Antiadrenergic autoimmunity in postural tachycardia syndrome

Artur Fedorowski1,2†, Hongliang Li3, Xichun Yu3, Kristi A. Koelsch3,4, Valerie M. Harris3,4, Campbell Liles3, Taylor A. Murphy3, Syed M. S. Quadri3,4, Robert Hal Scofield3,4, Richard Sutton5, Olle Melander1, and David C. Kem3†

1Department of Clinical Sciences, Lund University, Lund, Sweden; 2Department of Cardiology, Skåne University Hospital, Inga Marie Nilssons gata 46, Malmo 20502, Sweden; 3Department of Medicine, University of Oklahoma Health Sciences Center and VA Medical Center, Oklahoma City, OK, USA; 4Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; and 5National Heart & Lung Institute, Imperial College, London, UK

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Introduction

Postural tachycardia syndrome (POTS) is a functional cardiovascular disorder characterized by excessive heart rate increase and discomfort during orthostasis, usually accompanied by a spectrum of non-specific symptoms such as sporadic syncope, orthostatic intolerance, deconditioning, headache, cognitive impairment, and gastrointestinal dysfunction.1,2 The syndrome affects predominantly young women (70–80%) within a range of 15–40 years, first being defined in 1990s as a variant of orthostatic intolerance and dysautonomic syncope, but its aetiology remains unknown.3–5 Postural tachycardia syndrome is considered to be one of the most common autonomic disorders, with an estimated prevalence of 0.5 million in United States of America alone. Its onset may follow an acute

* Corresponding author: Tel.: +46 40331000; fax: +46 40336225. E-mail address: artur.fedorowski@med.lu.se
† A. Fedorowski and D.C. Kem are co-senior authors.
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Serum from patients diagnosed with postural tachycardia syndrome contains circulating autoantibodies with a direct stimulatory effect on adrenergic receptors.

The autoantibodies may also exert an allosterically mediated positive modulatory effect upon β1AR and a negative modulatory effect on α1AR activity.

These and possibly other yet-to-be identified immunoglobulins with different target epitopes might explain different constellations of symptoms in POTS.

This concept, if validated, would provide concrete support for novel therapeutic approaches against POTS based on immunotherapy.

What’s new?

- Serum from patients diagnosed with postural tachycardia syndrome contains circulating autoantibodies with a direct stimulatory effect on adrenergic receptors.
- The autoantibodies may also exert an allosterically mediated positive modulatory effect upon β1AR and a negative modulatory effect on α1AR activity.
- These and possibly other yet-to-be identified immunoglobulins with different target epitopes might explain different constellations of symptoms in POTS.
- This concept, if validated, would provide concrete support for novel therapeutic approaches against POTS based on immunotherapy.

Methods

Study patients

SYSTEMA (Syncope Study of Unselected Population in Malmö) is an ongoing single-centre project aiming at studying syncope epidemiology, pathophysiology, and prognosis in the general population. Within the SYSTEMA cohort, we randomly selected 17 patients with a confirmed diagnosis, by clinical history and tilt testing, of POTS (16 females; age, 26 ± 9 years), and 7 with recurrent VVS (5 females; age, 31 ± 9 years). These patients were investigated at the Syncope Unit of Skåne University Hospital between February 2011 and March 2014. The POTS patients reported at least 3 months duration of characteristic symptoms such as orthostatic intolerance, tiredness, headache, and were unresponsive to conventional therapy with persistent symptoms of orthostatic intolerance during blood collection. In contrast, VVS patients were selected among those who reported recurrent syncope prior to diagnosis, and were free from symptoms between the episodes of syncope. Eleven normal controls (10 females; age, 31 ± 6 years) were recruited at the study site in Sweden among healthy individuals without any history of syncope, autoimmune disease, and with a normal postural haemodynamics during active standing (i.e. pulse increase <30 bpm and absence of orthostatic hypotension). All the patients and controls were recruited from the same community of Malmö or neighbouring municipalities in Skåne, Sweden.

Examination protocol

The head-up tilt (HUT) test for SYSTEMA patients was performed according to the Italian protocol. The tilt table was raised at constant speed to 70° in ~18 s, and had the same lowering time. The patients’ haemodynamic parameters and electrocardiogram were monitored using a non-invasive beat-to-beat measurement device (Nexfin, BMEYE, Amsterdam, the Netherlands). Study participants were instructed to fast for 2 h before the test, and they were allowed to drink water ad libitum. Participants were also asked to complete a questionnaire, referring to their past medical history, duration, frequency and characteristics of syncope, smoking status, and current medication. The Regional Ethical Review Board in Lund, Sweden approved the study protocol (ref no 02/2008), and all study participants gave their written informed consent including serum analyses.

Diagnostic criteria of postural tachycardia syndrome and classic vasovagal syncope

The test was diagnostic when patients demonstrated a typical prodrome (such as palpitation, nausea, dizziness, lightheadedness, or intense perspiration) reproducing previous events experienced and/or precipitated syncope, and the haemodynamic parameters met the diagnostic criteria of POTS or VVS, respectively. Briefly, POTS was defined as characteristic symptoms of orthostatic intolerance (lightheadedness, dizziness, and/or discomfort) with a maximal and persistent heart rate increase of >30/min (or >40/min for those aged ≤19 years) or tachycardia of >120/min in standing position, and the absence of orthostatic hypotension, i.e. max systolic/diastolic BP drop was <20/10 mmHg on head-up tilt. Vasovagal syncope was defined as a reproduction of syncope during HUT associated with a characteristic pattern of pronounced hypotension, bradycardia, or asystole. In order to ascertain the accuracy of diagnoses, the digital test records were subsequently inspected off-line using Nexfin@PC (BMEYE, Amsterdam, the Netherlands), dedicated software provided by the manufacturer.

Sample preparation

Blood samples for the present study were obtained post-test (i.e. after the diagnostic HUT was performed) following overnight fasting. A trained nurse performed an antecubital venipuncture in a dedicated room after 10 min rest in supine position. Serum was separated by centrifugation and stored at −80 °C prior to shipping of duplicate aliquots in dry ice to the laboratory in Oklahoma City. The frozen integrity of each
Cell-based α1AR assay
Immunoglobulin G activation of α1AR in α1AR-NFAT-bla CHO-K1 cells was assessed using the GeneBlazer FRET-based β-lactamase reporter assay (Invitrogen) according to manufacturer’s instructions. Briefly, cells were plated in 96-well plates and incubated overnight. The individual IgG (0.1 mg/mL) samples and positive and negative controls were then added and incubated for 5 h. The α1AR blocker prazosin (10 μM) was used for specific blockade. The β-lactamase substrate CCF4-AM (LiveBLAzer-FRET B/G Loading Kit, Invitrogen) was then added and incubated for 2 h. The plates were read using a fluorescence microplate reader (BioTek Synergy 2 Multi-Detection Microplate Reader). All samples were assayed in triplicate. Negative (buffer) and positive (phenylephrine) controls were included in each assay. Data were expressed as the ratio of the emissions 460/530 nm (blue/green) after subtraction of the background values.

Full dose–response curves for phenylephrine (10⁻¹⁰–10⁻⁵ M) were generated in three known positive POTS subjects in the absence and presence of IgG (0.1 mg/mL) to examine the allosteric effect of IgG on the α1AR orthosteric ligand phenylephrine. We then used a simplified one dosage (10⁻⁶ M) phenylephrine response in the absence and presence of IgG from the POTS subjects and the normal controls to examine whether an apparent change in sensitivity existed irrespective of whether a direct activation of the receptor by the IgG was present.

Cell-based β1/2AR assay
Immunoglobulin G-mediated β1AR and β2AR activation of cAMP production in transfected CHO cells (with either β1AR or β2AR stimulation) was measured using the CAMP Hunter eXpress GPCR Assay Kit (DiscoverRx) as described.8,15 Briefly, β1AR-- or β2AR-CHO cells were dispensed into 96-well plates and incubated overnight. The medium was then removed and assay buffer containing the CAMP antibody and serum-derived IgG (0.1 mg/mL) was added in the presence and absence of the non-selective β1AR blocker propranolol (1 μM) and incubated for 30 min. The CAMP standard, negative (buffer), and positive (isoproterenol) controls were included in each assay. All samples were tested in triplicate. Following sample treatment, CAMP detection reagent and solution were added, and chemiluminescent signal was read on a TD-20/20 Luminometer (Turner BioSystems). The cAMP values generated for each assay were expressed as relative luminescence unit (RLU).

Dosage response curves for isoproterenol (10⁻¹⁰–10⁻⁶ M) in β1AR-CHO cells in the absence and presence of IgG (0.1 mg/mL) from three positive POTS subjects were constructed to examine the allosteric effect of IgG on the β1AR orthosteric ligand isoproterenol. Likewise, a simplified single dosage isoproterenol (10⁻⁶ M) response was examined using IgG from the POTS subjects and the normal controls to determine if an allosteric-mediated effect on the β1AR-CHO cells was present independent of any direct activation from the IgG.

Isolated cremaster arteriole assay
The vasoconstrictor effect of IgG activation of α1AR on resistance vessels was examined using an isolated rat cremaster arteriole assay as previously described.8 After equilibration in the presence of propranolol (1 μM) and the nitric oxide synthase inhibitor L-NNAME (1 μM) to eliminate any β2AR- and M3 muscarinic receptor-mediated vasodilation and to achieve steady-state myogenic tone, the arterioles were perfused with IgG (0.05 mg/mL) and their effects on vessel diameter were recorded. Phen tolamine (10 μM) was used to specifically block α1AR activity. The buffer baseline diameters were normalized to 100% and subsequent IgG-induced contractility was reported as percentage of baseline. This procedure was approved by the Oklahoma University Health Sciences Center Institutional Animal Care and Use Committee.

Statistical analyses
Data are expressed as mean ± SD. Group comparisons were performed using Student’s t-test for comparison of two groups, or one-way ANOVA followed by post hoc Tukey’s test for multiple group comparison. Pearson’s χ²-analysis was used to compare categorical variables. The positivity of bioactive autoantibodies was defined as values above the mean ± 2SD from the control group. A linear regression analysis was performed to examine the relationship between the haemodynamic parameters resting supine plus during head-up tilt and direct IgG-mediated receptor bioactivity. Analyses were performed using IBM SPSS Statistics version 22 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at P < 0.05.

Results
Clinical characteristics
The demographic and clinical characteristics for each group are shown in Table 1. The mean age for the POTS patients was not significantly different from those with VVS and control subjects. All patients in the POTS group had documented persistent increase in heart rate >30 bpm vs. supine during head-up-tilt with reproduction of typical orthostatic intolerance symptoms. There was no significant difference in BP response during the first 3 min of head-up tilt in the POTS compared with VVS and controls and there was no significant difference in catecholamine profile supine and 3-min HUT between POTS and VVS (see Supplementary material online, Table S1). The VVS group reported a longer history of syncopal symptoms (P = 0.017), but the number of syncope episodes, proportions of characteristic precipitating symptoms, and self-reported dizziness on standing did not differ between POTS and VVS patients.

Autoantibody activity
The activities of autoantibodies against the α1AR and β1/2AR were examined using IgG (0.1 mg/mL) in cell-based bioassays. These data are shown in Figure 1. Among the 17 patients with POTS, 8, 11, and 12 showed direct activation of α1AR (47%), β1AR (65%), and β2AR (71%), respectively (Figure 1A–C). Based on these direct assays, 6 of these 17 POTS patients (35%) harboured both α1AR- and β1AR-AAb (Table 2). None of these autoantibodies were found in the patients with VVS or healthy controls. The mean activity values of α1AR, β1AR, and β2AR autoantibodies were all significantly higher in the POTS group than for both VVS and normal controls (α1AR: POTS 0.31 ± 0.07(SD) vs. VVS 0.24 ± 0.04 and control 0.23 ± 0.04, P = 0.029 and 0.004, respectively; β1AR: POTS 1840 ± 186 vs. VVS 1578 ± 117 and control 1608 ± 127, P = 0.002 and 0.001, respectively; β2AR: POTS 1104 ± 230 vs. VVS 816 ± 73 and control 837 ± 72, P = 0.007 and 0.001, respectively) (Figure 1A–C). There was no significant difference between the latter two groups.

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The mean activities of α1AR, β1AR, and β2AR autoantibodies in 13 POTS patients were markedly suppressed by the α1AR blocker prazosin (10 μM, from 0.52 ± 0.06 to 0.46 ± 0.05, \( P = 0.0001 \)) and β-blocker propranolol (1 μM, β1AR: from 1068 ± 233 to 821 ± 71, \( P = 0.0013 \); β2AR: from 1221 ± 164 to 988 ± 37, \( P = 0.0001 \)) (Figure 1D–F). No significant effect of these blockers was observed in the control group.

To examine the effect of autoantibodies on orthostatic ligand activation of α1AR and β1AR, dosage response curves for phenylephrine (10⁻¹⁰–10⁻⁵ M) and isoproterenol (10⁻¹⁰–10⁻⁶ M) with and without POTS IgG (0.1 mg/mL) were constructed and compared. Immunoglobulin G from three POTS patients who were positive for α1AR and β1AR autoantibodies were used for curve comparisons. In the presence of POTS IgG, the phenylephrine response curve was shifted to the right, demonstrating a decreased α1AR response (Figure 2A). In contrast, the isoproterenol response curve was significantly shifted to the left, demonstrating an enhanced β1AR response with β1AR autoantibodies (Figure 2B). Neither shift was observed using control IgG. We then tested the effect of IgG (0.1 mg/mL) from all 17 POTS subjects on the responses to phenylephrine (1 μM) and isoproterenol (1 μM). There was a significant decrease in phenylephrine-induced α1AR activation with POTS IgG (0.13 ± 0.02) compared with phenylephrine alone (0.18 ± 0.03, \( P < 0.0036 \)) or IgG from normal controls (0.17 ± 0.02, \( P < 0.0005 \)) (Figure 2C). The isoproterenol-stimulated β1AR activation, on the other hand, was significantly increased with POTS IgG (1835 ± 309) compared with isoproterenol alone (1479 ± 86, \( P < 0.037 \)) or IgG from normal controls (1508 ± 80, \( P < 0.032 \)) (Figure 2D). Control IgG showed minimal modulating effects.

When each individual data was plotted, 8 of the 17 POTS patients demonstrated an inhibitory effect on phenylephrine-induced α1AR activation using a cut-off value of the mean—2SD from the control group (Figure 2E). Thirteen of the 17 POTS patients were positive for an enhanced effect on isoproterenol-induced β1AR activation using a cut-off value of the mean + 2D from the control group (Figure 2F). There was some overlap for the α1AR and considerable overlap for the β1AR with the other tests (Table 2). Overall, all POTS subjects had at least one positive test.

Immunoglobulin G (0.05 mg/mL) from each subject with POTS and 8 controls were tested for α1AR autoantibody-mediated contractile activity using an isolated rat cremaster arteriole assay. A total of 13 among the 17 POTS patients demonstrated significantly increased contractility (Figure 3A). There was a significant difference in the mean contractile activity between the POTS group and control group (91.0 ± 3.1% vs. 96.5 ± 1.3%, \( P < 0.0001 \)). The control subjects failed to produce any significant vasoactivity. Six POTS subjects with positive α1AR autoantibodies and 6 controls were then selected to test the effect of specific α1AR blockade. As shown in Figure 3B, POTS IgG-induced contractility was largely returned to baseline level by addition of the α1AR blocker phenolamine (10 μM, from 88.9 ± 3.0% to 96.5 ± 2.1%, \( P = 0.0006 \)). Phenolamine had no significant impact on control IgG activity.

In the linear regression analysis, IgG-mediated β1AR activity was strongly correlated with both 3 min (\( P = 0.0009 \)) and maximal upright heart rate (\( P = 0.0006 \)), whereas the latter was also correlated with α1AR activity (\( P = 0.039 \)) but not with β2AR activity (\( P = 0.11 \)). Neither SBP nor DBP were correlated with adrenergic receptor stimulation.

**Discussion**

We have demonstrated that serum from subjects with POTS contains circulating autoantibodies, some with a direct stimulatory
effect on adrenergic receptors. A majority also exerted an allosterically mediated positive modulatory effect upon β1AR and a negative modulatory effect on α1AR activity. These and possibly other yet-to-be identified immunoglobulins with different target epitopes may exert varying biological effects in particular patients, which may explain different constellations of symptoms in POTS. It is of interest that IgG-mediated β1AR and α1AR activation were correlated with the heart rate increase on standing. The relatively high correlation with the β1AR autoantibody activity would appear to be clinically relevant. The lower but still significant relationship of the allosterically mediated inhibitory action of the α1AR response would secondarily lead to the increase in heart rate by reflexly increasing circulating catecholamines.

The primary objective of this study was to determine if autoantibodies with the capacity to modify the activity of autonomic adrenergic receptors, previously reported in POTS patients in the USA, were likewise present in a cohort of POTS subjects from a European location. A secondary goal included usage of an ‘autonomic’ control group characterized by subjects with recurrent VVS. This permitted us to determine if such autoantibodies were different between normal subjects and POTS; and as well between POTS and a group with recurrent VVS. These latter two groups shared somewhat broadly...
based autonomic disorders but clearly were different from each other and from normal controls. A third goal was to develop bio-
activity assays that might permit larger scale testing for pathophysio-
logical and therapeutic study.

For the primary objective, we found that antiadrenergic $\alpha_{1A}$R autoantibody activity was evident in POTS, while the VVS subjects
were not different from normal subjects. In contrast to our initial
publication, $^8$ nearly half of the POTS subjects had direct
activation of the $\alpha_{1A}$R autoantibody activity. The $\alpha_{1A}$R activity in our previ-
ous study was assayed using IgG in an arteriolar assay and not the
cell-based assay. For this reason, we re-assayed IgG from
each POTS patient and a representative group of the controls in
the rat cremaster arteriole assay. These data showed a heteroge-
neous response in which the majority with increased
$\alpha_{1A}$R activity from the cell-based assay also demonstrated arteriolar contractile
activity. Eight subjects with no $\alpha_{1A}$R direct activity from the cell-
based assay also demonstrated positive contractile activity, while
three subjects with positive $\alpha_{1A}$R activity from the cell-based assay
demonstrated no arteriolar contractile activity.

We then examined the impact of representative IgG from POTS
and normal controls on phenylephrine dosage response curves. As
previously reported, $^8$ these data confirmed the active $\alpha_{1A}$R auto-
antibodies inhibited the phenylephrine response and shifted the
phenylephrine dosage response curve to the right. These autoanti-
bodies appear to function as partial agonists and the absence of
measurable direct activation of the transfected receptors in vitro in
the absence of their normal ligand does not exclude their presence.
It is possible this allosteric impact on the activity of the $\alpha_{1A}$R ligand
norepinephrine represents the distinct pathophysiological feature
characteristic of POTS. Consequently, identification of the antiadre-
nergic autoantibodies may additionally require testing for altered
dosage response curves using established receptor orthosteric
agonists.

$\beta_{1/2}$AR autoantibody activity was increased in POTS but not in
VVS. In the present study, approximately two-thirds activated
$\beta_{1/2}$AR in cell-based assays. Moreover, $\beta_{1}$AR autoantibodies
enhanced the isoproterenol response and most importantly shifted
the isoproterenol dosage response curves to the left, confirming
our previous observations. $^8$

Postural tachycardia syndrome continues to be a vexing dysauto-
nomia $^2$ that has long challenged afflicted patients, their families, and
caregivers. The variable presentation and seemingly associated
autonomic disorders, and other non-specific symptoms including
gastroparesis, migraine headaches, chest pain, chronic fatigue, and
mental confusion (brain-fog) have perplexed medical personnel.
Thus, it is important to define the aetiology and develop a rationale
for specific interventions for controlling the underlying pathophysi-
ology and thereby providing symptomatic relief. There has long
been speculation that the predilection for POTS in younger females
and occasional association with a viral/bacterial infection, vaccin-
ation or a stress event would support an autoimmune component
in its aetiology. $^6,^{16,17}$

Recognition that circulating autoantibodies interact with G
protein-coupled receptor targets and modify the autonomic system
has led our group to identify AAb to the $\beta_{2}$AR and M3 muscarinic
acetylcholine receptor in a group of subjects with idiopathic ortho-
static hypotension. $^{15}$ The present study focused on the activity ex-
pected with these receptors; but identified a unique property of
the studied IgG directed toward the $\alpha_{1A}$R which exerted allosteric
effects characteristic of a partial agonist since its presence shifted
the phenylephrine dosage response curve to the right. In contrast,
the $\beta_{1}$AR effect was just the opposite and shifted the $\beta_{1}$AR agonist

| Table 2 Test positivity (direct-activating and/or ligand-modulating activity) among patients diagnosed with POTS |
|---------------------------------------------------------------|
| Patient no. | $\alpha_{1A}$R Ab | $\beta_{1/2}$R Ab | $\beta_{2}$R Ab |
|---------------|----------------|----------------|----------------|
|               | Activating Modulating | Activating Modulating | Activating |
| 1             | x               |                  | x              |
| 2             | x               | x               |                |
| 3             | x               | x               |                |
| 4             | x               | x               |                |
| 5             | x               | x               |                |
| 6             | x               | x               |                |
| 7             | x               | x               |                |
| 8             | x               | x               |                |
| 9             | x               | x               |                |
| 10            | x               | x               |                |
| 11            | x               | x               |                |
| 12            | x               | x               | x              |
| 13            | x               |                |                |
| 14            | x               |                |                |
| 15            | x               |                |                |
| 16            | x               |                |                |
| 17            | x               |                |                |
| Total         | 8/17            | 11/17           | 12/17          |

$^6$ Recognition that circulating autoantibodies interact with G protein-coupled receptor targets and modify the autonomic system has led our group to identify AAb to the $\beta_{2}$AR and M3 muscarinic acetylcholine receptor in a group of subjects with idiopathic orthostatic hypotension. $^{15}$ The present study focused on the activity expected with these receptors; but identified a unique property of the studied IgG directed toward the $\alpha_{1A}$R which exerted allosteric effects characteristic of a partial agonist since its presence shifted the phenylephrine dosage response curve to the right. In contrast, the $\beta_{1}$AR effect was just the opposite and shifted the $\beta_{1}$AR agonist...
Figure 2 Effects of IgG (0.1 mg/mL) from POTS patients on PE and ISO responses in cell-based assays. IgG from 3 POTS patients with α1AR-activating activity shifted the PE dosage response curve to the right indicating an inhibitory allosteric effect (A). In contrast, IgG from 3 β1AR antibody-positive POTS patients shifted the ISO dosage response curve to the left compatible with a positive allosteric effect. Control IgG failed to alter these curves. When IgG from all 17 POTS patients was tested in the presence of PE (1 μM) or isoproterenol (1 μM), there was a significant decrease in the PE response compared with PE alone and control IgG (C) and a significant increase in the ISO response compared with ISO alone and control IgG (D). Control IgG showed minimal modulating effects. Individual data from (C) and (D) for the POTS and control subjects are shown in (E) and (F), respectively. The dashed line is the threshold derived from the control mean values—2SD for the α1AR-modulating effect or control mean values + 2SD for the β1AR-modulating effect.

do dosage response curve to the left. It is likely that under these conditions, the primary action of the autoantibodies is not an orthosteric direct activation or inhibition of the receptor but rather modulation of the activity and action of the ligand norepinephrine/epinephrine that normally is involved in the particular receptor transduction. These contrasting effects provide an appealing explanation for the cardiovascular effects associated with upright posture in those so afflicted.18

We believe that the present study helps affirm our concept that common cardiovascular dysautonomias may express a spectrum of autoantibodies, which contribute to a variety of clinical manifestations. This spectrum would certainly range from subclinical to overt expressions that may overlap with other entities such as inappropriate sinus tachycardia (IST), where circulating antibodies against cardiac β-receptors have been previously reported.18 Both POTS and IST share abnormal sinus tachycardia tendency, and differ in regard to resting heart rate control that is usually preserved in POTS but not in IST.2 Thus, some subjects who do not formally meet the diagnostic criteria of POTS may have partial presentation of such autoantibodies. From our studies, subjects with recurrent VVS and normal orthostatic tolerance would not be likely candidates for inclusion in this grouping. We have recently demonstrated an exaggerated catecholamine response in postural tachycardia among patients with syncope and dysautonomic cardiovascular response to orthostasis.19 The catecholamine surge may be seen as a compensatory mechanism to override the α1AR malfunction associated with the proposed autoimmune blockade as present in POTS patients but this explanation is unlikely in those with recurrent VVS.

Although it is likely that POTS has multiple causes, there has been a paucity of concrete evidence for other pathophysiological bases for an underlying pathophysiology to date for the vast majority of patients. It seems apparent that the high percentage of POTS subjects examined by our group present with an apparent autoimmune diathesis that supports the concept that these autoantibodies play important role in the pathophysiology of this entity.

Strengths and limitations

This report more than doubles the number of our previous observations, and focuses on a tightly characterized group of patients and controls. We have introduced a new direct assay to measure α1AR activity, which although similar in principle to the assay for β1/2AR in transfected cells was only positive in half of the POTS patients compared with the more demanding previous bioassay that measured contractility in the cremaster arterioles in vitro. This cremaster arteriolar bioassay is not suitable for use in large numbers of samples and the specific cell-based assays are an improvement with regard to numbers. Diagnostic criteria based on measuring the autoantibodies’ impact on an allosteric-mediated receptor response to the

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Finally, the study groups were not strictly matched. The marked presence of either or both α1AR and β1/2AR autoantibodies with their potent allosteric impact on the relevant natural ligand may provide a more favourable estimate of autoantibody involvement in the complex pathophysiology of POTS. This study has an important limitation as the parasympathetic tone was not evaluated during HUT, and possible autoantibodies against muscarinic receptors and/or acetylcholine system were not investigated. The vagal drive may have an important implication in related POTS symptomatology.20 Finally, the study groups were not strictly age matched, although the age difference was not significant.

**Conclusion**

The marked presence of either or both α1AR and β1/2AR autoantibodies with their potent allosteric impact on the relevant orthosteric ligands provides an appealing framework to support an autoimmune pathophysiology of POTS. This concept, if validated, would provide concrete support for novel therapeutic approaches against POTS based on immunotherapy. These could currently include suppression of autoantibody production using immunoglobulin infusions and in the foreseeable future by development of decoy peptides that are specific for autoantibodies that target the identified receptor epitopes.

**Supplementary material**

Supplementary material is available at Europace online.

**Conflict of interest:** none declared.

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First description of a Brugada phenocopy in the inferior leads in the context of an acute inferior myocardial infarction

Ahmet Taha Alper1, Ahmet Ilker Tekkesin1, Göksel Çinier1*, Ceyhan Türkcan1 and Adrian Baranchuk2

1Dr. Siyami Ersek Thoracic and Cardiovascular Surgery Training and Research Center, Tibbey Cad, 34107 Kadikoy-Istanbul, Turkey; and 2Division of Cardiology, Kingston General Hospital, Queen’s University, Kingston, Ontario, Canada

* Corresponding author. Tel: +90 532 4842350. E-mail address: cinierg@gmail.com

A 67-year-old male patient with no prior medical cardiac history presented to the emergency department with anginal chest pain lasting for 1 h. His electrocardiographic (ECG) revealed ST-segment elevation in leads II, III, and aVF (Figure, A). A diagnosis of acute inferior myocardial infarction was made and the patient was transferred to our Cath Lab for a coronary angiography that revealed right coronary artery (RCA) occlusion in the mid segment and 50% stenosis of the circumflex artery (CX). Personal or family history of unexplained syncope, sudden cardiac death, or implantable cardiac defibrillator implantation was denied. Initial blood tests were normal except increased high sensitive troponin I: 24.98 ng/mL (0.002–0.0342). The patient presented polymorphic ventricular tachycardia (VT) with haemodynamic deterioration (Figure B). He was successfully defibrillated. Following defibrillation, the ECG revealed typical type-1 Brugada pattern in leads II, III, and aVF (Figure C). Electrolytes were normal and the patient was not receiving any medication known to trigger a Brugada ECG pattern. A new coronary angiogram showed a patent stent to the RCA and no progression of the CX lesion. The ECG normalized spontaneously immediately after the angiogram (Figure, D). A provocative test with ajmaline was performed 2 days later which failed to induce a type-1 Brugada ECG pattern.

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