厚生労働科学研究費補助金

臨床研究基盤整備推進研究事業

各種高脂血症治療薬の糖尿病性心血管病進展予防効果の総合的検討に関する研究
(若手医師・協力者活用に要する研究)

平成18年度総括研究報告書

主任研究者　　井口昭久

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主任研究者　井口　昭久（名古屋大学大学院医学系研究科老年科学教授）

研究要旨　各種高脂血症治療薬の糖尿病性心血管病進展予防効果と作用機序を検討した。全体研究としては、主任研究者として、代謝内分泌学,循環器学,老年学,臨床薬理学専門医14名,12施設,40関連病院からなる研究班を結成した。2)17年3月末までに自立している糖尿病患者(4014名)を登録した。3)事務局として全登録患者を集計し、prospective cohort試験として,虚血性心疾患発症,死亡/同入院,CVD,ASO発症総死亡をエンドポイントに検討した。75.9%の脂質異常者を認めた。18年12月に登録後平均1.9年間の成績を回収し、解析をすすめた(追跡率初年度98.8、２年度92%)。心血管病発症率(虚血性心疾患IHD,脳血管障害CVD)は全糖尿病例では年2.2%と比較的高かった。 4)また、そのうち名古屋大学にて糖尿病患者215例、名古屋地区関連病院で糖尿病426例を登録し、上記研究に主体的に参加した。初年度イベント発症率は名古屋大学にてIHD2.8%, CVD1.4%、その他0.5%と高率であり、２年度イベント発症率はIHD2.4%, CVD1.5%、その他0.5%、登録者が高齢（平均74歳、ADLは自立）である事が関与していると推測された。関連病院では、各々0.2%、0.9%。0.7%で有った（平均年齢63歳）　現在, I)(本邦又は欧米の)血清脂質管理値達成によるイベント予防効果,II)高脂血症病態(メタボリック症候群,閉経等)による差異,III)新規高脂血症薬の安全性と多面的作用、IV)医療経済効果を検討している。糖尿病合併高脂血症薬の使用基準提示を目標とする。7)個別項目では糖尿病患者における認知症発症にTNF α とLDLcholesterol が関与している事、surrogate markerとして血中のNO代謝物濃度が有意である事を見いだした。

A. 採択された研究事業での研究概要

背景）本邦においては糖尿病罹患者が増加しており,高脂血症合併例の増加及び心血管合併症のリスクとしての大きさが注目されている。加齢そのものによっても高脂血症患者の頻度は増大する。糖尿病性心血管病変は耐糖能異常の段階から進行し,長期罹患者が増加している。糖尿病患者の死因としては心血管合併症によるものが最も多く予防法確立が急務である。一方、糖尿病合併高脂血症の治療効果は血糖降下療法を凌駕する可能性も欧米の大規模臨床試験で報告され、日本動脈硬化学会は糖尿病罹患者は血清LDL-Cholesterolの管理目標値をB３以上 120　mg/dl以下としている。さらに米国では100mg/dl以下と推奨している。さらに、スタチン製剤をはじめとする高脂血症薬には血管への直接作用がある可能性が報告されている。 複数の生活習慣病を合併する患者,心・脳血管障害合併者の増加に伴う治療方策が必要となっている。本研究は代謝内分泌学,循環器学,老年学,臨床薬理学医により研究班を結成し、エビデンスに基づく高脂血症合併糖尿病心血管病予防指針策定を目標とする。当該研究では名古屋大学を中心とし、一部関連病院での症例もあわせて登録し経過をフォローし、全体研究の中心となるように努力した。

B. 採択された研究事業での研究実績

対象は一昨年度登録した、全国12ケ所,40関連病院の共同研究機関より,当初計画より多い**糖尿病罹患者**4014名である。内、名古屋大学にて糖尿病患者215例、名古屋地区関連病院で糖尿病患者426例を登録し、上記研究に主体的に参加した。原則として外来通院者等の自立した成人で２型糖尿病患者で、上記の対象には心筋梗塞,脳梗塞罹患者も含めていない。特に名古屋大学では付属病院老年科で自立した高齢者を多く

含めた。全体のプロフィールは糖尿病群では平均年齢(64.5歳),男女比(1.12), HbA1C7.2%, TC 206.3,TG 144.1, HDL-C 55.5 mg/dlであった。一方、名古屋大学では平均年齢74.1歳(ADLは原則自立)、男女比0.78、HbA1C6.9%, TC209.1mg/dlであった（以下集計中)。名古屋地区関連病院で糖尿病426例を登録し他（平均年齢63歳)。当該年度より年齢階層別,性別, 薬剤別＜スタチン製剤(約84%),フィブラート製剤(9%)等＞、更に到達脂質濃度別(日本動脈硬化学会基準達成度, 総コレステロール値で32.2%)に各々分類しnested case control cohort studyとして評価検討を行っている。虚血性心血管病(心, 脳血管障害,ASO)発症,入院等をend pointとし, 一般所見, 脂質等の冠危険因子治療経過を追った。75才以上の高齢者（登録時自立）は自立度の変化も評価する事とした。初年度イベント発症率は全体研究では、部分集計(糖尿病2748例)では2.1%で、従来の高脂血症単独が対象の本邦の研究成績より高率であった。また、名古屋大学では、脳血管障害1.4%、虚血性心疾患2.8%、その他0.5%と高率であり、登録者が高齢である事が関与していると推測された。関連病院では、各々0.2%、0.9%。0.7%で有った。薬剤効果は現在解析中であるが、LDL濃度が低い場合(120mg/dl未満)及び高い場合(140mg/dl以上)に同一濃度範囲であってもスタチン使用者のIHD+CVD(2年間の解析からは特にIHD)罹患率が低い事が見いだされた。医療経済学的解析も施行し、概略的推計では現行のLDL濃度(平均120mg/dl)を90mg/dlに下げると, 虚血性心疾患発症率,10年後の罹患者総数とも約40%減少する可能性が示唆された。さらに脳血管障害も発症率を約24%, １０年後罹患者数を約25%減少させる可能性が示唆された。個別研究は, 血管内皮機能,TNFα,NO代謝物等のバイオマーカー,インスリン抵抗性,痴呆発症等との関係を検討し成果が出ている。安全管理モニター(名大鍋島, 浜医大中島両教授)の管理を頂いている。

（倫理面への配慮）

いずれの施設でも、研究対象者となる協力者に対してインフォームドコンセントを徹底し、協力者の利益が損なわれる事がないように十分に留意した。本研究は名古屋大学医学部附属病院をはじめ共同研究者が所属する施設の倫理委員会に申請,承認後に施行されている。被験者には同意を書面で頂き, いつでも取り消しが可能である事を明記し,認知機能障害のある方は対象外としている。プライバシーは匿名化を行い個人名が特定化されないよう細心の注意をはかっている。

C. 考察

本研究の意義は具体的な糖尿病、高脂血症の治療指針の策定にあるが、更に、長寿社会,日本で増加する生活習慣病自体の合併、心及び脳血管障害予防は、総合診療学、老年科学の領域でも重要と考え、代謝内分泌学、循環器学、老年学、臨床薬理学の専門家により,研究班を結成した。

具体的な成果及び今後の発展は全体研究では、１）糖尿病患者の重症度別評価に加え、高脂血症患者はメタボリック症候群罹患者,前期高齢者,閉経後女性（閉経後脂質上昇）等の層別の、目標脂質濃度、推奨薬剤を設定できる可能性を探る。当該研究で明らかになりつつ有るのは、糖尿病罹患者の血糖コントロールは高齢者ではむしろ良好に推移している（加齢による腎機能低下の影響か）点であり、血清脂質コントロールの意義がイベント数の現れる可能性がある。prospective cohort とい強力な手段をとり、全体の症例数を 4000 まで増やした事で、イベントに対する各種高脂血症薬の単独作用と、脂質低下作用におうところを直接, 間接作用として解析できる可能性が示唆されている。一方, 実態としては欧米はおろか本邦の学会ガイドラインでさえ４０%以下の準拠率である事が判明し、第２年度の班会議申し合わせが実行されればクロスオーバー試験的な作用が第３年度に期待される可能性もある。特に糖尿病合併高脂血症患者の心脳血管イベント発症率は部分集計では 2.1%強に上り, 特に名古屋大学では 4.7%と高率で、昨年末報告された MEGA,JELIS の約 0.5%に比し,リスクの大きさ,逆に言えば制御する必要性が示唆される。個別報告にも有るように

スーパースタチンは単剤でも目標値達成の可能性があるが，部分集計では50%前後に留まった。適応症例がかなり重症高脂血症患者に偏っている可能性も示唆される。2）脳血管障害は,脂溶性スタチンにのみ効果を認める可能性があり検討している。3）第２年度は、医療経済学者、疫学統計学者を班員に加えたため上記にみとめられる解析法が選択された。また医療経済学的にはMEGA studyと当該研究の医療経済効果の比較をお願いしている。4)第３年度は各エンドポイントを中心に本格的解析を進め、年々市場規模が増大している高脂血症薬の効果的,効率的な投与方法を提言する。個別研究では高齢者の自立度及びQOL改善に対する高脂血症薬治療の有効性の可能性を探りたい。バイオマーカーの分析により、高リスク群のスクリーニング及び治療効果の判定に応用したい。高脂血症薬の作用機序として、脂質低下作用に加え、NO利用化による血管内皮機能改善を直接的抗動脈硬化作用の一つとして推測しており、広義の分子標的治療薬としての可能性やテロメラーゼ等、老化関連酵素への関与の可能性も探りたい。

D. 健康危険情報

現在のところは認めない。

E. その他実施した臨床研究・治験の概要及び実績

厚生労働科学研究　難治性疾患克服研究事業原発性高脂血症に関する調査研究：高齢者糖尿病合併複合型高脂血症の実態調査（212例）

F. 研究発表

（1） 論文発表

2006年の私の業績と

梅垣君の業績を入れて下さい。

G. 知的財産権の出願、登録状況

特になし

臨床研究実施チームの組織

（1）臨床研究実施チーム（a組）

	①若手医師及び臨床研究協力者に対する指導者	②若手医師	③臨床研究協力者
氏　　名	林　登志雄	鈴木　麻里	平井　寿子
分担する研究項目	診療,同意取得、検査,群分け処方	診療,同意取得検査,データ解析	検査、データ解析
最終卒業学校・卒業年次・学位及び専攻科目	信州大学医学部医学科昭和59年卒・医学博士・老年科学	愛知医科大学医学部医学科平成12年卒・医学士・老年科学	岐阜大学農学部生命科学科修士平成10年卒・農学修士・老年科学

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Nitric oxide (NO) is a new clinical biomarker of survival in the elderly patients and its efficacy might be nearly equal to albumin

Masako Osawa [a], Toshio Hayashi [a,*], Hideki Nomura [a], Jun Funami [a], Asaka Miyazaki [a], Louis J. Ignarro [b], Akihisa Iguchi [a]

[a] *Department of Geriatrics, Nagoya University Graduate School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya City 466-8550, Japan*
[b] *Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, 650 Charles E. Young Drive South, Los Angeles, CA 90095, USA*

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Abstract

Background: For elderly patients, the consideration of prognostic factors is very important, but there have been few reports about the potential use of vasoactive substances as prognostic markers in the elderly.

Objective: We assessed endocrinological substances, such as plasma NO_x (metabolites of NO), as the prognostic marker in elderly. We compared their efficacy with that of such well-known markers as albumin and pro-inflammatory cytokines such as IL-6.

Methods: The patients were recruited consequently from the clinics of Nagoya University Hospital or related home care services facilities. One hundred and twenty seven elderly aged 65 and older were registered. Biochemical analyses such as albumin, total cholesterol, BNP, and NO_x were measured upon enrollment. The main outcome was the survival rate.

Results: Forty-six patients died during the follow-up period. Mann–Whitney's *U*-test showed that the levels of age, hemoglobin, total protein, serum albumin, serum creatinine, total cholesterol, HDL-cholesterol, LDL-cholesterol, high sensitive CRP, NO_x, IL-6, and TNF-α were significantly different between the living and deceased subjects. Among the dependent variables in the logistic regression analyses, only albumin and NO_x were significantly different. In the Kaplan–Meier analyses of mortality, the prognosis of patients in 3rd and 4th quartile of NO_x was significantly worse than that in 1st or 2nd quartile.

Conclusion: NO_x has potential both as a vascular marker and as a marker for predicting survival in elderly. In the latter role, it may be as effective as albumin.

Keywords: Nitric oxide; cGMP; Albumin; Biomarker; Elderly; Prognostic marker; Vascular functional marker

Many nations, including Japan, are experiencing rapid growth in their elderly populations. The main causes of death in Japanese elderly are heart disease, cerebro-vascular disease, and cancer. Several biochemical markers, such as albumin and cholesterol, have been identified as having prognostic value for mortality and hospitalization [1–3]. Recent studies also have indicated the potential role of the immune system in the pathophysiology of congestive heart failure (CHF) and malignancy [4,5]. Plasma levels of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) also have been reported to be significant prognostic predictors in patients with CHF or malignancy) [6–8]. TNF-α induces adhesion molecule expression such as ICAM-1 on endothelial cells, which promotes the progression of atherosclerosis [9]. In other words, in older populations, peripheral blood markers of nutrition or inflammation (albumin, cholesterol, IL-6, and TNF-α) have been individually shown to be increased risk for mortality [2,10,11].

In elderly people, the rate of CHF is important for predicting mortality and hospitalization rates. Brain natriuretic peptide (BNP) is a good marker of CHF, because the plasma BNP concentration is elevated according to the severity of CHF [12–15]. Binding of BNP to its receptors

* Corresponding author. Fax: +81 52 744 2371.
E-mail address: hayashi@med.nagoya-u.ac.jp (T. Hayashi).

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initiates natriuretic and vasorelaxant activities through an elevation in intracellular cyclic guanosine monophosphate (cGMP) [16,17]. Nitric oxide (NO) is also an important vasoactive substance, because it exerts anti-atherogenic effects by inhibiting the migration or proliferation of monocytes or smooth muscle cells and vasodilation mainly by cGMP dependent mechanism [18]. We reported that NO regulates cGMP in patients with renal insufficiency [19]. NO may be a useful prognostic marker for patients suffering from atherosclerotic diseases such as cerebral strokes or myocardial infarction, although as yet there have been no reports investigating the use of NO in this capacity. The source of NO is not only endothelial cells (endothelial NO synthase; eNOS) but also macrophages or T cells (inducible NO synthase; iNOS) and some neuronal cells (neuronal NO synthase; nNOS). The plasma level of NO_x (nitrite plus nitrate, metabolites of NO) may reflect the status of eNOS and, to some extent, the status of iNOS. Because iNOS is activated in patients with inflammations such as sepsis, advanced stages of malignancy, or progressed atherosclerotic lesions, the NO_x level may have potential as a marker of malignancy as well as atherosclerotic diseases [20,21].

For elderly patients, the consideration of prognostic factors is very important, but there have been few reports about the potential use of vasoactive substances. Therefore, in this study, we evaluated whether measurements of plasma levels of vasoactive factors such as NO_x, cytokines such as IL-6, and well-known markers such as albumin were useful as prognostic factors in the elderly.

Methods

Study sample

One hundred and twenty seven elderly subjects (48 males and 79 females; mean age, 81.3 ± 7.5 years; range, 65–101 years) were enrolled on August on 2002. The study was approved by the Ethics Committee of Nagoya University Graduate School of Medicine and written informed consent was obtained from all patients. Patients were selected consecutively among our geriatric clinics and related home care services. In brief, 91 participants were presented at Department of Geriatrics, Nagoya University Hospital and the related hospital as outpatients (31 from their homes, 31 from geriatric nursing care units, and 29 from other facilities such as private homes for the aged) and 36 were in home care services facility. At the baseline examination, participants underwent a review of their medical history, a physical examination, and assessment of cardiovascular disease risk factors. On registration, they were not suffering with acute or evident heart failure or acute inflammation whose serum.CRP is larger than 2 mg/dl. They were also not suffering with acute myocardial infarction or cerebral infarction within 3 months. We followed patients up to 2.8 years. All participants had a clinical visit each year of the study period, and their laboratory data were determined at each of these visits. We had telephone contact with the patients who could not have clinical visit, or their physicians.

Measurement

We measured fasting serum or plasma levels of biochemical products including lipids and plasma levels of neurohumoral factors and cytokines. Levels of general biochemical products were measured at SRL Laboratories, Tokyo, on an automated sequential multiple analyzer. Samples for the assay of plasma norepinephrine (NE), angiotensin-II, BNP, NO_x, cGMP, IL-6, and TNF-α levels were transferred to chilled disposable tubes containing EDTA-2Na. The blood samples were immediately placed on ice and centrifuged at −4 °C, and aliquots of plasma were immediately stored at −80 °C until assay. BNP levels were measured with a specific radioimmunoassay. NE levels were measured by HPLC. NO_x levels were measured using an NO detector-HPLC system (ENO10; Eicom Co., Kyoto, Japan) [22]. cGMP concentration was determined using a specific radioimmunoassay method (RPN226; Amersham, Buckinghamshire, England) [23]. Angiotensin-II levels were measured by radioimmunoassay. Both IL-6 and TNF-α measurements were performed using a commercially available radioimmunoassay kit (Quantikine HS; R&D Systems, Minneapolis, MN). Hypertension was defined as systolic BP ⩾ 140 mmHg, or diastolic BP ⩾ 90 mmHG or antihypertensive drugs were prescribed. Hyperlipidemia was defined as follows. Total cholesterol ⩾ 220 mg/dl or LDL cholesterol (total cholesterol − HDL cholesterol − triglyceride/5) ⩾ 140 mg/dl) or anti-hyperlipidemic drugs were prescribed. Diabetes mellitus was defined as in American Diabetes Society Guidelines [24] (in brief, fasting blood glucose ⩾ 126 mg/dl or hemoglobin A1C ⩾ 6.5 g/dl). Previously diagnosed hypertension, hyperlipidemia or diabetes were also included.

Statistical analysis

The results are presented as means ± SD. Values of $P < .05$ were considered to indicate statistical significance in all analyses. All statistical analyses were performed using Stat View software (SAS Institute Inc., Cary, NC). Characteristics of the survivors and the deceased subjects were compared using Mann–Whitney's *U*-test. Characteristics that were significantly different between the survivors and deceased by Mann–Whitney's *U*-test were further subjected to inherent multiple logistic regression analysis. As a result, adjusted odds ratios were calculated. Survival curves were calculated by the Kaplan–Meier method.

Results

Clinical characteristics

Table 1 shows the baseline characteristics of patients. There were no significant differences in age or coronary risk factors among the situations where the patients were

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

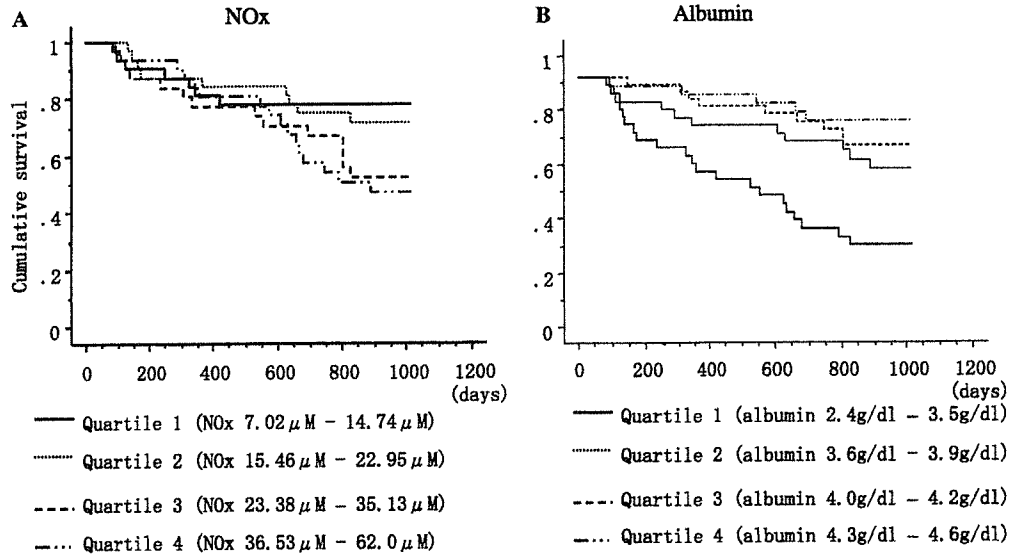

Fig. 1. Kaplan–Meier survival analysis. Circulating levels of NO_x (A) and albumin (B) were examined in relation to patient survival during follow-up. For this analysis, circulating levels of NO_x and albumin were arbitrarily divided into quartiles on registration.

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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