selective neuronal NOS (nNOS) inhibitor, were applied to examine the involvement of NOS in this process. L-NAME abrogated the effects of DHT on cognitive function (Figure 3F). In contrast, L-VNIO did not change the effect of DHT (Figure 3G). These results suggest that eNOS/SIRT1 in endothelial cells may play an important role in the protective effect of testosterone against senescence of the hippocampus.

## SIRT1 plays an important role in the protective effect of testosterone against endothelial senescence

Following the animal experiments, we examined whether testosterone inhibited endothelial senescence *in vitro* using cultured cells. We induced premature endothelial senescence by addition of $H_2O_2$ 100 μmol/L for 1 hour. DHT or testosterone treatment inhibited SA-βgal activity and the morphological appearance of senescence (Figure 4A). We observed that oxidative stress decreased eNOS and SIRT1 and increased PAI-1 expression, and DHT or testosterone treatment prevented these changes and increased the phosphorylation of eNOS at Ser1177 (Figure 4B). Overexpression of SIRT1 significantly inhibited oxidative stress-induced senescence, and DHT accelerated the effect of SIRT1 through phosphorylation of eNOS at Ser1177 (Figure 4C). To determine the role of endogenous SIRT1, DHT-treated endothelial cells were transfected with SIRT1 siRNA or treated with sirtinol, a chemical inhibitor of SIRT1. SIRT1 siRNA or sirtinol abrogated the effect of DHT on SA-βgal activity (Figure 4D). We previously reported that testosterone activated eNOS [18], and eNOS activation promoted SIRT1 expression [21]. Accordingly, we examined the role of eNOS in the protective effect of testosterone. We observed that DHT or testosterone treatment increased NOS activity that was reduced by oxidative stress (Figure 4E). Treatment with eNOS siRNA or L-NAME decreased the inhibitory effect of DHT on a senescent phenotype in parallel with SIRT1 expression (Figure 4F and G). These results indicate that eNOS/SIRT1 play an important role in the protective effect of testosterone and DHT against a senescent phenotype.

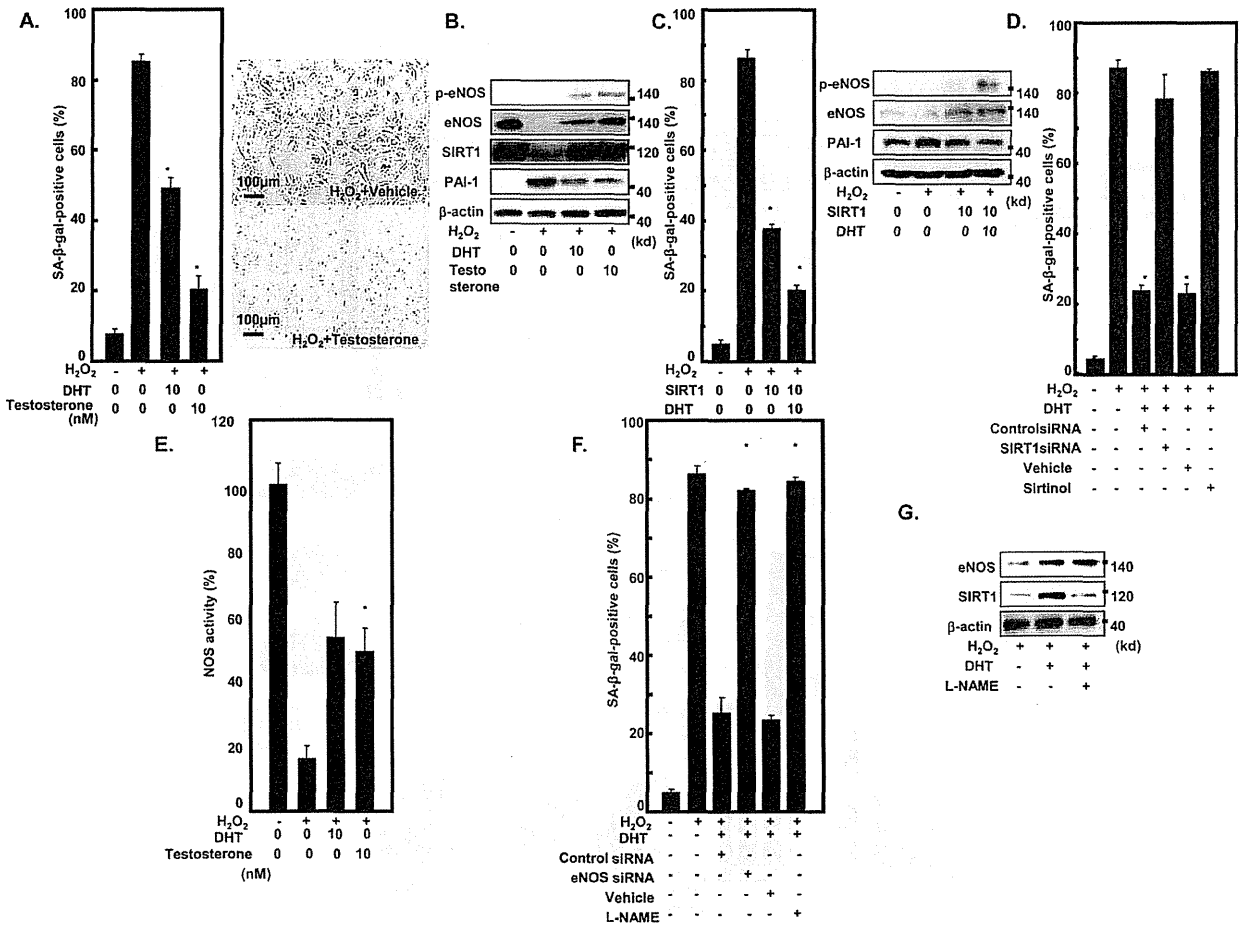


**Figure 4. Testosterone inhibits oxidative stress-induced endothelial senescence through eNOS/SIRT1. A.** Testosterone inhibited SA-βgal activity and senescent morphological appearance induced by hydrogen peroxide (100 μmol/L). **B.** Expression of eNOS, SIRT1, and PAI-1 in hydrogen peroxide (100 μmol/L)-treated HUVEC under treatment with DHT or testosterone. **C.** Overexpression of SIRT1 and DHT reduced SA-βgal activity. eNOS expression was increased by overexpression of SIRT1, and DHT increased phosphorylation of eNOS (Ser1177). **D.** SIRT1 inhibition by siRNA or sirtinol (100 μmol/L) abrogated the effect of testosterone on SA-βgal activity. **E.** Treatment with testosterone or DHT increased eNOS activity. **F.** eNOS inhibition by siRNA or L-NAME (10 mM) abrogated the effect of testosterone on SA-βgal activity. **G.** Treatment with L-NAME decreased SIRT1 expression in DHT-treated HUVEC. (*$p<0.05$, $N=3$).
doi:10.1371/journal.pone.0029598.g004

## Senescent endothelial cells induced by oxidative stress promoted neuronal senescence

Finally, we hypothesized that endothelial senescence promotes senescence of adjacent neuronal cells. To test this hypothesis, we used a co-culture system of endothelial cells (HUVEC) with neuronal cells (mouse hippocampal neuronal cells; MHC) (Figure 5A). Both cells were co-cultured, but were separated by a microporous polycarbonate membrane, for 10 days after endothelial cells were treated with hydrogen peroxide, and the senescent phenotype of MHC was analyzed. We found that the number of SA-βgal-positive cells and the senescent appearance of MHC were increased, and the concentration of acetylcholine in cells was decreased by co-culture with senescent endothelial cells (Figure 5B). In parallel with this, MHC showed increased PAI-1 and p53, and decreased SIRT1 expression (Figure 5C). We also found that senescent endothelial cells showed increased expression of inflammatory cytokines such as IL-6, IL-8, MCP-1, and TNF-α (Figure 5D). Both MHC and HUVEC, or HUVEC alone were treated with testosterone at 3 days before HUVEC were treated with hydrogen peroxide, and both cells were co-cultured for 10 days, and the senescent phenotype of MHC was analyzed. We found that the number of SA-βgal-positive MHC was decreased by treatment of HUVEC with testosterone irrespective of the treatment of MHC with testosterone (Figure 5E). In addition, we found that a SIRT1 activator, resveratrol treatment rescued the senescent phenotype of MHC (Figure 5F). These results suggest that senescent endothelial cells exhibit a senescence-associated secretory phenotype [22], induce neuronal senescence, and testosterone rescues it through up-regulation of SIRT1 (Figure 5G).

## Discussion

Testosterone level and cognitive function show a decline with age in men. A series of evidence suggests that this association is not just age related [23]. Results from cell culture and animal studies provide evidence that testosterone could have protective effects on brain function, especially in the hippocampus [24]. Here, we demonstrated that administration of testosterone restored cognitive function in male SAMP8 in association with improvement of the senescent phenotype in the hippocampus and cerebral vessels.

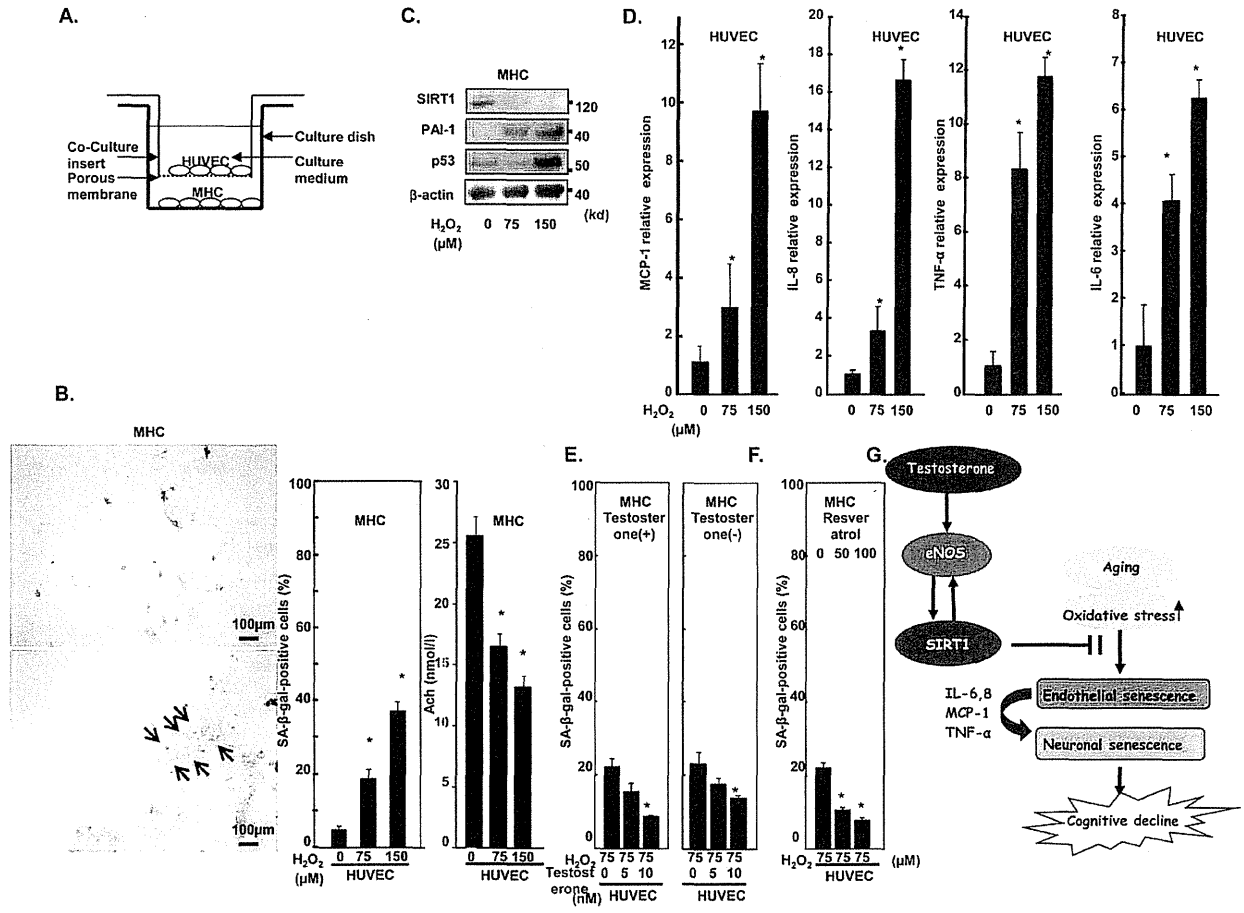


**Figure 5. Oxidative stressed-induced endothelial cell senescence promotes adjacent neuronal cell senescence. A.** Co-culture cell culture dish. **B.** Number of SA-βgal-stained MHC and senescent appearance of MHC were increased, and acetyl-choline concentration was decreased by co-culture with senescent endothelial cells. Senescent MHC are indicated by arrows. **C.** Expression of SIRT1, PAI-1, p53, and β-actin in MHC co-cultured with senescent endothelial cells. **D.** Expression of IL-6, IL-8, MCP-1, and TNF-α in endothelial cells were analyzed by RT-PCR. **E.** The number of SA-βgal-stained MHC was decreased by treatment with testosterone in both MHC and HUVEC (MHC, testosterone (+)), or HUVEC (MHC, testosterone (−)) alone. **F.** Resveratrol decreased the number of SA-βgal-stained MHC co-cultured with senescent endothelial cells. (*$p<0.05$, $N=3$). **G.** Hypothetical signal transduction pathways of testosterone in endothelial cells.
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We also showed that testosterone ameliorated endothelial senescence through eNOS/SIRT1-dependent mechanisms *in vitro*. The present study demonstrated that testosterone and SIRT1 interacts with each other and inhibited the senescence of hippocampal vascular and neuronal cells, suggesting that testosterone replacement therapy is a treatment option for cognitive decline with aging.

Testosterone may act in part through aromatase-dependent conversion to estradiol. To estimate a direct effect of androgens through AR, testosterone and DHT were used in this study. Both compounds showed significant protective effects on cognitive function.

In the present study, we used SAMP8 mice. SAMP is comprised of 14 strains derived from selective inbreeding of the AKR/J strain. SAMP8 exhibits age-related learning and memory deficits, as well as amyloid-like deposits in the brain [25]. Increased expression of hyperphosphorylated tau has also been detected in SAMP8 [26]. Given such features, SAMP8 has been proposed as a plausible age-associated AD animal model, and a suitable rodent model for studying the molecular mechanism underlying cognitive impairment [27]. A previous study has shown an age-related decrease in serum testosterone in SAMP8, and suggesting that impaired cognitive function in SAMP8 is due to reduced testosterone [28]. We observed that AR expression was abundant in the hippocampus of SAMR1 and SAMP8. Several studies have demonstrated that testosterone has a neuroprotective effect through AR in the hippocampus [29,30], and testosterone induced NO productions via AR-dependent activation of eNOS in endothelial cells [18,19].

Accumulating evidence suggests that $NAD^+$-dependent deacetylase SIRT1 play an essential role for cellular senescence and cognitive function. SIRT1 modulates endothelial cellular senescence [13], and overexpression of SIRT1 exhibits neuroprotective effects in hippocampus, and cognitive function of *Sirt1*-KO mice is markedly impaired [10,31,32].

The precise etiologic mechanism of the cognitive decline with aging is unclear, but it has been identified that cardiovascular risk factors are associated with a higher incidence of cognitive impairment [33]. In addition, age-associated vascular inflammation is an early manifestation of chronic stress responses, i.e. overloading of ROS on endothelial cells [34]. Indeed, SAMP8 showed enhancement of oxidative stress and a senescent phenotype in the hippocampus. Notably, senescent endothelial cells were increased in the hippocampus of SAMP8 accompanied by a reduction of SIRT1, and L-NAME abrogated the effect of DHT on cognitive function. Therefore, we hypothesized that testosterone influenced cerebral endothelial senescence via eNOS/SIRT1, and that pro-inflammatory cytokines, which were derived from senescent endothelial cells, promoted senescence in adjacent neuronal cells. Indeed, we observed that testosterone induced eNOS activity, and subsequently increased SIRT1 expression in endothelial cells. Inhibition of eNOS/SIRT1 abrogated the effect of testosterone on endothelial senescence. In a co-culture system, we found that senescent endothelial cells promoted senescence of adjacent neuronal cells, and treatment of endothelial cells with testosterone inhibited senescence of adjacent neuronal cells. It can reasonably be speculated, therefore, that SIRT1 may exert salutary actions against cognitive decline with aging by preventing a senescence-associated secretory phenotype of endothelial cells. Because L-NAME is a non-selective inhibitor of NOS, it is possible that the effect of L-NAME might be in part a result of inhibition of nNOS in concert with eNOS. However, a specific nNOS inhibitor, L-VNIO did not change the effect of DHT in SAMP8. In co-culture experiments, we found that treatment with resveratrol or testosterone did not change the expression or activation of nNOS in MHC (Figure S1C and D). Further studies are needed to address the differential role of eNOS and nNOS, and the exact role of SIRT1 *in vivo*.

In conclusion, supplementation of testosterone prevented cognitive impairment of SAMP8, in which testosterone secretion was decreased in association with the senescence of testis Leydig cells, through an eNOS/SIRT1-dependent mechanism. Unprecedented reversal of the senescent hippocampal changes and vascular protection may justify exploration of a neuronal rejuvenation strategy by utilizing testosterone for the prevention of cognitive decline with aging, particularly through up-regulation of eNOS/SIRT1.

## Methods

### Materials

Dihydrotestosterone (DHT), testosterone, and $N^G$-nitro-L-arginine methyl ester hydrochloride (L-NAME) were purchased from Sigma (St. Louis, MO). Hydrogen peroxide ($H_2O_2$) and resveratrol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Testosterone and placebo pellets were purchased from Innovative Research of America (Sarasota, FL). $N^5$-(1-Imino-3-butenyl)-L-ornithine (L-VNIO) was purchased from Enzo Life Sciences (Plymouth Meeting, PA).

### Cell culture

Human umbilical vein endothelial cells (HUVEC) were purchased from CAMBREX (Walkersville, MD). Population doubling levels (PDL) were calculated as described previously [35], and all experiments were performed at PDL of 10–11. In our preliminary experiments, HUVEC were cultured in EBM without phenol red (Clonetics, Walkersville,MD) with 10% dextran-charcoal-stripped serum to remove steroids from the culture medium. This condition, however, induced marked growth arrest and an increase in senescent cells. Consequently, we performed all experiments in EBM-2 (Clonetics) with 10% complete serum-supplemented medium.

### Animal experiments

The animal experiments were approved by our institutional review board (animal experiments ethics board, Graduate School of Medicine and Faculty of medicine, The university of Tokyo (approval ID: M-P-09-056)). Senescence-accelerated mice prone (SAMP) 8 and control senescence-accelerated mice resistant (SAMR) 1 male mice were all housed and maintained in a room at 22±2°C with automatic light cycles (12 h light/dark) and relative humidity of 40–60%. Mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). Food and tap water were provided ad libitum throughout the study. In the water maze test of this study, a group of male SAMR1 (N = 10) and SAMP8 (N = 10) was first tested. Male mice of 12 weeks of age were treated daily for 2 weeks with DHT (500 μg in 0.05 ml/mouse) by subcutaneous injection (s.c.) in the neck before the water maze test. Male mice of 18 months of age underwent subcutaneously implantation of a placebo (N = 5) or a 21-day-release 2.5 mg testosterone (N = 5) pellet into the dorsal neck region. L-NAME was given by gavage once a day (20 mg/kg) [36]. L-VNIO was given by intraperitoneal injection (0.5 mg/kg) [37]. Small fragments of testis tissue fragments from SAMR1 were grafted under the back skin of castrated male SAMP8 as previously described [38]. Briefly, after removal of the capsule and obvious connective tissue, donor testes were cut into small fragments. Testis fragments were kept in Dulbecco's modified Eagle's medium

(Gibco Lab Inc., Grand Island, NY, USA) on ice until grafting. SAMR1 were anesthetized and castrated, and testicular tissue fragments were grafted under the back skin of SAMP8. Mice were anesthetized with enflurane, killed by cervical dislocation, and trunk blood collected within 1 min. The blood was centrifuged and plasma testosterone was measured by radioimmunoassay method. The brain was removed for histological examination, after systemic perfusion with phosphate-buffered saline (PBS). For immunohistochemical studies, mouse brains were processed and labeled with anti-amyloid-β antibody (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) to visualize extracellular amyloid plaques, anti-NeuN antibody (Millipore, Billerica, MA) to assess pyramidal cell number, or DAPI (Dojindo Molecular Technologies, Inc., Tokyo, Japan) for nuclear staining. The primary antibody was purified rat anti-mouse CD31 (platelet endothelial cell adhesion molecule; PECAM-1) monoclonal antibody from Pharmingen (San Jose, CA, USA). Secondary antibodies (Alexa Fluor 488 donkey anti-rat IgG and Alexa Fluor 594 donkey anti-rat IgG) and antifade reagent were from Molecular Probes (Invitrogen). Fluorescent images were analyzed using a fluorescence microscope (BZ-9000, KEYENCE, Osaka, Japan).

## Plasmids and siRNA transfection

Proliferating cells were washed three times with growth medium and exposed to the indicated concentrations of testosterone or DHT diluted in medium. pIRES-SIRT1 plasmid was provided by Dr. M. Takata [39], and Dr. R.A. Weinberg [40]. Each plasmid was overexpressed by transfection using Lipofectamine LTX and PLUS reagents (Invitrogen) for HUVEC according to the manufacturer's instructions. Proliferating cells were transfected with each siRNA using silMPORTER (Upstate Cell Signaling Solutions). siRNAs for SIRT1 (GAT GAA GTT GAC CTC CTC A [41] and TGA AGT GCC TCA GAT ATT A), and eNOS were purchased from Santa Cruz Biotechnology, Inc.

## Immunoblotting and immunoprecipitation

Cells were lysed on ice for 1 hour in buffer (50 mmol/L Tris-HCl, pH 7.6, 150 mmol/L NaCl, 1% NP-40, 0.1% SDS, 1 mmol/L dithiothreitol, 1 mmol/L sodium vanadate, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 10 μg/mL leupeptin and 10 mmol/L sodium fluoride). Equal amounts of protein were separated by SDS/PAGE gel electrophoresis and transferred to nitrocellulose membranes. After blocking, the filters were incubated with the following antibodies; anti-SIRT1, anti-nNOS, anti-AR (Cell Signaling, Danvers, MA), anti-eNOS (BD Transduction Laboratories, San Jose, CA), anti-PAI-1 (Molecular Innovations, Southfield, MI), anti-PECAM-1 (Santa-Cruz Biotechnology, CA), and anti-ß-actin (Sigma). After washing and incubation with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG (Amersham, Piscataway, NJ) for 1 hour, antigen-antibody complexes were visualized by using an enhanced chemiluminescence system (Amersham).

## Senescence-associated ß-galactosidase (SA-ßgal) staining

HUVEC were pretreated with diluted EGM-2 medium for 3 day. HUVEC were then washed three times with EGM-2 and treated for 1 hour with 100 μmol/l $H_2O_2$ diluted in EGM-2. After treatment, HUVEC were trypsinized, re-seeded at a density of $1\times10^5$ in 60-mm dishes, and cultured with EGM-2 containing DHT or testosterone for 10 days. The proportion of SA-ßgal-positive cells was determined as described by Dimri et al [42].

## NOS activity assay

NOS activity was determined using an NOS assay kit (Calbiochem) according to the manufacturer's instructions.

## Measurement of acetylcholine

The concentration of acetylcholine was measured with a choline/acetylcholine quantification kit (BioVision, CA, USA) according to the manufacturer's instructions.

## Real-time quantitative reverse transcription PCR

Total RNA was isolated with ISOGEN (Nippon Gene Inc., Toyama, Japan). After treatment with Rnase-free Dnase for 30 min, total RNA (50 ng/μl) was reverse transcribed with random hexamers and oligo d(T) primers. The expression levels of SIRT1, IL-6, IL-8, MCP-1, and TNF-α relative to ß-actin were determined by means of staining with SYBR green dye and a LineGene fluorescent quantitative detection system (Bioflux Co., Tokyo, Japan). The following primers were used: SIRT1 F 5′-CCTGACTTCAGGTCAAGGGATGGTA-3′, R 5′-CTGATTAAAAATATCTCCTCGTACAG-3′; ß-actin F 5′-TGGGCATGGGTCAGAAGGAT-3′, R 5′-AAGCATTTGCGGTGGACCAT-3′; IL-6 F 5′-GGGAAGGTGAAGGTCGG-3′, R 5′-TGGACTCCACGACGTACTCAG-3′, IL-8 F 5′-CTGGCCGTGGCTCTCTTG-3′, R 5′-CCTTGGCAAAACTGCACCTTT-3′; TNF-α F 5′-GTAGCCCACGTCGTAGCAAAC-3′, R 5′-CTGGCACCACTAGTTGGTTGTC-3′; MCP-1 F 5′-CATTGTGGCCAAGGAGATCTG-3′, R 5′-CTTCGGAGTTTGGGTTTGCTT-3′.

## Co-culture system

For these experiments, co-culture dishes were used as outlined in Figure 5A. They were obtained from BD Biosciences (Erembodegem, Belgium) with a 6-well format. HUVEC were treated with $H_2O_2$ (100 μM) for 1 h and cultured on the permeable microporous (0.4 μm) membrane in the insert, and mouse hippocampus neuronal cells on the base of the culture dish, kept physically separated but allowing the passage of micromolecules through the porous membrane for 10 days. Mouse hippocampus neuronal cells were purchased from DS Pharma Biomedical Inc. (Osaka, Japan).

## Quantitative analysis of amyloid β

Measurement of amyloid β was performed using an amyloid β (1–40) (FL) assay kit (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) according to the manufacturer's instructions.

## Morris water maze test

The procedure of the Morris water maze test was described previously [43]. SAMR1 and SAMP8 mice were trained to find a visible platform with three trials on the first day, and then tested to find the hidden platform for 10 consecutive days. In each trial, the mice were allowed to swim until they found the hidden platform, or until 2 min had passed, and the mouse was then guided to the platform. On the test days, the platform was hidden 1 cm beneath the water. The escape latency was recorded by a video camera. The swim speed of each mouse was calculated by means of a video tracking system. Probe tests were performed on the $10^{th}$ day. During percent time quadrant test, the platform was removed from the pool. Mice were started in a position opposite the location of the platform position and allowed to swim for 60 seconds.

### Open field test

The open field test fear response to novel stimuli was used to assess locomotion, exploratory behavior, and anxiety. Open field test protocols were modified from that of Lukacs et al [44]. The open field test consisted of a wooden box (60×60×60 cm) and was indirectly illuminated by two fluorescent lights. A 10 cm area near the surrounding wall was delimitated and considered the periphery. The rest of the open field was considered the central area. The distance travelled, the ratio of the distance travelled in the central area/total distance travelled, and the time in the center of the open- field were analyzed as a measure of anxiety-like behavior. During the test, mice were allowed to move freely around the open field and to explore the environment for 15 min.

### Isolation of cerebral microvessels

Cerebral microvessels were isolated from the remaining brain tissue as previously described by Zhang et al [45] with minor modifications. Brain tissue, devoid of large vessels, was homogenized in ice cold PBS with Dounce homogenizer and centrifuged twice at 2000 g at 4°C. The supernatant, containing the parenchymal tissue, was discarded. The pellet was resuspended in PBS and centrifuged as described above. The resulting pellet was resuspended and layered over 15% Dextran (in PBS) (Sigma, St.Louis, MO) and centrifuged at 4500 g for 30 minutes at 4°C. The top layer was aspirated and discarded and the remaining pellet resuspended in 15% Dextran and centrifuged. The final pellet was resuspended in 1% bovine serum albumin (BSA), the suspension was then passed though a 40-µm nylon mesh (BD Falcon). Microvessels retained on the mesh were washed with BSA/PBS and collected by centrifugation at 900 g for 10 minutes at 4°C.

### Data analysis

Values are shown as mean ± S.E.M in the text and figures. Differences between the groups were analyzed using one-way analysis of variance, followed by Bonferroni test. Probability values less than 0.05 were considered significant.

## Supporting Information

**Figure S1 Testes of SAMP8 and SAMR1 mice and role of nNOS in neuronal senescence. A.** Testis weight of SAMR1 and SAMP8 with or without testosterone. **B.** Photographs of SAMR1 donor and SAMP8 recipient mice. White arrows indicate operation scar. **C.** Expression of nNOS in MHC treated with resveratrol or testosterone under the oxidative stress. **D.** Activity of nNOS in MHC treated with resveratrol or testosterone under the oxidative stress. ($^{*}p<0.05$, N = 3, *n.s:* not significant).
(TIF)

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## Author Contributions

Conceived and designed the experiments: HO MA YO. Performed the experiments: HO TA. Analyzed the data: HO SO KI ME MA. Contributed reagents/materials/analysis tools: TK MS. Wrote the paper: HO MA.

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ORIGINAL ARTICLE: PREVENTIVE GERIATRIC CLINICAL PRACTICE AND HEALTH

# Polypharmacy as a risk for fall occurrence in geriatric outpatients

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**Objective:** To investigate the predictors of falls, such as comorbidity and medication, in geriatric outpatients in a longitudinal observational study.

**Methods:** A total of 172 outpatients (45 men and 126 women, mean age 76.9 ± 7.0 years) were evaluated. Physical examination, clinical history and medication profile were obtained from each patient at baseline. These patients were followed for up to 2 years and falls were self-reported to their physicians. The factors associated with falls were analyzed statistically.

**Results:** A total of 32 patients experienced falls within 2 years. On univariate analysis, older age, osteoporosis, number of comorbid conditions and number of drugs were significantly associated with falls within 2 years. On multiple logistic regression analysis, the number of drugs was associated with falls, independent of age, sex, number of comorbid conditions and other factors that were significantly associated in univariate analysis. A receiver–operator curve evaluating the optimal cut-off value for the number of drugs showed that taking five or more drugs was a significant risk.

**Conclusion:** In geriatric outpatients, polypharmacy is associated with falls. Intervention studies are needed to clarify the causal relationship between polypharmacy, comorbidity and falls. **Geriatr Gerontol Int 2012; 12: 425–430.**

**Keywords:** bone/musculo-skeletal, elderly, falls, geriatric medicine, internal medicine, polypharmacy.

## Introduction

Previous studies have assessed the risk factors for falls in community-dwelling elderly,[1–3] but not in geriatric outpatients, and history of falls, physical ability and living environment were found to be predictors of falls. Outpatients have different characteristics from community-dwelling elderly, and previous studies have not assessed whether medical comorbidity and therapeutic drugs might be risk factors for falls. Falls in patients on medication are complicated, because some drugs, such as aspirin, can cause serious bleeding when they have injurious falls, and others, such as antihypertensive[4] and hypoglycemic[5,6] agents, can cause falls.

Previously, we reported that polypharmacy was associated with the tendency for falls using four indices of fall tendency in a cross-sectional setting in geriatric outpatients,[7] though that study did not evaluate fall occurrences, and also not in a longitudinal manner. Therefore, we aimed at investigating whether polypharmacy was predictive of fall occurrences in a prospective fashion. For this purpose, we followed geriatric outpatients for up to 2 years, and assessed whether polypharmacy is a risk for fall occurrence, together with other risks.

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The validity of two novel indices of fall tendency, the 22 items fall risk index[8] and the 13 points simple screening test,[3] which were used in our previous study, have been confirmed in community-dwelling elderly, but not in geriatric outpatients. Therefore, in the present investigation, the association of these two indices with falls was also evaluated to confirm their validity in geriatric outpatients in a longitudinal study.

## Methods

### *Patients*

From 2006 to 2007, a total of 190 consecutive patients aged 65 years or older who were receiving treatment for chronic diseases, such as hypertension, dyslipidemia, diabetes and osteoporosis, who were seen every 2–4 weeks at the outpatient clinic of the Research Institute of Aging Science, Tokyo, were enrolled. All the patients were able to walk independently and their condition was stable. Patients who had acute illness or overt dementia were excluded. Anthropometric and medical information including past history of stroke, myocardial infarction, malignancy and prescribed drugs was obtained from each patient at baseline from the medical chart recorded by the physician in charge. However, 18 patients were excluded, because they were lost to follow up soon after enrolment and the medical information was not fully obtained. All prescribed drugs had not been changed in the included patients for at least 2 months before enrolment. The patients were followed up for 2 years.

### *Occurrence of falls*

During the follow-up period, the patients and their family members responded to the annual questionnaire asking about the occurrence of falls within the past year. The questionnaire was repeated for 2 years.

### *Indices of fall tendency*

After enrolment, the patients were examined for two indices to investigate the fall tendency. These were (i) a questionnaire of the 22 items portable fall risk index;[8] and (ii) the 13 points simple screening test to assess the fall tendency.[3]

### *Ethical consideration*

The present study was approved by the Institutional Review Board of the Research Institute of Aging Science. We obtained written consent from all participants and/or their guardians.

### *Data analysis and statistical methods*

Values are expressed as mean ± standard deviation. In order to analyze the relationship between falls and comorbidity or drugs, variables were compared using Student's *t*-test or $\chi^2$-test as appropriate. Significant factors found in univariate analysis were included in multivariate logistic regression analysis to determine the association of falls with other variables. Receiver-operating curve (ROC) analysis was carried out to identify the optimal cut-off value of the number of drugs for predicting falls within 2 years. The value with the highest sum of sensitivity and specificity was used as the optimal cut-off value. Logistic regression analysis was carried out to assess the validity of the two indices of fall tendency, adjusted by age and sex. *P*-values <0.05 were considered statistically significant. Data were analyzed using JMP version 8.0.1 (SAS Institute, Cary, North Carolina, USA).

## Results

Baseline medical information and two indices of fall tendency were evaluated in 172 patients (Table 1). Drugs prescribed in less than 5% of the patients are not shown. Because only patients who were in a stable condition and were able to walk independently were included, patients with Parkinson's disease, severe paresis or painful arthralgia were not included. Calcium channel blockers prescribed in the present study were all long-acting agents, and the prescribed aspirin dosage was 100 mg in all cases. Only a few patients were receiving insulin therapy, sulfonylureas, angiotensin converting enzyme inhibitors, β-blockers, α-blockers, non-steroidal anti-inflammatory drugs or anticoagulants. No patients were taking neuroleptics or antiparkinsonian drugs.

After 1 year, all patients, except for one who died of congestive heart failure, were followed up ($n = 171$, follow-up rate 99.4%). Falls occurred in 22 patients. Only a higher age was associated with falls within 1 year on univariate analysis (non-fallers: 76.4 ± 6.8 years, fallers: 81.0 ± 6.9 years, $P = 0.004$).

After another year (2 years after enrolment), one patient had died of lung cancer, and five patients were lost to follow up. A total of 165 patients were evaluated (follow-up rate 95.9%), and 10 patients had fallen during the second year; thus a total of 32 patients had fallen within 2 years. As shown in Table 2, higher age, osteoporosis, number of comorbid conditions and number of drugs were significant factors associated with falls. To determine the association of falls with these significant factors, multivariate logistic regression analysis was carried out, and as shown in Table 2, the number of drugs was the only factor that was significantly associated with falls within 2 years.

As polypharmacy was assumed to be a risk for falls within 2 years, the cut-off of the number of the drugs was analyzed. Figure 1 shows the ROC curves to define the optimal cut-off point in relation to falls within

**Table 1** Characteristics and univariate analysis of association with fallers and non-fallers within 2 years and risk factors

| Total | | Non-fallers ($n = 133$) | Fallers ($n = 32$) | $P$-value (Fallers vs. Non-fallers) |
|---|---|---|---|---|
| Age (years) | 77.0 ± 7.0 | 76.3 ± 6.9 | 80.0 ± 6.9 | 0.007 |
| Body mass index (kg/cm$^2$) | 22.7 ± 3.2 | 22.7 ± 3.3 | 22.7 ± 3.1 | 0.98 |
| No. comorbid conditions | 1.9 ± 1.1 | 1.8 ± 1.1 | 2.3 ± 0.9 | 0.009 |
| No. drugs | 3.2 ± 2.8 | 2.8 ± 2.7 | 4.9 ± 2.5 | <0.0001 |
| Female ($n = 122$) | – | 72.9% | 78.1% | 0.66 |
| Hypertension ($n = 106$) | – | 62.4% | 71.8% | 0.41 |
| Dyslipidemia ($n = 76$) | – | 47.3% | 40.6% | 0.56 |
| Diabetes ($n = 23$) | – | 12.8% | 18.8% | 0.40 |
| Osteoporosis ($n = 59$) | – | 30.8% | 56.3% | 0.01 |
| History of stroke ($n = 6$) | – | 2.3% | 9.4% | 0.09 |
| History of myocardial infarction ($n = 3$) | – | 0.8% | 6.3% | 0.10 |
| History of cancer ($n = 8$) | – | 5.3% | 3.1% | 0.99 |
| Calcium channel blocker ($n = 59$) | – | 33.3% | 46.9% | 0.16 |
| Angiotensin II receptor blocker ($n = 56$) | – | 33.3% | 37.5% | 0.68 |
| Statin ($n = 40$) | – | 23.5% | 28.1% | 0.65 |
| Aspirin ($n = 31$) | – | 19.0% | 24.1% | 0.61 |
| Bisphosphonate ($n = 9$) | – | 4.6% | 9.4% | 0.38 |
| H2-blocker ($n = 9$) | – | 3.8% | 12.1% | 0.80 |
| Proton pump inhibitor ($n = 11$) | – | 5.3% | 12.1% | 0.23 |
| Hypnotic ($n = 31$) | – | 16.7% | 28.1% | 0.14 |

Values are expressed as mean ± SD ($n = 165$).

**Table 2** Logistic regression analysis of association of falls within 2 years with age, sex, other significant factors found in univariate analysis, and polypharmacy

| | Unadjusted odds ratio (95% CI) | Adjusted odds ratio (95% CI) | Adjusted odds ratio (95% CI) |
|---|---|---|---|
| Age (/1 year) | 1.08 (1.03–1.13)† | 1.06 (0.99–1.13) | 1.06 (0.99–1.13) |
| Sex (male = 0, female = 1) | 1.39 (0.56–3.48) | 0.98 (0.29–3.23) | 0.75 (0.23–2.38) |
| Osteoporosis ($n = 0$, Y = 1) | 3.12 (1.43–6.84)† | 2.76 (0.92–7.38) | 3.02 (0.96–6.15) |
| No. comorbid conditions (/disease) | 1.63 (1.14–2.32)* | 0.90 (0.55–1.47) | 0.99 (0.62–1.56) |
| No. drugs (/drug) | 1.29 (1.12–1.48)‡ | 1.30 (1.08–1.57)* | – |
| Five or more drugs ($n = 0$, Y = 1) | 5.04 (2.25–11.3)‡ | – | 4.50 (1.66–12.2)† |

*$P < 0.05$, †$P < 0.005$, ‡$P < 0.0005$. CI, confidence interval.

2 years: the area under the ROC was 0.731, and the optimal cut-off value of the number of drugs was five (sensitivity 0.576, specificity 0.788). Logistic regression analysis showed that taking five or more drugs was significantly associated with an increased risk of falls (odds ratio 4.5, 95% CI 1.7–12.2) after adjustment for age, sex, osteoporosis and number of comorbid conditions (Table 2).

Also, the association between falls and two indices of fall tendency was evaluated to confirm the validity of each index in geriatric outpatients. As both indices included the questionnaire asking whether patients were "taking five or more drugs," the number of drugs was excluded from this analysis because of duplication in the statistical model. As shown in Table 3, the 22 items fall risk index showed a tendency towards an association with falls within 2 years, odds ratio 1.12 (95% CI 1.00–1.26; $P = 0.05$), whereas the 13 points screening test was significantly associated with falls after adjustment for age, sex and other factors significantly associated in the univariate analysis. Therefore, these indices are considered to be good predictors of falls in geriatric outpatients, as has been shown in community-dwelling elderly subjects.

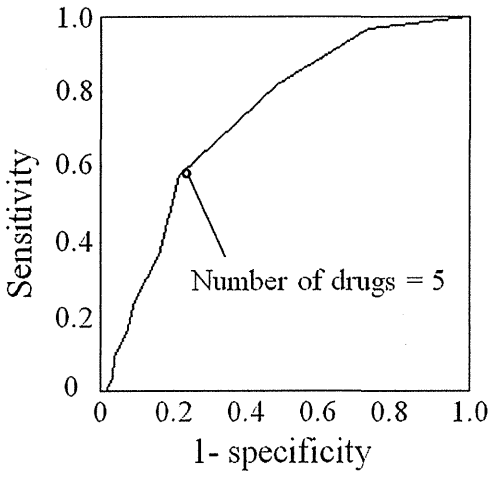


**Figure 1** Receiver–operating curves to define optimal cut-off value of number of drugs at baseline in relation to falls within 2 years. Area under the curve was 0.731, optimal cut-off value of the number of drugs was five (sensitivity = 57.6%, specificity = 78.8%).

## Discussion

The risk of falls has been assessed in community-dwelling elderly, and history of falls, physical ability and living environment were found to be predictors of falls. Also, in nursing home residents, cognitive function, gait disturbance and urinary incontinence are reported to be risk factors for falls,[9,10] and length of stay, disease condition, surgical procedures and some specific drugs are reported to be risk factors in hospital inpatients.[11,12]

Nevertheless, the risks in geriatric outpatients have not been sufficiently assessed, although assessment of fall risk in geriatric outpatients is important; their medical conditions or drugs might cause falls, and drugs, such as antiplatelet agents or anticoagulants, might cause critical bleeding after a fall. Also, physicians could prevent falls in their patients by giving advice during regular consultations, if risk factors are identified.

In our previous cross-sectional study assessing geriatric outpatients, polypharmacy was significantly correlated with indices of fall tendency, and the present follow-up study of geriatric outpatients showed the impact of polypharmacy on falls within 2 years. Statistical analyses showed that polypharmacy was a risk factor for falls, independent of age, sex and comorbidity.

Besides polypharmacy, several medications and comorbid conditions have been reported as risks for falls.[13–22] Among these, diabetes,[5,6] insomnia,[13] hypnotics,[13–15] antiarrhythmics[22] and antihypertensive agents[14] were not significantly associated with fall risk in the present study. Just 11 patients (45.9% of diabetic patients) were prescribed hypoglycemic agents, such as a sulfonylurea ($n = 8$) or insulin ($n = 3$), and the relatively low rate of prescription of hypoglycemic agents might have affected our result. Neither hypnotics nor antihypertensives were associated with falls. This result might be a result of the small sample size. Anti-arrhythmics were taken by just three patients (digoxin: $n = 2$, class IA anti-arrhythmic drug: $n = 1$). Other drugs, such as major tranquillizers,[14] antidepressants[17,18] and antiparkinsonian agents,[19,22] might increase fall risk; however, no patient used these drugs in the present study. In the present study, most of the patients were in a stable condition throughout the 2 years, though their drugs were changed gradually according to their medical conditions during the observation period. We only used the number of drugs at baseline for statistical analysis; however, the number of drugs increased from $3.2 \pm 2.8$ to $3.9 \pm 3.0$ during the 2 years. There were 17 patients whose number of drugs had been decreased, 70 patients not changed and 78 patients increased. The number of drugs after 2 years was also associated with falls ($P < 0.0005$). The optimal cut-off point for the number of drugs was again five (area under ROC curve 0.780, sensitivity 0.576, specificity 0.788). Furthermore, the changes in number of drugs were also associated with falls ($P < 0.05$), and the optimal cut-off point for the change in number of drugs was +1 (area under ROC curve 0.649, sensitivity 0.727, specificity 0.409).

**Table 3** Logistic regression analysis of association between 2-year fall occurrences with two indices of fall tendency; 22 items fall risk index and 13 points simple screening test

| | Unadjusted odds ratio (95% CI) | Adjusted odds ratio (95% CI) | Adjusted odds ratio (95% CI) |
|---|---|---|---|
| Age (/year) | 1.08 (1.03–1.15)** | 1.06 (0.99–1.13) | 1.06 (1.00–1.13) |
| Sex (male = 0, female = 1) | 1.39 (0.56–3.48) | 0.75 (0.23–2.43) | 0.79 (0.24–2.56) |
| Osteoporosis ($n = 0$, Y = 1) | 3.12 (1.43–6.84)** | 2.56 (0.96–6.82) | 2.61 (0.98–6.95) |
| No. comorbid conditions (/disease) | 1.63 (1.14–2.32)* | 1.24 (0.83–1.86) | 1.32 (0.88–1.97) |
| Fall risk index (/item) | 1.23 (1.11–1.37)*** | 1.12 (1.00–1.26) | – |
| Simple screening test (/point) | 1.19 (1.06–1.33)** | – | 1.14 (1.01–1.29)* |

*$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$. CI, confidence interval.

Consequently, polypharmacy, especially taking five or more drugs, should be considered a risk for falls.

There were several limitations of the present study. First, the falls were self-reported by the patients. Although all the patients had no overt dementia, they might have forgotten the incident of falling. We attempted to count the total fall occurrences in each patient; however, we could not differentiate the repeated falls in the second year from the fall occurrence in the first year. In fact, we asked 22 patients who reported falls in the first year about fall occurrence during the second year, but they did not accurately recall whether they experienced falls in the first or second year. Second, five patients were lost to follow up at 2 years for unknown reasons. The follow-up ratio was acceptable, although some of the patients might have fallen, have been no longer able to come to the clinic and moved to nursing homes. This might have slightly influenced the result. Also, the cause of falls in polypharmacy patients is not explained. Potentially inappropriate medications, which could cause adverse drug reactions, are usually seen in patients with polypharmacy, and falls might be the consequence of adverse drug reactions, such as dizziness, instability and light-headedness. Pathophysiological assessments and drug-reducing interventions are expected to elucidate the causal relationship.

Additionally, we showed that the 22-item fall risk index and its simple screening test were useful to predict falls in geriatric outpatients. Although both indices have been validated in community-dwelling elderly people, the present finding also showed their association with fall risk among geriatric outpatients. The difference of statistical significance between fall risk index and simple screening test might be a result of small sample size or the difference in the contribution of each item to total scores between the two indices. "Taking five or more drugs" accounts for only one item out of the 22-item fall risk index; in contrast, the same questionnaire accounts two points in the 13-point simple screening test. Because polypharmacy was a strong risk factor of falls in elderly outpatients in the present study, the proportion of polypharmacy in the scores might have caused the discrepancy. Taken together, it is likely that 13-point screening test was more suitable to our subjects who were taking several medicines.

In summary, the present study showed that geriatric outpatients with polypharmacy were at a high risk of falls, especially those receiving five or more drugs. Our finding might add new information for pharmacotherapy and geriatric research in elderly patients with chronic diseases. Intervention studies examining the effect of drug reduction for the prevention of falls are required in the future.

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## Disclosure statement

The authors declare no conflict of interest.

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**Author Contributions:** Paul Regal designed the study, assessed patients, served on the consensus panel, analyzed the data, and wrote the article. Eileen Heatherington performed cognitive tests and was a panel member for consensus diagnosis of dementia.

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## FACTORS ASSOCIATED WITH PROLONGED HOSPITAL STAY IN A GERIATRIC WARD OF A UNIVERSITY HOSPITAL IN JAPAN

*To the Editor*: We read with interest the article by Lakhan and colleagues,[1] which showed the high prevalence and worsening of geriatric syndrome during acute care hospitalization. Because falls, incontinence, impairment in activities of daily living (ADLs), and other geriatric syndrome components increase the care burden and limit discharge planning in acute care hospitals, geriatric syndrome might cause prolonged hospital stays. A prolonged hospital stay is one of the major determinants of medical cost and is thus a serious problem in geriatric medicine. Previous studies have shown that clinical events during hospitalization,[2,3] basic ADLs,[4] and nonmedical factors such as delayed transfer to a nursing facility or disagreement on the discharge plan among family members[5] are risk factors for prolonged hospital stay. Furthermore, because older adults have multiple comorbid conditions and are susceptible to adverse drug reactions (ADRs), these factors might be related to length of hospital stay. To test this hypothesis, the association between geriatric conditions such as geriatric syndrome, ADLs, and ADRs and prolonged hospital stay were comprehensively investigated using the database of the geriatric ward of the University of Tokyo Hospital from 1995 to 2010. The ethics committee of the Graduate School of Medicine, University of Tokyo approved this study.

All records of patients aged 65 and older from 1995 to 2010 were reviewed. Data on length of stay, acute hospitalization, ADRs, body mass index (BMI), number of diseases and drugs, geriatric syndrome, and Barthel Index were collected. Twenty-three components of geriatric syndrome such as falls, cognitive impairment, urinary incontinence, constipation, and insomnia were included in the analysis. Records lacking information on any of the variables were excluded. Cases of scheduled short-term hospitalization were excluded. Finally, the records of 1,616

**Table 1. Characteristics of Study Patients and Analyses for Length of Hospital Stay ($N$ = 1,616)**

| Characteristic | Value | Univariate Analysis ($R$ or Hospital Stay, Days, Mean ± SD) | Standardized Regression Coefficient |
|---|---|---|---|
| Age, mean ± SD | 78.3 ± 7.0 | 0.001 | −0.099[d] |
| Sex, *n* (%) | | | |
| Female | 778 (48.1) | 26.8 ± 20.2 | |
| Male | 838 (51.9) | 27.6 ± 24.6[a] | |
| Acute hospitalization, *n* (%) | | | |
| Yes | 300 (18.5) | 26.2 ± 21.0 | |
| No | 1,316 (81.5) | 31.8 ± 28.2[a,d] | |
| Adverse drug reaction, *n* (%) | | | |
| Yes | 190 (11.8) | 26.4 ± 19.5 | 0.078[c] |
| No | 1,426 (88.2) | 33.3 ± 38.1[a,d] | |
| Body mass index, kg/m$^2$, mean ± SD | 22.0 ± 4.1 | −0.59[d] | −0.062[b] |
| Barthel Index (points out of 100), mean ± SD | 83.1 ± 26.1 | −0.178[d] | −0.13[d] |
| Number of diseases, mean ± SD | 5.3 ± 2.3 | 1.43[c] | 0.082[c] |
| Number of drugs, mean ± SD | 6.8 ± 3.6 | 0.411[b] | – |
| Number of geriatric syndrome components, mean ± SD | 4.6 ± 3.6 | 1.66[d] | 0.19[d] |

All data were collected soon after admission. For sex, acute hospitalization, and adverse drug reactions, a simple *t*-test was performed for univariate analysis, and values are expressed as mean ± standard deviation (SD).

[a]*P*-values are for comparison to female or no. Pearson correlation coefficients ($R$) are shown for the remaining factors in univariate analysis. All variables shown were included in stepwise regression analysis, and factors significantly associated were analyzed in multiple regression analysis (coefficient of determination = 0.32).

[b]$P < .05$.

[c]$P < .005$.

[d]$P < .001$.

patients were analyzed (mean age 78.3 ± 7.0, 52% male). All data were obtained soon after admission. Values are expressed as means ± standard deviations and were analyzed using JMP version 9.0.2 (SAS Institute, Inc., Cary, NC). $P < .05$ was considered statistically significant.

Mean length of stay was 27.3 ± 22.6 days (range 1–322 days). The results of univariate and multivariate analyses for length of stay are shown in Table 1. Multiple stepwise regression analysis showed that ADRs, number of diseases, and number of geriatric syndrome components were positively associated with longer hospital stay, whereas age, BMI, and Barthel Index were negatively associated. The number of geriatric syndrome components was significantly associated with hospital stay independent of number of diseases.

The present analysis demonstrated that geriatric factors such as ADRs, multiple diseases, low BMI, ADL dependence, and number of geriatric syndrome components were associated with longer hospital stay in a large group. The finding that ADRs are a risk for prolonged hospital stay is consistent with a previous report,[6] and ADL dependence has been reported as a risk in a smaller group.[4] Furthermore, the number of geriatric syndrome components and undernutrition were risk factors for prolonged hospital stay in a large-scale study. Frailty, which is also known to be a risk factor,[7] was not examined independently in the present study, but ADL dependence and undernutrition, both of which are major components of frailty, were found to be risk factors, so it is reasonable to assume that frailty was associated with length of hospital stay in the current cohort as well. The present study revealed that the accumulation of geriatric syndrome components was a risk factor for prolonged hospital stay independent of multiple diseases and, presumably, frailty. Thus, geriatric syndrome should be comprehensively managed during hospitalization. The reason for the negative association between age and length of stay is unclear, but the presence of young-old patients with disability or complicated conditions on the geriatric ward might have influenced the results.

In summary, the present study provides new insight into the significance of geriatric conditions in relation to prolonged hospital stay in older adults. ADL dependence, undernutrition, ADRs, and geriatric syndrome should be carefully assessed and interventions provided when caring for older inpatients.

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## ACTIVITIES OF DAILY LIVING RATHER THAN DEPRESSIVE SYMPTOMS INCREASE THE RISK OF MORTALITY IN JAPANESE COMMUNITY-DWELLING ELDERLY PEOPLE: A 4-YEAR LONGITUDINAL SURVEY

*To the Editor:* The article entitled "Depressive Symptoms Increase the Risk of Mortality in Older Mexican Community-Dwelling Adults" by Piña-Escudero et al.[1] deeply impressed us. Although it has been shown that older adults with depressive symptoms (DSs) have fewer quality-adjusted life years than those with chronic medical conditions,[2] Piña-Escudero et al. in their 2-year longitudinal study, showed that DSs increase mortality risk regardless of multiple covariates such as medical conditions and disability in activities of daily living (ADL). Similarly, results of a meta-analysis of 25 studies suggest that depression increases the risk of mortality,[3] although those studies did not assess ADL in detail. The risk of mortality in Japanese community-dwelling elderly people is reported herein, focusing on DSs and ADLs in a 4-year longitudinal survey.

The study population included 1,818 community-dwelling individuals aged 65 and older in Tosa Town, Japan; 1,600 (88.0%) participants who completed self-reported geriatric questionnaires in 2006 were included in the study. The questionnaires consisted of questions on ADLs and the 15-item Geriatric Depression Scale (GDS-15).[4] For ADL assessment, participants rated their

## RELATIONSHIP BETWEEN TESTOSTERONE AND COGNITIVE FUNCTION IN ELDERLY MEN WITH DEMENTIA

*To the Editor:* A decrease in sex hormones with aging has been reported to be related to psychosomatic disorders such as late-onset hypogonadism syndrome, frailty, and cognitive impairment in adult men.[1] For example, a community-based cross-sectional study has shown that elderly men with a lower blood concentration of bioavailable testosterone have more-severe impairment of cognitive function.[2] Moreover, a longitudinal study indicated that serum free testosterone (FT) concentration could predict memory performance and cognitive status in elderly men,[3] but it is unknown whether lower testosterone concentration is related to cognitive impairment in individuals with dementia, because the previous studies primarily focused on a healthy community-based population. Also, few studies have addressed the relationship between testosterone and cognitive function in elderly Japanese men.

One recent cross-sectional study showed that total testosterone and FT concentration were associated with activities of daily living (ADLs) in institutionalized elderly men.[4] This study also revealed that a relationship between testosterone and cognitive function could be found even in institutionalized elderly men with physical or neuropsychiatric dysfunction. Thus, whether lower testosterone concentration is related to deterioration of ADL in elderly men with cognitive impairment was longitudinally investigated.

Fifty-two male outpatients attending the Center for Comprehensive Care on Memory Disorders at Kyorin University Hospital were recruited (mean age 77.0 ± 5.5, range 65–87). Participants' clinical backgrounds were hypertension, 48.9%; diabetes mellitus, 12.2%; and dyslipidemia, 38.1%. None had a history of stroke. Comprehensive geriatric assessment was performed based on basic ADLs (Barthel Index),[5] instrumental ADLs (Lawton and Brody IADLs, 0–5 points in men),[6] cognitive function (Mini-Mental State Examination (MMSE)),[7] mood (Geriatric Depression Scale (GDS), 15 items),[8] and vitality (Vitality Index, 10-point scale).[9] This assessment was repeated 1, 2, and 3 years after baseline assessment at the first visit to the clinic. At the first visit, blood was drawn after an overnight fast and FT concentration was measured using radioimmunoassay. FT values ranged from 1.0 to 53.0 pmol/L (mean ± SD 30.4 ± 11.0 pmol/L). Participants were classified into three groups according to tertile according to the baseline FT value (Figure 1), and the parameters from the comprehensive geriatric assessment were compared between groups and visits. Statistical data were analyzed using SPSS version 17.0 (SPSS, Inc., Chicago, IL). One-way analysis of variance (ANOVA) was applied for comparisons between groups, and the Fisher post hoc test was applied when significant ($P < .05$). One-way repeated ANOVA was used for comparisons between baseline and the 1-, 2-, and 3-year visits, and the Fisher post hoc test was applied when significant ($P < .05$).

There were no significant differences between groups in age (high, 75.3; middle, 76.6; low, 79.0), basic ADLs (high, 96.9; middle, 99.1; low, 95.3 points), MMSE (high, 23.2; middle, 25.1; low, 23.1 points), GDS-15 (high, 5.1; middle, 4.1; low, 4.6 points), and Vitality Index (high, 9.1; middle, 9.1; low, 8.8 points) at baseline, whereas IADLs tended to be lower (high, 4.1; middle, 4.1; low, 3.4 points, $P = .06$) in the low FT tertile group than in the other groups.

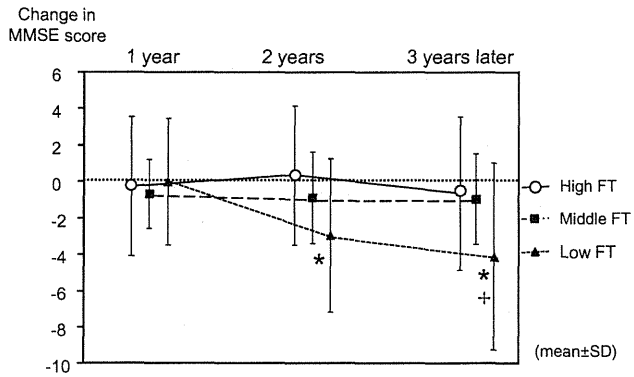


**Figure 1.** Change in Mini-Mental State Examination (MMSE) score according to tertile of serum free testosterone (FT) level in men. FT tertile: high, >36.1 pmol/L, n = 17; middle, 29.1–35.4 pmol/L, n = 17; low, <28.8 pmol/L, n = 18. *$P < 0.05$ vs highest FT group, +$P < 0.05$ vs middle FT group.

At the 1-year visit, there was no difference in change in MMSE score from baseline between the groups, although the decrease in MMSE score was larger in the low FT tertile group than in the middle and high tertile groups at the 2- and 3-year visits (Figure 1). Also, MMSE scores were lower in the low FT tertile group at the 2- ($P = .009$) and 3-year ($P < 0.001$) visits than at baseline, whereas they were not lower in the middle and high tertile groups. In contrast, there was no such trend in basic ADLs, IADLs, GDS scores, and Vitality Index.

Multiple regression analysis was performed with a decrease in MMSE score as a dependent variable and age; ADLs; body mass index; presence of hypertension, diabetes mellitus, or hyperlipidemia; and FT concentration as independent variables to consider factors affecting cognitive impairment, according to a previous report.[4] Blood FT concentration was found to be an independent predictor of decrease in MMSE score at the 3-year visit ($\beta = 0.492$, $P = .02$).

A number of investigations support the biological plausibility of a protective effect of testosterone against cognitive dysfunction. The present findings from memory clinic outpatients are consistent with previous findings observed in elderly community-based men, showing a relationship between FT concentration and cognitive performance.[3] Furthermore, the present findings indicate that a lower FT concentration could lead to a faster decline in cognitive function in elderly Japanese men who already show cognitive impairment. This study provides fundamental data for the future study of hormone replacement therapy for cognitive decline in elderly adults with low FT.

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**Author Contributions:** Nagai K designed the research; acquired, analyzed, and interpreted the data; and drafted the manuscript. Shibata S interpreted the data. Kobayashi Y, Yamada Y, Kimura S, Machida A acquired subjects and data and analyzed and interpreted the data. Akishita M and Toba K conceived and designed the research and interpreted the data. Kozaki K supervised the research.

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## BASELINE INSTRUMENTAL ACTIVITIES OF DAILY LIVING AND INCIDENT DEMENTIA

*To the Editor*: Sikkes et al.[1] have written an important paper showing that individuals without dementia with impairment in at least one of nine instrumental activities of daily living (IADLs) at baseline had a significantly higher incidence of dementia at 12 months (24.4%) than individuals without IADL impairment at baseline (16.7%) (P = .04). Their 531 participants who were followed for 12 months were relatively young (mean age 69.6), so it was decided to duplicate their study from prospective data from the Wyong Hospital Memory Clinic, 100 km north of Sydney. From 415 individuals attending a memory clinic, community-dwelling individuals aged 60 and older who were free of dementia at baseline and had a Mini-Mental State Examination score (MMSE[2]) of 25 to 30 and a follow-up MMSE and Montreal Cognitive Assessment (MoCA), range 0 (worst) to 30 (best)[3] at 12 months were selected in a consensus conference of a geriatrician (PJ) and a clinical nurse consultant (EH). Each individual's family rated IADLs on the Nottingham scale,[4] which ranged from 0 (worst) to 22 (best). Twenty-two of 82 (27%) converted to dementia at 12 months, compared with Sikkes conversion rate of 20.8% at 24 months—the most likely reason for this difference was that mean age (79.1) was 9.5 years older than theirs (69.6). Stats Direct Version 2.7.8b (StatsDirect Ltd, Altrincham, UK) from November 2011 was used to compare converters and nonconverters. Mean age of the 22 converters at baseline was significantly higher that that of the 60 nonconverters (82.0 ± 5.8 vs 78.0 ± 6.8, P < .01), mean IADL score at baseline was significantly lower (13.1 ± 5.3 vs 16.1 ± 4.0, P = .0236), MMSE score at baseline was by definition lower (25.6 ± 0.73 vs 27.5 ± 1.50, P < .001), and MoCA score at baseline was lower (19.2 ± 3.5 vs 22.8 ± 3.9, P < .001). At 12 months, IADL (11.4 ± 5.6 vs 15.4 ± 4.5, P = .004), MMSE score (21.6 ± 4.5 vs 27.4 ± 1.6, P < .001), MoCA (16.8 ± 3.6 vs 22.8 ± 4.2, P < .001) remained significantly lower in converters.

The Nottingham IADL covers seven of the nine IADL items that Sikkes used, excluding medications and finances. Women are more likely than men to perform five of the Nottingham IADL items unless the men live alone with no home care services: cleaning the kitchen, making a hot snack, washing small items of clothing, doing a full clothes wash, and doing housework.

Although the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*, criteria for dementia include a decline in social and occupational function, there is a surprising lack of research into IADLs as a predictor of incident dementia. This is an important topic for future research and ongoing studies are being conducted in three cohorts: Wyong Memory Clinic; general medical inpatients with delirium or subsyndromal delirium—a prospective randomized controlled trial, Central Coast Australia Delirium Intervention Study; and PhD study, PR DEFEAT DELIRIUM, in outpatients at high risk for incident delirium. One study[5] with 255 community-dwelling individuals attending a memory clinic who were followed an average of 13 months has been published. The 11.4% of participants with antithyroid antibodies had similar outcomes at 12 months with respect to IADLs, decline in IADLs, MMSE and MoCA scores, and transfer to residential care.

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The authors declare that they have no conflict of interest.

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# High risk of adverse drug reactions in elderly patients taking six or more drugs: Analysis of inpatient database

Dear Editor,

Polypharmacy is frequently seen in elderly patients, largely because of the existence of multiple comorbid conditions. All medications have the potential for harm as well as benefit, and thus, physicians must make difficult trade-offs between both sides of guideline-directed care.[1,2] Some drugs are reported to increase adverse drug reactions (ADR), and have been listed as potentially inappropriate medications (PIM), which should not be used generally in elderly patients.[3–5] However, it is still complicated for general practitioners to check PIM for each patient. As polypharmacy is a well-known risk for ADR,[6,7] and the frequency of PIM use rises sharply according to the number of drugs,[7] the optimal number of drugs defining polypharmacy might be of substantial help for physicians. Therefore, we aimed to determine the cut-off number of drugs in relation to ADR using the inpatient database of our geriatric department.

All records of patients aged 65 years or older who were admitted to the Department of Geriatric Medicine, The University of Tokyo Hospital, Tokyo, Japan, from 1995 to 2010 were reviewed. Retrospective use of the patient database was approved by the ethics committee of The University of Tokyo. Records lacking information on ADR or the number of drugs and patients taking no drugs were excluded. Finally, we analyzed the records of 2412 patients (mean ± SD age = 78.7 ± 7.3 years, male 51.3%). ADR was defined as unintended or undesired harmful effects presumably caused by drugs. The occurrence of ADR was assessed before discharge by the physician in charge, and other data were obtained soon after admission. Odds ratios with 95% confidence intervals for ADR were obtained by logistic regression analysis. The receiver operating characteristic (ROC) curve was assessed to define the optimal number of drugs in relation to ADR. Data were analyzed using JMP version 9.0.2 (SAS Institute, Cary, NC, USA).

The number of prescribed drugs per patient was 6.6 ± 3.6 (mean ± SD; range = 1–30), and ADR were observed in 252 patients (10.5%). Patients with ADR were taking more drugs than those without ADR (7.6 ± 3.8 *vs* 6.4 ± 3.5 drugs, $P < 0.0001$ by unpaired *t*-test). ADR was significantly associated with the number of drugs in unadjusted and age- and sex-adjusted logistic regression analysis (data not shown). When ADR were analyzed according to the number of drugs by quintile, the odds ratio of ADR was significantly higher in the groups taking six or more drugs (Fig. 1). Furthermore, ROC analysis showed that the optimal cut-off number of drugs was six, although the sensitivity of 0.560 and specificity of 0.710 were not high, with a small area under the curve of 0.591.

Previously, elderly outpatients taking five to eight drugs were reported to be at greater risk of ADR-related hospitalization than those taking zero to four drugs.[6] Also, we have reported that taking five or more drugs is a risk factor for falls in outpatients.[8] Taking these findings together, it might be reasonable to consider six or more drugs as the cut-off of polypharmacy in terms of ADR in elderly patients. The present study had some limitations; the results were obtained from inpatients managed by geriatricians, and thus might not extend to general outpatients. Next, this database did not have information for types of ADR; so they could not be clarified in detail in the present. According to our previous study, hematological, neurological and


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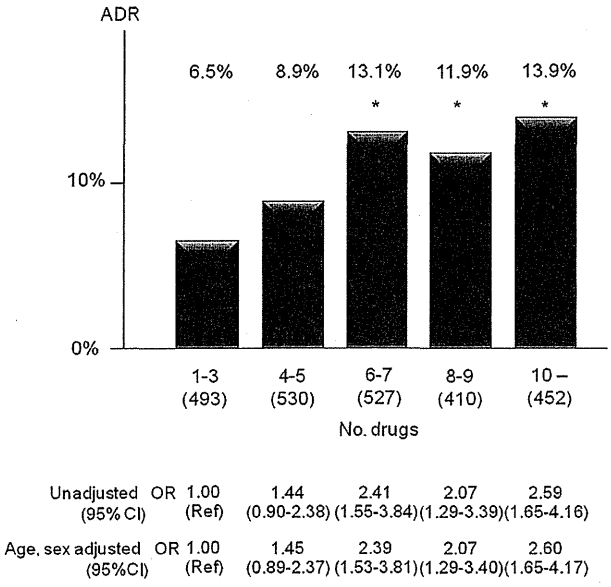


**Figure 1** Frequency of adverse drug reactions according to quintile of number of prescribed drugs. Unadjusted and age–sex adjusted odds ratios (95% confidence interval) of adverse drug reactions are shown. *$P < 0.05$ versus one to three drugs. OR, odds ratio.

cardiovascular events were reported to be more frequent than ADR in elderly inpatients,[9] and so, these are possibly the major types in the present study. Also, ROC analysis did not fit well for the present cohort.

In summary, the present study provided the cut-off number of drugs for screening of elderly patients at high risk of ADR. Prospective studies and intervention studies examining the effect of drug reduction on ADR and comorbid conditions are required to confirm this finding.

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# C-kit-positive acute myelogenous leukemia effectively treated with imatinib: A case report and review of the literature

It is highly advisable to choose a strategy to improve the quality of life (QOL), rather than a curative strategy, such as conventional chemotherapy, for very elderly patients with acute myelogenous leukemia (AML). Molecular targeted therapy might also be considered as an important strategy to take into account.[1]

An 88-year-old man was referred to Juntendo University Urayasu Hospital in Chiba, Japan, because of fever and headache in April 2004. The spleen was enlarged to 5 cm below the left costal margin. White blood cell (WBC) count was $61.1 \times 10^4/\mu L$, with 29% blasts and 6.5% basophils. Other data were hemoglobin (Hb) 10.6 g/dL, platelet (plt) $41.0 \times 10^4/\mu L$, lactate dehydrogenase (LDH) 685 IU/L, uric acid (UA) 10.0 mg/dL and C-reactive protein (CRP) 14.6 mg/dL. Bone marrow was myeloid hyperplasia with 27% blasts. Flow cytometer showed that the leukemic cells were positive for myeloperoxidase, CD7, CD13, CD15, CD33, CD34 and c-kit (CD117). Because the leukocytosis with blasts, mild basophilia and splenomegaly resembled blast crisis of chronic myeloid leukemia, and furthermore the patient was very old, imatinib 600 mg daily was tried. Fortunately, imatinib was effective before chromosome analysis later showed trisomy 8. Although the rate of blasts in the peripheral WBC was almost constant, the number of WBC decreased and red blood cells transfusion (RBCT) was not required soon. The patient could leave hospital on day 28 and he had a good QOL. On day 90, the WBC count was 5000/μL with 28% blasts, and Hb and plt were stable; furthermore, the spleen was not palpable. Although generalized edema and pleural effusion occurred as side-effects of imatinib on day 110, they improved with furosemide. However, on day 130, the number of WBC gradually increased,

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LETTERS TO THE EDITOR

# Gastrointestinal hemorrhage and antithrombotic drug use in geriatric patients

Dear Editor,

Recent guidelines recommend the aggressive use of antithrombotic medications in patients at high risk of thrombotic events. Although the risk of thrombosis increases with age, critical bleeding related to antithrombotic drug use is frequently seen in older patients.[1] Thus, guideline-directed use of antithrombotic medications might cause more harm than benefits among older patients with multiple comorbid conditions.[2,3] To increase the benefit-to-harm ratio, geriatricians might take care to stratify the risks and totally manage the patients. We hypothesized that such geriatricians' approaches lead to harmless use of antithrombotic medications. For this purpose, we carried out a case–control study to investigate the association between gastrointestinal hemorrhage and antithrombotic drug use.

We analyzed the inpatient registry of the Department of Geriatric Medicine, University of Tokyo Hospital between 1996 and 2007 (2249 patients) to identify patients ≥60 years-of-age who were admitted to the department as a result of gastrointestinal hemorrhage. The database was searched using the keywords of gastrointestinal hemorrhage, melena, hematemesis and anemia. Then, medical records of the extracted patients were reviewed. Finally, a total of 47 patients were defined to fulfil the criteria. Next, using risk-set sampling, we selected four controls per case matched for age, sex and the timing of hospitalization from the same inpatient registry. The data were obtained on prescriptions of antithrombotic drugs (aspirin, warfarin, cilostazol and ticlopidine) and anti-ulcer drugs (proton pump inhibitors and H2 blockers), and comorbid conditions.

Among the cases, causes of gastrointestinal hemorrhage were ulcer (48.9%), cancer (8.5%), ischemic colitis (6.3%), colon diverticulum (4.2%), Mallory–Weiss syndrome (4.2%) and hemorrhoid (2.1%), and 21.2% remained uncertain. As shown in Table 1, 17 cases and 71 controls were taking antithrombotic drugs. Of them, aspirin was most frequently prescribed both in case and control groups. There was no significant difference between case and control groups in the prescription rate of antithrombotic drugs ($\chi^2 = 0.20$, $P = 0.65$) and that of aspirin ($\chi^2 = 0.43$, $P = 0.51$). Furthermore, unadjusted logistic regression analyses showed that antithrombotic drug use and antiulcer drug use was not associated with gastrointestinal hemorrhage. The odds ratio of antithrombotic drug use for gastrointestinal hemorrhage was 0.91 (95% CI 0.46–1.81) after adjustment by age, sex and anti-ulcer drug use. Exclusion of the patients with cancer-related hemorrhage did not fundamentally influence the analytical results (data not shown).

This small case–control study showed no association of admission as a result of gastrointestinal hemorrhage with the use of antithrombotic drugs or aspirin among older patients. As most of the patients were managed by geriatricians in our department, the finding might be limited to the particular facility or cohort, but might not be extended to the general population. It is suggested, however, that geriatricians can make an appropriate decision on the indication and management of antithrombotic drugs for older patients. Although no studies have shown comparable findings in terms of gastrointestinal bleeding, geriatric evaluation and management has been reported to be effective to reduce serious adverse drug events.[4] A recent review on the management of antiplatelet agents[5] also recommended comprehensive strategies to reduce the risk of hemorrhagic complications. Prospective studies with a large sample size are required to confirm this issue. Nevertheless, it is certain that the use of antithrombotic

**Table 1** Age, sex and medication use in case and control subjects, and unadjusted odds ratios for gastrointestinal hemorrhage

| | Cases ($n = 47$) | Controls ($n = 189$) | Odds ratio (95% CI) |
|---|---|---|---|
| Age (years) | 78 ± 10 | 77 ± 9 | 1.02 (0.98–1.06) |
| Men (women = 0, men = 1) | 29 (61.7%) | 120 (63.5%) | 0.93 (0.48–1.79) |
| Antithrombotic drugs (no = 0, yes = 1) | 16 (34.0) | 71 (37.5) | 0.86 (0.44–1.68) |
| Aspirin (no = 0, yes = 1) | 10 (21.3) | 49 (25.9) | 0.77 (0.36–1.67) |
| Anti-ulcer drugs (no = 0, yes = 1) | 18 (38.2) | 45 (23.8) | 0.67 (0.35–1.29) |


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medi cations should be carefully determined by considering the risk/benefit balance of each patient.

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5 Kalyanasundaram A, Lincoff AM. Managing adverse effects and drug-drug interactions of antiplatelet agents. *Nat Rev Cardiol* 2011; **8**: 592–600.

# Pituitary insufficiency: A cause of hypoglycemia in an elderly diabetic patient

Dear Editor,

Hypoglycemia most likely occurs in the elderly as a result of poor glucose tolerance. The most common cause of hypoglycemia in elderly patients is antidiabetic drugs. Adrenal insufficiency, insulinoma and pituitary insufficiency are rare causes of hypoglycemia in older age.[1] Particularly in old patients, non-specific findings, such as weakness, fatigue and loss of appetite caused by pituitary insufficiency, might be attributed to aging.[2] Here, we reported an elderly patient with diabetes mellitus and hypopituitarism, presenting with refractory hypoglycemia and acute renal failure under therapy with oral antidiabetic drugs.

A 67-year-old woman was referred to geriatric clinic with symptoms of confusion, irritability, slowness of speech and movements, loss of appetite, nausea, and vomiting. A physical examination of her vital signs showed blood pressure 80/50 mmHg, pulse rate 104/min, body temperature 37.7°C and respiration 24/min. The patient was lethargic with incomplete cooperation (Karnofsky performance score of 30%). She had been taking metformin 2000 mg/day and gliclazide 30 mg/day with the diagnosis of diabetes for 2 years. In the biochemical examination, blood glucose, blood urea-nitrogen, creatinine, sodium and potassium were 32 mg/dL, 60 mg/dL, 3.2 mg/dL, 132 mmol/L and 4.9 mmol/L, respectively. After she was admitted to the geriatric clinic, her glucose infusion was given. Our initial evaluation of the clinical and laboratory parameters suggested that it could be acute renal failure as a result of dehydration and hypoglycaemia, which were the consequence of the prolonged effect of gliclazide. For this reason, oral antidiabetic drugs were discontinued, and glucose infusion was carried out. During her clinical follow up, we realized that her kidney functions had substantially increased. However, hypoglycemia persisted. Afterwards, all of the persistent hypoglycemia, hyponatremia and hypotension were evaluated, and the results were considered to be hypocortisolemia. The patient's other laboratory results, which were obtained during a hypoglycemia period, are presented in the Table 1. The basal serum cortisol (1.38 μg/dL) and adrenocorticototropic hormone levels (less than 0.3 U/L) showed strong evidence of cortisol deficiency. Due to these results, pituitary insufficiency was diagnosed. However, magnetic resonance imaging and magnetic resonance angiography did not show any structural or vascular abnormalities in the hypophysis and brain. Once prednisolone (7.5 mg/day) treatment

**Table 1** Endocrinological laboratory results

| Parameters | | Normal range |
|---|---|---|
| Blood cortisol | 1.38 ug/dL | 6.2–19.4 ug/dL |
| TSH | 0.055 uIU/mL | 0.4–4.2 uIU/mL |
| Free T4 | 13.24 pmol/L | 10.3–23.2 pmol/L |
| IGF-1 | 1.00 mg/L | 1.73–5.11 mg/L |
| GH | <3 μg/L | |
| PRL | 0.57 ng/mL | 3–20 ng/mL |
| FSH | 2.02 mIU/mL | 25.8–134.8 mIU/mL |
| LH | 1.36 mIU/mL | 7.7–58.5 mIU/mL |
| Estradiol | 27.96 pg/mL | 5–54.7 pg/mL |
| C peptide | 1.02 ng/mL | 0.9–7.1 ng/mL |
| Insuline | 2.83 μU/mL | 3–28 μU/mL |

All the laboratory results were measured between 08.00 hours and 09.00 hours, and confirmed by a second determination.
FSH, follicule stimulating hormone; GH, growth hormone; IGF1, insulin-like growth factor-1; LH, luteinizing hormone; PRL, prolactin; TSH, thyroid stimulating hormone; T4, thyroxine.

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