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ORIGINAL ARTICLE

Biomarkers of response to alpha-lipoic acid ± palmitoiletanolamide treatment in patients with diabetes and symptoms of peripheral neuropathy

Silvia Pieralice[1] · Riccardo Vari[1] · Alessandra Minutolo[1] · Anna Rita Maurizi[1] · Elvira Fioriti[1] · Nicola Napoli[1] · Paolo Pozzilli[1] · Silvia Manfrini[1] · Ernesto Maddaloni[1]

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Abstract

Purpose To evaluate the effect of oral alpha-lipoic acid (ALA) ± palmitoyl-ethanolamide (PEA) on neuropathic symptoms in patients with diabetic peripheral neuropathy (DPN) and to identify factors related to the efficacy of the treatment.

Methods This is a retrospective observational pilot study evaluating 49 patients with diabetes and positive Neuropathy Symptoms Score (NSS). Clinical and biochemical variables, including NSS, were compared between untreated patients and patients treated with oral 600 mg/day ALA ± 600 mg/day PEA at baseline (first occurrence of NSS ≥ 3) and at least 2 months after baseline. Number of days between treatment initiation and symptoms' relief and related factors were also investigated.

Results Thirty subjects were treated with ALA ± PEA and 19 subjects did not receive any specific treatment for neuropathy symptoms. Follow-up visits occurred after 98 ± 46 days. NSS significantly decreased in patients treated with ALA ± PEA (5.4 ± 1.3 at baseline vs. 1.7 ± 2.4 at follow-up, $p < 0.001$), but not in untreated patients ($p = 0.164$). Subjects treated with ALA ± PEA reported a mean time from treatment initiation to symptoms' relief of 18.4 ± 9.0 days. The number of days of treatment needed for symptoms' relief was inversely related to HDL-cholesterol levels ($r = -0.503$, $p = 0.010$) and to eGFR ($r = -0.428$, $p = 0.033$), whereas there was no significant relationship between time to symptoms' relief and age, HbA1c, lipid profile and the severity of symptoms at baseline.

Conclusions This study documents that oral administration of ALA ± PEA helps in controlling neuropathy symptoms in diabetes. Moreover, our data show that higher HDL-c levels and better renal function are associated to a faster therapeutic effect, suggesting them as biomarkers of response to therapy with ALA ± PEA.

Keywords Diabetic neuropathy · Alpha-lipoic acid · Palmitoiletanolamide · PEA, HDL

Introduction

Diabetic neuropathies are common, symptomatic, long-term complications of type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), encompassing a broad spectrum of clinical and pathophysiological frameworks and affecting both peripheral and autonomic nervous system [1, 2]. In this context, diabetic peripheral neuropathy (DPN) affects approximately 50% of patients with diabetes and its prevalence increases with the duration of disease [3]. The etiology is multifactorial [4] and pathogenic factors include persistent hyperglycemia, microvascular insufficiency, oxidative stress, defective neurotropism, and autoimmune nerve destruction [5]. The clinical features vary enormously, and patients may present a variety of complaints including paraesthesias, sensory loss, motor deficits, and severe neuropathic pain (burning, lancinating, tingling or shooting) that seriously compromises their quality of life [5].

However, despite the prevalence and the impact of DPN on morbidity and quality of life of patients with diabetes, there is a lack of studies investigating new treatment for tackling symptoms of DPN [6]. Oxidative stress is thought to play a key role in the pathogenesis of diabetic neuropathy

These authors contributed equally: Silvia Pieralice, Riccardo Vari

✉ Ernesto Maddaloni
e.maddaloni@unicampus.it

[1] Department of Medicine, Unit of Endocrinology and Diabetes, Campus Bio-Medico University of Rome, Rome, Italy

[7, 8]. Alpha-lipoic acid (ALA), a potent thiol-containing endogen antioxidant, has been shown to improve nerve blood flow, reduce oxidative stress, and improve distal nerve conduction [9], and appears effective in reducing symptoms of neuropathic pain in DPN [9, 10]. Consequently, daily integration of 600/800 mg of alpha-lipoic acid is often prescribed by physicians to treat neuropathic symptoms in patients with diabetes. Some supplements include palmitoyl-etanolamide (PEA), an endogenous fatty acid amide that inhibits the mast cells in releasing of inflammatory mediators [11]. PEA is also an endogenous CB1 and PPAR-α agonist, reducing the expression of COX-2, iNOS, and preventing NF-kB nuclear translocation [12]. The addition of PEA to ALA treatment may be of some benefit in relieving chronic pain. Nonetheless there is no strong evidence in literature about the effect of PEA on metabolic neuropathies.

The aim of the current study was to retrospectively investigate the effect of 600 mg/day alpha-lipoic acid ± 600 mg/day palmitoyl-ethanolamide administered orally once a day on neuropathic symptoms in patients with DPN and to identify factors related to the efficacy of the treatment.

Patients and methods

Study design and population

In this retrospective observational pilot study, data of patients affected by T1DM or T2DM screened for DPN symptoms in the Unit of Endocrinology and Diabetes, Department of Medicine, Campus Bio-Medico University of Rome from October 2016 to June 2018, were retrieved. The first visit within the specified period with an available Neuropathy Symptom Score (NSS) was considered as baseline visit. The first re-evaluation of NSS occurring 2 to 12 months after the baseline visit was considered as follow-up visit.

Data from subjects meeting the following inclusion criteria were included in this analysis: (1) established diagnosis of T1DM or T2DM; (2) age >18 years; (3) diagnosis of DPN; (4) Neuropathy Symptom Score (NSS) ≥ 3 at baseline visit; (5) at least one NSS evaluation within 12 months from baseline. Exclusion criteria comprised: (1) causes of neuropathy other than diabetes; (2) estimated glomerular filtration rate (eGFR) <45 ml/min/1.73 m^2 using the Modification of Diet in Renal Disease formula; (3) current or previous treatment of diabetic neuropathy with pharmacological agents.

Definition of neuropathy

DPN was defined as the presence of signs and/or symptoms of peripheral nerve dysfunction in patients with diabetes after the exclusion of other causes of neuropathy [5]. Symptoms of somatic neuropathy were assessed using the NSS [13], whereas the neuropathy disability score (NDS) was performed to quantify signs of diabetic peripheral neuropathy.

Assessment of neuropathy

At baseline, neurological deficits were assessed using the NDS, which includes tuning fork vibration perception, pin prick perception, temperature perception, and the examination of ankle reflexes, ranging between 0 and 10. NDS was considered positive if ≥3 [14].

Neuropathy symptoms were evaluated at baseline visit and at the follow up visit using the NSS, a validated questionnaire about neuropathy symptoms, with a score ranging from 0 (no neuropathic symptoms) to 9 (severe neuropathic symptoms) [13]. Patients were considered to have mild symptoms when the NSS was 3 or 4, moderate symptoms when the score was 5 or 6, and severe symptoms when the score was between 7 and 9.

Data collection

The following clinical and biochemical data collected at the baseline visit were extracted from the electronic medical records of our hospital: age, gender, type of diabetes mellitus, height, weight, body mass index (BMI), blood pressure, history of diabetic complications (neuropathy, nephropathy, retinopathy, peripheral artery disease, cardiovascular disease), history of other diseases, diabetes medications, other medications, alcohol consumption and smoking habit; creatinine, eGFR, microalbuminuria, triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c). HbA1c and NSS were extracted for both the baseline and the follow-up visit. If an improvement of NSS from the baseline visit was recorded among patients treated with ALA ± PEA, patients were asked to self-report the number of days between treatment initiation and improvement in symptoms.

Statistical analyses

Values are expressed as mean ± standard deviation (SD) for continuous variables and as proportions for categorical

Table 1 Population features by treatment allocation

	All ($n = 49$)	Treated group ALA ± PEA ($n = 30$)	Untreated group ($n = 19$)	P value
Gender				
Males	28 (57.1)	19 (63.3)	9 (47.5)	0.271
Females	21 (42.9)	11 (36.7)	10 (52.6)	
Age (years)	51.9 ± 17.6	58.4 ± 16.0	41.7 ± 15.4	**<0.001**
Years of disease	13.6 ± 8.1	13.1 ± 9.0	14.4 ± 6.7	0.209
BMI (Kg/m^2)	25.6 ± 4.3	26.3 ± 4.2	24.5 ± 4.4	0.086
eGFR, ml/min/1.73 m^2	96.5 ± 21.9	91.7 ± 23.3	103.4 ± 18.2	0.074
HbA1c (%)	7.6 ± 1.1	7.5 ± 1.1	7.6 ± 1.2	0.707
Total cholesterol (mg/dl)	170.9 ± 40.4	166.3 ± 43.8	177.6 ± 34.9	0.354
HDL cholesterol (mg/dl)	60.1 ± 17.4	57.2 ± 15.5	64.2 ± 19.4	0.180
LDL cholesterol (mg/dl)	91.8 ± 38.1	89.3 ± 38.7	95.3 ± 37.9	0.604
Triglycerides (mg/dl)	93.2 ± 61.3	99.0 ± 68.1	84.7 ± 50.1	0.381
NSS	5.1 ± 1.2	5.4 ± 1.3	4.6 ± 1.0	**0.022**
NDS	1.8 ± 2.4	2.4 ± 2.6	0.9 ± 1.6	**0.039**

Values are expressed as mean ± standard deviation for continuous variables and as absolute value (%) for categorical variables

BMI body mass index, *eGFR* estimated glomerular filtrate, *HbA1c* glycated hemoglobin, *NSS* neuropathy symptoms score, *NDS* neuropathy disability score

variables (%). Data were checked for normal distribution with the Shapiro-Wilk test. Comparisons were done using Student's t test, Kruskal–Wallis, and Chi square or Fisher exact test depending on distribution and sample size. Paired t-test and Wilcoxon signed-rank test were used to evaluate changes in variables between the baseline and the follow-up visit. Linear models were used for multivariable analyses to adjust for covariates. Non parametric variables were converted to natural logarithm before being tested with the model. Furthermore, a propensity score analysis using 1-to-1 matching has been used to test the primary outcome also in groups balanced for baseline features. A sample size of 20 subjects per group (treated vs. untreated) was needed to detect a 40% difference in a two-sample comparison of proportions test with a 80% power. Two-sided tests at the 0.05 level of significance were used for all statistical comparisons. Stata/IC 12.1 software (StataCorp) and Prism 7.0d Software (GraphPad Software) used for data analysis and graphical representations.

Results

Population features

A total of 49 patients (28 males, 21 females) with a baseline NSS ≥3 were enrolled in the study. Twenty-eight (57%) were affected by T1DM and 21 (43%) by T2DM, with a mean age of 51.9 ± 17.6 years. Among patients with T2DM, 6 patients received insulin +/− oral hypoglycamic agents, 10 patients were on non-insulin therapies, and 5 patients were on diet only. All patients with T1DM were on insulin therapy. Retinopathy was present in 17% of the enrolled subjects, while only 2% of patients had nephropathy. History of coronary heart disease was reported in 22% of subjects, whereas 7% had a diagnosis of carotid stenosis.

Thirty subjects (18 with T1DM and 12 with T2DM) had been treated with oral ALA ± PEA because of NSS ≥ 3, while the remaining 19 patients (15 with T1DM and 4 with T2DM) did not receive any specific therapy despite the positive NSS. Baseline population features by treatment allocation are shown in Table 1. Compared to the untreated group, patients treated with ALA ± PEA had a higher mean age (58.4 ± 16.0 vs. 41.7 ± 15.4 years, $p < 0.001$), higher NSS (5.4 ± 1.3 vs. 4.6 ± 1.0, $p = 0.02$) and higher NDS (2.4 ± 2.6 vs. 0.95 ± 1.6 years, $p = 0.03$). Retinopathy was present in 24% of patients treated with ALA ± PEA and in 5% of the untreated group, while coronary disease was reported, respectively, in 31% and 11% of subjects. History of nephropathy was reported in 4% of treated subjects, whereas no patient in the untreated group presented the disease. Other variables are comparable at baseline with no significant differences between the two groups.

Response to therapy

Follow-up visits occurred 98 ± 46 days after the baseline visit. Twenty (66.7%) subjects treated with ALA ± PEA, and 5 (26.3%) untreated subjects showed an NSS < 3 at the follow-up ($p = 0.009$ for difference between groups). In the multivariate model accounting for age, baseline NSS, and baseline NDS as confounders, the difference in the percentages of subjects with NSS at the follow-up was still significant ($p = 0.008$). After propensity score matching, with balanced baseline features also in terms of age ($p = 0.564$), NSS ($p = 0.137$) and NDS ($p = 0.347$), results were consistent with those in the unmatched (p for differences between groups = 0.007). A subgroups analysis failed to

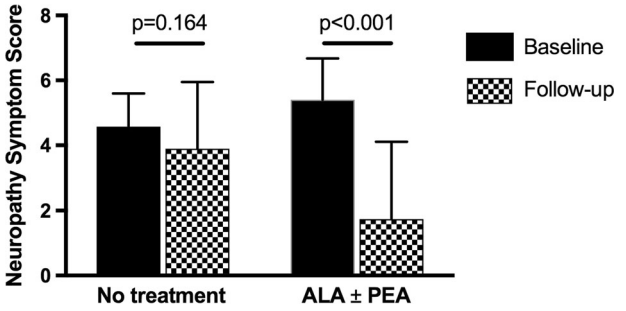

Fig. 1 Neuropathy Symptom Score (NSS) at baseline (black bars) and at the follow-up visit (checkered bars) by treatment allocation

show differences between subjects treated with ALA alone and those treated with ALA + PEA (p-value for the interaction >0.05).

At follow-up visit, no changes in HbA1c levels were found in patients treated with ALA ± PEA vs. patients who did not receive any treatment [mean ± SD, HbA1c 7.5 ± 1.3 vs. HbA1c 7.5 ± 1.2] ($p = 0.891$). Likewise, a subgroups analysis did not find significant changes of HbA1c values between patients treated with ALA alone and those treated with ALA + PEA ($p = 0.382$).

Mean NSS significantly decreased from 5.4 ± 1.3 at the baseline to 1.7 ± 2.4 at follow-up in patients treated with ALA ± PEA ($p < 0.001$), while there was no significant reduction of NSS in the untreated group (4.6 ± 1.0 vs. 3.9 ± 2.0, $p = 0.164$) (Fig. 1).

Among patients treated with ALA ± PEA, the mean time from treatment initiation to symptoms' relief was 18.4 ± 9.0 days. Possible biomarkers associated to a faster response to therapy were evaluated among subjects treated with ALA ± PEA. The time from treatment initiation to symptoms' relief was shorter in women than in men (13.6 ± 7.8 vs. 20.6 ± 8.8 days, $p = 0.050$). There was no difference between subjects with T1DM and T2DM (19.6 ± 11.3 vs. 17.7 ± 7.7 days, $p = 0.596$) and between subjects with and without personal history of cardiovascular disease (21.5 ± 10.6 vs. 17.6 ± 9.2 days, $p = 0.356$) or of retinopathy (17.4 ± 7.8 vs. 19.0 ± 9.7 days, $p = 0.702$). The number of days of treatment needed for symptoms' relief was inversely related to HDL-c levels ($r = -0.503$, $p = 0.010$) and to eGFR ($r = -0.428$, $p = 0.033$) (Fig. 2a, b). There was no significant relationship between time to symptoms' relief and age ($r = -0.017$, $p = 0.931$), Hba1c ($r = 0.267$, $p = 0.178$) total cholesterol ($r = -0.102$, $p = 0.619$), LDL-c ($r = -0.084$, $p = 0.691$), triglycerides ($r = 0.184$, $p = 0.366$). The severity of symptoms at baseline also did not impact the rapidity of response to treatment ($r = -0.095$, $p = 0.632$).

Among subjects treated with ALA ± PEA, women had higher mean values of HDL-c than men (69.5 ± 2.3 vs. 50.0 ± 12.6 mg/dl, $p < 0.001$), while no differences were found in age, Hba1c, total cholesterol, LDL-cholesterol, triglycerides, and eGFR. The Adj R^2 of a bivariate linear regression model accounting for HDL-c and gender as independent variables was similar to the Adj R^2 of the univariate model having only HDL-c as independent variable (0.219 vs. 0.222).

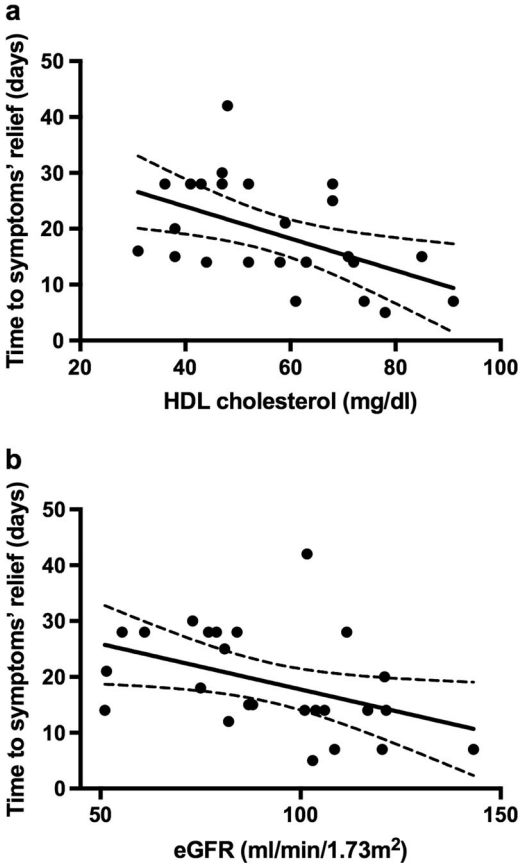

Fig. 2 Correlation between self-reported time to symptoms' relief and baseline HDL-c concentration (**a**) and baseline eGFR values (**b**)

Discussion

In this study, treatment with 600 mg/day ALA ± 600 mg/day PEA orally administered was found to be associated with a clinically significant reduction in neuropathy symptoms, with a mean treatment response time of 18 days. Furthermore, we showed that subjects with higher baseline concentrations of HDL-c and those with higher eGFR have a faster response to treatment with ALA ± PEA. Our results contribute in clarifying whether orally administered ALA may be useful in diabetes with neuropathy symptoms. Indeed, while there is a substantial agreement about the effectiveness of intravenous ALA in reducing neuropathy symptoms [15, 16], studies investigating oral administration

of ALA showed mixed results. In a recent meta-analysis by Mijnhout et al., authors concluded that the mild improvements in DPN symptoms observed in SIDNEY [17] and ORPIL [18] trials after 3–5 weeks of oral administration of ALA ≥ 600 mg were probably not clinically relevant, because of a reduction in total symptom score (TSS) lower than 30% [19]. In our study, the 67% of patients treated with oral ALA ± PEA reported a NSS < 3, that is considered as absence of diabetic neuropathy symptoms; these data support the potential benefit of using ALA ± PEA in patients with diabetes and symptoms of peripheral neuropathy. A growing body of evidence suggests that oxidative stress may play a central role in causing nerve damage [20]. Alpha-lipoic acid, and its reduced form, dihydrolipoic acid (DHLA), act as potent antioxidants, essential co-enzyme in mitochondrial bioenergetic reactions, metal chelators, and modulator of the signaling transduction of antioxidant and anti-inflammatory pathways [21]. As a result, ALA should appear as a pathophysiological-oriented treatment approach.

We also found that the time needed to symptoms' relief was remarkably shorter than what was previously reported by other authors [19, 22]. We postulated that this outcome may be partly explained by the addition of PEA to ALA treatment in some patients. PEA has important neuroprotective, anti-inflammatory, and analgesic effects, both in the central and the peripheral nervous system. It has been shown effective in reducing neurogenic inflammation and non-metabolic neuropathic pain [23, 24]. A pooled data meta-analysis evaluating the efficacy and safety of PEA on pain intensity in patients suffering from chronic and/or neuropathic pain, concluded that pain relief induced by PEA is progressive, age-independent, and gender-independent and not related to the aetiopathogenesis of chronic pain [25]. PEA may therefore represent a new promising compound for the management of neuropathic pain. However, there is no strong evidence in literature about the effect of PEA on diabetic neuropathies. In this study, a sensitivity analysis did not find differences between ALA or ALA ± PEA. Nevertheless, this study was not powered to detect differences between these two different treatment strategies and ad hoc randomized trials should be performed to clarify the eventual benefits of adding PEA to ALA for treatment of neuropathic symptoms.

In our cohort, higher eGFR seems to be associated with a faster treatment response, supporting data from previous studies showing an association between peripheral neuropathy and eGFR in patients with diabetes [26]. Diabetic neuropathy is a microvascular complication of diabetes, often associated to the other microvascular complications, diabetic nephropathy in particular. In the case of renal impairment, the associated increased risk of axonal damage could be due to a multifactorial etiology including renal impairment associated endothelial dysfunction, oxidative stress, and inflammation [27], and to uremic toxins in case of advanced stage kidney disease.

Similarly, HDL-c levels at baseline appears to impact the time of response to therapy, suggesting a potential role of HDL in facilitating the action of ALA ± PEA in patients with DPN. Consistently, among subjects treated with ALA ± PEA, women, who had higher mean values of HDL-cholesterol than men, showed a faster response to therapy. While this observation should be tested in larger samples, taking into account measures of markers of oxidative stress, as well as endothelial function and leukocyte activation, a number of plausible mechanisms may support a role of HDL in the management of diabetic neuropathies. It is well-known that HDL has anti-inflammatory, anti-oxidative, and anti-thrombotic properties, protecting against diabetic macrovascular complications [28, 29]. However, its anti-oxidant activity seems to act on microvascular circulation too. On that note, the European Diabetes (EURODIAB) Prospective Complications Study [30] and the Pittsburgh Epidemiology of Diabetes Complications [31] studies found associations between higher triglycerides, lower HDL-c, and neuropathy. Similarly, a recent prospective cohort study conducted in 2777 children and adolescents found that lower HDL-c was a risk factor for DPN in both T1DM and T2DM youth. In this study the authors postulated that lower HDL-c could act in the pathogenesis of DPN [32]. We hypothesize that higher HDL-c levels may be associated with a prompter response to anti-oxidant administration. Furthermore, the acting role of HDL in the pathogenesis of nerve damaging is suggested by Tangier disease, a rare human genetic disorder caused by mutations in the ATP-binding cassette (ABCA1) transporter gene, which mediates the transport of intracellular cholesterol to extracellular lipid-free apolipoprotein A-I (ApoA-I), allowing HDL synthesis [33]. Tangier disease is characterized by a nearly complete absence of normal HDL (<5 mg/dL) and ApoA1 (5 mg/dL) in plasma and cholesterol accumulation in the cells of the reticuloendothelial system, leading to the classic clinical manifestations of enlarged orange tonsils, hypersplenism and nerve disfunction, such as transient peripheral neuropathy or syrongomyelia, as well as an increased risk of accelerated atherosclerosis and cardiovascular events. The wide nerve impairment in Tangier disease, supports the hypothesis of the existing association between HDL cholesterol levels and neuropathy.

Our study has several limitations. The low sample size did not provide enough power to test whether the relationship between gender, HDL-c and response to treatment are independent, neither if there is an interaction between gender and HDL-c in relation to the outcome (days of response to therapy). However, the fact that the Adj R^2 of

the regression model accounting for HDL-c did not change when introducing gender in the model could suggest that the role of HDL-c as a biomarker of response to treatment is not influenced by gender. Since this is a retrospective observational study, we cannot exclude a placebo effect of treating subjects with ALA ± PEA. A number of recent randomized, double-blind clinical trials in neuropathic pain have observed that a greater magnitude of placebo response may make it difficult to demonstrate efficacy of a treatment for neuropathic pain [34]. This issue should be addressed by future randomized placebo-controlled trial.

In conclusion, our investigation may serve as a pilot study, suggesting that 600 mg/day ALA ± 600 mg/day PEA administered orally to subjects with symptomatic DPN may be useful in controlling neuropathy symptoms in both T1DM and T2DM. However, whether the recorded improvements could be augmented further by the addition of PEA to ALA supplements, remains to be determined. In addition, our data shows that higher HDL-c levels and higher eGFR are associated to a faster therapeutic effect, suggesting an important role of both HDL and kidney function in determining response to therapy of diabetic neuropathy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the ethics committee of Campus Bio-Medico University of Rome, Italy. For this retrospective study, formal consent is not required. No patient identifiable information has been used in this study and only data from which identifying factors have been removed were used for statistical analysis.

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