Clin Auton Res (2017) 27:97–101
DOI 10.1007/s10286-017-0404-z

SHORT COMMUNICATION

Pure autonomic failure without synucleinopathy

Risa Isonaka[1] · Courtney Holmes[1] · Glen A. Cook[2] · Patti Sullivan[1] · Yehonatan Sharabi[3] · David S. Goldstein[1]

Received: 10 January 2017 / Accepted: 1 February 2017 / Published online: 10 February 2017
© Springer-Verlag Berlin Heidelberg (outside the USA) 2017

Abstract Pure autonomic failure is a rare form of chronic autonomic failure manifesting with neurogenic orthostatic hypotension and evidence of sympathetic noradrenergic denervation unaccompanied by signs of central neurodegeneration. It has been proposed that pure autonomic failure is a Lewy body disease characterized by intraneuronal deposition of the protein alpha-synuclein in Lewy bodies and neurites. A middle-aged man with previously diagnosed pure autonomic failure experienced a sudden, fatal cardiac arrest. He was autopsied, and tissues were harvested for neurochemical and immunofluorescence studies. Post-mortem microscopic neuropathology showed no Lewy bodies, Lewy neurites, or alpha-synuclein deposition by immunohistochemistry anywhere in the brain. The patient had markedly decreased immunofluorescent tyrosine hydroxylase in sympathetic ganglion tissue without detectable alpha-synuclein even in rare residual nests of tyrosine hydroxylase-containing ganglionic fibers. In pure autonomic failure, sympathetic noradrenergic denervation can occur without concurrent Lewy bodies or alpha-synuclein deposition in the brain or sympathetic ganglion tissue.

Keywords Pure autonomic failure · Synuclein · Sympathetic · Catecholamine · Norepinephrine

Abbreviations

AAG	Autoimmune autonomic ganglionopathy
CSF	Cerebrospinal fluid
DA	Dopamine
DHPG	3,4-Dihydroxyphenylglycol
DOPAC	3,4-Dihydroxyphenylacetic acid
ILBD	Incidental Lewy body disease
MSA	Multiple system atrophy
NE	Norepinephrine
OCT	Optimal cutting temperature
OH	Orthostatic hypotension
PAF	Pure autonomic failure
PBS	Phosphate-buffered saline
PD	Parkinson's disease
PET	Positron emission tomography
THir	Immunoreactive tyrosine hydroxylase

Introduction

According to the consensus definition published in 1996 [4], pure autonomic failure (PAF) is a rare disease characterized by chronic neurogenic orthostatic hypotension (OH), no clinical signs of central neurodegeneration, and low plasma norepinephrine (NE) levels, the latter reflecting generalized sympathetic noradrenergic denervation [1].

Since publication of the consensus definition, post-mortem studies have noted the occurrence of Lewy bodies, a pathologic hallmark of Parkinson's disease, in sympathetic

Electronic supplementary material The online version of this article (doi:10.1007/s10286-017-0404-z) contains supplementary material, which is available to authorized users.

✉ David S. Goldstein
goldsteind@ninds.nih.gov

[1] Clinical Neurocardiology Section, Clinical Neurosciences Program, Division of Intramural Research, National Institute of Neurological Disorders and Stroke, National Institutes of Health, 9000 Rockville Pike MSC-1620, Building 10 Room 5N220, Bethesda, MD 20892-1620, USA

[2] Naval Medical Center, Portsmouth, VA, USA

[3] Tel Aviv University Sackler Faculty of Medicine, Tel HaShomer, Israel

ganglion tissue from PAF patients [3]. Lewy bodies contain an abundance of the protein, alpha-synuclein, and PAF patients have also been reported to have ganglionic alpha-synuclein deposition [5]. The view has evolved that PAF is in a family of autonomic synucleinopathies that also includes multiple system atrophy (MSA) and Parkinson's disease (PD) with autonomic failure [1].

Here we report the case of a patient who had the characteristic clinical triad seen in PAF—chronic neurogenic OH, no clinical signs of central neurodegeneration, and generalized sympathetic noradrenergic denervation—but without Lewy bodies or alpha-synuclein deposition in the brain, brainstem, sympathetic ganglion tissue, or myocardium. The results fit with the concept of PAF being a heterogeneous disorder and not necessarily associated with synucleinopathy.

Case report

The patient felt well until he was about 50 years old, when he began to experience erectile dysfunction and urinary frequency and retention. These were followed soon afterward by symptomatic OH. All these symptoms progressed gradually. He had his first episode of OH-related syncope about 5 years later, and he had to self-catheterize because of urinary retention. He had frequent orthostatic lightheadedness and occasional syncopal episodes. The patient also reported dream enactment behavior, constipation, heat intolerance, decreased sweating, and angina-like chest pain during exertion. He did not have cognitive changes, visual hallucinations, slurred speech, altered handwriting, decreased sense of smell, or any motor complaints. His University of Pennsylvania Smell Identification test score was normal at 35/40.

The patient's maternal grandmother had died of PD. The patient grew up on a farm and had long-term, frequent pesticide exposure. He served in the military during a period of ground water contamination at the base camp. He also had chronic, repeated industrial exposures as a crew member in a *Sturgeon* class nuclear powered attack submarine. Physical examination showed an adult white man who appeared healthy. During the examination he fell asleep and evinced obstructive sleep apnea. Neurological examination was unremarkable, except for mild length-dependent sensory neuropathy, and routine laboratory tests were generally normal or negative. Brain magnetic resonance imaging was normal.

The patient was first evaluated at the NIH Clinical Center at 57 years old. He had severe, rapid OH evoked by head-up tilt (Fig. 1) and a markedly abnormal pattern of beat-to-beat blood pressure associated with the Valsalva maneuver, indicating neurogenic OH.

Low concentrations of norepinephrine (NE) and of its intra-neuronal metabolite 3,4-dihydroxyphenylglycol (DHPG) in venous and arterial plasma and skeletal muscle microdialysate (Supplementary Table) signified generalized sympathetic noradrenergic denervation. Low cerebrospinal fluid (CSF) concentrations of both NE and DHPG pointed to concurrent central noradrenergic deficiency.

Between the initial evaluation and a follow-up evaluation 3 years later, plasma norepinephrine and DHPG levels decreased proportionately similarly, while plasma dopamine levels increased so that at follow-up the plasma DA/NE ratio during supine rest was 19 times and during orthostasis 33 times that at the time of the initial evaluation (Supplementary Table).

During both evaluations the patient had normal results of the quantitative sudomotor axon reflex test.

Thoracic 18F-dopamine positron emission tomographic (PET) scanning showed decreased radioactivity in the left ventricular apex and free wall and normal radioactivity in the interventricular septum. Skin biopsy revealed decreased immunoreactive tyrosine hydroxylase (THir) in *arrector pili* muscle, without detected alpha-synuclein (data not shown). The patient did not have an elevated level of antibodies to the neuronal nicotinic receptor.

Several months after the follow-up evaluation, the patient had a sudden cardiac arrest. He was resuscitated from asystole, but died a few days later. He was autopsied at the NIH Clinical Center, and tissues from body organs were harvested for research purposes. The post-mortem interval was about 14 h.

Gross and microscopic neuropathology were done according to standard techniques. The neuropathology report noted evidence of anoxic encephalopathy. There was no depigmentation of the substantia nigra or locus ceruleus, and there was no cerebellar or brainstem atrophy. Microscopic analyses did not detect Lewy bodies, Lewy neurites, glial cytoplasmic inclusions, or intra-neuronal alpha-synuclein deposits anywhere in the brain or brainstem.

Frozen tissue samples were assayed for catechols by batch alumina extraction followed by liquid chromatography with electrochemical detection. Substantially decreased myocardial NE and DHPG contents (Supplementary Table) confirmed cardiac sympathetic denervation. NE and DHPG concentrations in sympathetic ganglion tissue also seemed low (normal values have not yet been established). Putamen dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) contents were decreased.

For immunofluorescence confocal microscopy, frozen samples were sliced into 10 μm thick sections. Nonspecific binding was blocked by incubation with glycine and then with 1% bovine serum albumin, 0.3% Triton-X, and 10% normal donkey serum in phosphate-buffered

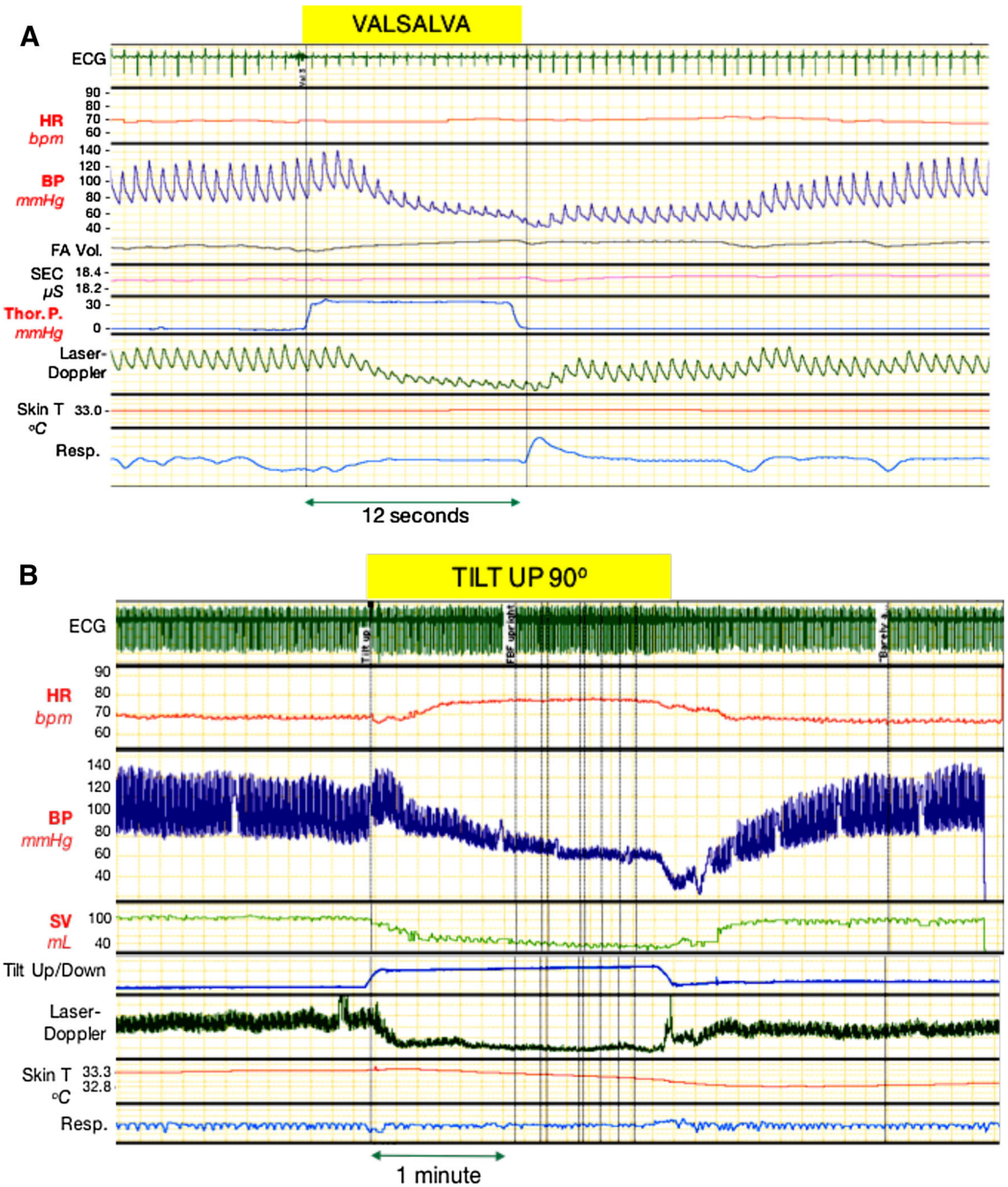

Fig. 1 Hemodynamics associated with **a** the Valsalva maneuver and **b** head-up tilting. The tracings show the electrocardiogram and beat-to-beat heart rate (HR) and blood pressure (BP) at the time of initial evaluation. Other channels show cardiac stroke volume (SV), forearm volume as indicated by impedance plethysmography (FA Vol.), skin electrical conductance (SEC), thoracic pressure (Thor. P.), laser-Doppler microcirculatory flow, skin temperature, and respiration (Resp.). The patient was tilted head-up at 90° from *horizontal*. Blood pressure rapidly decreased, confirming OH. The patient had a progressive fall in blood pressure in Phase II during the Valsalva maneuver and an absence of a pressure overshoot in Phase IV, indicating that the OH was neurogenic

saline. The samples were then incubated for 24 h at 4 °C with rabbit anti-tyrosine hydroxylase antibody (Pel-Freez Biologicals, Rogers, AR, USA) and mouse monoclonal anti-alpha-synuclein (Santa Cruz Biotechnology, Santa Cruz, CA, USA), followed by incubation for 1 h at room temperature with Cy3-conjugated anti-rabbit secondary antibody (Jackson Immune Research Labs), Alexa 488-conjugated anti-mouse IgG secondary antibody (Thermo Scientific, Inc., Rockford, IL, USA), and Hoechst 33342 trihydrochloride trihydrate (Thermo Scientific, Inc.).

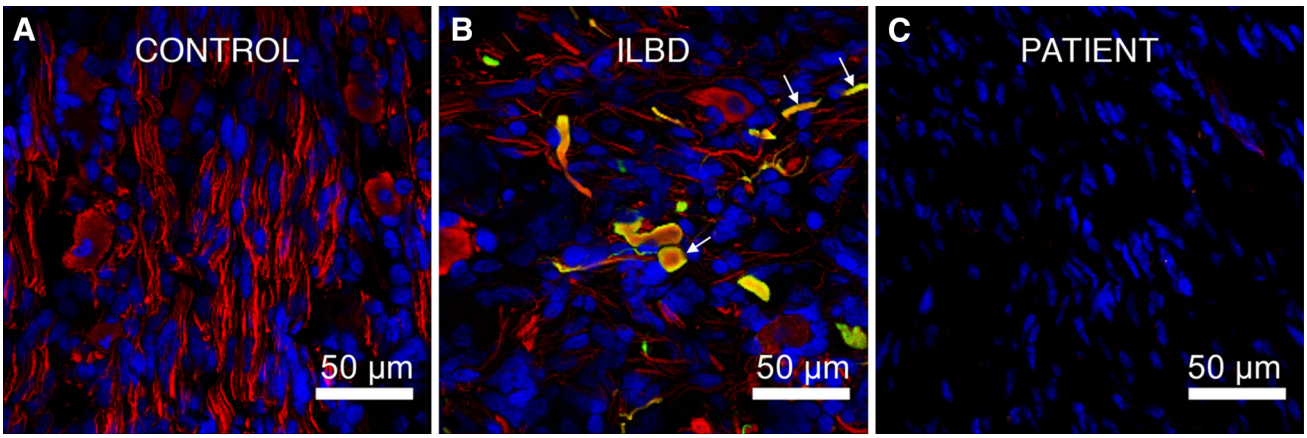

Fig. 2 High magnification immunofluorescence microscopic images of post-mortem sympathetic ganglion tissue. The panels show DAPI (*blue*), immunoreactive tyrosine hydroxylase (THir, *red*), and alpha-synuclein (*green*) in a control subject (**a**), a patient with incidental Lewy body disease (ILBD) (**b**), and our patient (**c**). The patient with ILBD had alpha-synuclein co-localized with THir (*yellow*, Lewy neurites and a possible Lewy body indicated by *arrows*). Our patient had decreased THir and no alpha-synuclein

The immunostained tissues were mounted on slides with anti-fade reagent and subjected to immunofluorescence confocal microscopy using a Zeiss LSM 510 confocal laser scanning microscope (Carl Zeiss, Germany).

Sympathetic ganglion tissue from a control subject showed abundant, diffusely distributed immunoreactive tyrosine hydroxylase (THir)-containing fibers and no alpha-synuclein (Fig. 2a). Tissue from a positive control patient with incidental Lewy body disease (ILBD) showed alpha-synuclein co-localized with TH in neurons and neurites (Fig. 2b). In marked contrast, sympathetic ganglion tissue from our patient had generally decreased THir with foci of intense THir and no alpha-synuclein (Fig. 2c). Myocardial tissue from a comparison patient with MSA demonstrated THir-positive fibers, whereas our patient had virtually no myocardial THir. In the scarce remaining TH-positive fibers in myocardial tissue there was no evidence of co-localization of alpha-synuclein with TH. Myocardial alpha-synuclein deposition was not found in either patient, including near myocardial axons identified by the TH immunostaining (data not shown). In contrast with skin biopsy samples from patients with Lewy body dementia, our patient had no alpha-synuclein deposition in skin biopsy tissue (data not shown).

Discussion

Our patient had the characteristic clinical features of PAF—neurogenic orthostatic hypotension, no clinical evidence of central neurodegeneration, and neurochemical evidence of generalized sympathetic noradrenergic denervation [4]. In vivo and post-mortem data and the patient's clinical course excluded secondary causes such as medications, diabetes mellitus, amyloidosis, and multiple myeloma and also excluded PD, MSA, autoimmune autonomic ganglionopathy (AAG), DA-beta-hydroxylase deficiency, and autoimmunity-associated autonomic failure with sympathetic denervation.

Regarding differentiating PAF from AAG, AAG was excluded both by negative anti-nicotinic receptor antibody testing and by the particular pattern of plasma catechols. In AAG, interference with ganglionic neurotransmission attenuates exocytotic release of norepinephrine (NE) from intact post-ganglionic nerves. Accordingly, the ratio of NE to its neuronal metabolite, 3,4-dihydroxyphenylglycol (DHPG), is low. In PAF, because of denervation and consequently decreased vesicular NE stores, DHPG production and plasma DHPG levels are low. Meanwhile, probably due to compensatorily increased nerve traffic to the remaining terminals and decreased neuronal reuptake of released NE, plasma NE levels can be near normal, and the NE/DHPG ratio is high. Moreover, in AAG cardiac sympathetic innervation is intact, whereas in PAF there is cardiac sympathetic denervation (as in our patient).

Several previously reported autopsied PAF cases had Lewy bodies, intra-neuronal synucleinopathy, or both [3, 5]. These findings contributed to the current view that PAF is a form of autonomic synucleinopathy [1]. Unexpectedly, our patient had no Lewy bodies, Lewy neurites, or alpha-synuclein deposits in the brain, brainstem, sympathetic chain, myocardium, or skin. That is, the patient had PAF without synucleinopathy.

The severity of sympathetic noradrenergic denervation as indicated by plasma NE and DHPG levels worsened between the initial and 3-year follow-up evaluations. The patient, therefore, had a progressive neurodegenerative process in sympathetic noradrenergic nerves.

In contrast, plasma DA levels increased. The plasma DA/NE ratio at follow-up was 19–33 times higher than at

the time of the initial evaluation. The differential changes in levels of the two catecholamines would be consistent with compensatorily augmented nerve traffic to residual terminals and preferential release of vesicles containing DA that had not yet been converted to NE. Alternatively, since NE is produced within vesicles by the action of intravesicular DA-beta-hydroxylase after vesicular uptake of DA from the cytoplasm, increased DA/NE ratios might reflect deficient vesicular storage, which seems to be a common theme in catecholaminergic neurodegeneration [2]. Low cerebrospinal fluid levels of NE and DHPG provided evidence of concurrent central noradrenergic deficiency.

The patient had normal sense of smell, which is not typical of Lewy body diseases. Since our patient had a non-Lewy body form of PAF, this result raises the possibility that olfactory dysfunction in Lewy body diseases may be causally related to synucleinopathy.

Considering the patient's history of having grown up on a farm with multiple, repeated pesticide exposures and well water, and of having been a crew member in an attack submarine, we speculate that PAF in this case could have resulted from one or more environmental agents that produced oxidative stress in catecholaminergic neurons.

In summary, we report a case of PAF without Lewy bodies. The relative frequencies of PAF with vs. without synucleinopathy are unknown. It is reasonable to hypothesize that retention of the PAF phenotype for a number of years may indicate absence of synucleinopathy. It is hoped that ongoing natural history studies with definitive autopsy data will test this hypothesis in the future.

Acknowledgements The research reported here was supported by the Division of Intramural Research, NINDS, NIH.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Financial support Division of Intramural Research, NINDS, NIH.

References

1. Garland EM, Hooper WB, Robertson D (2013) Pure autonomic failure. Handb Clin Neurol 117:243–257
2. Goldstein DS, Holmes C, Sullivan P, Mash DC, Sidransky E, Stefani A, Kopin IJ, Sharabi Y (2015) Deficient vesicular storage: a common theme in catecholaminergic neurodegeneration. Parkinsonism Relat Disord 21:1013–1022
3. Hague K, Lento P, Morgello S, Caro S, Kaufmann H (1997) The distribution of Lewy bodies in pure autonomic failure: autopsy findings and review of the literature. Acta Neuropathol 94:192–196
4. Kaufmann H (1996) Consensus statement on the definition of orthostatic hypotension, pure autonomic failure and multiple system atrophy. Clin Auton Res 6:125–126
5. Kaufmann H, Hague K, Perl D (2001) Accumulation of alpha-synuclein in autonomic nerves in pure autonomic failure. Neurology 56:980–981