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ORIGINAL RESEARCH

Sudoscan in ATTRv Amyloidosis: A Potential Marker of Disease Progression?

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ABSTRACT

***Introduction*:** Hereditary transthyretin amyloidosis (ATTRv amyloidosis) is a severe, autosomal dominant disease resulting from multisystemic extracellular deposition of amyloid fibrils, leading to progressive organ damage and death. Sudoscan is a reproducible tool investigating sweat gland function and, indirectly, small nerve fiber impairment. The aim of this study was to evaluate any changes over time in electrochemical skin conductance (ESC) measured by Sudoscan in a cohort of late-onset patients with ATTRv from a single Italian center. Additionally, we investigated the role of Sudoscan as a marker of disease severity to confirm previous literature data.

***Methods*:** We enrolled 61 patients with a late-onset ATTRv amyloidosis harboring different *TTR* variants with at least one clinical and instrumental evaluation including Sudoscan. Correlations with clinical data (including both clinical scales and questionnaires) were investigated to confirm the role of Sudoscan as a marker of disease severity. Moreover, a longitudinal analysis was performed in the subgroup of patients with at least 4 complete yearly evaluations ($n=23$) to assess the role of Sudoscan as a marker of disease progression.

***Results*:** At each yearly assessment, ESC values from both feet and hands significantly correlated with disease duration and neuropathy severity, as assessed by common clinical scales and questionnaires. No correlation was found with age at evaluation. Moreover, we observed a statistically significant change over time in ESC values measured at the feet (fESC) but not at the hands (hESC).

***Conclusions*:** Sudoscan may represent a reliable marker of dysautonomia in ATTRv amyloidosis, displaying a potential role as a marker of both disease severity and progression. It could, therefore, serve as an outcome measure in future clinical trials. In addition, feet ESC seems to be a

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significant, independent predictor of autonomic dysfunction.

Keywords: Hereditary transthyretin amyloidosis; Autonomic neuropathy; Sudomotor; Sudoscan; Electrochemical skin conductance

Key Summary Points

Why carry out this study?
Hereditary transthyretin amyloidosis (ATTRv, v for variant) is a multisystemic disease characterized by amyloid deposition that primarily affects the peripheral nervous system, heart, kidney and gastrointestinal tract
Autonomic nervous system (ANS) involvement in ATTRv is quite frequent and can represent an early manifestation of the disease
What was learned from the study?
Sudoscan is a quick and non-invasive diagnostic test able to provide an indirect objective evaluation of small nerve fiber involvement by assessing sweat gland function
Sudoscan may represent a reliable marker of dysautonomia in ATTRv amyloidosis
Sudoscan may represent a reliable marker of both disease severity and progression in ATTRv amyloidosis

INTRODUCTION

Hereditary transthyretin amyloidosis (ATTRv, *v* for 'variant') is a rare, adult-onset, autosomal-dominant disease with variable penetrance, caused by mutations in the *TTR* gene encoding transthyretin. Pathogenic *TTR* variants decrease the stability of the TTR tetramer and prompt the start of the amyloid cascade in several organs and tissues, causing progressive organ dysfunction [1, 2].

Clinically, ATTRv amyloidosis manifests as a progressive multisystemic disease with great phenotypic heterogeneity. Among the variegated manifestations of the disease, impairment of the autonomic nervous system (ANS) is quite frequent and can represent an early manifestation of the disease, especially in endemic areas [1]. Common autonomic manifestations include cardiovascular autonomic dysfunction (e.g., orthostatic hypotension), gastrointestinal symptoms, urinary symptoms, xerophthalmia and/or xerostomia, or sweating abnormalities. Moreover, in men, erectile dysfunction can be an early feature of the disease that may precede the onset of sensory neuropathy. Gastrointestinal symptoms often lead to progressive, unintentional weight loss, and cachexia represents a major cause of death in endemic regions [1, 3, 4]. However, although dysautonomia is considered a less prominent feature in late-onset ATTRv, it is not uncommon; indeed, autonomic disturbances are almost constant during the course of the disease, having a great impact on the patient's quality of life [5].

In the past, the disease prognosis was typically severe, as involved organs, in the absence of treatment, relentlessly move toward life-threatening progressive failure [1]. Nowadays, the availability of new drugs potentially able to prevent or delay disease progression, especially if started early [6], has prompted the search for reliable biomarkers aimed at improving early diagnosis. In parallel, the identification of markers useful for monitoring disease severity and progression and assessing response to treatment is equally fundamental.

Sudoscan is a quick, non-invasive diagnostic test able to provide an objective indirect evaluation of small nerve fiber involvement through the assessment of sweat gland function. Its role in ATTRv amyloidosis has already been assessed [7–9], showing a high diagnostic accuracy as a screening test in *TTR* mutation carriers, especially in the early-onset population [7]. Conversely, its role as a marker of disease progression is still considered uncertain.

In this single-center study, we aimed to assess the role of Sudoscan as a measure of

disease progression by evaluating any potential changes over time in electrochemical skin conductance (ESC) values in a cohort of patients with ATTRv from central Italy, a non-endemic area.

METHODS

Data were collected from a cohort of adult patients with ATTRv-PN in follow-up at our Department of Neurology in Rome, Italy, between March 2016 and August 2024.

For each patient, detailed demographic, genetic and clinical data were recorded, including pathogenic *TTR* variant, sex, age at onset and evaluation, clinical phenotype and any concomitant diseases. Patients with any medical disorder that could affect the peripheral nervous system (e.g., diabetes mellitus) or sweat gland function (e.g., Sjögren syndrome) were not included.

Patients were assessed on a yearly basis (±3 months) through an extensive neurological and neurophysiological evaluation according to standard clinical practice.

Disease severity was estimated based on common clinical outcome measures [2, 10], such as the Familial Amyloid Polyneuropathy (FAP) stage [11], Polyneuropathy Disability (PND) score [12], Neuropathy Impairment Score (NIS) [13] and its subscores at the lower (NIS-LL) and upper limbs (NIS-UL). The total score on the Norfolk Quality of Life-Diabetic Neuropathy (QoL-DN) questionnaire [14] and the score on the Compound Autonomic Dysfunction Test (CADT) [15] were recorded as measures of quality of life and autonomic impairment, respectively. Specifically, we defined a *modified*, four-item version of the CADT (*mCADT* hereafter), obtained by removing the erectile dysfunction item to align the score for both male and female patients.

Any change in blood pressure (BP) upon standing was recorded to look for orthostatic hypotension [clinically defined as a drop in systolic BP (SBP) of at least 20 mmHg and/or diastolic BP (DBP) of at least 10 mmHg within 3 minutes of standing] [16].

Focusing on the degree of systolic blood pressure (SBP) decrease, *postural BP scoring* was calculated and recorded as a measure of autonomic cardiovascular function, with a higher score reflecting a worse degree of orthostatic intolerance (namely, SBP decrease < 20 mmHg = 0 points; decrease of 20 to < 30 mmHg = 1 point; decrease of ≥ 30 mmHg = 2 points) [17, 18].

Patients with at least one symptom or sign suggestive of autonomic dysfunction (postural hypotension; slow digestion/early satiety, nausea and/or vomiting; severe diarrhea or constipation, or alternating diarrhea and constipation; unintentional weight loss; sphincter disturbances; erectile dysfunction in male patients) were labeled as *patients with dysautonomia*.

The presence and severity of neuropathy were evaluated through conventional nerve conduction studies (NCSs), according to standard protocols [19]. Age- and sex-adjusted normative values of our laboratory were used [20].

The sudomotor function, as an indirect measure of small nerve fiber and autonomic involvement, was assessed by Sudoscan™ (Impeto Medical, Paris, France). Sudoscan is a non-invasive rapid test measuring electrochemical skin conductance (ESC) of the feet and hands in response to a low voltage electrical stimulus (< 4 V) [21]. Mean scores for both feet (fESC) and hands (hESC) were automatically calculated by the device and considered for analysis.

The study was approved by the Ethics Committee of "Fondazione Policlinico Agostino Gemelli IRCSS" (Prot. ID Prot. ID 5470), and written informed consent was obtained from all participants. All procedures were carried out in accordance with the principles of the 1964 Declaration of Helsinki.

Statistical Analysis

The study sample was described in its demographic and clinical characteristics using descriptive statistics techniques.

To compare the distribution of categorical variables between groups, the Chi-squared test or Fisher's exact test were used, as appropriate.

For quantitative data, the normal distribution was assessed by both graphical and statistical

methods (visual inspection of normal *Q*–*Q* plots and Shapiro-Wilk test, respectively). Given the nature of data and violation of normality, non-parametric tests were adopted.

Comparisons between independent groups were performed using the Mann-Whitney *U* test or Kruskal-Wallis *H* test, as appropriate. Pairwise comparisons, where applicable, were performed using Dunn's procedure. A Friedman test (and post hoc tests, where pertinent) was run to evaluate whether there were any significant changes over time in fESC and hESC values. Given the small sample size available at T4 and T5, the repeated measures analysis was limited to those patients with a complete 3-year follow-up (i.e., from T0 to T3). For both between- and within-group analyses, post hoc tests were adjusted by a Bonferroni correction for multiple comparisons. Adjusted *p* values (*adj p*) are reported in these cases.

A Spearman's rank-order correlation coefficient was used to evaluate the relationship between ESC values and the main demographic and clinical variables at baseline and each subsequent evaluation (i.e., from T0 to T4).

A binomial logistic regression analysis was carried out to identify any potential independent predictors for the presence of dysautonomia. fESC and hESC values, as well as other potential confounding variables, were evaluated for this purpose after assessing assumptions (linearity, multicollinearity, absence of significant outliers). A stepwise method with backward elimination was used to perform variable selection.

For fESC values, a receiver-operating characteristic (ROC) curve was created by plotting the true-positive rate (TPR; i.e., "sensitivity") versus the false-positive rate (FPR; i.e., "1 – specificity") across different cut-off points to evaluate the optimal threshold able to discriminate between patients with and without dysautonomia. The area under the curve (AUC) was calculated. Youden's *J* index was used to identify the optimal cut-off that best maximized the difference between TPR and FPR.

Statistical analysis was performed using IBM® SPSS® Statistics version 25.0 (IBM Corp., Armonk, NY, USA). For all analyses, statistical significance was set at $\alpha \leq 0.05$.

RESULTS

Study Population

The study enrolled 61 symptomatic patients with ATTRv with at least one clinical and neurophysiological evaluation including Sudoscan. Most patients were male (46/61, 75.4%), and all were Caucasian. Pathogenic *TTR* variants were exclusively associated with a late-onset phenotype. p.Val50Met was the most common *TTR* variant (30/61, 49.2%), followed by the p.Phe84Leu variant (19/61, 31.1%). At baseline, 21/61 patients (34.4%) were treated [namely, 12 with tafamidis 30 mg, 4 with patisiran, 3 with inotersen and 2 with diflunisal with a mean therapy duration of 24.14 ± 27.01 (range 1–105) months]. The main demographic and clinical features of the study cohort at baseline are summarized in Table 1.

For follow-up, patients were assessed on a yearly basis (± 3 months). A yearly evaluation with Sudoscan was available for 48 patients at 1 year since baseline (T1), 31 patients at 2 years (T2), 23 patients at 3 years (T3) and 14 patients at 4 years (T4). Only a small percentage of patients (6 out of 61) had a follow-up with Sudoscan of at least 5 years (up to 7 years), but these data were not included in any analyses because of the extremely low count.

Sudoscan as a Marker of Disease Progression

Longitudinal Analysis

A repeated measures test was run in the subgroup of patients with at least 4 yearly evaluations (i.e., from T0 to T3; $n = 23$) to detect any change over time in ESC values measured on both feet and hands.

fESC values were statistically significantly different at the different time points ($p = 0.001$). Post-hoc analysis revealed that fESC values decreased significantly from baseline (T0: median = 54.00) to the 2-year evaluation (T2: median = 36.00) (adjusted $p = 0.005$) and from baseline to the 3-year evaluation (T3: median = 33.00) (adjusted $p = 0.002$) but not

Table 1 Demographic and clinical features of the whole study cohort ($n = 61$) at the baseline evaluation (T0)

	ATTRv patients ($n = 61$)
Sex	
Male	46 (75.4%)
Female	15 (24.6%)
TTR variant	
p.Val50Met	30 (49.2%)
p.Phe84Leu	19 (31.1%)
p.Ile88Leu	3 (4.9%)
p.Val142Ile	3 (4.9%)
p.Thr79Lys	2 (3.3%)
p.Ala140Ser	2 (3.3%)
p.Ala129Ser	1 (1.6%)
p.Glu74Gln	1 (1.6%)
Age at onset (years)	$M = 64.11 \pm 11.31$; $Mdn = 66.00$ [59.00 to 72.00]
Age at the baseline evaluation (years)	$M = 68.38 \pm 8.59$; $Mdn = 69.00$ [64.00 to 73.50]
Baseline disease duration (i.e., time since symptom onset to T0) (months)	$M = 53.51 \pm 65.03$; $Mdn = 38.00$ [7.00 to 66.50]
Phenotype at baseline	
Mixed	48 (78.7%)
Neuropathic	9 (14.8%)
Predominantly/exclusively cardiac	4 (6.6%)
FAP stage at baseline ($n = 57$)[a]	
FAP1 (ambulatory without assistance)	40 (70.2%)
FAP2 (ambulatory with assistance)	17 (29.8%)
PND score at baseline ($n = 57$)[a]	
PND I (sensory disturbances but preserved walking capability)	22 (38.6%)
PND IA (symptomatic SFN with normal NCSs)	9 (15.8%)
PND IB (sensory disturbances and large fiber loss as confirmed on NCSs)	13 (22.8%)
PND II (impaired walking capability but ability to walk without a stick or crutches)	18 (31.6%)
PND IIIa (walking only with the help of 1 stick or crutch)	10 (17.5%)
PND IIIb (walking with the help of 2 sticks or crutches)	7 (12.3%)

Table 1 continued

	ATTRv patients (*n* = 61)
NIS total score at baseline[b]	*M* = 48.85 ± 39.74; *Mdn* = 36.00 [16.00 to 80.00]
NIS-LL at baseline[c]	*M* = 25.66 ± 20.73; *Mdn* = 21.75 [6.00 to 41.75]
NIS-UL at baseline[d]	*M* = 23.19 ± 20.57; *Mdn* = 18.00 [6.50 to 37.50]
Norfolk QoL-DN total score at baseline[e]	*M* = 45.87 ± 34.25; *Mdn* = 40.00 [18.00 to 72.50]
Dysautonomia at baseline (at least one symptom/sign)	43 (70.5%)
Orthostatic hypotension at baseline (drop in either SBP or DBP upon standing)	22 (36.1%)
Systolic BP scoring at baseline	
0 points (SBP decrease < 20 mmHg)	43 (70.5%)
1 point (SBP decrease ≥ 20 to < 30 mmHg)	8 (13.1%)
2 points (SBP reduction ≥ 30 mmHg)	10 (16.4%)
"Modified" CADT (*mCADT*) at baseline[f]	*M* = 13.08 ± 2.81; *Mdn* = 14.00 [11.00 to 16.00]
fESC at baseline (μS)	*M* = 50.95 ± 24.98; *Mdn* = 57.00 [25.50 to 75.00]
hESC at baseline (μS)	*M* = 51.85 ± 23.02; *Mdn* = 56.00 [32.50 to 70.00]

Quantitative variables are summarized as mean (*M*) ± standard deviation (SD); median (*Mdn*) [interquartile range, IQR]. Qualitative variables are expressed as absolute frequencies (*n*) and percentages (%). Percentages may not total 100 due to rounding

ATTRv hereditary transthyretin amyloidosis, *FAP* familial amyloid polyneuropathy, *mPND* modified polyneuropathy disability score, *SFN* small fiber neuropathy, *NCSs* nerve conduction studies, *NIS* neuropathy impairment score, *NIS-LL* subscore of the NIS at lower limbs, *NIS-UL* subscore of the NIS at upper limbs, *Norfolk QoL-DN* Norfolk Quality of Life-Diabetic Neuropathy questionnaire, *BP* blood pressure, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *CADT* compound autonomic dysfunction test, *fESC* electrochemical skin conductance values at feet, *hESC* electrochemical skin conductance values at hands

[a]The count refers to patients with evidence of polyneuropathy (i.e., pure neuropathic plus mixed phenotypes)

[b]NIS composite total score ranges from 0 to 244, with higher scores indicating greater neuropathic impairment

[c]NIS-LL subscore ranges from 0 to 88, with higher scores indicating greater neuropathic impairment of the lower limbs

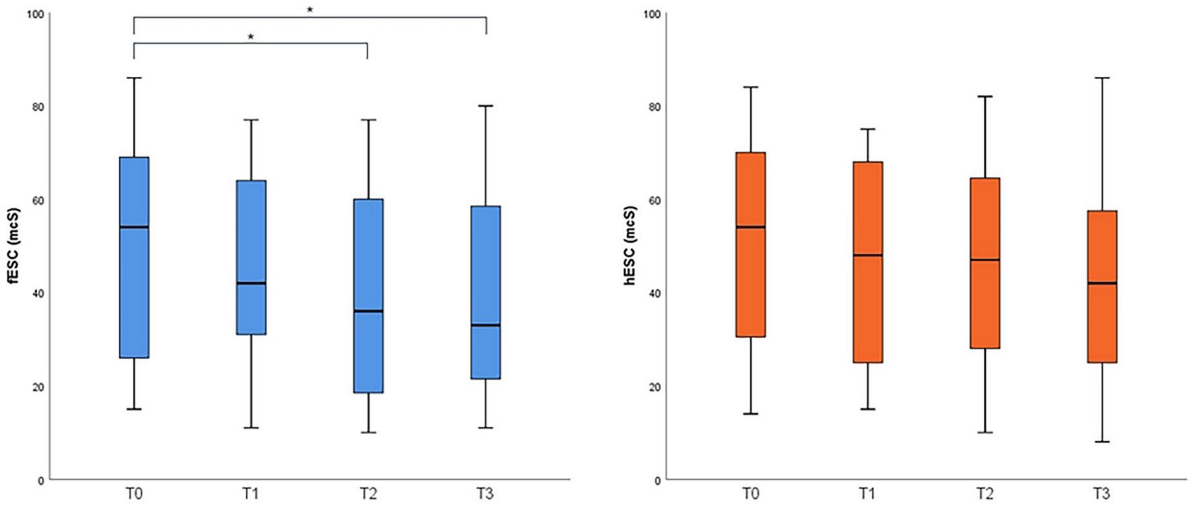
[d]NIS-UL subscore ranges from 0 to 156, with higher scores indicating greater neuropathic impairment of the upper limbs

[e]The total score on the Norfolk QoL-DN questionnaire ranges from − 4 to 136, with higher scores indicating poorer quality of life

[f]A "modified", 4-item version of the CADT (*mCADT*), obtained by removing the erectile dysfunction item, was used to align the score for both males and females (range 0–16, with lower scores suggesting greater autonomic dysfunction)

from baseline to the 1-year evaluation or other timepoints combinations (Fig. 1).

Conversely, there were no significant within-group differences in hESC values ($p = 0.116$)



	T0	T1	T2	T3	p^a	Pairwise comparisons[b] *adj. p*
fESC (*μS*)	54.00 [26.00 to 69.00]	42.00 [31.00 to 64.00]	36.00 [18.50 to 60.00]	33.00 [21.50 to 58.50]	**0.001**	T0 *vs* T2: adj. p = **0.005** T0 *vs* T3: adj p = **0.002**
hESC (*μS*)	54.00 [30.50 to 70.00]	48.00 [25.00 to 68.00]	47.00 [28.00 to 64.50]	42.00 [25.00 to 57.50]	0.116	N/A

Fig. 1 Electrochemical skin conductance values over time measured on feet (fESC) and hands (hESC). The longitudinal analysis was conducted in the subgroup of patients with at least 4 yearly evaluations ($n = 23$). T0 refers to the baseline evaluation, T1 to the 1st year evaluation and so on. Top: each boxplot represents the area between the first ($Q1$) and the third ($Q3$) quartile (interquartile range, IQR), with the horizontal line inside the boxes representing the median ($Q2$). Whiskers extend to the lowest and highest values within 1.5 times the IQR. *$p < 0.05$. Bottom: quantitative data in the table are reported as median [interquartile range] and expressed in microSiemens (μS). Bold values denote statistical significance at the $p < 0.05$ level. *fESC* feet electrochemical skin conductance, *hESC* hands electrochemical skin conductance, *N/A* not applicable. [a]Differences over time were evaluated by the Friedman test. [b]Post-hoc pairwise comparisons were performed where pertinent, and significance values adjusted using the Bonferroni correction for multiple tests. Only statistically significant post-hoc tests are displayed. Adjusted *p*-values (*adj p*) are reported

despite a downward trend over time (Fig. 1).

Sudoscan as a Marker of Disease Severity

Correlations Between ESC Values and Demographic-Clinical Variables

To further validate the role of the Sudoscan as a marker of disease severity in ATTRv amyloidosis, Spearman's rank-order correlations were run at each available timepoint (T0 to T4) to evaluate any correlations between either fESC and hESC values and demographic and clinical outcome measures in the whole study cohort.

At baseline ($n = 61$), fESC values showed a statistically significant negative correlation with NIS ($r_s = -0.625$, $p < 0.001$), Norfolk total score ($r_s = -0.510$, $p < 0.001$) and disease duration

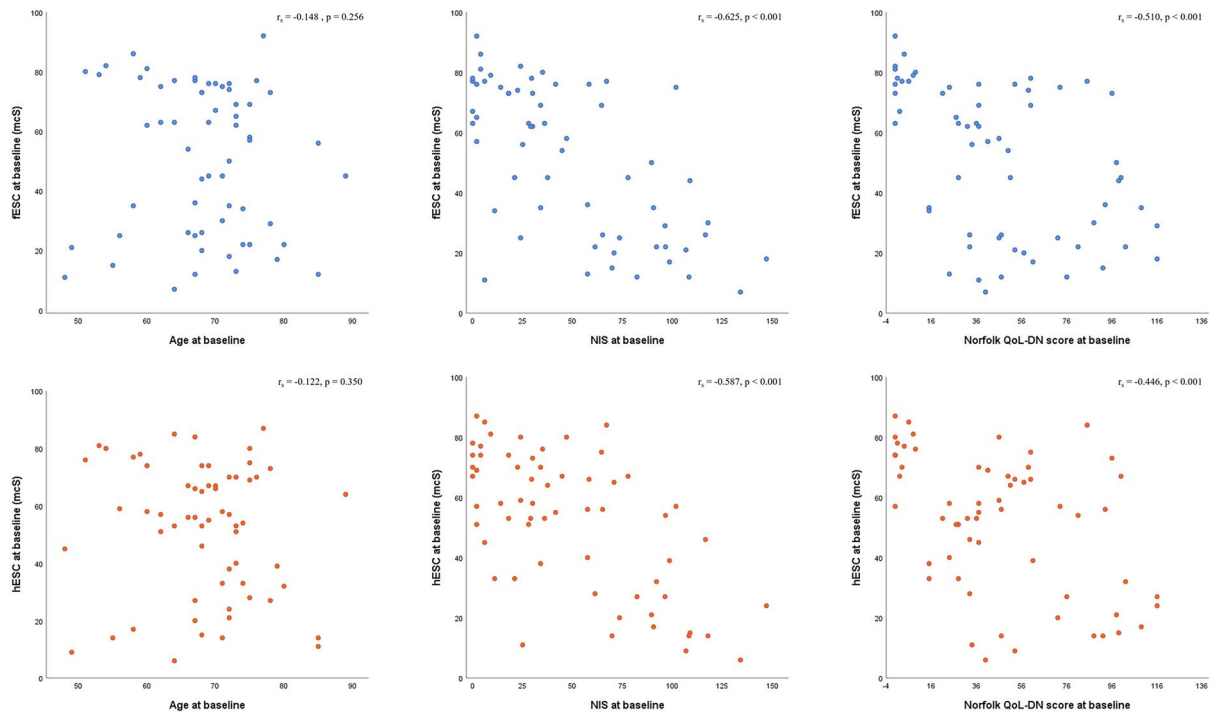


Fig. 2 Scatterplots investigating the relationship between electrochemical skin conductance (ESC) values and the main demographic and clinical variables at the baseline evaluation (T0). ESC measured on both feet (fESC) and hands (hESC) significantly inversely correlated with disease severity (as measured by Neuropathy Impairment Score [NIS]) and quality of life (as expressed by the Norfolk total score) but not with age at evaluation

($r_s = -0.661$, $p < 0.001$). A significant direct correlation was observed between fESC values and mCADT ($r_s = 0.607$, $p < 0.001$) (Fig. 2, and Supplementary Table S1). Equally, for hESC values, a statistically significant negative correlation was identified with disease duration ($r_s = -0.473$, $p < 0.001$), NIS ($r_s = -0.587$, $p < 0.001$) and Norfolk ($r_s = -0.446$, $p < 0.001$), and a positive correlation was observed with mCADT ($r_s = 0.654$, $p < 0.001$) (Fig. 2, and Supplementary Table S1). Conversely, neither fESC nor hESC values displayed any statistically significant correlation with age at evaluation ($r_s = -0.148$ and $r_s = -0.122$, respectively; $p > 0.05$) (Fig. 2 and Supplementary Table S1).

Analogous results were detected for each following timepoint (results not shown).

Comparisons Between Subgroups

A Mann-Whitney *U* test was run to determine whether there were any differences in fESC and hESC values at baseline between patients with and without dysautonomia (Supplementary Table S2). The two subgroups did not differ in terms of either age at evaluation ($p = 0.563$) or gender ($p = 0.340$). Median fESC values were statistically significantly lower in patients with dysautonomia (35.00 µS) than in patients without any autonomic disturbance (73.50 µS) ($p < 0.001$). Analogous results were observed regarding median hESC values (51.00 µS in the first subgroup vs 72.00 µS in the second one; $p < 0.001$).

When comparing patients with and without postural hypotension (intended as a significant decrease in either SBP or DBP), a statistically significant difference was detected for both fESC (25.50 vs 65.00 µS, respectively; $p < 0.001$) and hESC (36.00 vs 66.00 µS, respectively; $p < 0.001$). The two subgroups were not different in terms of either age at evaluation ($p = 0.701$) or gender ($p = 0.216$) (Supplementary Table S3).

A further comparison was made by stratifying patients based on their systolic BP scoring at baseline (Supplementary Table S4). No differences between the three subgroups were detected in terms of either age ($p=0.362$) or sex ($p=0.118$). A Kruskal-Wallis test showed that median fESC values were statistically significantly different between subgroups with different BP scoring ($p=0.001$). Specifically, post-hoc analysis revealed a statistically significant difference in median fESC values between patients with a BP score of 0 (65.00 µS) and those with a BP score of 2 (19.50 µS) (adjusted $p=0.001$) but no significant differences between the first subgroup and those with a BP score of 1 (35.50 µS) (adjusted $p=0.281$) or between those with a BP score of 1 and 2 (adjusted $p=0.514$). Significant differences between subgroups were also observed for hESC ($p<0.001$). Post-hoc analysis detected a statistically significant difference between the subgroup with a BP score of 0 (66.00 µS) and the subgroups with either a BP score of 1 (25.00 µS; adjusted $p=0.005$) or 2 (27.50 µS; adjusted $p=0.001$) but not between the latter two subgroups (adjusted $p=1.000$).

Potential Predictors of Dysautonomia

Finally, we evaluated whether fESC and hESC values, as well as other relevant demographic and clinical measures (age, gender, NIS, Norfolk QoL-DN total score), could be predictive of the presence of dysautonomia.

Performing a binomial logistic regression analysis using a stepwise method (backward elimination), only fESC and Norfolk total score were identified as having a statistically significant impact on the likelihood of dysautonomia (*for fESC*: OR 0.952, 95% CI 0.913–0.993, $p=0.023$; *for Norfolk*: OR 1.036, 95% CI 1.005–1.067, $p=0.021$).

Regarding fESC values, a cut-off of 57.50 µS was able to discriminate between patients with and without dysautonomia with a sensitivity of 88.9% and specificity of 67.4% (AUC 0.826, 95% CI 0.724–0.928, $p<0.001$).

DISCUSSION

ATTRv amyloidosis is a severe progressive disease with a poor prognosis if untreated. The recent advent of new effective therapies has made it essential to monitor disease progression as part of evaluating treatment response.

Typically, disease monitoring is based on common clinical outcome measures, such as FAP stage, PND score, NIS and its subscores, and patient-reported questionnaires, supported by traditional NCSs [22, 23]. However, conventional electrophysiological studies, while exploring the large nerve fibers, are lacking in the assessment of small nerve fibers [24, 25].

Currently, skin biopsy is still considered the gold standard for the diagnosis of small fiber neuropathy [24, 26, 27], providing insights about both somatic and autonomic small nerve fibers. However, it is an invasive technique available only in a few highly specialized centers.

For this reason, several non-invasive investigations assessing small fiber and autonomic function have been proposed in ATTRv amyloidosis, such as the Sympathetic Skin Response (SSR) [10, 28, 29], Quantitative Sensory Testing (QST) [10, 30, 31], Quantitative Sudomotor Axonal Reflex Test (QSART) [30] and Heart Rate Variability (HRV) evaluation [10]. Nevertheless, most of them are time-consuming tests with limited reproducibility, restricting their use in routine clinical practice.

Sudoscan is a quick, reproducible, non-invasive test of autonomic function, whose application in ATTRv amyloidosis has been largely explored [7–9]. This simple instrumental tool has shown high diagnostic accuracy in the early diagnosis of ATTRv, especially in the early-onset population [7], prompting its use as a screening test in the monitoring of presymptomatic *TTR* mutation carriers [32]. In parallel, in clinically affected patients, Sudoscan has also been shown to be a reliable measure of disease severity, even in non-endemic countries [8, 9]. However, longitudinal studies, able to investigate the role of Sudoscan in monitoring disease progression over time, are still lacking.

In the present study, we evaluated the application of Sudoscan in the follow-up of an Italian

cohort of late-onset patients with ATTRv. We observed significant changes over time in ESC values measured on the feet. Specifically, a significant reduction in fESC values became evident at both 2 and 3 years from the baseline evaluation. Conversely, we did not detect an equally statistically significant change in ESC values measured on the hands despite a downward trend over time. This discrepancy may be partially explained by the length-dependent pattern of neuropathy in most cases [33]. Thus, ESC values measured on the feet may represent a more sensitive marker than those measured on the hands.

In addition to a potential role in monitoring disease progression, our findings confirmed the importance of Sudoscan as a marker of disease severity [8, 9]. Both fESC and hESC values showed a significant correlation with different clinical scales, such as the NIS and Norfolk, which are commonly used as markers of disease severity, confirming previous literature data [8, 9]. These correlations, evident at the baseline evaluation, were consistently observed over time and confirmed at each subsequent yearly evaluation.

A significant correlation for both fESC and hESC was also detected with mCADT. The CADT is a simple questionnaire specifically developed to evaluate the main symptoms of autonomic dysfunction in ATTRv amyloidosis [15]. In our study, we adopted a "modified" version of the questionnaire, including four domains for both male and female patients, to align the scores independently from the sex. These correlations highlight how the ANS may be as significantly impaired as other organs and systems in ATTRv amyloidosis, even in the late-onset population, underlining the importance of investigating autonomic involvement through both patient-reported questionnaires and objective tests. Sudoscan, being a simple, rapid, quantitative test, may represent a valid instrument for this purpose and should be performed at each evaluation, especially but not exclusively in presymptomatic carriers, guiding further investigations (e.g., skin biopsy) in any doubtful cases.

To further confirm the role of Sudoscan as a measure of autonomic involvement and disease severity, we compared patients with and without dysautonomia. Both fESC and hESC values differentiated patients with at least one symptom and/or sign of autonomic dysfunction from patients without any evidence of dysautonomia, showing significantly lower values in the former group. Analogous results were also observed when comparing patients with and without postural hypotension. These findings are consistent with previous pathological evidence of sudomotor denervation in patients with ATTRv and the association between reduced sudomotor innervation and symptoms of orthostatic hypotension [34].

Moreover, ESC values measured on the feet proved to be a significant independent predictor of dysautonomia. This finding is in line with the previous results by Castro et al. in a cohort of early-onset p.Val50Met patients [7] but expands the application of fESC to the evaluation of late-onset patients harboring different *TTR* variants. We also observed an analogous predictive role for the Norfolk questionnaire, a patient-reported outcome measure exploring quality of life, not investigated in the above-mentioned study.

In our cohort, a fESC cut-off of 57.50 µS could discriminate between patients with and without dysautonomia with a sensitivity of almost 89% and specificity of about 67%. This threshold differs from that previously reported by Castro et al., which detected a fESC cut-off of 66 µS for confirming dysautonomia (sensitivity 76%, specificity 85%) [7]. However, it should be considered that, as already mentioned, the paper by Castro et al., including only patients with a p.Val50Met variant associated with early-onset disease, refers to a different ATTRv population as compared to our study. It is indeed well known that the typical "Portuguese" phenotype is characterized by early, more prominent autonomic dysfunction compared to the late-onset disease observed in non-endemic areas, where autonomic impairment is usually less relevant and often delayed. In our case, we decided to prioritize sensitivity over specificity to better identify patients with actual dysautonomia who may require more advanced tests for further confirmation.

An important measurable feature of autonomic dysfunction in patients with ATTRv amyloidosis is orthostatic hypotension, which can significantly affect quality of life by increasing

the risk of falls and injuries. In the pivotal patisiran trial (APOLLO), autonomic function was assessed using the postural blood pressure scoring, based on changes in systolic BP upon standing [17, 18]. In our study, stratification based on BP score showed a statistically significant difference in fESC and hESC values. Namely, patients without postural hypotension (BP score of 0) had significantly higher fESC values than those with severe orthostatic intolerance (BP score of 2). Similarly, in terms of hESC values, the subgroup without evidence of orthostatic hypotension significantly differed from patients with either moderate or severe impairment.

Despite these promising results, Sudoscan has some relevant limitations since it is not suitable for patients with advanced stages of the disease (e.g., those confined to a wheelchair or bed or with severe hypotrophy of the intrinsic hand muscles with contractures preventing the adherence to the device's electrodes). This limitation reduced the number of patients in our cohort available for long-term follow-up. Additionally, some patient dropout during the follow-up, due to the disease's severe prognosis and related mortality, further reduced our overall sample size over time. Therefore, the relatively limited number of patients with complete long-term follow-up may have partially influenced our results.

Our study has some other limitations. As a measure of autonomic dysfunction, we adopted a revised version of the CADT, a questionnaire specifically designed for ATTRv amyloidosis [15]. However, this brief, simple questionnaire may not fully reflect all the variegated dysautonomic features of the disease as it is limited to a few domains. Surely, the additional use of a more comprehensive questionnaire, such as the Composite Autonomic Symptom Score, specifically in its refined, abbreviated 31-item version (COMPASS-31) [35], could have further supported the role of Sudoscan as a measure of autonomic involvement. However, since this more time-consuming questionnaire was not available for all enrolled patients at each follow-up, it was not included in our analysis.

CONCLUSION

Despite the above-mentioned limitations, Sudoscan may represent a simple and objective measure of autonomic dysfunction in ATTRv amyloidosis, displaying a potential role not only as a measure of both disease onset and severity but also as a marker of disease progression. Moreover, ESC values measured on the feet seem to be significant independent predictors of autonomic dysfunction.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest. Angela Romano received financial grants (honoraria and speaking) from Akcea and travel grants to attend scientific meetings from Akcea, Alnylam, Pfizer and Csl Behring. Marco Luigetti received financial grants (honoraria and speaking) from Ackea, Alnylam, AstraZeneca, Sobi and Pfizer and travel grants from Ackea, Alnylam, AstraZeneca, Sobi, Pfizer, Kedrion and Grifols. All other authors (Valeria Guglielmino, Francesca Vitali, Maria Ausilia Sciarrone, Giovanni Siconolfi, Andrea Di Paolantonio, Guido Primiano) declare no disclosures.

Ethical Approval. The study was approved by the Ethics Committee of "Fondazione Policlinico Agostino Gemelli IRCSS" (Prot. ID 5470), and a written informed consent was obtained from all participants. All procedures were carried out in accordance with the principles of the 1964 Declaration of Helsinki.

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