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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

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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

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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 $-4\,^{\circ}C$, and aliquots of plasma were immediately stored at $-80\,^{\circ}C$ 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 $\geqslant 140$ mmHg, or diastolic BP $\geqslant 90$ mmHG or antihypertensive drugs were prescribed. Hyperlipidemia was defined as follows. Total cholesterol $\geqslant 220$ mg/dl or LDL cholesterol (total cholesterol $-$ HDL cholesterol $-$ triglyceride/5) $\geqslant 140$ mg/dl) or anti-hyperlipidemic drugs were prescribed. Diabetes mellitus was defined as in American Diabetes Society Guidelines [24] (in brief, fasting blood glucose $\geqslant 126$ mg/dl or hemoglobin A1C $\geqslant 6.5$ g/dl). Previously diagnosed hypertension, hyperlipidemia or diabetes were also included.

Statistical analysis

The results are presented as means $\pm$ 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