrenomedullin. *P-values for log-transformed concentrations.

Paper III

The distribution of age at first syncope showed a bimodal pattern, with a maximum peak at 15 years and a smaller peak at 70 years (Figure 10). The distribution of age at examination was also bimodal, with a maximum peak at 75 years and a smaller peak at 20 years (Supplementary material online to paper III). The proportion of HUT diagnoses stratified according to age at first syncope group are shown in Figure 11 (the entire study population) and Figure 12 (patients aged ≥60 years).

Table 6 shows clinical characteristics and diagnoses after cardiovascular autonomic testing in 1928 patients, stratified according to age at first syncope-group. Compared with late-onset (≥60 years) syncope, patients with early-onset (<30 years) reported a higher proportion of prodromes (64 vs. 26%, p<0.001) and palpitations (41 vs. 15%, p<0.001) and had a higher frequency of VVS (59 vs. 19%, p<0.001) and PPS (4 vs. 0.2%, p<0.001). In the late-onset group, orthostatic hypotension (23 vs. 3%, p<0.001), CSH/CSS (9 vs. 0.6%, p<0.001) and complex syncope (26 vs. 14%, p<0.001) were more common. However, the proportion of no definitive diagnosis after HUT was not different between early-onset and late-onset syncope patients.

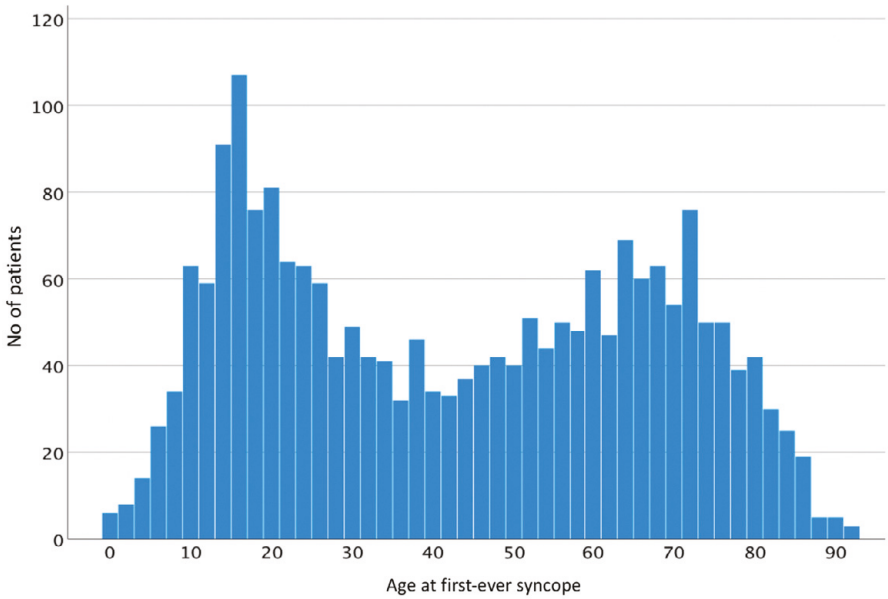

Figure 10. The age distribution of first syncope in 1928 patients. Reprinted from paper III, European Heart Journal, open access under CC BY-NC.

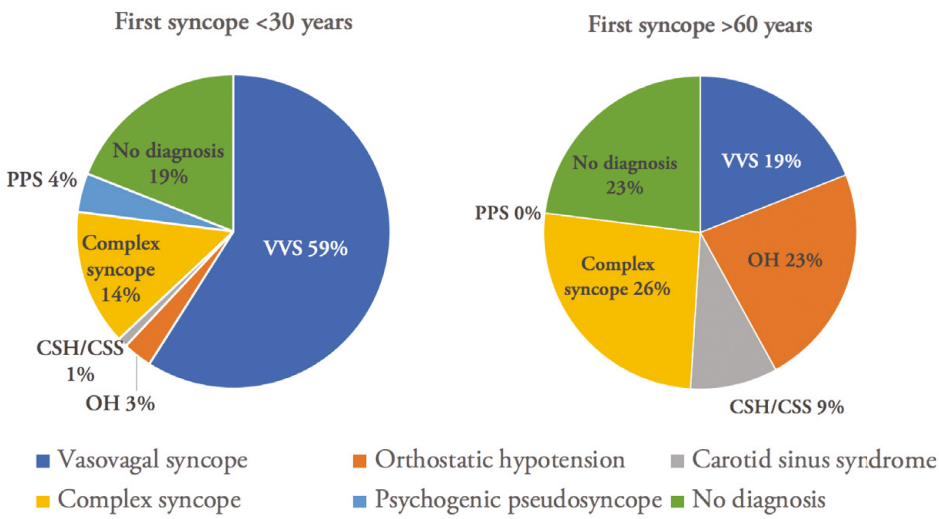

Figure 11. Percentage of head-up tilt diagnoses in 1928 syncope patients. Adapted from paper III, European Heart Journal, open access under CC BY-NC.

Table 6. Clinical features and HUT diagnoses of 1928 syncope patients according to age at first syncope group.

Clinical features	First syncope <30 years n=773	First syncope 30-59 years n=570	First syncope ≥60 years n=585	P-value <30 vs 30-59 years	P-value <30 vs ≥60 years	P-value 30-59 vs ≥60 years
Age at examination, median [IQR]	28 [22-43]	52 [44-60]	74 [69-80]	<0.001	<0.001	<0.001
Female sex, n (%)	578 (75)	324 (57)	276 (47)	<0.001	p<0.001	p<0.001
Years between first syncope and investigation, median [IQR]	10 [2-30]	3 [1-10]	2 [1-5]	<0.001	p<0.001	<0.001
No. of syncope episodes, median [IQR]	7 [3-20]	4 [2-10]	3 [2-6]	<0.001	p<0.001	0.136
Supine SBP	124±17	133±20	144±23	<0.001	<0.001	<0.001
Supine DBP	71±10	75±11	74±12	<0.001	<0.001	0.209
Supine HR	70±12	70±12	70±12	0.519	0.996	0.542
Prodromes, n (%)	496 (64)	269 (47)	149 (26)	<0.001	<0.001	p<0.001
Palpitations, n (%)	290 (41)	166 (34)	71 (15)	0.001	<0.001	<0.001
Dizziness upon standing, n (%)	590 (76)	380 (67)	398 (68)	<0.001	0.001	0.562
Hypertension, n (%)	59 (8)	145 (26)	341 (59)	<0.001	<0.001	<0.001
Coronary artery disease, n (%)	13 (2)	29 (5)	79 (14)	<0.001	<0.001	<0.001
VVS, n (%)	455 (59)	251 (44)	108 (19)	<0.001	<0.001	<0.001
OH, n (%)	24 (3)	54 (10)	133 (23)	<0.001	<0.001	<0.001
CSS/CSH, n (%)	5 (0.6)	14 (2.5)	54 (9)	0.006	<0.001	<0.001
Complex syncope, n (%)	106 (14)	97 (17)	152 (26)	0.096	<0.001	<0.001
PPS, n (%)	33 (4)	9 (2)	1 (0.2)	0.005	<0.001	0.010
No HUT diagnosis, n (%)	148 (19)	144 (25)	135 (23)	0.007	0.072	0.404

SBP= systolic blood pressure, DBP=diastolic blood pressure, HR= heart rate, VVS=vasovagal syncope, OH=orthostatic hypotension, CSH/CSS= carotid sinus hypersensitivity/syndrome, PPS= psychogenic pseudosyncope. Data are presented as mean ± SD unless indicated otherwise. The Bonferroni-adjusted significance level is set at p=0.017.

In a subgroup of 836 patients aged ≥60 years at the investigation (Table 7), 12% reported having early-onset syncope whereas 70% reported late-onset syncope. Prodromes (52 vs. 26%, p<0.001), VVS (39 vs. 19% p<0.001) and complex syncope (37 vs. 26%, p=0.023) were more common in in early-onset compared with late-onset syncope. In the late-onset group, OH (23 vs. 7%, p<0.001) and hypertension (59 vs. 40%, p=0.001) were more common compared with early-onset syncope. In patients aged ≥60 years with complex syncope, 85% had two concomitant diagnoses and 15% had 3 diagnoses. The most common combination was VVS and OH.

In patients aged ≥60 years (Table 8), older age at first syncope was a significant predictor of OH (+31% higher odds per 10-year difference, p<0.001) and CSH/CSS (+26%, p=0.004). Conversely, younger age at first syncope predicted the presence of prodromes (+23%, p<0.001), VVS (+22%, p<0.001) and complex syncope (+9%, p=0.018).

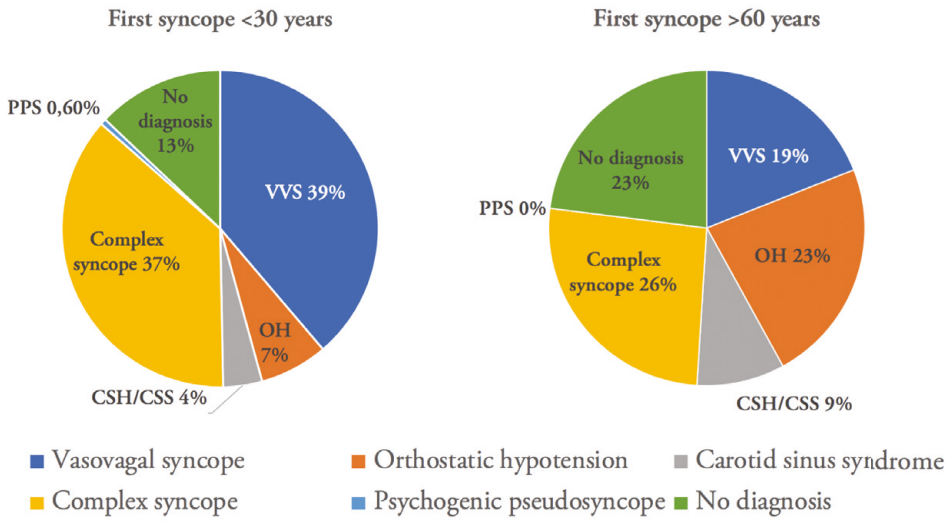

Figure 12. Percentage of head-up tilt diagnoses in 836 syncope patients aged ≥60 years. Adapted from paper III, European Heart Journal, open access under CC BY-NC.

Table 7. Clinical features and HUT diagnoses of 836 patients examined at ≥60 years according to age at first syncope group.

Clinical features	First syncope <30 years n=100	First syncope 30-59 years n=151	First syncope ≥60 years n=585	P-value <30 vs 30-59 years	P-value <30 vs ≥60 years	P-value 30-59 vs ≥60 years
Female sex, n (%)	71 (71)	83 (55)	276 (47)	0.011	<0.001	0.088
Age at investigation, median [IQR]	70 [65-75]	64 [61-70]	74 [69-80]	<0.001	<0.001	<0.001
Years between first syncope and investigation, median [IQR]	54 [46-60]	11 [5-20]	2 [1-5]	<0.001	<0.001	<0.001
No. of syncope episodes, median [IQR]	8 [3-20]	6 [3-10]	3 [2-6]	0.001	<0.001	0.003
Supine SBP	145±19	141±22	144±23	0.256	0.910	0.143
Supine DBP	77±11	75±12	74±12	0.316	0.026	0.222
Supine HR	70±12	70±12	70±12	0.804	0.689	0.902
Prodromes, n (%)	51 (52)	52 (34)	149 (26)	0.020	<0.001	0.113
Palpitations, n (%)	13 (13)	28 (19)	71 (12)	0.146	0.186	0.136
Dizziness upon standing, n (%)	69 (69)	98 (65)	398 (68)	0.500	0.884	0.431
Hypertension, n (%)	40 (40)	70 (47)	341 (59)	0.307	0.001	0.009
Coronary artery disease, n (%)	8 (8)	15 (10)	79 (14)	0.609	0.129	0.241
Heart failure, n (%)	2 (2)	8 (5)	50 (9)	0.198	0.024	0.186
Atrial fibrillation, n (%)	9 (9)	13 (9)	113 (20)	0.921	0.013	0.002
VVS, n (%)	39 (39)	47 (31)	108 (19)	0.198	<0.001	0.001
OH, n (%)	7 (7)	21 (14)	133 (23)	0.089	<0.001	0.017
CSS/CSH, n (%)	4 (4)	8 (5)	54 (9)	0.637	0.082	0.120
Complex syncope, n (%)	37 (37)	45 (30)	152 (26)	0.234	0.023	0.351
PPS, n (%)	0 (0)	1 (1)	1 (0.2)	0.415	0.697	0.302
No HUT diagnosis, n (%)	13 (13)	29 (19)	135 (23)	0.197	0.023	0.299

SBP= systolic blood pressure, DBP=diastolic blood pressure, HR= heart rate, VVS=vasovagal syncope, OH=orthostatic hypotension, CSH/CSS= carotid sinus hypersensitivity/syndrome, PPS= psychogenic pseudosyncope. Data are presented as mean ± SD unless indicated otherwise. The Bonferroni-adjusted significance level is set at p=0.017.

Table 8. Association between age at first syncope and cardiovascular autonomic testing in 836 unexplained syncope patients aged ≥60 years.

	Odds ratio*	95% CI	P-value
Vasovagal syncope	0.78	0.71-0.86	<0.001
Orthostatic hypotension	1.31	1.18-1.43	<0.001
Carotid sinus hypersensitivity/syndrome	1.26	1.08-1.45	0.004
Complex syncope	0.91	0.84-0.98	0.018
Psychogenic pseudosyncope	0.89	0.29-1.53	0.730
No diagnosis	1.09	0.99-1.18	0.069
Prodromes	0.77	0.69-0.85	<0.001

*Odds ratios are presented per 10-year increment of first-ever syncope age.

Paper IV

Cardiovascular autonomic testing was performed in 2663 patients with unexplained syncope after initial evaluation, leading to a diagnosis in 79% of cases, including VVS (42%), complex syncope (16%), OH (10%), POTS and CSS (4% each), PPS (2%) and iOH (1%). In 21% of patients CAT showed normal hemodynamic values and no diagnosis could be made (Figure 13). Table 9 shows the clinical characteristics and haemodynamic values stratified according to CAT diagnosis group.

Figure 14 shows the distribution of age at examination in the CAT diagnosis groups. Vasovagal syncope (as a single diagnosis) had the highest prevalence at age 20 years that decreased with older age. Complex syncope had a bimodal age distribution with the highest peak at ages 70-75 years and a smaller peak at ages 20-25 years. The prevalence of OH increased with age, reaching a peak at age 70 years and the prevalence of CSS showed a similar pattern, with a peak at age 75 years. Postural orthostatic tachycardia syndrome and PPS had the highest prevalence at age 20 years. Immediate OH showed an even prevalence across ages 20-60 years that decreased with older age. Negative CAT showed an even prevalence in age groups 20-50 years and a slightly higher prevalence at ages 60-70 years.

Predictors of negative CAT

Older age at first syncope and at examination, higher systolic blood pressure and heart rate, absence of prodromal symptoms and a history of diabetes, hypertension, atrial fibrillation, heart failure and coronary artery disease were univariate predictors of negative CAT (Table 10). After adjusting for age and sex (Table 11); older age at first syncope (+8% higher odds per 10-year increase, p=0.042), higher baseline heart rate (+12% per 10 beats-per-minute, p=0.003), absence of prodromal symptoms (+48%, p<0.001) and a history of hypertension (+45%, p=0.003), diabetes (+82%, p<0.001),

heart failure (+98%, p=0.014) and coronary artery disease (+51%, p=0.027) were predictors of negative CAT.

Table 12 shows clinical characteristics and haemodynamic values in VVS patients compared with negative CAT. Patients with negative CAT were older (median age 54 vs 40 years, p<0.001), had higher baseline blood pressure and heart rate, reported fewer previous syncope episodes, had a lower proportion of prodromal symptoms (44% vs 59%, p<0.001) and a higher proportion of diabetes and cardiovascular disease (hypertension, heart failure, atrial fibrillation and coronary artery disease). Syncope with traumatic injuries tended to be more frequent in the negative CAT group.

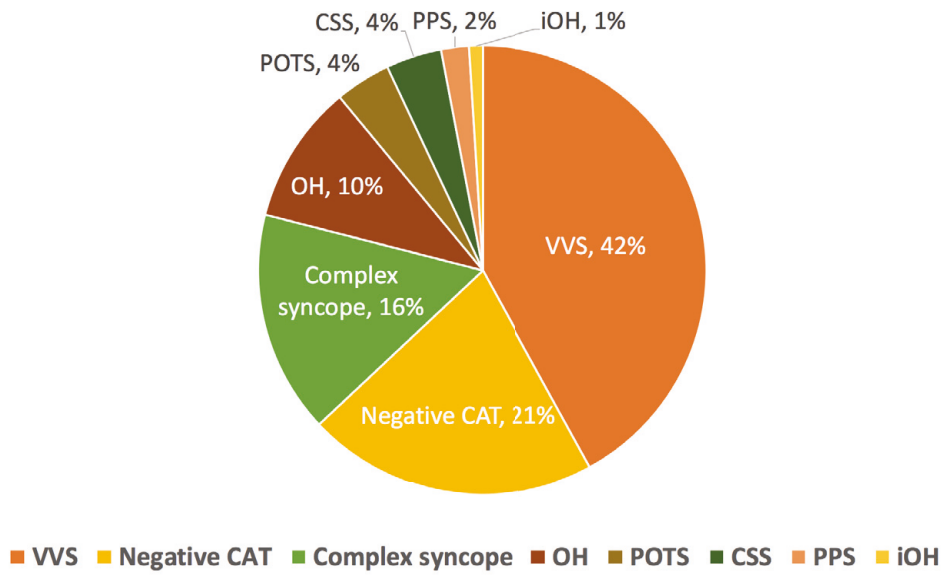

Figure 13. The proportion of CAT diagnoses in 2663 patients with unexplained syncope.

Table 9. Clinical characteristics and haemodynamic values in 2663 patients with unexplained syncope according to final CAT diagnosis group.

Clinical features	VVS n= 1111 (42%)	Neg CAT n=546 (21%)	Complex syncope n=440 (16%)	OH n=268 (10%)	POTS n=107 (4%)	CSS n=96 (4%)	PPS n=58 (2%)	iOH n=37 (1%)	P-value Omnibus test
Age, median [IQR]	40[26-57]	54[35-71]	67[52-75]	72[61-79]	28[22-36]	74[66-80]	31[23-42]	54[38-67]	<0.001
Female sex, n(%)	724 (65)	347 (64)	230 (52)	130 (49)	88 (82)	35 (37)	50 (86)	26 (70)	<0.001
Supine SBP	129 ±19	135±21	139±22	139 ±24	125±15	138±18	125±19	129±18	<0.001
Supine DBP	74 ±11	76 ±12	75 ±11	75 ±12	76 ±12	73 ±11	75 ±13	74 ±9	0.013
Supine HR	69±11	72±12	70±12	72±11	82±15	69±12	72±13	70±14	<0.001
No. of syncope episodes, median [IQR]	5[2-10]	4[2-10]	4[2-10]	5[2-10]	3[2-10]	4[2-10]	30[11-100]	5[2-10]	<0.001
Prodromes, n(%)	652 (59)	238 (44)	196 (45)	89 (34)	69 (65)	29 (32)	37 (64)	19 (51)	<0.001
Palpitations, n(%)	270 (24)	119 (22)	82 (19)	40 (15)	34 (32)	10 (11)	27 (47)	8 (22)	<0.001
Dizziness, n(%)	750 (68)	393 (72)	302 (69)	212 (79)	96 (90)	63 (66)	50 (86)	35 (95)	<0.001
Syncope with traumatic injuries, n(%)	574 (52)	310 (57)	216 (49)	184 (69)	52 (49)	57 (61)	40 (69)	19 (51)	<0.001
Supine syncope, n(%)	215 (19)	89 (16)	62 (14)	15 (6)	17 (16)	7 (7)	41 (71)	2 (5)	<0.001
Hypertension, n(%)	159 (14)	179 (33)	179 (41)	113 (43)	4 (4)	48 (50)	3 (5)	10 (28)	<0.001
Diabetes, n(%)	44 (4)	65 (12)	42 (10)	33 (12)	3 (3)	15 (16)	2 (3)	0 (0)	<0.001
Heart failure n(%)	13 (1)	21 (4)	7 (2)	14 (5)	1 (1)	5 (5)	0 (0)	0 (0)	<0.001
Atrial fibrillation, n(%)	40 (4)	56 (10)	36 (8)	54 (20)	0 (0)	14 (15)	0 (0)	2 (6)	<0.001
Coronary artery disease, n(%)	31 (3)	48 (9)	33 (8)	34 (13)	1 (1)	17 (18)	1 (2)	3 (8)	<0.001

CAT=cardiovascular autonomic testing, VVS=vasovagal syncope, OH=orthostatic hypotension, iOH=immediate orthostatic hypotension, CSS= carotid sinus syndrome, PPS= psychogenic pseudosyncope, SBP= systolic blood pressure, DBP=diastolic blood pressure, HR= heart rate. Data are presented as mean ± SD unless indicated otherwise.

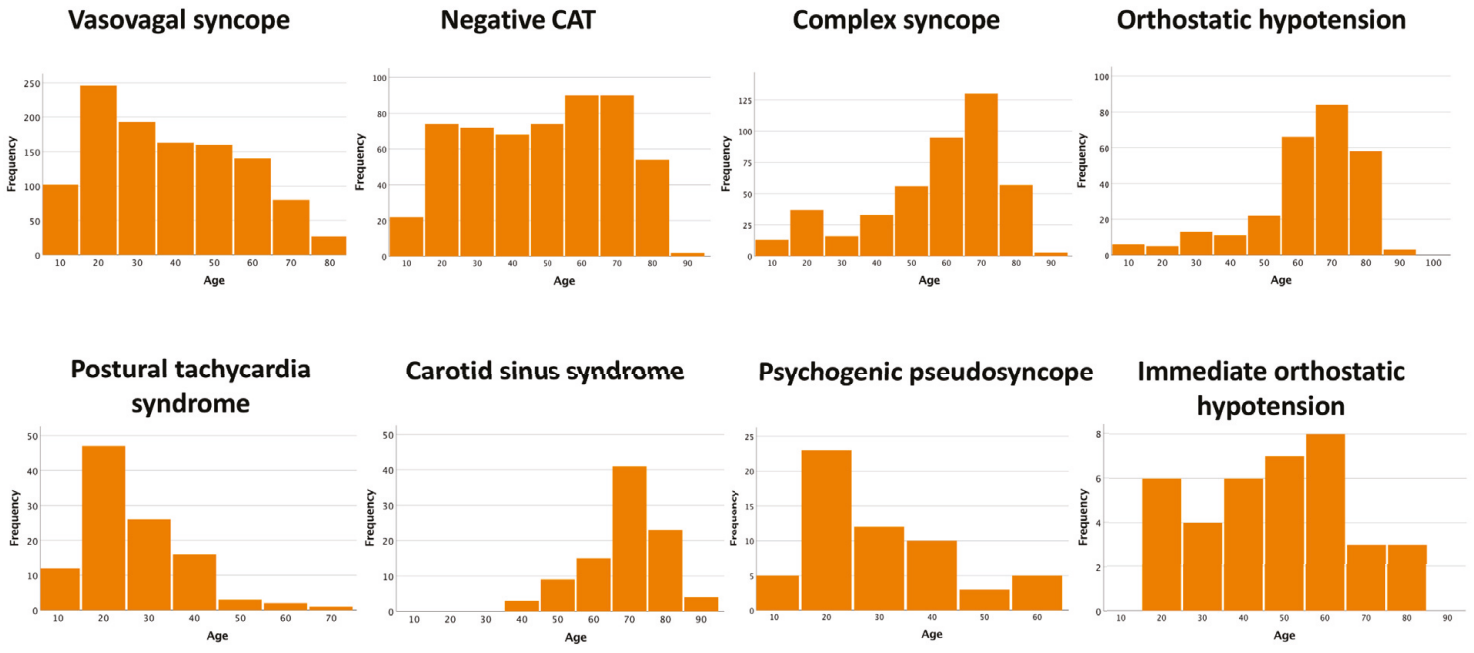

Figure 14. Age distribution (number of patients by age decade) according to CAT diagnosis group.

Table 10. Predictors of negative CAT.

	Odds ratio	95% CI	P-value
Age (per ten-year increment)	1.06	1.02-1.11	0.008
Age at first syncope (per ten-year increment)	1.07	1.03-1.11	0.001
Sex	1.13	0.93-1.38	0.208
Supine SBP (per 10 mmHg)	1.06	1.01-1.10	0.013
Supine HR (per 10 beats-per-minute)	1.12	1.04-1.20	0.002
No of previous syncope episodes	1.00	0.99-1.00	0.650
Absence of prodromes	1.56	1.28-1.90	<0.001
Palpitations	1.07	0.83-1.37	0.607
Dizziness	1.05	0.85-1.29	0.675
Supine syncope	0.90	0.70-1.16	0.424
Syncope with traumatic injuries	1.12	0.93-1.36	0.232
Hypertension	1.51	1.23-1.85	<0.001
Diabetes	1.93	1.41-2.63	<0.001
Heart failure	2.08	1.22-3.56	0.007
Atrial fibrillation	1.54	1.12-2.13	0.009
Coronary artery disease	1.61	1.13-2.28	0.008

CAT= cardiovascular autonomic testing, SBP= systolic blood pressure, HR=heart rate.

Table 11. Predictors of negative CAT (adjusted for age and sex).

	Odds ratio	95% CI	P-value
Age at first syncope (per ten-year increment)	1.08	1.00-1.02	0.042
Supine SBP (per 10 mmHg increment)	1.00	0.99-1.01	0.222
Supine HR (per 10 beats-per-minute)	1.12	1.04-1.20	0.003
Absence of prodromes	1.48	1.20-1.83	<0.001
Hypertension	1.45	1.14-1.85	0.003
Diabetes	1.82	1.32-2.51	<0.001
Heart failure	1.98	1.15-3.42	0.014
Atrial fibrillation	1.40	0.99-1.98	0.053
Coronary artery disease	1.51	1.05-2.18	0.027

CAT= cardiovascular autonomic testing, SBP= systolic blood pressure, HR=heart rate.

Table 12. Clinical characteristics and hemodynamic values in patients with VVS compared with negative CAT.

Clinical features	VVS n= 1111	Negative CAT n=546	P-value
Age, median [IQR]	40 [26-57]	54 [35-71]	<0.001
Female sex, n(%)	724 (65)	347 (64)	0.518
Supine SBP	129 ± 19	135 ± 21	<0.001
Supine DBP	74 ±11	76 ±12	0.001
Supine HR	69 ± 11	72 ± 12	<0.001
No. of syncope episodes, median [IQR]	5 [2-10]	4 [2-10]	<0.001
Prodromes, n(%)	652 (59)	238 (44)	<0.001
Palpitations, n(%)	270 (24)	119 (22)	0.585
Dizziness, n(%)	750 (68)	393 (72)	0.050
Syncope with traumatic injuries, n(%)	574 (52)	310 (57)	0.047
Supine syncope, n(%)	215 (19)	89 (16)	0.070
Hypertension, n(%)	159 (14)	179 (33)	<0.001
Diabetes, n(%)	44 (4)	65 (12)	<0.001
Heart failure, n(%)	13 (1)	21 (4)	<0.001
Atrial fibrillation, n(%)	40 (4)	56 (10)	<0.001
Coronary artery disease, n(%)	31 (3)	48 (9)	<0.001

CAT= cardiovascular autonomic testing, VVS=vasovagal syncope, SBP= systolic blood pressure, DBP=diastolic blood pressure, HR= heart rate. Data are presented as mean ± SD unless indicated otherwise.

Discussion

Paper I

Epinephrine and norepinephrine

We found that an increase in epinephrine concentration at 3 minutes of HUT predicted shorter time to syncope, however supine and orthostatic norepinephrine levels did not. These findings are in accordance with previous studies showing a surge of epinephrine prior to HUT-induced VVS compared with HUT-negative controls (68, 71, 74-77). Furthermore, near the time of HUT-induced VVS, the ratio of epinephrine/norepinephrine was higher than at baseline in the same patients or compared with HUT-negative controls (68, 74, 111-113).

Whether the rise in epinephrine reflects a compensatory response to orthostatic stress or is a direct trigger of VVS is unknown. A study showed that patients with positive passive HUT had higher orthostatic epinephrine concentrations compared with those in whom VVS was induced by nitroglycerine (78), indicating that higher epinephrine concentrations are associated with increased VVS susceptibility. Other studies have shown that the increase in epinephrine occurs before any hemodynamic changes are noticeable in HUT-induced VVS (68, 76), suggesting that the rise in epinephrine is directly involved in VVS pathophysiology and not only a compensatory mechanism.

Potential mechanisms for epinephrine as a trigger of VVS have previously been suggested, including the hypothesis that catecholamine overstimulation of left ventricle contractility could activate myocardial afferent mechanoreceptors, leading to withdrawal of sympathetic activity, vasodilatation and hypotension (114). However, several studies have shown that withdrawal of sympathetic activity causing vasodilatation is not a prerequisite for hypotension in VVS (115-117), instead a fall in cardiac output, caused by venous pooling is the culprit (37, 118, 119). Another hypothesis that is more plausible in light of these observations concerns selective vasodilatation in certain vascular beds, such as the splanchnic bed, leading to reduced preload and cardiac output (71, 120).

Similar to our observations, a previous study of 33 patients showed that elevated levels of baseline and orthostatic epinephrine predicted shorter time to syncope (72),

indicating that epinephrine may be a trigger for VVS. However, there was substantial data scatter of time to syncope at low epinephrine concentrations, a finding that was also present in our study population (Figure 6). This implies that VVS pathophysiology is heterogenous and for some individuals the increase in epinephrine might be the trigger, while in others, other mechanisms are prevailing.

Vasopressin

We found that an early orthostatic increase in vasopressin correlated with shorter time to syncope. Some authors have reported increased baseline vasopressin levels in VVS patients compared with controls (68, 111, 121), while others found no differences in baseline concentrations (90, 122). However, most studies reported a large increase in vasopressin prior to syncope. Vasopressin is secreted in response to haemodynamic crisis such as haemorrhage and hypovolemic shock (84), and the vasopressin surge observed prior to syncope indicates that it is a compensatory response caused by severe hypotension in VVS. Patients with positive passive HUT had higher early orthostatic concentrations of vasopressin compared with those with nitroglycerine-induced VVS (78), suggesting that higher vasopressin levels are associated with increased VVS susceptibility. Aside from antidiuretic and vasoconstrictive effects, vasopressin can modulate the baroreflex through central pathways, increasing the sensitivity of cardiac baroreceptors to increase vagal outflow and decrease sympathetic activity, an effect that may promote VVS (84, 90, 114, 123).

Adrenomedullin

Previous studies on ADM were mostly small and showed inconsistent results. One study reported that ADM increased during orthostasis in healthy subjects (124), another found no difference in supine and orthostatic ADM levels (125). Studies comparing HUT-positive patients with HUT-negative controls reported no significant difference in baseline or orthostatic ADM concentrations (121, 126). A previous study from the SYSTEMA cohort showed that patients with VVS had lower supine levels of ADM compared with HUT-negative controls and lower concentrations predicted cardioinhibitory VVS (69). We found that higher supine and early orthostatic ADM concentrations predicted longer time to syncope. These findings might seem contradictory knowing that ADM is released in response to volume overload and is a vasodilator and natriuretic peptide (99, 101), however it also prevents vascular leakage by maintaining the barrier function of the endothelium (101). This could have a protective effect against the trigger of orthostatic VVS; decreased intravascular volume and reduced cardiac output.

Endothelin-1 and atrial natriuretic peptide

We found that endothelin-1 and atrial natriuretic peptide did not predict time to syncope. Previous studies have shown inconsistent results, with increased endothelin in patients with VVS (93, 94, 127), decreased endothelin in VVS (70, 95) and no difference between VVS patients and controls (122). Atrial natriuretic peptide decreased during HUT but was higher in VVS patients compared with controls (68). Low supine levels of atrial natriuretic peptide were associated with VVS diagnosis (70) and patients with cardioinhibitory VVS had higher levels of atrial natriuretic peptide compared with vasodepressor VVS (98). The roles of endothelin-1 and atrial natriuretic peptide in VVS are uncertain and warrant further research.

Age and blood pressure

We found that older age and higher supine SBP predicted longer time to syncope. In a study of 5236 patients with suspected VVS, the positivity rate of drug-free HUT declined with advancing age, however the positivity rate of nitroglycerine-potentiated HUT remained stable, indicating that older patients need stronger stressors to trigger VVS (128). Older patients also had longer HUT duration before syncope compared with younger patients, consistent with our findings.

In a report of 288 patients comparing positive passive with nitroglycerine-potentiated HUT, the former group had lower orthostatic blood pressure at the beginning of HUT (78). A study evaluated the effect of age and baseline blood pressure on the blood pressure level at syncope, and patients aged >60 years had higher baseline orthostatic blood pressure and tolerated a longer duration of passive HUT before syncope occurred. The blood pressure level at syncope was not significantly different between age groups and this was interpreted as older patients having a greater "blood pressure reserve" for maintaining consciousness compared with younger patients (129). Two recent studies evaluated ambulatory blood pressure monitoring in VVS patients compared with controls and showed that the former group had lower 24-hour systolic blood pressure and more frequent systolic blood pressure drops (130, 131).

Paper II

Epinephrine and norepinephrine

The physiological response to orthostasis involves doubling plasma norepinephrine levels within a few minutes of standing (79). The release of norepinephrine is impaired in OH (79, 81-83, 94), however in dOH norepinephrine concentrations can be normal

or increased (132). Consistent with previous reports and supporting the idea that dOH is a milder form of ANS dysfunction (133), we found that the orthostatic norepinephrine concentration was higher in dOH compared with cOH.

During orthostasis, the concentration of epinephrine initially increased 3-fold and returned to baseline after prolonged standing in healthy subjects (79). There was no significant difference in epinephrine concentrations at the end of HUT comparing OH patients with controls (83). We found that the orthostatic increase in epinephrine was higher in cOH compared with dOH. At the 3-minute time point in HUT where blood samples were collected, blood pressure had significantly decreased in cOH, but not in dOH. The higher level of epinephrine in cOH can be interpreted as a compensatory response to pronounced hypotension. This adrenomedullary response might be augmented in light of ANS dysfunction and reduced postganglionic norepinephrine release (81).

Vasopressin

Previous studies have shown that plasma vasopressin levels increased 3-fold during HUT in healthy subjects (79, 94) and in patients with OH, however the increase was considered insufficient in relation to the extent of hypotension (94). It was reported that patients with a lesion in the afferent central or peripheral parts of the baroreflex arc had a subnormal increase in vasopressin levels during HUT, whereas OH patients with lesions in the efferent pathway had normal or increased levels of vasopressin (89). Patients with diabetic neuropathy and OH had reduced vasopressin release compared with controls and diabetics without neuropathy (87).

We found that supine and early orthostatic concentrations of vasopressin were higher in cOH compared with dOH. Classical OH patients had a larger orthostatic blood pressure fall which stimulated a compensatory vasopressin response, and after 3 minutes of HUT when blood was sampled, dOH patients had not yet experienced a significant decrease in blood pressure. The higher supine vasopressin level in cOH patients might be related to chronic frequent blood pressure falls in these patients.

Endothelin-1, adrenomedullin and atrial natriuretic peptide

Previous studies have shown that plasma endothelin increased during HUT in normal controls, however it did not increase in OH patients (94) and a higher supine concentration of endothelin predicted OH during HUT (70). We did not find a significant difference in supine or orthostatic endothelin-1 levels comparing cOH with dOH. The role of endothelin-1 in OH is uncertain and requires further study.

It was reported that there was no difference in supine and standing atrial natriuretic peptide levels comparing OH patients with controls (82). Adrenomedullin and atrial natriuretic peptide are released in volume overload states and it is not unexpected that cOH and dOH do not differ regarding these neurohormones.

Clinical and hemodynamic variables

As previously reported (134), we found that patients with dOH were younger compared with cOH, indicating that dOH could be an early presentation of cOH. Previous studies showed that cOH patients had more severe autonomic dysfunction (134-136), in line with our findings that cOH patients had greater orthostatic blood pressure fall and higher prevalence of pathologic Valsalva manoeuvre. It was reported that Parkinson's disease and reduced renal function were frequently associated with autonomic dysfunction (137-140) and we observed that both conditions were more common in cOH compared with dOH, however there was no difference in prevalence of diabetes in the two groups. Supine hypertension was a common finding in patients with OH (53, 54) and we observed a tendency towards higher supine blood pressure in the cOH group compared with dOH. Classical orthostatic hypotension was associated with increased cardiovascular morbidity (44, 47, 141), which can explain the higher prevalence of pacemaker treatment in this group. Patients with cOH reported palpitations prior to syncope less frequently, this finding could be explained by cardiac sympathetic denervation (142).

Delayed orthostatic hypotension was first described by Streeten and Anderson in 1992 (132). Gibbons and Freeman reported 10-year follow up on 90 patients diagnosed with either cOH or dOH; 54% of dOH patients progressed to cOH and 31% were diagnosed with alpha-synucleinopathies. Ten-year mortality was increased in dOH compared with controls and highest in cOH patients (143). These observations suggest that dOH is not a benign condition as previously assumed, but in many cases a progressive condition with increased mortality.

The findings in paper II show that compared with dOH, cOH is associated with more severe abnormalities of autonomic and neuroendocrine control mechanisms and add to the growing evidence that dOH is an early form of cardiovascular autonomic dysfunction.

Paper III

Age distribution of first syncope

Previous reports on the age distribution of syncope have included smaller samples and focused on specific populations (22-24, 33, 144-146) and studies including large populations with a range of ages are scarce. Ganzeboom reported that the median age at first syncope was 15 years in 394 medical students, and 39% had previously experienced syncope (22). Peak age at first syncope was 15 years and 35% had previous syncope in 549 middle aged individuals in the general population (23). A bimodal syncope incidence pattern with a second peak after the age of 70 years was reported in the Framingham heart study (26) and in a general practitioner setting (36). Paper III confirms the bimodal distribution of age at first syncope in a large population of patients with unexplained syncope evaluated in a specialized syncope unit.

Prodromes and head-up tilt diagnoses

Prodromes are less common in older syncope patients compared with younger patients (28, 30, 147-149). This could be the result of amnesia (30, 150) or degeneration of the autonomic nervous system in the ageing patient (149). We showed that elderly patients with early-onset syncope (predominantly of vasovagal origin) had a higher proportion of prodromes compared with those experiencing only recent syncope, implying that syncope characteristics do not change throughout life in these patients.

The prevalence of OH and CSS increase with older age (36, 151). We found that older patients with late-onset syncope had a higher probability of CSH/CSS and OH compared with early-onset syncope. Hypertension, a comorbidity that correlates with OH (152, 153) was more common in the late-onset syncope group.

Carotid sinus hypersensitivity and syndrome

Carotid sinus syndrome was defined as symptomatic response (a fall in SBP of ≥50 mmHg and/or ventricular pause of ≥3 seconds) to CSM in a patient with previous syncope (20). In paper III and IV, all included patients in this group presented syncope in their history but when challenged with CSM not all had reproduction of syncope. Some had no prodrome for their previous syncope and as older patients can have amnesia for syncope (150, 154), symptom reproduction may not be possible. However, all had symptoms, usually presyncope. Consequently, we felt confident that these patients would in many centres be classified as CSS.

Complex syncope

The term complex syncope has been mentioned in the literature (155) but is not widely used. Studies have shown that about 20% of older patients have multiple potential causes of syncope as VVS often overlaps with OH and CSS (155-157). Ageing causes impaired autonomic function, which affects baroreflex sensitivity and regulation of heart rate and blood pressure, and accompanied by the presence of cardiovascular comorbidities and polypharmacy, predispose the older individual to syncope (36, 155, 158-160). Rafanelli reported that complex syncope was present in 23% of 873 syncope patients evaluated by HUT and the prevalence increased with older age. Consistent with our results, the most common combination was VVS and OH (155). In a study of 987 syncope patients, 18% had complex diagnosis and older age and cardiac comorbidities were predictors of complex syncope. Complex syncope was an independent predictor of increased mortality (156).

The impact of age at first syncope on the prevalence of complex syncope has not been previously reported. We have shown that the frequency of complex syncope increased with older age at first syncope. In older patients, complex syncope was more common in those with early-onset syncope compared with late-onset, as patients with pre-existing VVS susceptibility developed OH and CSH/CSS with age. The coexistence of multiple syncope mechanisms has implications for treatment, recurrence rate and prognosis of syncope patients. We found that 28% of patients aged ≥60 years had complex syncope. The high prevalence of complex diagnoses in older patients underlines the need for a comprehensive syncope evaluation including cardiovascular autonomic testing.

Negative head-up tilt

Cardiovascular autonomic testing did not provide a syncope diagnosis in 22% of patients, similar to previously reported figures (156, 161, 162). The proportion negative HUT did not differ between patients with early-onset and late-onset syncope, however in patients aged ≥60 years, no diagnosis after HUT was more common in the late-onset group. Older patients with late-onset syncope also had a higher proportion of hypertension, atrial fibrillation and heart failure, indicating that they may have undetected cardiac syncope.

Paper IV

The age distribution of negative cardiovascular autonomic testing

The age distribution of negative CAT was different from the other diagnoses (Figure 14). The prevalence of VVS, POTS and PPS was highest in younger patients, whereas OH and CSS increased with age. The prevalence of negative CAT was even across age groups 20-50 years and increased slightly in age groups 60-70 years. These findings are consistent with a previous study in which the frequency of unexplained syncope after evaluation in 503 patients was even across age groups (163). If most patients with negative CAT had undetected cardiac syncope, we would expect them to be older, however as patients referred to the syncope unit were highly selected, it is possible that arrhythmia as syncope aetiology was missed during initial evaluation. We previously reported that prior to referral to the syncope unit, patients with negative CAT had been evaluated by echocardiography and/or Holter monitor in 60% and by exercise test in 30% of cases (164). However, as the diagnostic yield of Holter monitoring in syncope is low (165), arrhythmia as the cause of syncope is not improbable.

Negative cardiovascular autonomic testing compared with vasovagal syncope

If patients with VVS and negative CAT were similar with regard to history and clinical characteristics, it would indicate that VVS could be the aetiology of syncope in the latter group. Previous studies have shown that compared with VVS, negative HUT patients were older (155) and experienced prodromes less frequently (155, 166). Unexplained syncope and VVS shared many similarities in a study of 341 patients, however the former group were more often male and had a shorter history of previous syncope (167). We found that compared with the VVS group, patients with negative CAT were older, had higher resting blood pressure and heart rate, fewer syncope episodes, a higher proportion of syncope without prodromes and a higher frequency of hypertension, diabetes, atrial fibrillation, coronary artery disease and heart failure, indicating that the two groups do not share a common aetiology. However, the ISSUE 3 study showed that older patients with VVS presented differently with short or absent prodromes and negative HUT, yet implantable loop recorder (ILR) revealed VVS as the cause of syncope (168).

Predictors of negative cardiovascular autonomic testing

After evaluation with CAT in the syncope unit, the aetiology of syncope remained unexplained in 21% of patients. A similar figure was reported in a study of 1058 patients evaluated with HUT in a syncope unit setting (169).

We found that older age at first syncope, higher resting heart rate, lack of prodromes, a history of hypertension, diabetes, coronary artery disease and heart failure were predictors of negative CAT. In a previous study, we showed that VVS was the predominant cause of early-onset syncope (164). Syncope without prodromes and cardiovascular comorbidities are risk factors for cardiac syncope (170). A meta-analysis of 38 843 patients showed that unexplained syncope was associated with increased mortality and the association was stronger in older patients with diabetes or hypertension (171), emphasizing the need for continued evaluation of patients with negative CAT.

The predictors of negative CAT in paper IV are mainly those that also increase the risk of cardiac syncope. Consequently, syncope patients that present with these factors may benefit from evaluation with ILR rather than CAT as the first step in the investigation (172). Syncope patients with negative CAT should receive an ILR, as emphasized by the European Society of Cardiology syncope guidelines (20) and further supported by a recent study (172). Even with the use of an ILR, syncope aetiology will not be determined in a subset of patients, an obvious challenge for future research.

Methodological considerations

Head-up tilt

Head-up tilt has been criticised for having low sensitivity and specificity (173), however there is no diagnostic gold standard for evaluating syncope to which it can be compared and the positivity rate of HUT has often been used as sensitivity.

In a meta-analysis of 4361 syncope patients and 1791 controls, the positivity rate for nitroglycerine potentiated HUT was 66%. The positive rate in controls (false positives) was 11%. HUT protocols using nitroglycerine had the highest positivity rate (174). The positivity rate was higher in patients with a history typical of VVS and lower in those with atypical clinical features (175).

Another concern is whether syncope induced by HUT is equivalent to the patient's spontaneous episodes. The ISSUE-2 trial included 392 patients implanted with ILRs and showed that ECG findings during HUT were poorly correlated to ILR observations during patients' spontaneous syncope, where an asystolic pause was more common (176). The ISSUE-3 trial included 504 syncope patients implanted with ILRs. Head-up tilt was positive in 56% of patients who had evidence of VVS on ILR. Among those patients with an arrhythmic cause of syncope on ILR; 43% had a positive HUT (177). These findings led to a reinterpretation of HUT as exposing orthostatic hypotensive

susceptibility in patients that predispose them to VVS and this may overlap with cardiac syncope (175).

Reproducibility of positive HUT has been reported to around 80% in the second test (178-180). The decreasing frequency of positive responses on repeat testing may be explained by the patient becoming aware of imminent syncope and trying to avoid it, for example by leg movements.

Despite these concerns, HUT and cardiovascular autonomic testing have proven to have clinical value and used in specialized syncope units, can increase the diagnostic yield, reduce costs and improve patient care (62, 181, 182). However, the interpretation of HUT requires experience to avoid low diagnostic precision. Head-up tilt can identify OH, POTS and PPS and with regard to VVS, a positive HUT must reproduce the patient's spontaneous syncope.

Assessment of neurohormones

The concentration of neurohormones were measured at supine rest and after 3 minutes of HUT. In paper I, further changes in neurohormone concentrations that might have occurred at syncope or in the post-syncopal phase were not assessed. In paper II, at the timepoint 3 minutes of HUT, blood pressure had significantly decreased in cOH, but not in dOH. Serial sampling during orthostasis would allow better comparisons of levels of neurohormones in cOH and dOH. The concentration of plasma norepinephrine represents spill over from the synaptic cleft and is determined by release from the presynaptic neuron and removal by reuptake and might not be an accurate reflection of sympathetic nerve traffic (79). Multiple other cardiovascular neurohormones that were not included in the studies may contribute to VVS and OH pathophysiology (65).

Strengths and limitations

Papers I-IV have several important limitations. The patients included in SYSTEMA were referred to the specialized syncope unit for cardiovascular autonomic testing and were therefore highly selected and may not represent patients with orthostatic intolerance or syncope in the general population.

There was no healthy control group. Some studies use HUT-negative patients as the control group, however these patients have orthostatic intolerance or syncope and in paper IV we demonstrated that some of them possibly have undetected cardiac syncope and cannot be considered healthy controls.

The main parameter in paper III, age at first syncope, required patients to recall events that occurred many years earlier and in elderly patients this can be a source of error.

In papers III and IV, there was no follow-up data on the final syncope diagnosis in patients with negative CAT. Some patients received ILR following evaluation with CAT, however we do not have complete data on the findings.

The main strength of the papers is the inclusion of a large number of patients examined in the setting of a tertiary syncope unit.

Conclusions

This thesis examined the pathophysiological mechanisms involved in vasovagal syncope and orthostatic hypotension with focus on cardiovascular biomarkers, and how history and clinical characteristics are associated with the outcome of syncope evaluation in a specialized syncope unit. Based on the results of the four studies included in this thesis, the following conclusions were made:

Paper I

Susceptibility to head-up tilt-induced vasovagal syncope is inversely related to age, higher supine blood pressure and higher adrenomedullin concentration. Greater orthostatic increase in epinephrine and vasopressin are associated with shorter time to syncope. These results confirm, with a greater number of patients, previous observations and add to the understanding of the pathophysiology of vasovagal syncope.

Paper II

Compared with delayed orthostatic hypotension, patients with the classical form are older, have pacemaker-treated arrhythmia, lower glomerular filtration rate, pathologic Valsalva test and Parkinson's disease. Classical orthostatic hypotension is associated with increased orthostatic levels of vasopressin and epinephrine, but blunted increase in norepinephrine. These findings add to the increasing evidence that classical orthostatic hypotension, compared with the delayed form, is associated with more severe abnormalities of neurohormonal and autonomic regulation and can be viewed as a more advanced form of the condition.

Paper III

In patients with unexplained syncope, the first-ever syncope incidence has a bimodal lifetime pattern with peaks at 15 and 70 years. The majority of older syncope patients have their first episode when older and orthostatic hypotension and carotid sinus syndrome are more common in this group. In contrast, among older patients with life-long duration of syncope; prodromes, vasovagal and complex syncope are more common. This study confirms the bimodal pattern of first syncope incidence in a large group of patients and highlights the value of a thorough history in the evaluation of elderly syncope patients.

Paper IV

Cardiovascular autonomic testing provides a syncope diagnosis in 79% of patients investigated in a syncope unit. Syncope without prodromes and cardiovascular comorbidities are predictors of failure to establish syncope aetiology. These are known risk factors for cardiac syncope and therefore patients with inconclusive CAT require further evaluation. These findings may contribute to improved syncope evaluation and patient care.

Future perspectives

Vasovagal syncope is considered a benign condition without excess mortality (20), however for a minority of affected individuals with severe recurrent episodes and minor or no prodromes, it has major negative implications. Because of the episodic nature of the condition, finding an effective treatment has been difficult. To help these patients, further research regarding the pathophysiology of VVS, including haemodynamic and neurohormonal aspects, is needed. With knowledge of factors that predispose to and trigger VVS, diagnostic and treatment options will improve, leading to better patient care.

Orthostatic hypotension can lead to falls, traumatic injuries and increased mortality but is often overlooked in elderly individuals. Orthostatic hypotension poses a therapeutic challenge in elderly, frail patients with cardiovascular comorbidities (43, 48, 49). Future studies should focus on improved detection and treatment of patients with OH.

The head-up tilt test is valuable in syncope evaluation but has some limitations. Further research on how to improve the diagnostic precision of HUT can focus on patient selection and improved, more practical protocols, making HUT more widely accessible. Further research on biomarkers and their role in different syncope aetiologies may lead to improved diagnostics through a stratification pathway for different diagnostic modalities, such as CAT/HUT or ILR.

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