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Inflammatory Markers and Frailty in Long-Term Care Residents

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Abstract

OBJECTIVES—Frail elders in long-term care (LTC) facilities are at increased risk for impaired mobility and falls. Little data are available on the relationship of pro-inflammatory cytokines to measures of frailty, function, mobility, and falls in this cohort. Our objective was to determine if pro-inflammatory biomarkers were associated with increased frailty as assessed by functional status, mobility, mental health, and falls over 24 months.

DESIGN—Secondary analysis of a 2-year double-blind clinical trial for osteoporosis.

SETTING—Nursing homes and assisted living facilities.

PARTICIPANTS—178 women age 65 with osteoporosis in LTC.

MEASUREMENTS—Baseline serum concentrations of pro-inflammatory cytokines and soluble receptors including high sensitivity CRP (HS-CRP), tumor necrosis factor alpha (TNF α) and its two receptors (TNF α -R1 and TNF α -R2), interleukin-6 (IL-6), soluble interleukin-6 receptor (sIL-6R), and interleukin-10 (IL-10), functional status as assessed by activities of daily living, the Nursing Home Physical Performance Test (PPT), gait speed, cognitive status, mental health and falls.

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RESULTS—At baseline, increased age was moderately associated with higher serum concentrations of HS-CRP ($r = 0.22$), TNF α -R1 ($r = 0.36$), TNF α -R2 ($r = 0.34$) and IL-10 ($r = 0.16$) (all $p < 0.05$). Frail participants had significantly higher levels of HS-CRP, TNF α -R1, TNF α -R2, IL-6 and IL-6-sR (all $p < 0.05$) than those non-frail. Higher baseline levels of HS-CRP and IL-6 were associated with worse physical performance and gait speed at 12 months independent of age, zoledronic acid use and comorbidity ($|r| = 0.25$ to 0.30 ; all $p < 0.05$). Inflammatory markers were not significantly associated with incident falls.

CONCLUSIONS—Higher pro-inflammatory biomarkers are associated with frailty, and decreased function and mobility in older women residing in LTC facilities.

Keywords

Frailty; long-term care; inflammation; cytokines; falls; gait speed; zoledronic acid

INTRODUCTION

Frailty is a prevalent geriatric syndrome that encompasses a number of varying phenotypic characteristics in old age, including muscle weakness, bone fragility, and increased fall risk, which result in increased vulnerability for functional decline and mortality.^{1–4} Frailty is characterized by multi-organ system dysfunction and maladaptive responses to stressors, leading to functional decline and other serious adverse health outcomes such as infection, delirium, and trauma, among others.^{5,6} Although there is not a single operational definition of frailty at the present time,⁷ several definitions have been proposed, most notably the frailty phenotype described by Fried¹ as well as the frailty index proposed by Rockwood.^{8–10} The Fried model uses five traits (weight loss, weakness, poor endurance/exhaustion, slowness, and low physical activity) to define an underlying physiologic state of multi-system decline, with three of these required for a positive frailty phenotype.¹ The Rockwood model looks at the proportion of accumulation of deficits such as symptoms, signs, functional impairments, and laboratory abnormalities, the composite of which reflects the severity of frailty-associated morbidity and mortality.^{8–10} Of note, it is important to be aware that much of the information about frailty in older adults has been gleaned through investigations of community-dwelling older adults taking part in large epidemiologic studies.^{11–14} The characterization of frailty in older adults residing in long-term care (LTC), as well as the applicability of these criteria for this special population, is even less well-defined.¹⁵

Another phenotypic characteristic of old age that has received attention in recent years is the sub-clinical chronic inflammatory state seen in older adults. Investigators have used the term “inflammaging,” defined by Franceschi et al. to be “a global reduction in the capability to cope with a variety of stressors and a concomitant progressive increase in the proinflammatory status,¹⁶” to describe this phenomenon. Several studies have demonstrated that both the innate and adaptive components of the immune system exhibit dysfunction in older adults considered frail by the previously discussed definitions.^{17–19} Circulating levels of pro-inflammatory markers such as interleukin-6 (IL-6) and C-reactive protein (CRP) are elevated in older adults^{20–22} and have been linked to a number of conditions, particularly cardiovascular disease,^{23,24} though relatively fewer studies have examined the effect of

elevated pro-inflammatory markers specifically on frailty. A recent meta-analysis reported that in cross-sectional studies, when compared to robust (i.e. non-frail) participants, frail and pre-frail participants had significantly high CRP and IL-6 levels, though the three longitudinal studies included in this meta-analysis did not bear out this association.²⁵ The studies included in this meta-analysis, among others which have also explored the relationship of pro-inflammatory markers and measures of frailty such as function, mobility, mental health, and falls^{20,21,26,27} primarily include community-dwelling older adults. There are fewer data regarding this relationship in older adults who reside in LTC facilities, who are often older and frailer than those who reside in the community at baseline.

The ZEST (Zoledronic acid in frail Elders to STrengthen bone) study demonstrated that over 2 years, a one-time infusion of zoledronic acid positively affected spine and hip bone mineral density (BMD) and bone turnover in frail female LTC residents.²⁸ Zoledronic acid has previously been shown to reduce the rate of new hip fractures and improve survival in patients who had undergone repair of a prior hip fracture.²⁹ Intravenous bisphosphonates such as zoledronic acid have been known to activate a transient acute phase response characterized by fever, myalgias, and other flu-like symptoms in the 24–36 hours following infusion, and can be associated with a transient fall in the number of circulating lymphocytes.³⁰ Other studies have reported a reduction in the number of gamma-delta T lymphocytes one year following exposure to zoledronic acid in patients with osteoporosis,³¹ though there is a dearth of additional literature regarding the potential effects of intravenous bisphosphonate exposure on other immune-regulatory markers. In the present study, we have tested the hypothesis that elevated serum levels of pro-inflammatory biomarkers were associated with increased frailty as assessed by functional status, mobility, mental health, and falls in the ZEST cohort. The aims of this investigation were (1) to determine if the baseline measurement of serum levels of pro-inflammatory markers would serve as a potential marker for frailty as assessed by functional status (ADLs, IADLs, PPT), mobility, mental health, and history of falls; and (2) to examine whether baseline levels of pro-inflammatory markers were associated with functional decline and incident falls over time.

METHODS

Study Design

This prospective secondary analysis reports the baseline levels of inflammatory cytokines and changes in measures of frailty over 24 months in 178 women with inflammatory biomarker data enrolled in a double-blind, placebo-controlled, randomized clinical trial.²⁸ Briefly, participants were randomly assigned to either active treatment with one-time infusion of zoledronic acid 5 mg or matching placebo as previously described.

Participants

Women age ≥ 65 residing in long-term care facilities in the greater Pittsburgh area were screened for this study and enrolled if they had low bone mineral density (BMD), defined as T-score less than or equal to −2.0 SD or previous adult fragility fracture.²⁸ The protocol was approved by the University of Pittsburgh Institutional Review Board and the Pennsylvania

Department of Health, and participants provided written informed consent before participation in the study.

Inflammatory Biomarkers

Serum samples were collected non-fasting in the morning, processed, frozen at -80°C , and run using the same assays to reduce inter-batch variability. We analyzed serum biomarkers known to play a role in the inflammatory response that have been previously examined to determine their potential association with incident fracture risk in large epidemiologic studies of community-dwelling adults.^{26,27} These included high sensitivity C-reactive protein (HS-CRP), tumor necrosis factor alpha (TNF α) and its receptors (TNF α -R1 and TNF α -R2), interleukin 6 (IL-6) and its soluble receptor (sIL-6R), and interleukin 10 (IL-10). Serum levels of cytokines and soluble cytokine receptors were measured in duplicate using enzyme-linked immunosorbent assay (ELISA) kits. The detectable limit for the HS-CRP ELISA (ALPCO Diagnostics, Salem, NH) was 1.9 ng/ml, for IL-6 (using Human IL-6 Ultra-Sensitive Kit, Meso Scale Discovery, Rockville, MD) was 0.7 pg/ml, for sIL-6R (using DR600 kit, R&D Systems, Inc., Minneapolis, MN) was 10 pg/ml, for TNF α (using Human TNF α Ultra-Sensitive Kit, Meso Scale Discovery) was 0.3 pg/ml, for TNF α -R1 (using DRT100 kit, R&D Systems, Inc.) was 3 pg/ml, for TNF α -R2 (using DRT200 kit, R&D Systems, Inc.) was 1 pg/ml, and for IL-10 (using Human IL-10 Ultra-Sensitive Kit, Meso Scale Discovery) was 23.4 pg/ml. Assays of blind duplicates collected for our participants yielded average inter-assay coefficients of variation of 2.22% for HS-CRP, 2.79% for IL-6, 3.54% for sIL-6R, 7.07% for TNF α , 8.95% for TNF α -R1, 5.93% for TNF α -R2, and 4.31% for IL-10.

Outcomes

Outcome measures of function, mobility, cognition, and mental health were used to describe a phenotypic picture of frailty in our cohort as previously described.^{28,32} To examine function and mobility, activities of daily living (ADLs, including eating, toileting, transferring, bathing, and dressing), instrumental activities of daily living (IADLs, including shopping, meal preparation, or heavy housework), gait speed, and Nursing Home Physical Performance Test (PPT)³³ were used. Cognitive status was assessed using the Short Portable Mental Status Questionnaire (SPMSQ),³⁴ and mental health was evaluated with the Patient Health Questionnaire (PHQ-9).³⁵ Additionally, we developed a modified version of the Fried frailty index¹ (Table 1) to categorize participants as non-frail (0 positive frailty criteria), pre-frail (1–2 positive frailty criteria), and frail (≥ 3 positive frailty criteria). Additional outcome variables of interest include the history of falls at baseline as determined by self-report (history) as well as in the Minimum Data Set (MDS), charts and/or medical records at the long-term care facilities (any or recurrent), as well as incident falls over the course of the study period. We assessed comorbidity using the Duke comorbidity index.³⁶

Statistical Analysis

Statistical analysis was performed using SAS[®] version 9.3 (SAS Institute, Cary, NC). We used appropriate descriptive statistics to summarize baseline participant characteristics by frailty categorization. Additionally, medians and interquartile ranges for skewed continuous variables measuring timed performances and inflammatory markers were used. Age,

treatment group (zoledronic acid/placebo) and comorbidity-adjusted partial correlation coefficients (r) were used to measure the association of serum inflammatory markers and each continuous variable representing outcomes at baseline and change over time. Means comparisons using analysis of variance (ANOVA) were used to determine whether serum inflammatory markers differed across frailty categories. Timed performance (sit-to-stand, walking) and inflammatory markers were log transformed prior to correlation analyses and ANOVA.

RESULTS

Baseline Characteristics and Associations

The mean age of participants at baseline was 85.6 ± 5.0 years (mean $\pm$ SD), 42% had experienced at least one fall at baseline (Table 2), and 63.5% reported a fracture as an adult.

Eleven participants were classified as non-frail, 53 as pre-frail, and 114 as frail using our modified version of the Fried Frailty Index (Table 2). Frail participants were older and had lower scores for ADLs, IADLs, PPT, and SPMSQ than those who were not frail. Walk time, sit-to-stand time, and gait speed were slower in frail patients. Additionally, frail participants had worse PHQ-9 scores and were more likely to have had prior falls (Table 2). Frail participants had significantly higher levels of HS-CRP, TNF α -R1, TNF α -R2, IL-6 and IL-6-sR than those non-frail, whereas those classified as pre-frail had significantly higher levels of TNF α -R1 and TNF α -R2 than those non-frail (all $p < 0.05$, Figure 1). Additionally, frail participants had significantly higher levels of HS-CRP and IL-6 compared to pre-frail participants (both $p < 0.05$).

At baseline, age was moderately associated with higher serum concentrations of HS-CRP, TNF α -R1, TNF α -R2 and IL-10 ($r = 0.16$ to 0.36 ; all $p < 0.05$). Participants who had experienced at least one fall had higher serum concentrations of TNF α -R2 ($p = 0.0478$), but the difference was slightly attenuated after controlling for age and comorbidity ($p = 0.0688$).

Associations of Baseline Inflammatory Markers and Future Function Over Time

Higher HS-CRP and IL-6 levels at baseline were associated with worse activities of daily living, physical performance, walk time, and gait speed at 12 months independent of age, zoledronic acid use and baseline comorbidity ($|r| = 0.19$ to 0.32 ; all $p < 0.05$). Higher IL-6 was associated with physical performance, walk time and gait speed at 12 months ($|r| = 0.23$ to 0.29 ; all $p < 0.05$). But these associations generally did not persist at 24 months, with the exception of instrumental activities of daily living and IL-6 ($r = -0.30$; $p < 0.05$). The strength of associations was in the mild to moderate range, as indicated by the magnitudes of correlations (Table 3). Controlling for age, comorbid burden and zoledronic acid use, inflammatory markers did not significantly differ between incident fallers and non-fallers, but a suggestive difference was seen in IL-6 (median [interquartile range] = 2.4 [1.6 – 4.8] vs 2.0 [1.2 – 3.2]; $p = 0.0995$).

In terms of baseline to follow-up changes in functional status, higher baseline levels of the anti-inflammatory cytokine IL-10 were moderately associated with declines in IADL scores over 12 months ($r = -0.20$, $p < 0.05$) and TNF α with declines in walking performance ($|r|$

=0.22–24; $p<0.05$), independent of age, comorbid burden, and zoledronic acid use. TNF α -R2 was associated with decline in IADL over 24 months ($r=-0.22$; $p<0.05$).

DISCUSSION

This prospective secondary analysis of older female adults in LTC facilities suggested that women with higher pro-inflammatory cytokine levels including HS-CRP and IL-6 at baseline had poorer baseline as well as 12- and 24-month functional measures, as well as greater declines in these functional measures over time, independent of baseline comorbidity. However, a history of falls at baseline and incident falls were not associated with higher pro-inflammatory cytokine levels after adjustment for comorbid conditions.

Although many studies have reported the negative effects of so-called “inflammaging”^{16,24,37,38}, few data are available on the impact of subclinical chronic inflammation seen in the frailest older adults on their global functional state, which we consider to be inclusive of not only activities of daily living, but also mobility, gait, number of falls, mood, and cognitive function. In an analysis of the Health, Aging, and Body Composition (Health ABC) study consisting of a large cohort of community-dwelling older adults, Penninx et al. found that participants who developed incident mobility limitation during the study period had significantly higher serum levels of CRP, IL-6, and TNF α at baseline (all $p<0.001$).³⁹ Furthermore, even after adjustment for incident severe illness and other comorbidities, participants with one or two elevated levels of inflammatory markers showed greater risk of developing incident mobility limitation, with risk ratios 1.32 (for one) and 1.37 (for two) than those with no high levels. For participants with high serum levels of all three markers (i.e. CRP, IL-6, and TNF α), the relative risk of developing incident mobility limitation was 1.84.³⁹ Our study is unique in that we examined the effect of elevated inflammatory markers at baseline on multiple measures of function in women residing in long-term care who were known to be frail at baseline with a number of functional limitations.

In the Study of Osteoporotic Fractures, which included older community-dwelling women and utilized similar inflammatory markers, women with a high inflammatory burden (those in the highest quartile) had a greater risk of hip fracture over approximately 6 years.⁴⁰ In the Women’s Health Initiative, from a nested case-control study, elevated levels of TNF α -R1, TNF α -R2, and IL-6-sR were associated with an increased risk of hip fracture in a median follow-up of 7 years.²⁷ Although our study was smaller and shorter in duration compared to these studies, the cytokine trends we found with higher levels of the TNF- α receptors as well as the soluble IL-6 receptor being associated with frailty and worsening function support potential explanation for increased risk of hip fractures in these other cohorts.

There are some general limitations to this study. Due to the fact that the study population consisted of frail elders residing in long-term care facilities, selected outcomes were limited to assessments that could be performed at each individual facility. In regards to our assessment of frailty using a modified Fried frailty index, there may have been implicit bias introduced into our results based on these modifications, as explored previously in a systematic review by Theou et al.⁴¹ Although the associations between higher levels of pro-

inflammatory cytokines and markers of frailty were adjusted for comorbidity, we did not distinguish between participants who had a diagnosis characterized by chronic inflammation (e.g. rheumatoid arthritis, systemic lupus erythematosus, etc.) and/or participants who have taken anti-inflammatory agents such as TNF α inhibitors. Additionally, we do not have data regarding inflammatory biomarkers at follow-up time points in these patients, which precludes us from commenting on the potential effect of zoledronic acid exposure on inflammatory biomarkers over time. For both of these reasons, our ability to focus on clinically significant outcomes related to the sub-clinical chronic inflammation seen in older adults may have decreased. It is worthwhile to note, however, that after excluding the 18 (10%) study participants who were on systemic glucocorticoids at baseline, we did not find significant differences in our results. Moreover, because ours was an ancillary and exploratory analysis mainly to generate hypotheses rather than draw definitive conclusions, we did not consider strict multiplicity corrections for obtaining statistical significance. Additionally, a longer study would be better able to examine effects of inflammation of markers of frailty over time, which may be valuable given the chronicity of sub-clinical inflammation seen in frail older adults. Another limitation is that men were not included in this study, making our results less generalizable to the population of older adults as a whole. Finally, this was a secondary analysis of a clinical trial that examined the impact of zoledronic acid on skeletal integrity rather than one specifically designed prospectively for the present investigation.

Despite these limitations, there are several important strengths to this study. For instance, the Fried frailty criteria do not include other domains such as declines in cognition, mood disorders, and poor function, all of which are common findings in long-term care residents. Our inclusion of these additional domains in the current analysis allows for a fuller characterization of a frailty phenotype while still remaining clinically feasible and relevant. Additionally, the assay methods used to evaluate levels of pro-inflammatory cytokines are state of the art. In particular, we measured soluble receptor levels of some of these cytokines, which is an important distinction as previous studies have shown that elevations of soluble receptor levels for these cytokines are more constant and may be more representative of chronic and/or severe inflammation.^{42–44} Some of these soluble cytokine receptors (e.g. TNF α -R1) have been shown in large cohort studies to be associated with morbidity, independently of their corresponding ligands.⁴⁵ Finally, while large epidemiologic studies have examined inflammatory biomarkers in community-dwelling older adults and had excluded frail older adults in long-term care, we were able to examine these associations by performing the study on site in the long-term facilities.

In summary, higher pro-inflammatory biomarkers were associated with decline in function and mobility independent of baseline comorbidity in frail female long-term care residents. Additional confirmatory studies are needed in broader target populations that also include men.

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Elements of Financial/ Personal Conflicts	*Author 1 GA Langmann		Author 2 KS Perera		Author 3 MA Ferchak		Author 4 DA Nace		Author 5 NM Resnick		Author 6 SL Greenspan	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Employment or Affiliation		X		X		X		X		X		X
Grants/Funds		X		X		X		X		X		X
Honoraria		X		X		X		X		X		X
Speaker Forum		X		X		X		X		X		X
Consultant		X		X		X		X		X		X
Stocks		X		X		X		X		X		X
Royalties		X		X		X		X		X		X
Expert Testimony		X		X		X		X		X		X
Board Member		X		X		X		X		X		X
Patents		X		X		X		X		X		X
Personal Relationship		X		X		X		X		X		X

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Figure 1.

Baseline log-transformed inflammatory markers (mean $\pm$ SD) for (A) HS-CRP (high sensitivity C-reactive protein), (B) IL-6-sR (interleukin-6 soluble receptor), (C) tumor necrosis factor alpha receptor 1 (TNFα-R1) and (D) tumor necrosis factor alpha receptor 2 (TNFα-R2) by frailty category

^ap<0.05 for frail vs. non-frail

^bp<0.05 for pre-frail vs. non-frail

^cp<0.05 for frail vs. pre-frail

Table 1Modified Fried Frailty Index¹

Fried Frailty Domain	ZEST Frailty Domain and Characterization
Shrinking/weight loss	Weight loss from baseline to 6 months. If ≥ 5 pounds, receive 1 point for frailty
Weakness (grip strength)	Quad strength assessed by sit-to-stand time from Physical Performance Test (PPT). If ≥ 2 seconds, receive 1 point for frailty
Poor endurance/exhaustion	PHQ-9 question: "Are you feeling tired or have little energy?" If score ≥ 1, receive 1 point for frailty
Low activity	IADL question #2: "Can you get to places out of walking range?" If help is needed, receive 1 point for frailty
Slowness	Measured time 6 meter walk. If ≥ 0.8 m/s, receive 1 point for frailty

Score: Not frail (Fried category of robust) = 0; pre-frail = 1–2; frail = ≥ 3 of a total of 5.

PPT: Nursing Home Physical Performance Test

SPMSQ: Short Portable Mental Status Questionnaire

PHQ-9: Patient Health Questionnaire

Table 2

Baseline clinical characteristics and cytokines (mean±standard deviation, median [interquartile range] or N (%))

	Total	Non-frail	Pre-frail	Frail
N	178	11	53	114
Age *	85.6±5.0	81.6±7.2	84.9±4.9	86.3±4.7
BMI	27.6±5.3	26.0±2.4	27.5±5.6	27.8±5.4
ADL (0–14) * [†]	11.7±2.4	13.5±0.9	13.3±1.0	10.7±2.4
IADL (0–14) * [†]	8.0±3.9	12.5±1.2	10.5±3.4	6.4±3.3
PPT(0–24) * [†]	19.7±4.4	23.8±0.4	22.1±1.9	18.2±4.7
Walk time (s) * ^L	14.7±14.2 11.6 [8.6–17.1]	6.3±0.9 6.5 [5.7–6.8]	10.9±4.8 9.6 [7.7–11.8]	17.7±17.4 14.2 [10.1–18.6]
Sit-to-stand time (s) * ^L	3.2±3.1 2.2 [1.5–3.8]	1.3±0.3 1.2 [1.0–1.6]	1.6±1.1 1.5 [1.1–1.8]	4.2±3.6 3.2 [2.3–5.3]
Gait speed (m/s) * [†]	0.55±0.24	0.97±0.15	0.63±0.22	0.45±0.17
SPMSQ score (# correct) * [†]	8.1±2.4	9.5±1.2	8.8±1.7	7.7±2.6
PHQ-9 score (0–27) *	3.8±4.3	0.9±1.4	2.2±2.9	4.9±4.7
Previous fall(s) *	75 (42%)	2 (18%)	17 (32%)	56 (50%)

* $p < 0.05$ across frailty categories

^L log transformed for obtaining p-value

[†] indicates a higher value is better

indicates a lower value is better

ADL: activities of daily living

IADL: instrumental activities of daily living

PPT: Nursing Home Physical Performance Test

SPMSQ: Short Portable Mental Status Questionnaire

PHQ-9: Patient Health Questionnaire

Table 3

Associations of baseline cytokines and absolute values of 12-month or 24-month function tests independent of treatment group, age and baseline comorbidity

	HS-CRP (ng/ml) ^L		TNFa-R1 (pg/ml) ^L		TNFa-R2 (pg/ml) ^L		IL-10 (pg/ml) ^L		IL-6 (pg/ml) ^L		IL-6R (pg/ml) ^L	
	M12	M24	M12	M24	M12	M24	M12	M24	M12	M24	M12	M24
ADL (score)	-0.19 [*]	-0.1	-0.12	-0.19	-0.05	-0.17	0.09	-0.10	-0.18	-0.09	0.06	0.11
IADL (score)	-0.14	-0.05	-0.07	-0.15	-0.05	-0.2	-0.01	-0.05	-0.27 [*]	-0.30 [*]	0.02	-0.03
PPT (score)	-0.25 [*]	-0.06	-0.11	-0.11	-0.07	-0.07	-0.03	0.10	-0.29 [*]	-0.16	-0.08	0.03
Walk time (6m) ^L	0.32 [*]	0.16	0.11	0.19	0.09	0.17	-0.06	-0.01	0.23 [*]	0.17	0.06	0.11
Sit to stand (s) ^L	0.16	0.2	0.05	0.13	-0.05	0.09	-0.03	-0.10	0.22 [*]	0.07	-0.07	-0.05
SPMSQ (correct)	0.01	0.14	-0.07	-0.1	-0.03	-0.07	0.06	0.08	-0.04	-0.09	-0.09	-0.1
PHQ-9 (score)	0.05	0.02	-0.09	-0.08	-0.09	0.03	-0.07	-0.18	0.04	-0.07	-0.09	-0.22 [*]
Gait speed (m/s)	-0.30 [*]	-0.08	-0.16	-0.08	-0.15	-0.06	0.04	-0.03	-0.27 [*]	-0.09	-0.12	-0.08

^LLog transformed

^{*} p<0.05

M12=after 12 months (N=125)

M24=after 24 months (N=100)

ADL: activities of daily living

IADL: instrumental activities of daily living

PPT: Nursing Home Physical Performance Test

SPMSQ: Short Portable Mental Status Questionnaire

PHQ-9: Patient Health Questionnaire