Table 1
Baseline characteristic of patients on registration

Characteristics $n = 127$			
Profiles		Chronic medications	
Age (years old)	81.31 ± 7.48	Angiotensin converting enzyme inhibitor	24
Male	48	Angiotensin II receptor antagonist	18
Female	79	β-Blocker	9
		Ca antagonist	37
Medical history			
Hypertension	57	Diuretics	29
Hyperlipidemia	27		
Diabetes mellitus	36	Nitrate	29
		Digitalis	16
Prior congestive heart failure	24		
Ischemic heart disease	21	Antiplatelet agents	49
Arrhythmia	23	Warfarin	8
Stroke	51	Statins	16
Arteriosclerosis obliterans	6		

enrolled. Their complicating diseases were hypertension (44.9%), hyperlipidemia (21.3%), diabetes mellitus (28.3%), congestive heart failure (18.9%), ischemic heart disease (16.5%) and cerebral vascular disease (40.2%, including patients with only lacuna). Table 1 also shows the chronic medications of patients. Anti-hypertension drugs ⟨angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blocking agents (ARBs), β-adrenergic receptor blocking agents and calcium antagonists⟩, diuretics, antiplatelet agents, and nitrates [isosorbide dinitrate 20 mg/day, $n = 14$, 40 mg/day $n = 3$, nitroglycerin patch 25 mg/day $n = 12$] were prescribed frequently.

Survival

During the 2.8-year follow-up period, 46 of the 127 patients died (Table 2). Seventeen, seventeen, and twelve patients died during the 1st, 2nd, and 3rd year of the follow-up period, respectively. The causes of death were as follows: cardiovascular disease ($n = 15$ patients: 6 cases of heart failure, 3 of acute myocardial infarction, 4 of renal failure related to cardiovascular disease, and 2 of cerebro-vascular disease), infection ($n = 13$), malignancy ($n = 2$), cardiopulmonary arrest without evident cause ($n = 6$), liver cirrhosis ($n = 1$), intestinal twist ($n = 1$), dehydration ($n = 1$), senility ($n = 1$), burned to death ($n = 1$), and unknown ($n = 5$). Four patients cannot be followed by medical record or telephone contacting. There are no significant differences in gender ratio (male/female) between participants being alive after 2.8 years and those dead during 2.8 years. There are also no significant differences between them in the drugs or the suffered diseases including ischemic heart disease, congestive heart failure, and smoking.

Table 2
Biochemical characteristic of patients on registration

	On registration ($n = 127$)	Participants being alive after 2.8 years ($n = 77$)	Participants dead during 2.8 years ($n = 46$)	*P* value
Hemoglobulin (g/dl)	11.6 ± 1.9	12.2 ± 1.7	10.7 ± 1.8	.0001
Total protein (g/dl)	7.04 ± .71	7.15 ± .69	6.84 ± .69	.0129
Albumin (g/dl)	3.83 ± .52	3.98 ± .42	3.56 ± .55	.0001
Total cholesterol (mg/dl)	197.8 ± 46.0	210.6 ± 45.3	178.1 ± 41.4	.0003
HDL-cholesterol (mg/dl)	50.1 ± 20.5	53.2 ± 20.1	44.6 ± 19.7	.0078
LDL-cholesterol (mg/dl)	123.8 ± 35.2	131.9 ± 35.9	111.7 ± 31.7	.0038
Triglyceride (mg/dl)	113.3 ± 62.3	114.6 ± 55.7	111.9 ± 74.4	.2577
Creatinine (mg/dl)	.88 ± .6	.80 ± .38	.94 ± .69	.4518
cGMP (pmol/ml)	4.89 ± 3.2	4.74 ± 3.2	5.40 ± 3.0	.1114
Hs-CRP (ng/ml)	8224.9 ± 17224	6493.1 ± 15669	11123.7 ± 19391	.0150
HANP (ng/ml)	43.1 ± 56.3	38.1 ± 36.7	52.5 ± 79.4	.2673
BNP (ng/ml)	79.1 ± 81.1	71.3 ± 81.6	92.6 ± 77.9	.0565
NO_x (μmol/l)	28.3 ± 19.3	25.1 ± 18.4	33.7 ± 19.8	.0059
Norepinephrine	541.0 ± 439.7	493.0 ± 431.7	619.2 ± 478.4	.3103
Angiotensin II	9.03 ± 8.90	8.74 ± 7.78	9.50 ± 10.57	.7047
Il-6 (pg/ml)	7.81 ± 10.0	7.17 ± 9.6	8.99 ± 11.0	.0174
TNF-α (pg/ml)	1.94 ± 6.021	2.177 ± 7.7	1.58 ± .89	.0001

Values are expressed as means ± SD. $P < .05$: significant difference between alive and dead. Hs-CRP: high sensitive C reactive protein, HANP: human atrial natriuretic peptide, BNP: brain natriuretic peptide, NO_x; NO metabolites.

Biochemical analyses

The biochemical characteristics of all participants are shown in Table 2. The age, hemoglobin, albumin, total cholesterol, HDL cholesterol, and LDL cholesterol levels on registration were significantly higher in those who survived than in those who died during the study period. Gender or creatinine was not significant in this study.

Markers profile

The plasma concentrations of the various makers are also depicted in Table 2. Plasma NO_x and IL-6 were significantly lower in those who were alive after the study period than in those who died during the period. Plasma TNF-α was also higher in the survivors than in the patients who died. BNP level on registration was not different significantly ($P = .0565$).

Multivariate survival analyses

Logistic regression was applied to analyze significant characteristics: age, hemoglobin total protein, albumin, total cholesterol, HDL-cholesterol, LDL-cholesterol, IL-6, TNF-α, and NO_x, which showed significant differences between the survivors and the deceased by using Mann–Whitney's *U*-test, which was described as above. They were subjected to logistic regression analysis. The results are depicted in Table 3. The adjusted odds ratios of albumin and NO_x were .236 and 1.027, respectively. Based on this result, 1SD decrease of albumin level from a certain level is estimated to be 2.11 in odds ratio. 1SD increase of NO_x level from a certain levels is to be 1.67 in odds ratio. As the effect of albumin and that of NO_x are independent, the odds ratio increases synergistically. NO_x was shown to correlate the serum hemoglobin, HDL-cholesterol, creatinine, and HS-CRP on baseline level, and NO_x level was known to be affected by age, gender, and creatinine (renal function). So, we thought creatinine and gender as independent risk factor, and re-applied logistic regression including creatinine and gender as well as the conditions above. The result is almost same as before that albumin and NO_x are significant, and that the adjusted odds ratios of albumin and NO_x were .195 ($P = .045$) and 1.031 ($P = .034$) respectively.

Table 3
Multivariate logistic regression analysis

	Adjusted odds ratio	95% CI	*P* value
Age (year)	1.047	.977–1.122	.194
Hemoglobulin (g/dl)	.766	.562–1.044	.0917
Total protein (g/dl)	.997	.458–2.170	.9932
Albumin * (g/dl)	.236	.058–.955	.0429
Total Cholesterol(mg/dl)	.994	.949–1.042	.8083
HDL-Cholesterol (mg/dl)	1.000	.948–1.055	.9972
LDL-Cholesterol (mg/dl)	.993	.943–1.046	.7988
Hs-CRP (ng/ml)	1.001	1.000–1.002	.0924
NO_x * (μmol/l)	1.027	1.010–1.541	.0394
Il-6 (pg/ml)	.975	.904–1.051	.5072
TNF-α (pg/ml)	.967	.727–1.288	.8197

Values are expressed as means ± SD. $P < .05$: significant difference between alive and dead. Hs-CRP: high sensitive C reactive protein, HANP: human atrial natriuretic peptide, BNP: brain natriuretic peptide, NO_x; NO metabolites.
CI: confidence interval. * means the significant marker ($P < .05$).

These data showed that NO is a useful prognostic marker with efficacy almost equal to that of albumin, the best-known prognostic marker to date. BNP and other cytokines such as IL-6 or TNF-α were not significant prognostic markers in the present study. Further, in the patient who died by cardiovascular diseases, multivariate survival analyses showed that NO_x and the history of ischemic heart disease were significant prognostic markers. In patients who died by other causes than cardiovascular diseases, NO_x and albumin were significant markers.

Survival rate

The survival rates are shown in Fig. 1. Panel (A) shows the relation between survival rate and NO_x levels on entry, and panel (B) shows the relation between survival rate and albumin levels on entry. The survival rate goes up in proportion to NO_x levels and goes down in proportion to albumin levels. Kaplan–Meier analyses of mortality show that the prognosis of patients was dependent on NO_x levels. The prognosis of patients in 3rd and 4th quartile of NO_x was significantly worse than that in 1st or 2nd quartile of NO_x. The prognosis of patients in first quartile of albumin is worse than that of other patients, which there is no difference in prognosis between 2nd, 3rd or 4th quartile of albumin. In other words, NO_x levels reflect the viability of all patients; however,in albumin level, the patients in 4th quartile only have worst prognosis.

Discussion

The present data demonstrate that NO is a new clinical biomarker of survival in elderly patients and that its efficacy might be nearly equal to that of albumin, the best known prognostic marker to date. This is the first report of the use of a vascular functional marker such as NO as a prognostic marker in the elderly.

We assessed for around 3 years prognosis to expect short and mid term prognosis. Because elderly patients might have many illnesses—e.g., occult congestive heart failure, arteriosclerosis, latent malignancy, etc.—we evaluated nutrition markers, pro-inflammatory cytokines, natriuretic peptide and vascular endocrinological substances.

Serum albumin and cholesterol levels were reported as prognostic marker and it was reported recently that elderly individuals with low cholesterol constitute a heterogeneous group with regard to health characteristics and mortality risk [1–3]. It is needless to say that inflammatory markers or cytokines are valuable, but these substances are often

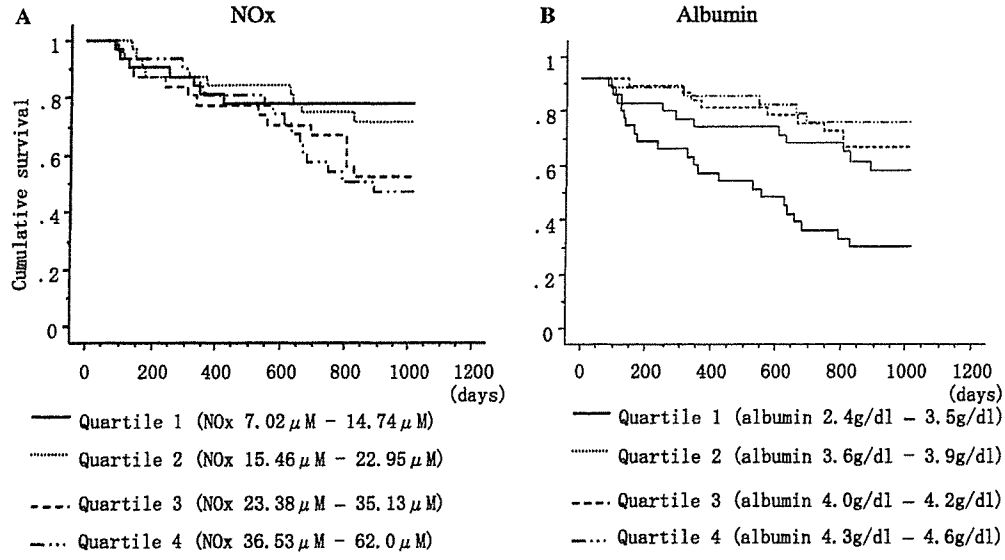

Fig. 1. Kaplan–Meier survival analysis. Circulating levels of NO_x (A) and albumin (B) were examined in relation to patient survival during follow-up. For this analysis, circulating levels of NO_x and albumin were arbitrarily divided into quartiles on registration.

affected by acute infections or chronic collagen disease such as rheumarthritis, which is very common in elderly people.

The present study revealed that low albumin, low cholesterol, high IL-6 and high sensitive-CRP indicate poor prognosis. However, logistic regression analysis indicated that only albumin and NO_x were predictive.

NO is produced by L-arginine and NO synthases (eNOS in endothelial cells, iNOS mainly in inflammatory cells, and nNOS in the nervous system). Atherosclerosis is an inflammatory disease [25] characterized by vascular endothelial cell dysfunction and diminished production of NO [26]. eNOS gene transfer can reduce atherogenesis in hypercholesterolemic animals [27]. NO is a widespread signaling molecule in the cardiovascular system, and functions in multiple ways to protect against the progression of atherosclerosis [28–30]. Plasma NO_x was difficult to assay and estimate, because protein of plasma affects the NO_x value. HPLC developed for measurement of NO_x specially is convenient and seemed to be accurate and reliable in those points. Plasma concentrations of NO_x [31] are higher in patients with CHF, and NO_x concentrations may vary according to the severity of heart failure [32]. Both the natriuretic peptide family and NO mediate their physiological action through a second messenger, cGMP [33–35]. NO increases production of cGMP by activation of soluble guanylate cyclase [33–35], while the natriuretic peptide family increases production of cGMP by activation of particle guanylate cyclase. In the present study, NO_x increased not only in the patients with heart failure but also in the patients with other atherosclerotic diseases.

In animal experiments including ours, the mRNA and protein of eNOS were increased in atherosclerotic vessels [36]. Further, coronary risk factors such as hyperlipidemia were also shown to increase mRNA of eNOS of vessels. In plasma drawn from vein, it is possible that NO_x amount increased because of high eNOS in vessels, although arterial endothelial dysfunction occurred because of intimal thickening and increased reactive oxygen species intra arteries. Taken together, we speculate NO_x levels reflects pre-clinical and clinical situation of both the vessels (atherosclerosis induced ischemia etc.) and heart (heart failure etc.) and thus NO_x was most sensitive in this study.

There were no patients with sepsis or advanced malignancy at registration. No patients died within the 3 months after registration, and the maximum levels of NO_x were at most 3 times the mean, indicating the less contribution of iNOS in this study and NO_x levels principally reflect eNOS derived one. In fact, we observed iNOS only in some macrophage derived foam cells and T lymphocytes in the peripheral of necrotic core of advanced atherosclerosis, not in mild or moderate atherosclerosis or vein [37]. Although the detailed mechanisms should be elucidated, a continuous increase in NO was suggested to have a deteriorating effect on the prognosis and might be used to predict the prognosis.

Study limitations

We measured the plasma NO_x level in order to assess basal NO production. We were afraid that plasma NO_x concentration might be affected by exogenous NO sources such as diet or drugs (NO donors or eNOS activator like angiotensin converting enzyme inhibitors; ACE-I) [38,39]. We collected fasting blood samples. We evaluated the effect of NO donors (nitroglycerin etc.), eNOS activators (ACE-Is etc.) and patients' diseases on plasma NO_x level. The Mann–Whitney's *U*-test showed no significant effects (ACEI .219, HMG-CoA reductase inhibitor .391, NO donors .291, ischemic heart diseases .307 etc). The effect of drugs may be masked between elderly patients because of

individual difference of plasma NO_x levels by other factors such as severity of diseases [40,41]. These data are consistent with previous data, however, we should suppose the effect of aging on renal function, which increase NO_x levels. The number of participants is small and it should be elucidated more for larger participants in future.

Conclusively, this study first suggests the importance of the NO related responses in the prognosis of elderly, which is as strong as that of albumin, past well-known marker. Vascular factor might be important as much as nutritional factor in elderly.

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