Granulocyte Colony-Stimulating Factor for Amyotrophic Lateral Sclerosis: A Randomized, Double-Blind, Placebo-Controlled Study of Iranian Patients

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Background and Purpose The aim of this study was to determine the efficacy and tolerability of granulocyte colony-stimulating factor (G-CSF) in subjects with amyotrophic lateral sclerosis (ALS).

Methods Forty subjects with ALS were randomly assigned to two groups, which received either subcutaneous G-CSF (5 μg/kg/q12h) or placebo for 5 days. The subjects were then followed up for 3 months using the ALS Functional Rating Scale-Revised (ALSFRS-R), manual muscle testing, ALS Assessment Questionnaire-40, and nerve conduction studies. CD34+/CD133+ cell count and monocyte chemoattractant protein-1 (MCP-1) levels were evaluated at baseline.

Results The rate of disease progression did not differ significantly between the two groups. The reduction in ALSFRS-R scores was greater in female subjects in the G-CSF group than in their counterparts in the placebo group. There was a trend toward a positive correlation between baseline CSF MCP-1 levels and the change in ALSFRS-R scores in both groups (Spearman's ρ=0.370, p=0.070).

Conclusions With the protocol implemented in this study, G-CSF is not a promising option for the treatment of ALS. Furthermore, it may accelerate disease progression in females.

Key Words amyotrophic lateral sclerosis, ALS Functional Rating Scale, granulocyte colony-stimulating factor, CD34+/CD133+ cells, monocyte chemoattractant protein-1, compound motor action potential.

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INTRODUCTION

The effect of granulocyte colony-stimulating factor (G-CSF) on the course of neurological diseases has drawn considerable attention over the past decade. G-CSF mobilizes hematopoietic (CD34+) stem cells and—with repeated administration—recruits immature CD34+/CD133+ stem cells that are capable of differentiating into neuronal progenitors.[1,2] G-CSF receptor expression may play an autocrine protective role in various cells of the nervous system,[3] and G-CSF may protect against ischemic neuronal damage by inhibiting apoptosis and inflammation, mobilizing stem cells, or enhancing neuronal differentiation.[3,4]

Recent studies have suggested a role for G-CSF in the treatment of amyotrophic lateral sclerosis (ALS).[5-9] In a mouse model of ALS, G-CSF significantly improved motor performance and motoneuron survival, and reduced denervation atrophy.[10] A study involving subjects with ALS found significantly reduced G-CSF receptor expression in motoneurons and increased G-CSF expression in reactive astrocytes.[8] The authors suggested that reduced G-CSF receptor expression on motor neurons might account for the pathophysiol-

ogy of ALS. Simultaneously the levels of monocyte chemoattractant protein-1 (MCP-1) were increased, suggesting that reduced−CSF receptors can exacerbate the ALS course.

Most studies of G-CSF efficacy in subjects with ALS have been limited in several ways. A nonrandomized study involving 13 subjects with ALS demonstrated a significantly lower monthly reduction in ALS Functional Rating Scale-Revised (ALSFRS-R) scores and compound motor action potential (CMAP) amplitude following 5 days of G-CSF treatment.[9] Another uncontrolled study involving 24 individuals did not show any significant effect of G-CSF on clinical outcomes.[11] In a placebo-controlled study of G-CSF treatment in ten subjects with ALS, G-CSF treatment failed to improve any of the clinical outcomes, although it significantly reduced the fractional anisotropy on diffusion tensor imaging.[12] Finally, a double-blind study involving 39 subjects found no statistically significant benefit with G-CSF, although there was a trend toward slowing of disease progression following two cycles of G-CSF treatment.[13] One problem with that study was that more than 50% of the subjects were lost to 1-year follow-up.

The present study was a randomized double-blind, placebo-controlled trial of G-CSF with assessment of various clinical, functional, electrophysiological, and molecular outcomes, the aim of which was to provide a thorough view of the efficacy of G-CSF and its tolerability in Iranian subjects with ALS, and to address some of the caveats noted in previous studies.

METHODS

Trial design and setting
This was a single-center, 3-month, randomized, double-blind, placebo-controlled, and parallel-group study that was conducted in a tertiary referral center affiliated with Tehran University of Medical Sciences between November 2012 and November 2013.

Participants
Male and female subjects with a diagnosis of probable or definite ALS based on the revised El Escorial World Federation of Neurology criteria were screened for inclusion in the study. The following inclusion criteria were applied: aged 18−85 years, symptom history not exceeding 2 years, and ALSFRS-R score ≥20. Although ethnicity was not an inclusion criterion, all of the subjects were ethnically Iranian. Riluzole treatment was not an exclusion criterion if the patient had been stable on the therapeutic protocol for at least 30 days prior to study entry. The following exclusion criteria were applied: ALS in first-degree relatives; pregnancy or lactation; history

of neoplasia, myeloproliferative, or any other disorders that could be exacerbated by G-CSF; active immunological disease; spleen diameter ≥180 mm; severe heart, kidney, or liver disease, positive HIV status, forced vital capacity ≤50% of that predicted; cognitive disorders that interfere with the study procedure; and history of hypersensitivity reaction to G-CSF or *Escherichia-coli*-derived proteins.

After receiving a complete explanation of the study procedures, all subjects (or their representatives) provided written informed consent to participate prior to study entry. The study protocol was approved by the Ethics Committee and the Institutional Review Board of Tehran University of Medical Sciences (approval no. 91-01-54-17265). The trial was conducted in accordance with the last revision of the Declaration of Helsinki and was registered at ClinicalTrials.gov (registration no. NCT01825551).

Study procedures
The subjects underwent a complete physical and neurologic examination, peripheral blood smear, complete blood count with differentials, liver enzymes, serum lactate dehydrogenase (LDH), uric acid, CD34+ and CD133+ cell count, serum and cerebrospinal fluid (CSF; in a subset of subjects) MCP-1 levels, abdominal ultrasound (if the spleen was palpable), and pulmonary function testing (if significant respiratory dysfunction was present). Scores for the ALS Assessment Questionnaire-40 (ALSAQ-40), ALSFRS-R, and manual muscle testing (MMT) were assessed every month. Nerve conduction velocity (NCV) studies were performed by an expert (A.T.) at baseline and at the study endpoint. The white blood cell (WBC) count was measured every day during the treatment phase. WBC differentials, liver enzymes, and CD34+ and CD133+ cell counts were measured on days 4 and 6, and LDH and uric acid levels were assessed on day 4 of the trial. MCP-1 concentration was only tested at baseline.

Subjects were randomly assigned to receive either subcutaneous G-CSF (containing PDgrastim, filgrastim, 300-μg recombinant G-CSF, equal to 30,000,000 IU of filgrastim, mannitol, and sodium acetate; Pooyesh Darou, Tehran, Iran) 5 μg/kg/q12h or normal saline for five consecutive days. The subjects were hospitalized and closely monitored for any possible serious adverse events during the first few days. Treatment was discontinued if the leukocyte count rose to more than 50,000/μL; the remaining doses were administered when levels had returned to lower values (below 15,000/μL). The participants were then followed up in the outpatient clinic.

Assessments
Amyotrophic lateral sclerosis Functional Rating Scale-Revised is a 12-item (total score of 0−48) physician-administered

measure of ALS severity that assesses function in three major domains: bulbar, motor, and respiratory. Each item is scored on a five-point scale (0−4), with higher scores reflecting better function.[14]

Amyotrophic lateral sclerosis Assessment Questionnaire-40 is a 40-item subjective measure of health status for subjects with ALS that is categorized into five domains: eating/drinking, communication, activities of daily living/independent activities of daily living, physical mobility, and emotional functioning. The total ALSAQ-40 score and those of its domains are converted into a 100-point scale, with lower scores reflecting a better health status.[15,16]

Manual muscle testing was based on the examination of 34 muscles, converted to a 10-point scale. The final MMT score is the mean of the scores for all 34 muscles. MMT appears to be the preferred method for measurement of global strength.[17]

The NCV studies involved a belly-tendon montage of CMAP amplitude recordings from the median (abductor pollicis brevis), ulnar (abductor digiti minimi), tibial (abductor hallucis brevis), and common peroneal nerves (extensor digitorum brevis). CMAPs were recorded in response to supramaximal stimulation with 0.2-ms duration.

MCP-1, CD34+, and CD34+/CD133+ cells
The method for evaluating CD34+, CD34+/CD133+, and MCP-1 has been described in detail elsewhere.[1,18]

Outcome measures
The primary outcome measure was the monthly rate of decline in ALSFRS-R score. Secondary outcome measures were the changes from baseline in the ALSAQ-40 and MMT scores, and the CMAP amplitude of the nerves. Furthermore, the correlations between MCP-1 levels, counts of CD34+, CD133+, and CD34+/133+ cells, and ALSFRS-R scores were calculated. Safety issues were systematically assessed using both a checklist for clinical symptoms and laboratory values.

Randomization, allocation concealment, and blinding
A randomization list was prepared using a computerized random-number generator in a 1:1 ratio and block size of four. Allocation concealment was achieved using sequentially numbered and opaque envelopes. Treatment allocation, evaluation of side effects, and possible changes in treatment protocol, clinical rating, and electrophysiological assessment of the subjects were conducted by separate researchers. The subjects, the evaluator, the person responsible for administering the intervention, and the statistician were all blind to the treatment allocation.

Statistical analysis
STATA version 12.0 (StataCorp, College Station, TX, USA) was used for the data analysis. All analyses were carried out on the data from participants with at least one postbaseline measurement. Per-protocol and linear mixed model analyses were both used for comparison of outcomes. Repeated-measures linear mixed model analysis was performed using the STATA module for analyzing repeated-measures data (xtmixed), which can account for unbalanced data. Except where stated otherwise, the data are presented as mean±SD values, and the cutoff for statistical significance was set at $p<0.05$.

The sample size was calculated based on a between-group difference of three (with a standard deviation of three) for the change in ALSFRS-R scores from baseline. This yielded a sample size of 16 in each group, and accounting for a potential 20% loss to follow-up, a sample size of 40 was reached.

RESULTS

Baseline characteristics and laboratory values
Sixty-seven subjects were screened for eligibility criteria and 40 subjects were randomized into either the G-CSF (n=20) or placebo (n=20) group. All subjects had at least one postbaseline measurement, and 35 subjects completed the study (n=18 and 17 for the G-CSF and placebo groups, respectively). The baseline characteristics of the subjects are summarized in Table 1. One patient experienced transient fever and chills at day 4 of treatment.

The WBC count in the G-CSF group rose from 7,892± 2,009/μL at baseline to 49,689±14,613/μL at day 6 ($p<0.001$, repeated-measures ANOVA), whereas no significant change was observed in the placebo group (7,041±1,613/μL and 6,862±1,312/μL at baseline and day 6, respectively; p=0.985). The numbers of neutrophils, CD34+, CD133+, and CD34/133+ cells increased significantly in the G-CSF group ($p<0.01$ for all), but not the placebo group. Of the laboratory tests, alkaline phosphatase and LDH levels increased significantly in the G-CSF group.

ALSFRS-R scores
Both groups exhibited a progressive decline in the ALSFRS-R score ($p<0.0001$ for time effect). In the mixed model analysis, no significant time-treatment interaction was observed (Fig. 1, Table 2). The per-protocol analysis (Table 3) revealed that the monthly reduction in ALSFRS-R scores did not differ significantly between the G-CSF group (1.53 points/month) and the placebo group [1.61 points/month; mean difference (95% CI)=0.074 (-0.952 to 1.101), t (34)=0.146, p=0.884].

Table 1. Baseline characteristics of the patients in the two groups

Variable	G–CSF (*n*=20)	Placebo (*n*=20)
Gender, male (*n*, %)	13 (65)	12 (60)
Age (years)	51.3 (8.6)	52.5 (11.6)
Duration of disease (months)	17.0 (6.4)	15.7 (6.3)
Time since diagnosis (months)	9.7 (8.2)	9.8 (7.6)
History of riluzole treatment (*n*, %)	14 (70)	14 (70)
Duration of riluzole treatment (months)	5.5 (6.7)	5.4 (6.1)
History of smoking (*n*, %)	7 (35)	6 (30)
History of alcohol use (*n*, %)	3 (15)	0 (0)
BMI (kg/m²)	24.3 (4.7)	25.1 (4.5)
Baseline ALSFRS-R score	33.3 (7.9)	36.6 (4.6)
Bulbar domain	9.6 (2.9)	10.5 (1.9)
Motor domain	11.8 (6.8)	14.4 (4.6)
Respiratory domain	11.9 (0.3)	11.6 (0.7)
Baseline ALSAQ-40 score	63.0 (17.5)	60.3 (12.1)
Physical mobility domain	71.7 (20.2)	72.2 (19.0)
ADL/IADL	69.6 (25.1)	66.0 (22.4)
Eating disorder	44.7 (30.4)	33.7 (19.0)
Communication	52.3 (31.5)	50.1 (29.0)
Emotional functioning	60.9 (26.6)	57.7 (18.6)
Baseline MMT score	6.6 (2.5)	7.9 (1.2)
Baseline WBC count (/μL)	7,892 (2,009)	7,041 (1,613)
Baseline neutrophil count (/μL)	4,605 (1,320)	4,139 (1,129)
Baseline lymphocyte count (/μL)	2,442 (887)	2,193 (793)
Baseline aspartate aminotransferase (U/L)	26.9 (7.3)	23.9 (8.2)
Baseline alanine aminotransferase (U/L)	30.0 (13.0)	28.0 (17.0)
Baseline alkaline phosphatase (U/L)	174.6 (55.3)	160.4 (50.0)
Baseline lactate dehydrogenase (U/L)	336.7 (83.2)	317.8 (69.5)
Baseline CD34+ cell count (/μL)	5.9 (4.7)	4.6 (1.2)
Baseline CD133+ cell count (/μL)	3.2 (1.4)	3.0 (1.5)
Baseline CD34+/133+ cell count (/μL)	0.2 (0.2)	0.4 (0.2)
Baseline serum MCP-1 (pg/mL)	95.0 (32.3)	91.5 (40.2)
Baseline CSF MCP-1 (pg/mL)	162.6 (30.9)	144.1 (42.5)

Except where indicated otherwise, the data are presented as mean (SD) values.
ADL: activities of daily living, ALS: amyotrophic lateral sclerosis, ALSAQ-40: ALS Assessment Questionnaire-40, ALSFRS-R: ALS Functional Rating Scale-Revised, BMI: body mass index, CSF: cerebrospinal fluid, G-CSF: granulocyte colony-stimulating factor, IADL: independent activities of daily living, MCP-1: monocyte chemoattractant protein-1, MMT: manual muscle testing, WBC: white blood cell.

ALSAQ-40 and MMT

A progressive change in the ALSAQ-40 and MMT scores was observed in both groups, corresponding to a worsened health status ($p<0.0001$ for time effect). The change in either outcome did not differ significantly between the two groups (Fig. 1, Table 2 and 3).

NCV

Except for one of the measurements (right common peroneal), changes from baseline did not differ significantly between the two groups (Table 4).

Association between CD34+ (CD133+) cell count, MCP-1 level, and ALSFRS-R score

In the G-CSF group, changes in the CD34+ cell count in the first 6 days were negatively correlated with the ALSFRS-R score reduction (r=-0.485, p=0.041). Further analysis revealed that those with large changes in CD34+ (>100/μL) cell count did not differ significantly from the placebo group in terms of reduction in ALSFRS-R scores. The elevation in the CD34+ cell count was lower in the females than the males of the G-CSF group, with the difference tending toward significance (55.9±45.7/μL vs. 106.2±61.8/μL; p=0.062, Mann-Whitney U-test). Further exploratory analysis demonstrated that female

subjects in the G-CSF group tended toward a greater reduction in ALSFRS-R scores than their counterparts in the placebo group (p=0.073). Changes in CD133+ (r=-0.102, p=0.687) or CD133+/CD34+ (r=-0.323, p=0.190) cell counts were not significantly associated with ALSFRS-R reduction.

Baseline CSF MCP-1 levels were positively correlated with the change in ALSFRS-R scores in both groups (Spearman's ρ=0.370, p=0.070). No significant correlation was observed between the serum levels of MCP-1 and ALSFRS-R scores.

DISCUSSION

In the present study, although G-CSF treatment increased
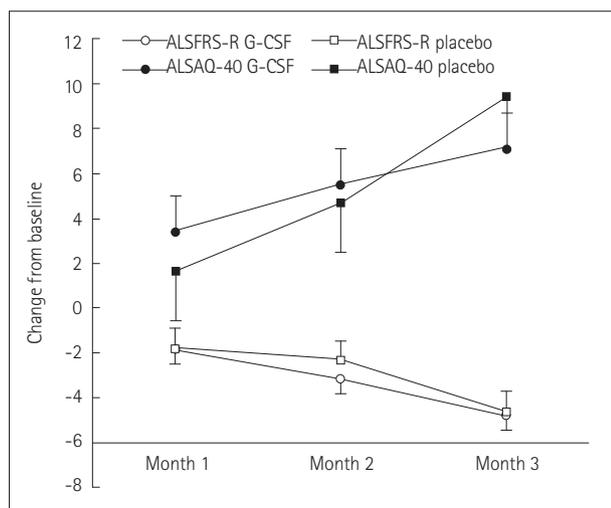
Fig. 1. Changes from baseline (mean±standard error of mean) in ALSFRS-R and ALSAQ-40. ALSAQ-40: ALS Assessment Questionnaire-40, ALSFRS-R: ALS Functional Rating Scale-Revised, G-CSF: granulocyte colony-stimulating factor.

the WBC (and CD34+/CD133+ cells) count to the expected range, it failed to improve any of the study outcomes in the subjects. Furthermore, an elevation in the CD34+ and CD133+ counts was not correlated with better outcomes. These findings, together with most previous studies demonstrating little clinical advantage with G-CSF use in subjects with ALS, suggest that G-CSF administration with current protocols is unlikely to be of any clinical benefit in subjects with ALS.

An important question is thus raised as to why, despite promising preliminary data, G-CSF failed to improve clinical outcomes in subjects with ALS. G-CSF can pass through the blood-brain barrier (BBB) in rats, but human data are not yet available.[13,19] However, the neuroradiological changes observed following G-CSF administration in humans could be taken as evidence of the ability of this growth factor to exert its effects beyond the BBB.[12] Furthermore, the findings of a separate line of studies suggest that G-CSF has direct neuroprotective properties through immunomodulation or counteraction of apoptotic pathways.[5,10] Another reason for the lack of a G-CSF effect might be inadequacy of the duration or dose of treatment.[12] All clinical studies to date have used one or only a few cycles of G-CSF with up to 1 year of follow-up, and found only minor differences in clinical outcomes. Therefore, within the range of currently routine protocols, it is unlikely that extended follow-up periods will yield additional clinical benefits of G-CSF administration. Conversely, studies of G-CSF administration in a mouse model of ALS employed much higher doses (up to 100 μg/kg body weight), which may account for the observed difference in the outcomes between human and animal studies.[7,10]

In the present study, the elevation in CD34+ cell count was positively correlated with a better clinical response. In

Table 2. Results of mixed-effects analyses

Variable	Coefficient	Standard error	Z	p>|z|	95% CI	p[†] joint group×time
ALSFRS-R total						0.964
G-CSF group×month 1*	0.00	1.10	0.00	1.000	-2.15–2.15	
G-CSF group×month 2	-0.38	1.12	-0.34	0.734	-2.58–1.81	
G-CSF group×month 3	0.22	1.14	0.18	0.856	-2.03–2.44	
ALSAQ-40						0.378
G-CSF group×month 1	1.8	2.4	0.74	0.461	-3.0–6.6	
G-CSF group×month 2	0.5	2.5	0.19	0.847	-4.4–5.4	
G-CSF group×month 3	-2.6	2.5	-1.01	0.311	-7.5–2.4	
MMT						0.771
G-CSF group×month 1	0.11	0.16	0.71	0.478	-0.20–0.43	
G-CSF group×month 2	0.16	0.16	0.97	0.333	-0.16–0.48	
G-CSF group×month 3	0.04	0.17	0.26	0.792	-0.28–0.37	

*In the first column "G-CSF group×month 1" means the interaction of being in the G-CSF group versus the placebo group×measurement at month 1 versus at baseline visit, [†]The p value of the last column combines the p values from all interactions.
ALS: amyotrophic lateral sclerosis, ALSAQ-40: ALS Assessment Questionnaire-40, ALSFRS-R: ALS Functional Rating Scale-Revised, G-CSF: granulocyte colony-stimulating factor, MMT: manual muscle testing.

Table 3. Comparison of outcomes between the two groups

Variable	Month 1	Month 2	Month 3	Change from baseline at endpoint	t	p
ALSFRS-R					-0.147	0.884
G-CSF	31.5 (8.8)	30.8 (8.9)	29.0 (9.6)	4.8 (4.2)		
Placebo	34.8 (7.0)	34.6 (4.3)	32.5 (5.5)	4.6 (4.9)		
ALSFRS-R bulbar domain					0.000	1.000
G-CSF	9.2 (3.6)	9.2 (3.8)	8.9 (4.1)	0.8 (1.7)		
Placebo	10.0 (2.2)	10.0 (2.0)	9.8 (2.6)	0.8 (1.3)		
ALSFRS-R motor domain					0.666	0.510
G-CSF	11.0 (6.5)	10.6 (5.7)	9.4 (5.6)	3.5 (3.5)		
Placebo	13.6 (4.6)	12.9 (3.9)	11.3 (4.5)	2.8 (2.4)		
ALSFRS-R respiratory domain					-1.358	0.183
G-CSF	11.3 (1.5)	11.0 (2.0)	10.7 (2.5)	0.3 (1.2)		
Placebo	11.3 (2.2)	11.6 (0.7)	11.4 (1.0)	1.2 (2.5)		
ALSAQ-40					-0.681	0.501
G-CSF	66.4 (17.9)	67.4 (18.6)	69.0 (19.5)	-7.1 (8.7)		
Placebo	61.9 (12.4)	64.3 (14.8)	69.4 (17.4)	-9.4 (11.7)		
ALSAQ-40 physical mobility domain					0.508	0.618
G-CSF	75.5 (18.7)	78.4 (19.2)	79.2 (19.2)	-7.3 (11.6)		
Placebo	73.9 (18.5)	75.6 (21.1)	79.2 (20.8)	-9.3 (12.0)		
ALSAQ-40 ADL/IADL domain					0.627	0.535
G-CSF	76.2 (24.7)	78.8 (24.0)	81.9 (22.2)	-12.2 (12.3)		
Placebo	66.0 (22.4)	71.1 (24.8)	76.4 (24.6)	-9.6 (13.2)		
ALSAQ-40 eating/drinking domain					-0.834	0.410
G-CSF	47.3 (30.0)	50.5 (31.8)	54.8 (34.4)	-8.5 (16.5)		
Placebo	38.0 (18.2)	44.6 (22.3)	45.2 (27.6)	-14.8 (6.5)		
ALSAQ-40 communication domain					-0.754	0.456
G-CSF	54.0 (32.7)	54.4 (33.8)	56.7 (34.5)	-5.1 (9.0)		
Placebo	52.8 (27.6)	51.7 (28.1)	56.7 (33.0)	-9.7 (24.3)		
ALSAQ-40 emotional functioning domain					-1.829	0.076
G-CSF	62.1 (24.7)	59.1 (25.4)	59.0 (25.4)	-0.8 (13.9)		
Placebo	57.6 (18.2)	61.0 (22.8)	68.7 (23.2)	-9.7 (15.3)		
MMT					0.122	0.903
G-CSF	6.3 (2.6)	6.2 (2.4)	5.6 (2.6)	20.4 (12.1)		
Placebo	7.5 (1.3)	7.3 (1.1)	6.9 (1.2)	20.9 (12.4)		

Data are presented as mean (SD) values.
ADL: activities of daily living, ALS: amyotrophic lateral sclerosis, ALSAQ-40: ALS Assessment Questionnaire-40, ALSFRS-R: ALS Functional Rating Scale-Revised, G-CSF: granulocyte colony-stimulating factor, IADL: independent activities of daily living, MMT: manual muscle testing.

Table 4. Comparison of CMAP amplitude between the two groups

Nerve	CAMP amplitude at baseline		CAMP amplitude at endpoint		Change from baseline		
	G-CSF	Placebo	G-CSF	Placebo	G-CSF	Placebo	p
Right median	1.4 (0.4–5.4)	3.4 (1.4–5.0)	0.0 (0.0–1.0)	2.0 (1.0–4.0)	0.8 (0.1–2.1)	0.6 (0.1–2.2)	0.934
Left median	1.3 (0.7–5.0)	3.8 (1.8–5.3)	0.0 (1.0–4.0)	2.0 (1.0–4.0)	0.2 (0.1–1.0)	0.7 (0.2–1.1)	0.371
Right ulnar	0.5 (1.5–4.0)	3.0 (2.0–5.0)	1.0 (0.0–3.0)	2.0 (1.0–4.0)	0.4 (0.1–1.1)	0.9 (0.2–1.3)	0.437
Left ulnar	2.0 (1.0–5.0)	3.5 (2.0–6.5)	1.0 (0.0–3.0)	2.0 (2.0–4.0)	0.6 (0.0–2.2)	0.5 (0.1–1.1)	0.816
Right common peroneal	1.0 (0.0–1.5)	0.5 (0.1–2.5)	1.0 (0.0–1.0)	1.0 (0.0–2.0)	0.1 (0.0–0.3)	0.4 (0.1–0.7)	0.020
Left common peroneal	0.6 (0.1–2.3)	0.8 (0.0–2.5)	0.2 (0.0–1.7)	0.5 (0.0–2.0)	0.3 (0.1–1.1)	0.1 (0.0–0.3)	0.184
Right tibial	6.7 (1.7–11.1)	5.8 (1.3–7.9)	4.6 (1.2–8.4)	4.6 (2.1–6.8)	0.8 (0.1–2.0)	0.9 (0.1–2.0)	0.882
Left tibial	5.0 (1.0–10.0)	6.5 (1.5–10.0)	3.6 (0.4–8.3)	5.7 (2.8–8.9)	0.9 (0.5–2.3)	0.6 (0.1–1.1)	0.165

Data are presented as median (interquartile range) values. Mann-Whitney U test.
CMAP: compound motor action potential, G-CSF: granulocyte colony-stimulating factor.

further exploratory analysis it was determined that the lower CD34+ count and lower clinical response in the females of the G-CSF group accounted for this finding. G-CSF did not improve the ALSFRS-R score in the females; indeed, it appeared to aggravate it compared to placebo. The lower CD34+ cell count observed in the females is probably an innocent-bystander phenomenon, because healthy females generally have a lower CD34+ cell yield following G-CSF treatment.[20] Furthermore, the greater symptom progression in the females of the G-CSF group is unlikely to be related to the predictive role of female gender in disease progression, because similar findings were not observed in the females of the placebo group.[21] Together these exploratory findings raise the possibility that G-CSF exacerbates the course of ALS in female subjects. However, our study was not designed to analyze outcomes based on gender subgroups, and therefore further investigation is required to address this finding.

The present study demonstrated a trend toward a faster symptom progression in subjects with higher baseline CSF MCP-1 concentrations. As a multifunctional chemokine, MCP-1 is involved in several inflammatory, allergic, and immunodeficiency conditions.[22] Importantly, MCP-1 can increase BBB permeability to monocytes and dendritic cells, and can thus exacerbate inflammation.[23] Accordingly, elevated MCP-1 serum and CSF concentrations may hasten neuronal death by enhancing inflammation in ALS.[8,23]

This study was limited by its short duration. However, findings of previous studies suggest that a longer duration of follow-up is unlikely to confer any important clinical benefit with currently administered doses of G-CSF. We recruited a relatively large number of subjects compared to other published trials of G-CSF in subjects with ALS. However, there were still near-significant p values in some of the secondary outcomes, and it is possible that type II errors were present due to the smallness of the sample.

While this study is not entirely novel, it has some distinctive features. Given that ALS exhibits a diverse risk profile across ethnicities,[24] genetic background and therefore response to treatment might also differ across populations. Thus, this study can be considered an account of how Iranian ALS subjects respond to G-CSF. This might also be the reason for some of the subtle differences between the findings of this study and others. This study had several strengths. The stringent eligibility criteria led to the recruitment of a homogeneous patient population. Data from all subjects were entered into intention-to-treat analysis, and the final dropout rate was only 10%. Although the short study duration is probably one reason for the low number of dropouts, at the same time the short-spaced follow-ups provide a clearer view of the disease response in the short term. Moreover, the assessment of several aspects (e.g., clinical and functional) of ALS in our subjects ensures that the lack of G-CSF efficacy is not due to insensitivity of some of the measurement instruments.

In summary, although short-term G-CSF treatment was found to be safe in this study, there was little evidence of its efficacy in subjects with ALS. If G-CSF is to be tested further in studies of subjects with ALS, it probably should be administered with a different dose or delivery protocol (e.g., high dose or intraspinal delivery) and with greater caution in females.

Conflicts of Interest

The authors have no financial conflicts of interest.

Acknowledgements

REFERENCES

1. Tarella C, Rutella S, Gualandi F, Melazzini M, Scimè R, Petrini M, et al. Consistent bone marrow-derived cell mobilization following repeated short courses of granulocyte-colony-stimulating factor in patients with amyotrophic lateral sclerosis: results from a multicenter prospective trial. *Cytotherapy* 2010;12:50-59.
2. Zangiacomi V, Balon N, Maddens S, Lapierre V, Tiberghien P, Schlichter R, et al. Cord blood-derived neurons are originated from CD133+/CD34 stem/progenitor cells in a cell-to-cell contact dependent manner. *Stem Cells Dev* 2008;17:1005-1016.
3. Solaroglu I, Jadhav V, Zhang JH. Neuroprotective effect of granulocyte-colony stimulating factor. *Front Biosci* 2007;12:712-724.
4. Lu CZ, Xiao BG. Neuroprotection of G-CSF in cerebral ischemia. *Front Biosci* 2007;12:2869-2875.
5. Pollari E, Savchenko E, Jaronen M, Kanninen K, Malm T, Wojciechowski S, et al. Granulocyte colony stimulating factor attenuates inflammation in a mouse model of amyotrophic lateral sclerosis. *J Neuroinflammation* 2011;8:74.
6. Henriques A, Pitzer C, Dittgen T, Klugmann M, Dupuis L, Schneider A. CNS-targeted viral delivery of G-CSF in an animal model for ALS: improved efficacy and preservation of the neuromuscular unit. *Mol Ther* 2011;19:284-292.
7. Henriques A, Pitzer C, Dupuis L, Schneider A. G-CSF protects motoneurons against axotomy-induced apoptotic death in neonatal mice. *BMC Neurosci* 2010;11:25.
8. Tanaka M, Kikuchi H, Ishizu T, Minohara M, Osoegawa M, Motomura K, et al. Intrathecal upregulation of granulocyte colony stimulating factor and its neuroprotective actions on motor neurons in amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* 2006;65:816-825.
9. Zhang Y, Wang L, Fu Y, Song H, Zhao H, Deng M, et al. Preliminary investigation of effect of granulocyte colony stimulating factor on amyotrophic lateral sclerosis. *Amyotroph Lateral Scler* 2009;10:430-431.
10. Pitzer C, Krüger C, Plaas C, Kirsch F, Dittgen T, Müller R, et al. Granulocyte-colony stimulating factor improves outcome in a mouse model of amyotrophic lateral sclerosis. *Brain* 2008;131(Pt 12):3335-3347.
11. Chiò A, Mora G, La Bella V, Caponnetto C, Mancardi G, Sabatelli M, et al. Repeated courses of granulocyte colony-stimulating factor in amyotrophic lateral sclerosis: clinical and biological results from a

prospective multicenter study. *Muscle Nerve* 2011;43:189-195.

12. Duning T, Schiffbauer H, Warnecke T, Mohammadi S, Floel A, Kol-patzik K, et al. G-CSF prevents the progression of structural disintegration of white matter tracts in amyotrophic lateral sclerosis: a pilot trial. *PLoS One* 2011;6:e17770.

13. Nefussy B, Artamonov I, Deutsch V, Naparstek E, Nagler A, Drory VE. Recombinant human granulocyte-colony stimulating factor administration for treating amyotrophic lateral sclerosis: a pilot study. *Amyotroph Lateral Scler* 2010;11:187-193.

14. Franchignoni F, Mora G, Giordano A, Volanti P, Chiò A. Evidence of multidimensionality in the ALSFRS-R Scale: a critical appraisal on its measurement properties using Rasch analysis. *J Neurol Neurosurg Psychiatry* 2013;84:1340-1345.

15. Jenkinson C, Fitzpatrick R, Brennan C, Bromberg M, Swash M. Development and validation of a short measure of health status for individuals with amyotrophic lateral sclerosis/motor neurone disease: the ALSAQ-40. *J Neurol* 1999;246 Suppl 3:III16-III21.

16. Shamshiri H, Eshraghian MR, Ameli N, Nafissi S. Validation of the Persian version of the 40-item amyotrophic lateral sclerosis assessment questionnaire. *Iran J Neurol* 2013;12:102-105.

17. Great Lakes ALS Study Group. A comparison of muscle strength testing techniques in amyotrophic lateral sclerosis. *Neurology* 2003;61:1503-1507.

18. Wilms H, Sievers J, Dengler R, Bufler J, Deuschl G, Lucius R. Intrathecal synthesis of monocyte chemoattractant protein-1 (MCP-1) in amyotrophic lateral sclerosis: further evidence for microglial activation in neurodegeneration. *J Neuroimmunol* 2003;144:139-142.

19. Zhao LR, Navalitloha Y, Singhal S, Mehta J, Piao CS, Guo WP, et al. Hematopoietic growth factors pass through the blood-brain barrier in intact rats. *Exp Neurol* 2007;204:569-573.

20. Martino M, Callea I, Condemi A, Dattola A, Irrera G, Marcuccio D, et al. Predictive factors that affect the mobilization of CD34(+) cells in healthy donors treated with recombinant granulocyte colony-stimulating factor (G-CSF). *J Clin Apher* 2006;21:169-175.

21. Chiò A, Logroscino G, Hardiman O, Swingler R, Mitchell D, Beghi E, et al. Prognostic factors in ALS: A critical review. *Amyotroph Lateral Scler* 2009;10:310-323.

22. Yadav A, Saini V, Arora S. MCP-1: chemoattractant with a role beyond immunity: a review. *Clin Chim Acta* 2010;411:1570-1579.

23. Henkel JS, Engelhardt JI, Siklós L, Simpson EP, Kim SH, Pan T, et al. Presence of dendritic cells, MCP-1, and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. *Ann Neurol* 2004;55:221-235.

24. Cronin S, Hardiman O, Traynor BJ. Ethnic variation in the incidence of ALS: a systematic review. *Neurology* 2007;68:1002-1007.