DOI 10.1007/s00702-004-0220-1
J Neural Transm (2005) 112: 649–660

Journal of Neural Transmission
Printed in Austria

High dose vitamin E therapy in amyotrophic lateral sclerosis as add-on therapy to riluzole: results of a placebo-controlled double-blind study

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Vitamin E levels measurements

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Received May 26, 2004; accepted July 31, 2004
Published online October 27, 2004;

Summary. Increasing evidence has suggested that oxidative stress may be involved in the pathogenesis of amyotrophic lateral sclerosis (ALS). The antioxidant vitamin E (alpha-tocopherol) has been shown to slow down the

onset and progression of the paralysis in transgenic mice expressing a mutation in the superoxide dismutase gene found in certain forms of familial ALS. The current study, a double blind, placebo-controlled, randomised, stratified, parallel-group clinical trial, was designed to determine whether vitamin E (5000 mg per day) may be efficacious in slowing down disease progression when added to riluzole. *Methods*. 160 patients in 6 German centres with either probable or definite ALS (according to the **El Escorial Criteria**) and a disease duration of less than 5 years, treated with riluzole, were included in this study and were randomly assigned to receive either alpha-tocopherol (5000 mg per day) or placebo for 18 months. The **Primary outcome measure** was survival, calculating time to death, tracheostomy or permanent assisted ventilation, according to the *WFN-Criteria of clinical trials*. **Secondary outcome measures** were the rate of deterioration of function assessed by the modified Norris limb and bulbar scales, manual muscle testing (BMRC), spasticity scale, ventilatory function and the Sickness Impact Profile (SIP ALS/19). Patients were assessed at entry and every 4 months thereafter during the study period until month 16 and at a final visit at month 18. Vitamin E samples were taken for compliance check and **Quality Control** of the trial. For **Safety**, a physical examination was performed at baseline and then every visit until the treatment discontinuation at month 18. Height and weight were recorded at baseline and weight alone at the follow-up visits. A neurological examination as well as vital signs (heart rate and blood pressure), an ECG and VEP's were recorded at each visit. Furthermore, spontaneously reported adverse experiences and serious adverse events were documented and standard laboratory tests including liver function tests performed. For **Statistical Analysis**, the population to be considered for the primary outcome measure was an ''intent-to-treat'' (ITT) population which included all randomised patients who had received at least one treatment dose ($n = 160$ patients). For the secondary outcome measures, a two way analysis of variance was performed on a patient population that included all randomised patients who had at least one assessment after inclusion. *Results*. Concerning the primary endpoint, no significant difference between placebo and treatment group could be detected either with the stratified Logrank or the Wilcoxon test. The functional assessments showed a marginal trend in favour of vitamin E, without reaching significance. *Conclusion*. Neither the primary nor the secondary outcome measures could determine whether a megadose of vitamin E is efficacious in slowing disease progression in ALS as an add-on therapy to riluzol. Larger or longer studies might be needed. However, administration of this megadose does not seem to have any significant side effects in this patient population.

Keywords: Alpha-tocopherol (vitamin E), ALS, riluzole.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly evolving, fatal neurodegenerative disease resulting from the degeneration of cortical, bulbar and spinal motor neurones (Williams and Windebank, 1991). The disease progresses inexorably to death, usually due to failure of respiratory function, with a median duration of

three years (Norris et al., 1993; Ringuel et al., 1993; Brooks, 1996). Although the aetiology of ALS is unknown, two hypotheses that have received wide attention in recent years are excitotoxicity (Shaw and Ince, 1997) and oxidative stress (Cookson and Show, 1999). The latter hypothesis suggests that failure of antioxidant defences in motor neurons leads to attack of sub-cellular components by oxygen free radicals, causing lipid peroxidation, cytoskeletal disruption and mitochondrial damage. This hypothesis has received support form studies showing oxidative damage to motor neurons in post mortem tissue from ALS-patients (Beal et al., 1997; Ferrante et al., 1997), changes in the levels of antioxidant enzymes (Przedborski et al., 1996; Moumen et al., 1997) and free radical damage to mitochondrial DNA (Vielhaber et al., 2000). The oxidative stress hypothesis of the aetiology of ALS received a boost in 1993 when Rosen and coworkers (1993) identified mutations in gene encoding the antioxidant enzyme CU/Zn superoxide dismutase (SOD) in certain familial forms of ALS.

In 1994 Gurney and colleagues (1996) reported the development of a transgenic mouse strain that expressed the mutant SOD gene found in familial ALS and went on to develop a fatal progressive degenerative motor neuron disease. Treatment of these mice with alpha-tocopherol and selenium delayed the onset of the disease and slowed the progression of the motor deficit. The development of the oxidative stress hypothesis for the aetiology of ALS has stimulated renewed interest in the use of antioxidants in the treatment of this condition. One of the most suitable antioxidants for the use in clinical trials is vitamin E (alpha-tocopherol). The chemical structure of this liquid-soluble vitamin was discovered in 1938. It is found naturally in a wide variety of foodstuffs, particularly vegetable oils. In the body, vitamin E acts as a highly efficient antioxidant, scavenging free radicals and preventing peroxidation of polyunsaturated fatty acids in cell membranes, including neurons (Favier, 1995). As oxidative stress is thought to play a role in the pathogenesis of several neurodegenerative disorders, alpha-tocopherol has received much interest as a potential drug for the treatment of these diseases. A very large study demonstrated that alpha-tocopherol at a daily dose of 2000 mg had no apparent benefit in treating patients with newly diagnosed Parkinson's disease (Parkinson Study Group, 1996; Shoulson, 1998). On the other hand, 2000 mg alpha-tocopherol was shown to be beneficial in slowing down functional deterioration of patients with Alzheimer's disease (Sano et al., 1997). In 1987, Norris and colleagues described a trend towards a lessening of fatigue, fasciculations and cramps in a small number of ALS patients who were included in an open study and administered alpha-tocopherol (Norris et al., 1989). A French study administrating 1000 mg of alpha-tocopherol in 289 patients could not detect any effect neither on survival nor on the deterioration of function assessed by the modified Norris limb scale (Desnuelle et al., 2001). However, patients given alpha-tocopherol were less likely to progress from the milder state A to the more severe state B of the ALS Health State Scale.

In this situation and as no drug was officially approved for treatment of ALS yet, an increasing number of patients started to self-administer vitamin E in various regimens, and in part with striking success. One 79 years old patient, for example, himself a M.D. (C.H.), started in 1993 on a megadose of 5×1000 mg

vitamin E/day after having been diagnosed as suffering from ALS, initially of the spinal type with typical symptoms. He developed muscle atrophy especially of the hands, right arm and shoulders with fasciculations, weakness and loss of muscular strength in this regions, followed by similar symptoms in the legs (including fasciculations and typical EMG signs).

Transcranial magnet stimulation had revealed a prolongation of impulse conduction to the right leg. This patient who is still alive has been tolerating vitamin E quite well and has, so far, although never on riluzole, experienced only very slight disease progression (now including swallowing difficulties). He is still able to have a very active and independent life (at the age of 79 years) which includes medical work and especially counselling of other ALS patients. He has in addition collected safety information from a total of 1120 patients regarding this unusually high dose, and has reported a very good tolerability of this therapy (Hager, 1994, 1996).

We therefore considered a prospective placebo-controlled randomised trial a necessity, with the principal aim to investigate the safety of such a unusual high dose of vitamin E in combination with riluzole in this elderly and weak patient population. This clearly is also of interest for other potential medical uses of this interesting lipophilic compound with strong antioxidant properties.

2. Material and methods

2.1 Patients and treatment

A total of 160 patients with probable or definite ALS (according to the El Escorial Criteria) (Brooks, 1994) treated with riluzole were randomly assigned to either receive vitamin E (α-tocopherol, 5×1000 mg/day, Schwarzhaupt, Cologne, Germany) or placebo in identical capsules on a double-blind basis over a period of 18 months. The patients took 5 capsules distributed over the active day.

The primary study endpoint was defined as death (or need for permanent assisted ventilation or tracheostomy whatever occurred first).

During the study visits (screening/baseline, month 4, 8, 12, 16, 18) physical examinations including ECG's, VEP's, spirometry and blood taking for standard laboratory investigations and determinations of vitamin E levels were performed, and the status of the disease was assessed by a Sickness Impact Profile adjusted to ALS patients (Bergner et al., 1981; McBuire et al., 1996), a spasticity scale (Smith et al., 1994), the manual muscle testing according to the BMRC and the modified Norris bulbar (theoretical range 0–39) (Norris et al., 1974) and limb scale (theoretical range 0–63) (Lacomblez et al., 1989).

Patients were asked about side effects during visits as well as at phone interviews in months 2, 6, 10 and 14.

2.2 Determination of vitamin E plasma concentrations

Plasma concentrations of reduced vitamin E were determined by HPLC with electrochemical detection according to a method previously described for brain tissue (Ringuel et al., 1993). Briefly, 500 µGL aliquots of plasma samples were transferred into glass screw-topped conical centrifuge tubes containing 0.5 ml 0.1% (w/v) 2,6-di-tertiary-butyl-p-cresol (Fluka, Buchs, Switzerland) in absolute ethanol. The contents were processed under argon and on dry ice, capped and mixed. A known amount (80 µg/ml) of the internal standard – tocopherol (Sigma, St. Louis, USA) and 1.0 ml n-hexane was added, and the solution was processed under argon and dry ice, capped, mixed for 1 min and centrifuged at 362 g at 4°C for 5 min. Five hundred µl of the organic layer were dried under N_2 gas, and redissolved in mobile phase. Samples (1 µl) were injected into

a HPLC system consisting of a AGILENT 1100 series (Bio-Rad, Munich, Germany), a Nucleosil 120-5C_{18} reverse-phase (250 × 4.6 mm) analytical column (Machery-Nagel, Düren, Germany), an electrochemical detector (model 1640; Bio-Rad, Munich, Germany), and a mobile phase (flow rate: 1.5 ml/min) containing 96% (v/v) methanol and 4% (v/v) aqueous 25 mM sodium perchlorate. The detector potential was set at +0.75 V relative to the Ag–AgCl reference electrode. The signal from the detector was recorded and data analyses were performed using an AGILENT Chem Statian for LC9D (Bio-Rad, Munich, Germany). Concentrations were calculated from the peak height with the aid of an external standard.

2.3 Statistical methods

For the primary endpoint (survival) a log-rank test using the SAS Lifetest procedure (method: Kaplan Meier product limit estimation) has been used on an intention-to-treat basis. The secondary endpoints were analysed with a bivariate one-way analysis of variance, using the GLM procedure (change to baseline). The primary endpoint as well as the secondary endpoints were evaluated for the total groups (Vitamin E and placebo) as well as for subgroups (bulbar and limb forms of ALS), which clearly differ in their progression. The study size had been the calculated based upon the results from the first riluzol study and was powered to detect a 50% improvement.

A number of other results as well as adverse events were dealt with on a descriptive basis (mean, standard deviation, frequency).

3. Results

3.1 Patient population

The first patient was included in December 1998, all 160 patients had been recruited by end of June 2001 (71 at Ulm, 27 at Wiesbaden, 17 at Homburg, 19 at Hannover, 22 at Halle and 4 at Erlangen).

83 patients (54 males) were randomised to vitamin E and 77 (50 males) to placebo; average age was 59 and 57 years and average body weight was 73 and 71 kg. For patients' characteristics see Table 1.

Familial forms of ALS were very rare as expected (2 in both groups), and the bulbar form of ALS was also less frequent than the limb form (18 vs. 65 in the vitamin E group, 18 vs. 59 in the placebo group).

98% of the patients were on riluzole; 23% had already had some vitamin E therapy, 18% of the patients were treated with magnesium and 12% with creatine.

Table 1. Demographic characteristics of the trial patients

	$n =$	m	f	Age	F-ALS	Smoker	Cigarettes
All							
Vitamin E	83	54	29	59 ± 11	2	20	16 ± 7
Placebo	77	50	27	57 ± 11	2	17	16 ± 10
Limb							
Vitamin E	65	46	19	59 ± 11	1	19	16 ± 7
Placebo	59	40	19	56 ± 11	2	13	16 ± 10
Bulbar							
Vitamin E	18	8	10	60 ± 9	1	1	5
Placebo	18	10	8	58 ± 11	0	4	14 ± 12

Mean disease duration was 20 ± 13 months in the vitamin E group and 25 ± 17 in the placebo group.

All these differences at baseline were not significant.

3.2 Primary endpoint

During the study, 32 patients from each group reached the primary endpoint (31 patients died on vitamin E therapy and 28 in the placebo group). Furthermore, there were 4 events with patients requiring permanent assisted ventilation or tracheotomy in the vitamin E group and 10 in the placebo group (with 5 patients subsequently having ventilation, tracheotomy, and one of them dying). The median survival time was approximately 300 days. Endpoints have been reached after an overall time to event of 315 ($\pm$13) in the vitamin E vs. 278 ($\pm$15) days in the placebo group.

Overall, the 'survival' rate was rather similar in the two treatment groups at 18 months (61.4% and 58.4% in the vitamin E and placebo group respectively for all events). No significant difference between placebo and treatment group was detected either with the stratified Log rank test or the stratified Wilcoxon test. The hazard ratio was calculated as 1.145 (95% confidence intervals 0.701 to 1.872). The life test analysis is shown in Fig. 1.

3.3 Secondary endpoints

The functional assessments according to the scales showed a trend in favour of vitamin E, without, however, reaching significance. The change to baseline of the Norris limb scale, for example, showed an overall decrease of -17.0 points in the vitamin E group and -19.4 in the placebo group. Broken down by strata, the values were at -11.3 and -18.9 (vitamin E/placebo) for the bulbar patients and at -18.6 and -19.5 for the limb patients respectively, suggesting a more beneficial effect in bulbar patients (see Fig. 2).

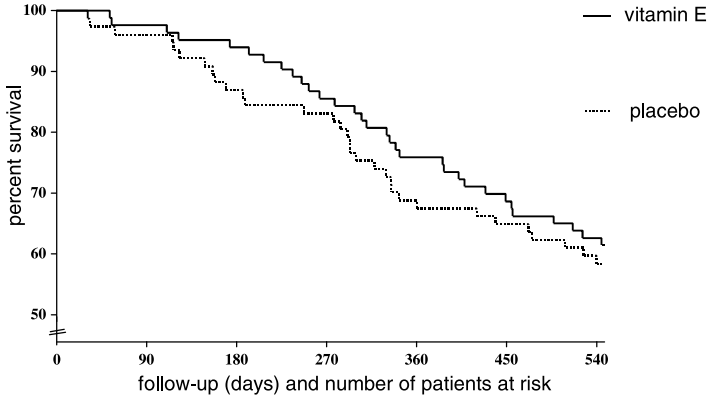

Fig. 1. Kaplan-Meier curves of cumulative survival for vitamin E versus placebo treatment ($p = 0.58$)

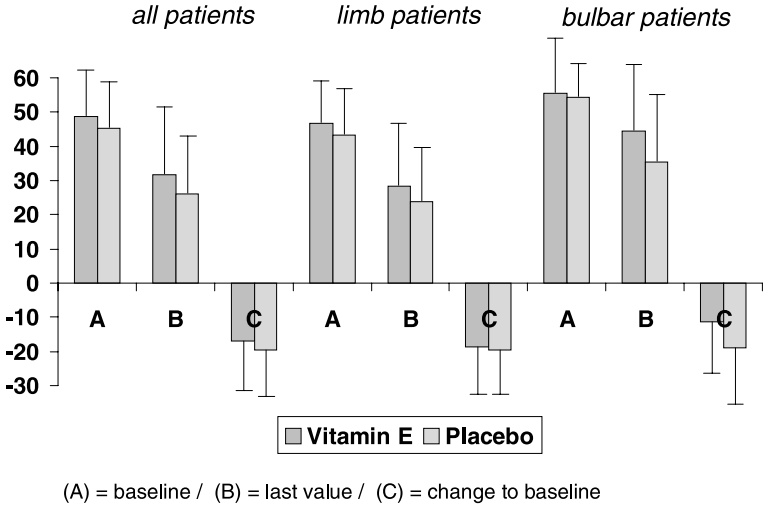

Fig. 2. Norris limb score – changes to baseline, means and standard deviations ($p = 0.32$)

Only the vital capacity outcome measure showed a marginal trend against vitamin E ($p = 0.07$). This was somewhat compensated for by less patients needing intermittent assisted ventilation (1 in 33 after 18 months in the vitamin E group vs. 8 in 24 on placebo, i.e. 3% vs. 33%). In addition, the spirometers used in the centres were not standardised.

3.4 Safety results

No differences between the treatment groups could be detected as regards vital signs (weight, heart rate, blood pressure), serious and other adverse event reporting, concomitant medication, ECG's and VEP's. Also standard laboratory tests did not indicate of toxic or otherwise harmful effects caused by vitamin E.

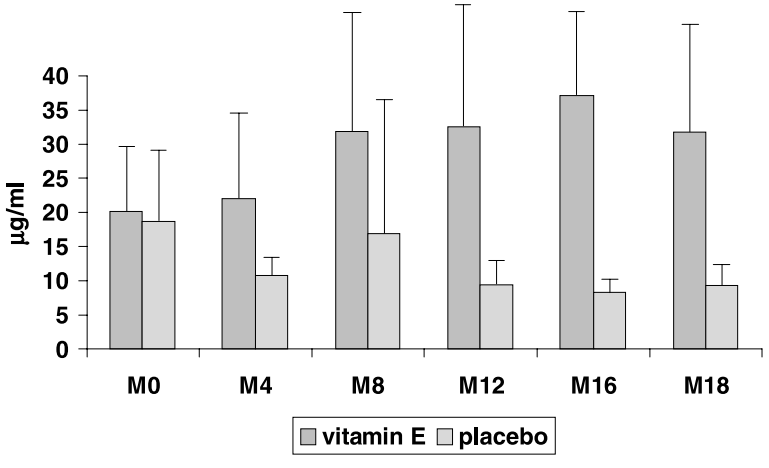

Fig. 3. Vitamin E plasma levels (means and standard deviations)

3.5 Vitamin E plasma levels

Vitamin plasma levels were, as expected, significantly higher in the high dose vitamin E group than in the placebo group (see Fig. 3).

At baseline, both groups had levels around 20 μg/ml during therapy, the levels went down to about 10 μg/ml in the placebo group whilst in the vitamin E group, plasma levels ranged between 30 and 40 μg/ml. In a recent study (Rosen et al., 1993), lower mean vitamin E serum levels were reported in a control population (14.80 ± 0.57 μg/ml) than we found at baseline. This could be a result of the fact that one quarter of the patients included in this study previously used tocopherol.

4. Discussion

As a first important result of this study must be considered the fact that it has been possible to perform a large prospective randomised double-blind study in such a devastating disease such as ALS just with dedicated clinicians supported only by a patients' charity (with only very limited further support from the pharmaceutical industry, see acknowledgments).

The project turned out to be extremely difficult as vitamin E is quite an old product, and so there was nearly no interest from the pharmaceutical industry.

It is obvious that independent studies become of increasing importance, especially when older drugs – which as a rule are cheaper, being out of patents – need to be explored in new indications, especially when it comes to rare and very severe medical conditions.

Also drug authorities have expressed a need for this type of investigations which they intend to support by a special, so-called orphan legislation and reduction in registration fees (but funding remains an unresolved issue) in contrast to, e.g. the US with the funding potential and expertise at the NIH, NCI etc. The feasibility and importance but also the actual limitations of performing this kind of 'orphan studies' in rare and life-threatening diseases is in addition an important issue recently taken up also by the EMEA with its 'orphan drug act' of 2001 (Horowski, 1995), EC act 141/2000.

A first result of medical importance of the study is, that high-dose administration of vitamin E in doses quite exceeding nutritional needs is safe at least under the conditions of our study. This may become of great importance as there is increasing evidence that oxidative stress and free radicals play an important role in a number of diseases. Even in Parkinson's disease, where a very large and expensive NIH-sponsored study of 500 mg vitamin E per day (the DATATOP study; Parkinson Study Group, 1996) has failed to show benefits in newly diagnosed patients, new epidemiological and other data seem to indicate again some protective effect of nutritional vitamin E (Zhang et al., 2002), and the same seems to hold true for Alzheimer's disease (Zandl et al., 2004).

It is however quite possible that timing or duration of exposure to high doses of vitamin E is critical, and this might be one of the reasons that in our study no significant efficacy on the primary endpoint could be observed. It can also be argued that our dosing schedule did not increase vitamin E levels sufficiently as measurement of vitamin E plasma levels showed only a 3–4 fold increase

compared to the control group. Vitamin E serum levels are well recognised as reflecting the short-term tocopherol intake. However, little is known about the ability of supplemental vitamin E to alter human brain vitamin E levels. A recent study demonstrated in Parkinson's disease patients that oral vitamin E supplementation, even at high doses (4,000 IU/days), fails to increase ventricular cerebrospinal fluid vitamin E levels (Pappert et al., 1996) This lack of change may be due to limited passage across the blood-brain barrier or very rapid vitamin E metabolism.

There is, however, obviously a need for further investigation of the fate and disposition of vitamin E in humans and especially in patients with ALS, and in case of limited bioavailability to the relevant motoneurones, intravenous or even intrathecal injection or some form of drug targeting should be considered.

As vitamin E, after single dose oral administration, increases vitamin E levels in plasma for over 20 hours, timing in plasma sampling should play only a minor role. If a depot is formed within the body, it may become necessary to prolong the observation period very much and also, if one wants a 'real' placebo control, a long wash-out period with controls before starting a trial becomes necessary.

As one quarter of patients in this study reported to have been on vitamin E before the trial, since this is rather easily available to patients in Central Europe, this consideration becomes important, and even more so, as also within the study, 7 patients on placebo openly decided ''off-label'' use of vitamin E (but remained in the placebo group for the 'intention-to-treat'-analysis). This indeed leads to an additional bias against vitamin E. Even by best efforts, however, the study could not be conceived as large as might have been necessary from an efficacy point of view; our statistical considerations were only based upon numbers from the first successful therapeutic study in ALS with riluzole 50 mg $2\times$/day p.o. where over a period of 18 months with 155 patients, significant prolongation of survival of ALS patients (by 2–3 months average) had been described (Bensimon et al., 1994). With vitamin E as an add-on therapy to this only licensed product, our study, due to its highly restricted funding, could be powered only to detect a further 50% improvement, an ambitious goal for any new therapy (as must be said with the benefit of hindsight).

When discussing efficacy, it should therefore be made very clear that the failure of this study to show a significant prolongation in survival cannot be considered convincing evidence that vitamin E has no effect and is not useful in ALS (''absence of evidence is not evidence of absence'') especially if one takes into account our limited knowledge about vitamin E intake and kinetics.

Furthermore, the mortality in the patients enrolled into this study was definitively less than expected from the literature (which had been used for our calculations). This might have been due to the accumulated clinical experience in the study centres.

Finally, all patients had to be on riluzole therapy for ethical reasons, reducing again the discriminative power and thus the theoretical chances of a significant result of this study.

In conclusion, our results indicate that much larger and longer studies might be needed, with close monitoring of vitamin E intake. These studies include a withdrawal period before starting the trial as well as a longer treatment period. In this context we intend to follow up the surviving patient cohort regardless of post trial open label treatment in order to improve our database as regards possible long term effects.

Such an endeavour might also be useful for identification of 'vitamin E responders' if such a subgroup exists; it may be interesting that in the open case collection preceding this trial, at least two 'responders' did not, for different reasons, use riluzole at all. Indeed, if riluzole acts by reducing glutamate-induced excitotoxicity, it can be assumed that the very oxidative stress vitamin E is supposed to be acting upon is avoided or at least reduced in patients on riluzole, thus preventing vitamin E from exerting its antioxidant properties to the full benefit of the patients.

This dilemma may come up with any new therapy: for simple statistical reasons, new therapeutic studies now will need a much larger number of patients if any significant add-on effect is to be seen, but furthermore pharmacological intervention within a same pathogenetic cascade may not always be synergistic (as we tend to hope) but might even become neutralized or antagonized by a second drug. In such an event, a direct comparison of a new treatment vs. riluzole and placebo might be the best way – a strategy which however, for ethical reasons, appears to be extremely difficult.

5. Conclusion

Overall, neither the primary nor the secondary outcome measures of this placebo-controlled randomised prospective trial could determine whether a megadose of vitamin E is effective in slowing disease progression in ALS although there was a small trend in favour of this drug. There were similar trends in the secondary outcome measures. Administration of the megadose of vitamin E does not seem to have any significant side effects. This last result might become of greater importance if we will be able to identify responders in ALS, or if vitamin E is to be used in other indications as discussed.

Acknowledgements

The authors want to thank H.D. Klinke from Schwarzhaupt, Cologne, for supplying high-dosed vitamin E for the study, Schering, Berlin, for logistic support, S. Poli and R. Burger for their valuable technical assistance in carrying out the vitamin E analyses and B. Gelhar for very efficient data management and statistical analysis. The study would not have been possible without the continuous support from the Charcot Foundation, Ulm, and the dedicated work by all participants. We also want to thank all patients who took part in our study.

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