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Research Article

# A Challenge Identifying Essential Factors for Avoiding Diseases from the Confirmed Information of Survival / Death Using Community Health Check-ups Data: Focusing on BNP, $FLC_S$, HbA1c, and LDL-C

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## Abstract

**Purpose:** We aimed to identify critical factors for long-term health maintenance and disease prevention by analyzing biomarkers and health data from community health check-ups. **Methods:** We analyzed data from 1,105 residents participating in a community health examination in August 2007. After applying propensity score matching to adjust for age and gender, we selected 400 participants (200 men and 200 women) for detailed analysis. We utilized logistic regression to identify mortality risk factors based on survival data collected 11 years post-examination. **Results:** Our analysis revealed that Brain Natriuretic Peptide (BNP), free light chains ($FLC_L$), and glycated haemoglobin (HbA1c) are significant mortality risk factors. We found that BNP is linked to heart failure, HbA1c to diabetes, and $FLC_L$ to immune function. Higher levels of these biomarkers are associated with increased mortality risk, highlighting their predictive value. **Conclusion:** We believe incorporating these biomarkers into routine health check-ups is essential for enhancing early detection and prevention of chronic diseases. By identifying high-risk individuals through these measures, healthcare providers can implement timely interventions promoting healthier aging and longevity. Our study underscores the necessity of proactive health management strategies to mitigate risks associated with chronic conditions, ultimately contributing to better health outcomes in the population. We are confident that integrating these biomarkers into health assessments can surely help in preventing undiscovered diseases and improve overall public health.

**Keywords:** BNP; FLCs; Chronic inflammation; Asymptomatic cardiac target organ damage (TOD); Asymptomatic cardiovascular disorder

## Introduction

Predictors of health and disease play a crucial role in long-term health maintenance and the development of preventive medicine. According to statistics from the Japanese Ministry of Health, Labour, and Welfare in 2023, malignant neoplasms (cancer), cardiovascular diseases, and lung diseases remain the leading causes of death, among which cancer accounts for about 30% of all deaths, and heart disease keeps a high proportion [1]. In addition, the incidence of diabetes continues to increase, and diseases related to chronic inflammation are also on the rise. Under these circumstances, preventive medicine and early diagnosis are becoming more and more important. Survival studies based on follow-up after health check-ups and early detection of diseases through subsequent detailed examinations are highly significant in the real world. In the future, it is necessary to expand more active education to inform the importance of health check-ups to residents as a practical strategy, such as efforts to prevent diseases by estimating asymptomatic diseases and predicting survival probability using rapidly evolving AI technology based on longitudinal health check-ups data.

### Purpose of the Study

Our study aimed to identify factors contributing to long-term health maintenance and disease prevention using physical findings, biomarkers, and other examination data from community health examinations and subsequent survival confirmation data and to link these with preventive strategy.

### Subjects and Methods

The subjects of this study were residents who underwent a community health check-up in August 2007 as a part of a health promotion project for town development. From the registration data of 1105 participants (403 males and 702 females), we conducted a propensity score matching to adjust the age and gender of the participants. This process led to the selection of 400 matched participants (200 men and 200 women) as the final study subjects. Then, survival analysis was limited to the population whose survival we could confirm in 2018, 11 years after the medical check-ups.

## Methods of Data Analysis

### Extraction of Mortality-Related-Risk Factors by Logistic Regression Analysis

Based on the survival confirmation 11 years after the medical check-ups, in a total of examinees, we applied multiple logistic regression analysis to find out mortality risk factors for 400 examinees extracted by adjusting and matching the covariates: age and sex, and we used the odds ratios to predict their influence on survival. In the analysis, we checked multicollinearity using the Variance Inflation Factor (VIF) and considered items with a VIF of <5 non-collinear. The collinear items were similarly analyzed separately in the same models to confirm their validity.

The health metrics incorporated into the multivariate logistic regression model are of significant importance. These include body mass index (BMI), Max blood pressure (Max BP), Min blood pressure (Min BP), Body fat (BF), Red blood cells (RBC), Haemoglobin (Hb), Haematocrit( Ht), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), gamma-Glutamyl-transpeptidase (gamma-GTP), Creatinine (CRE), High-sensitivity C-reactive protein (hs-CRP), low-density lipoprotein (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), glycated haemoglobin (HbA1c), cardiac Troponin T (cTnT), Brain Natriuretic Peptide (BNP), Free Light Chain Kappa ($FLC_K$), Free Light Chain Lambda ($FLC_L$) , Death causes (disease 0-5), Smoking habits (1-5), and Drinking habits (1-5). These metrics play a crucial role in our analysis and understanding of mortality risk factors. The serum concentrations of $FLC_K$ and $FLC_L$, which were the focus of this study, were measured by enzyme-linked immunosorbent assays [2].

### ROC curve analysis and IDI survival analysis of survival/ death-related factors

We calculated the AUC using ROC curve analysis for items significantly extracted by logistic regression analysis and predicted their impact on survival. The AUC is an indicator that evaluates the model's overall discriminative power. It is suitable for capturing changes in sensitivity and specificity at specific cut off values.

However, the AUC has limitations in capturing subtle improvements. In response to this, a new predictive method has been successful in capturing these nuances. To demonstrate further improvement in the model by adding predictive factors [3-5], we evaluated the items using IDI, a method that has recently become widely used.

### Visualization of Mutual Relations among Significant Variables by Decision Tree Analysis

We conducted a decision tree analysis using the significant variables extracted by the logistic regression model. Using the calculated G2 coefficient (G-squared coefficient), we repeatedly processed a decision tree analysis by the optimal branching for the analysis model and visualized mutual relations among significant variables.

### Estimation of Mortality Risk Factors and Survival Analysis Using the Cox Proportional Hazards Model

The Cox proportional hazards model was used to analyze the

relationship between mortality-related risk factors, survival time, and survival and death at 11 years after the date of the medical health check-ups as the objective variables. This analysis assessed the impact on survival time by predicting the mortality from hazard ratios of the identified factors. After excluding examinees whose survival time could not be confirmed, we conducted propensity score matching by adjusting age and gender in the entire target population. We chose 244 subjects (109 males, mean age 74.8 years / 135 females, mean age 75.8 years) for the analysis. As in the logistic regression analysis, we incorporated the variables found significant in the univariate analysis into the multivariate model. We divided the group into two subgroups by the cut-off value of the objective variable calculated from the ROC curve analysis, and the two subgroups were visualized separately as survival curves by the Kaplan-Meier method.

### Statistical Analysis Software

We used the following statistical analysis software: EZR: R Commander ver. 2.7-1 (Saitama Medical Center, Jichi Medical University), Jamovi ver. 2.5.3 (Jamovi Project) and JMP Ver14 (SAS Institute). Through our statistical analysis, we regarded $p<0.05$ as statistically significant.

### Ethical Considerations

The protocol for this study has been reviewed and approved by the Clinical Research Ethics Committee of Shizuoka General Hospital (approval number: SGHIRB #2024044).

## Results

### Patient Background

The entire target data of this study was derived from the health check-ups data of residents in the mountainous area of Shimane Prefecture, consisting of 403 males and 702 females, for a total of 1105 patients. The average age was 71.2 (29-99) for men and 68.5 (23-93) for women. The average BMI for men was 23.2 (15.1-39.0) and 23.0 (14.4-39.7) for women. We used data from 400 persons (Table 1) in the analysis after adjusting background to remove the effects of confounding factors: age and gender by propensity score matching. We conducted survival analysis using the data after propensity score matching after excluding the examinees whose death date could not be confirmed 11 years after the health examination.

| | **survivor（n＝200）** | | | | | | | **dead（n＝200）** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex(M) | | n | 92 | | | | | | n | 92 | | | | | |
| (F) | | | 108 | | | | | | | 108 | | | | | |
| Age | | Mean | 75.2 | | | | | | Mean | 75.2 | | | | | |
| | Mean | SD | Median | IQR | | | | Mean | SD | Median | IQR | | | | **P-Value** |
| BF | 24.3 | 6.7 | 23.6 | 20 | − | 27.9 | | 23.4 | 7.6 | 23.4 | 18 | − | 28.6 | | 0.2 |
| BMI | 23.1 | 3.2 | 22.9 | 21 | − | 25 | | 22.8 | 3.2 | 23.1 | 20.3 | − | 25.1 | | 0.413 |
| Max BP | 137.8 | 16.2 | 137 | 126.8 | − | 147.3 | | 137.9 | 19.1 | 136.5 | 125 | − | 148 | | 0.944 |
| Min BP | 74.1 | 8.8 | 74 | 69 | − | 80 | | 74.1 | 11.5 | 74 | 67 | − | 83 | | 0.988 |
| cTnI | 0.058 | 0.075 | 0.036 | 0.017 | − | 0.07 | | 0.062 | 0.081 | 0.031 | 0.019 | − | 0.077 | | 0.624 |
| BNP | 41.2 | 37 | 29.7 | 15.8 | − | 55 | | 62.7 | 95.7 | 35.5 | 19.3 | − | 64.7 | ※ | 0.003 |
| hsCRP | 0.12 | 0.3 | 0.041 | 0.023 | − | 0.09 | | 0.143 | 0.518 | 0.039 | 0.024 | − | 0.079 | | 0.587 |
| RBC | 413.2 | 43.2 | 415 | 381.8 | − | 438.3 | | 406.9 | 44 | 407 | 378.8 | − | 429.3 | | 0.152 |
| Hb | 13.5 | 1.4 | 13.5 | 12.6 | − | 14.5 | | 13.3 | 1.4 | 13.3 | 12.4 | − | 14.1 | | 0.076 |
| Ht | 38.4 | 3.7 | 38.3 | 35.7 | − | 40.7 | | 37.6 | 3.7 | 37.6 | 35.4 | − | 39.7 | | 0.053 |
| TCho | 190 | 28.5 | 189.5 | 171.8 | − | 206 | | 185.4 | 29.4 | 185 | 165 | − | 206.3 | | 0.109 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HDL-C | 57.8 | 13.3 | 57.5 | 49 | − | 66 | | 58 | 15.9 | 57 | 47 | − | 66 | | 0.87 |
| LDL-C | 111.6 | 23 | 111 | 97.8 | − | 124 | | 106.6 | 25.2 | 107 | 91 | − | 122.3 | ※ | 0.039 |
| TG | 114.1 | 54.1 | 99 | 75.8 | − | 140.3 | | 118.5 | 124.8 | 93 | 65.8 | − | 128.3 | | 0.649 |
| CRE | 0.718 | 0.156 | 0.7 | 0.6 | − | 0.8 | | 0.726 | 0.25 | 0.7 | 0.6 | − | 0.8 | | 0.719 |
| UA | 4.97 | 1.38 | 4.75 | 4.1 | − | 5.7 | | 5 | 1.47 | 4.8 | 3.9 | − | 5.8 | | 0.834 |
| AST | 25.4 | 6.2 | 24 | 21 | − | 29 | | 27.1 | 9.7 | 25 | 22 | − | 29 | ※ | 0.034 |
| ALT | 20.1 | 8.5 | 18 | 15 | − | 22 | | 20.6 | 8.6 | 18 | 15 | − | 24.3 | | 0.558 |
| γ-GTP | 29.9 | 31.9 | 20 | 14 | − | 33 | | 33.3 | 38.3 | 20.5 | 14 | − | 34 | | 0.329 |
| FBS | 116.1 | 30.6 | 109 | 97 | − | 123 | | 121.5 | 38.6 | 110 | 96 | − | 134.3 | | 0.118 |
| HbA1c | 5.39 | 0.51 | 5.3 | 5.1 | − | 5.53 | | 5.58 | 0.77 | 5.4 | 5.18 | − | 5.8 | ※ | 0.004 |
| $FLC_k$ | 48.3 | 13.7 | 47.7 | 37.9 | − | 57.3 | | 52.3 | 17.6 | 50.7 | 39.9 | − | 62.1 | ※ | 0.012 |
| $FLC_L$ | 50.6 | 13.9 | 49.6 | 40.2 | − | 57.3 | | 55.2 | 18.2 | 51.4 | 43.1 | − | 64.8 | ※ | 0.004 |
| smoking | 35 | (cases) | | | | | | 53 | (cases) | | | | | | 0.111 |
| alcoholic drinker | 102 | (cases) | | | | | | 106 | (cases) | | | | | | 0.701 |

BF: Body Fat, BMI: Body Mass Index, BP: Blood Pressure, cTnI: high-sensitivity Troponin I, BNP: Brain Natriuretic Peptide, hsCRP: high-sensitivity C-Reactive Protein, $FLC_k$: Free Light Chain Kappa, $FLC_L$: Free Light Chain Lambda

**Table 1:** Patient background data after propensity score matching process by sex and age.

### Survival/Mortality-Related Factors and Survival Prediction

### Logistic regression analysis

The results of logistic regression analysis showed that HbA1c [OR = 1.620, 95% CI (1.150 - 2.270), p = 0.0058], AST [OR = 1.030, 95% CI (1.000 - 1.060), p = 0.0491], $FLC_L$ [OR = 1.020, 95% CI (1.000 - 1 .030), p=0.0212], and BNP [OR=1.010, 95% CI (1.000 - 1.010), p=0.0119] were significant. We identified diabetes, heart failure, and immune-related biomarkers as death risk factors.

### Survival Prediction Power-up of Mortality Risk Factors Using AUC from ROC Curve Analysis and IDI

Using the ROC curve analysis, we predicted survival based on mortality risk factors and the results evaluated by AUC. The AUC calculated by ROC curve analysis to predict survival was HbA1c [AUC = 0.571, 95% CI (0.514 - 0.627)] > BNP [AUC = 0.567, 95% CI (0.511 - 0.623)] >$FLC_L$ [AUC = 0.561, 95% CI (0.505 - 0.617)], in order. The AUC increased to 0.621 [95% CI (0.566 - 0.676)] when combined with BNP, HbA1c, and $FLC_L$.

To evaluate the power of improvement in survival prediction, when we added BNP, HbA1c, and $FLC_L$ to the usual health check items and compared the predictive power before and after the addition. Figure 1 shows that the AUC increased from 0.572 (95% CI: 0.516 - 0.628) to 0.655 (95% CI: 0.601 - 0.709) by adding the three items. Figure 2 shows the results by IDI. The distance between the dotted and solid lines represents the degree of separation between the basal model (before adding items) and the added model in the death and survival groups. A wider gap indicates that the added item enhances the basal model's predictive power. The IDI value was 0.052 (95% CI: 0.031 - 0.074, $p < 0.001$), indicating that the model adding HbA1c, BNP, and $FLC_L$ to the basal model significantly enhanced predictive power in comparison. An IDI greater than 0.01 significantly improves predictive power; an IDI greater than 0.02 is considered more substantial evidence. Therefore, these results show that the model adding HbA1c, BNP, and $FLC_L$ is significantly superior to the basal model in predicting mortality.

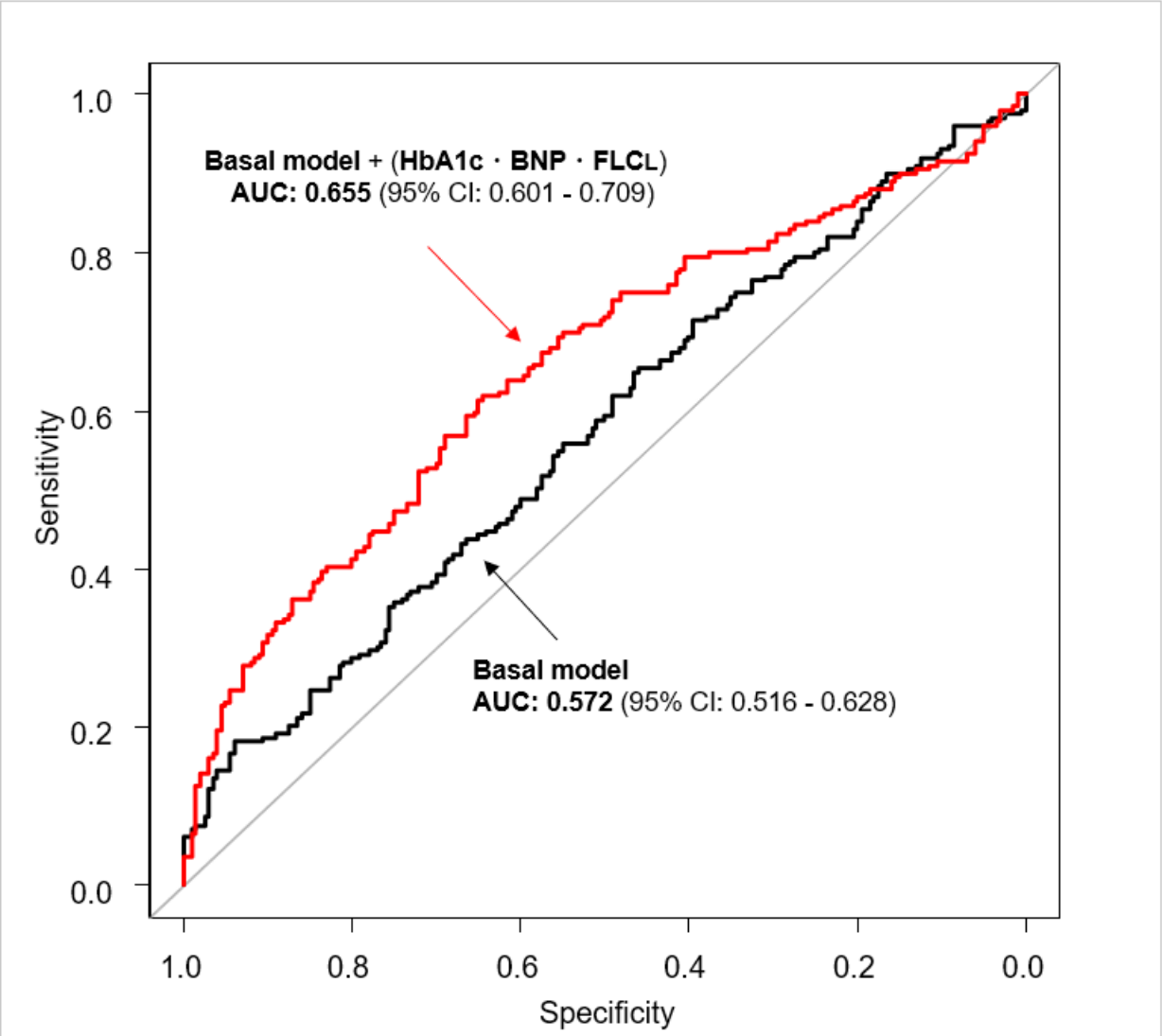


**Figure 1:** The impact of predictive power in the enhanced model in which we add specific parameters for conducting ROC curve analysis.

We compared the power to predict survival between the basal and enhanced models, adding BNP, HbA1c, and $FLC_L$ to the basal model. As shown in Figure 1, the AUC increased from 0.572 (95% CI: 0.516 - 0.628) to 0.655 (95% CI: 0.601 - 0.709) by adding the three parameters.

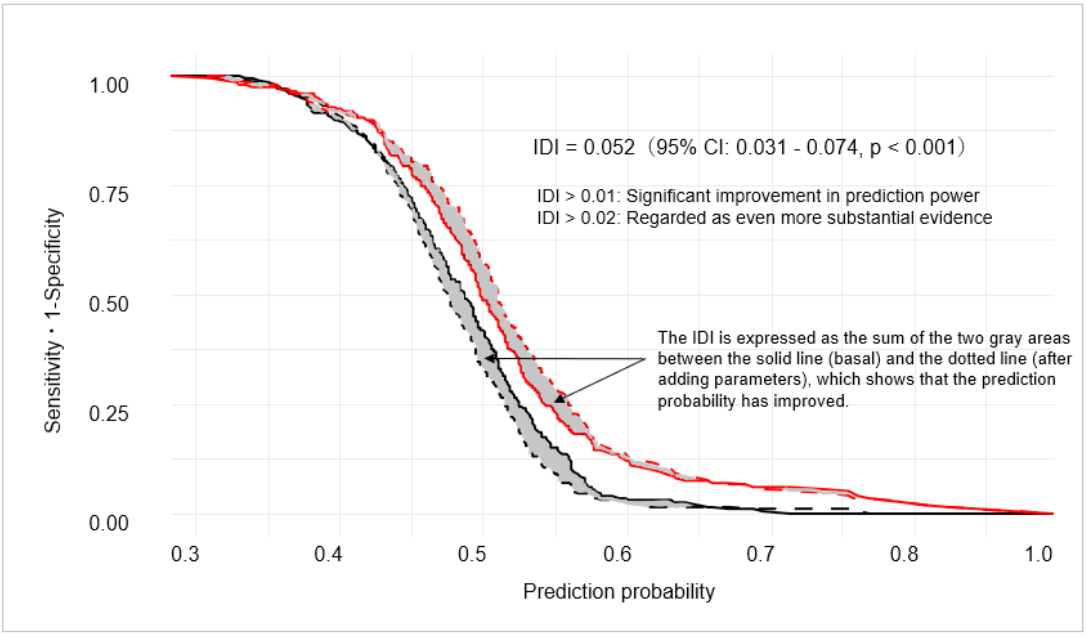


**Figure 2:** The impact of survival prediction using the advanced model, IDI.

The graph shows the survival prediction power of mortality risk factors using IDI. The X-axis represents the predicted probability, and the Y-axis represents the sensitivity (true positive rate) for the event group (dead group) and 1-specificity (false positive rate) for the non-event group (survivor group). In the dead group, the curve shifts upwards because the predicted probability in the new model after adding the parameter is higher than that of the basal model (reference). Conversely, in the survivor group, the curve shifts downwards because the lower probability of predicting death improves the false positive rate. The motion of these two curves indicates that adding the parameter has enhanced the discriminative power.

**Estimation of Mortality Risk Factors Using G2 Coefficients in Decision Tree Analysis**

The G2 coefficient for $FLC_L$, a substantial 14.120, emerged as the factor with the most significant impact on the split in this analytical model. This finding is of paramount importance in our understanding of mortality risk factors. $FLC_K$, HbA1c, and BNP also showed high G2 coefficients, further underlining their potent influence on survival/death.

Based on these results, we evaluated mortality risk factors, AUC by ROC curve, and G2 coefficient in decision tree analysis, and Table 2 shows the results. Although there were some differences among the three methods, BNP, a marker of heart failure; HbA1c, a diabetes-related marker; and $FLC_L$, related to immunocompetence, were identified as common and essential factors. LDL-C and AST were also consistently identified as relevant factors.

| | Logistic regression analysis odds ratio | | ROC Curve (AUC) | | Candidate |
|---|---|---|---|---|---|
| | | | | | G^2 |
| **HbA1c** | **1.620,** 95%CI (1.150-2.270), p=0.0058 | **HbA1c** | **0.571,** 95%CI (0.514-0.627) | **$FLC_L$** | **14.12** |
| **AST** | **1.030,** 95%CI (1.000-1.060), p=0.0491 | **BNP** | **0.567,** 95%CI (0.511-0.623) | **$FLC_k$** | **11.569** |
| **$FLC_L$** | **1.020,** 95%CI (1.000-1.030), p=0.0212 | **$FLC_L$** | **0.561,** 95%CI (0.505-0.617) | **HbA1c** | **10.326** |
| **BNP** | **1.010,** 95%CI (1.000-1.010), p=0.0119 | **AST** | **0.536,** 95%CI (0.480-0.593) | **BNP** | **9.829** |

**Table 2:** Results of survival prediction by each analysis method when the objective variable is survival/death.

### Identification of Factors Associated with Mortality Risk and Survival Analysis Using Cox Proportional Hazards Model

Cox proportional hazards analysis identified two risk factors for mortality: BNP [hazard ratio 1.003, 95% CI (1.000 - 1.005), P = 0.0283] and LDL-C [hazard ratio 0.988, 95% CI (0.980 - 0.996), P = 0.0041]. Figure 3 shows survival curves for both factors. The log-rank test results showed significant differences between the low and high BNP and LDL-C groups, p=0.019 and p<0.001, respectively.

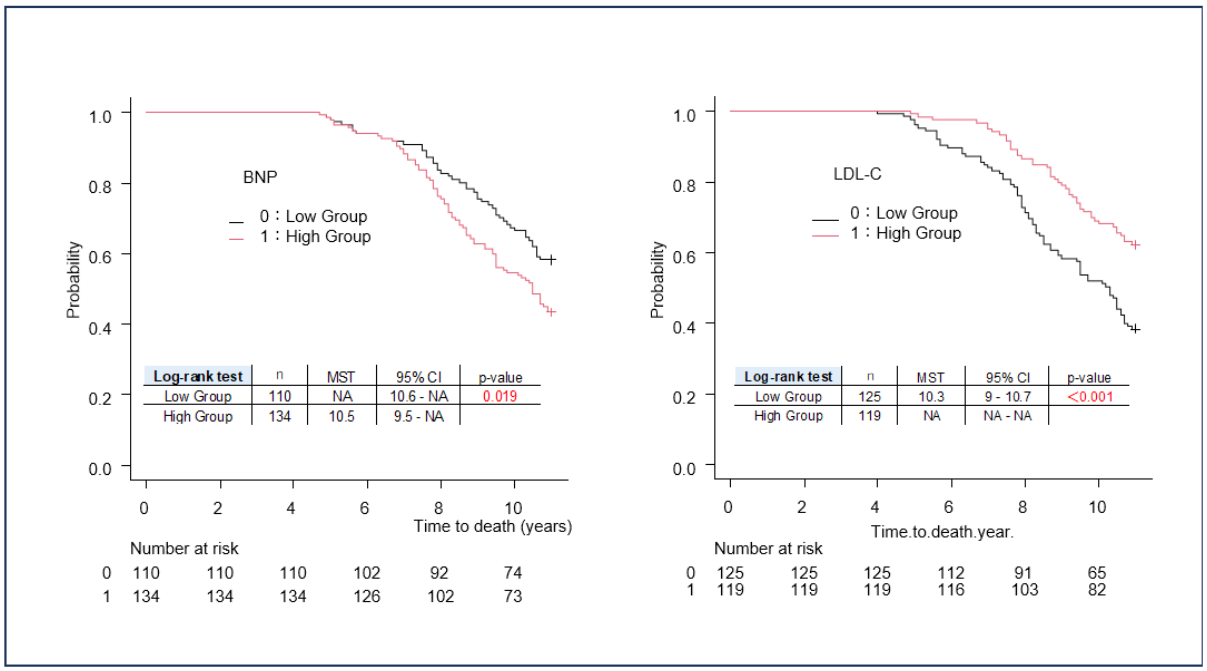


**Figure 3:** Survival curve analyses for mortality risk factors.

Figure 3 shows the results of Kaplan-Meier survival curves and log-rank tests for BNP and LDL-C, identified as risk factors for mortality by Cox proportional hazards analysis. For BNP, the mortality risk was higher (p=0.019) in the high group, and also, for LDL-C, it was significantly higher (p<0.001) in the low group.

## Discussion

Predicting health and disease is crucial in long-term health maintenance and prevention. This study, which aimed to identify disease risk factors, used a robust methodology. We utilized the data of residents who participated in the 2007 health check-ups of a town in a mountainous area of Shimane Prefecture and conducted a survey to track their survival confirmation 11 years later. This study identified various mortality risk factors using logistic regression analysis. BNP, a biomarker of heart failure, $FLC_L$, related to immune function, and HbA1c, a diabetes-related factor, emerged as pivotal factors related to survival and death. From the ROC curve analysis, we proved BNP, HbA1c, and $FLC_L$ to be influential mortality risk factors. Notably, the IDI results made the influence of these factors more apparent, as the IDI is considered a much better indicator than the AUC of the ROC curve for evaluating a model's discriminative power.

The mortality risk factors identified in this study are related to heart failure, diabetes, and immune function, and we discuss their significance for predicting survival in the residents' health check-ups in light of their involvement.

HbA1c is an essential marker for diabetes diagnosis and treatment assessment. Diabetes mellitus is known to cause macrovascular and microvascular disorders and to induce chronic inflammation through increased secretion of inflammatory cytokines associated with cardiovascular disorders and abnormal lipid metabolism. Therefore, our results show that BNP, a biomarker of heart failure, and FLCs, biomarkers associated with inflammatory diseases, are closely linked with HbA1c, a marker of diabetes mellitus.

**Diabetes Biomarker HbA1c and Heart Failure Biomarker BNP**

Diabetes mellitus is a disease that progresses slowly, and its symptoms are difficult to recognize, so many cases of cardiovascular disorders and chronic inflammation occurring in the body are left unnoticed and untreated. In 2022, heart disease was the second leading death cause in Japan, with 40-50% of deaths due to TOD, such as acute Cardiac Events (CEs) and Sudden Cardiac Deaths (SCDs). These patients are often suffering from cardiac disease despite the absence of obvious cardiac symptoms. Many diabetic patients, in particular, are thought to be often among those with TOD.

Allan Struthers et al. report that they have targeted an asymptomatic CVD and are confident about the potential for improved primary prevention using BNP [6].

Although we widely use BNP in clinical practice as an established biomarker for heart failure, several cohort studies of asymptomatic cardiovascular disorders have also reported an essential role for BNP in predicting CVD progression [7-10].

Echocardiography plays a pivotal role as a diagnostic tool for CVD. It provides invaluable information on the structure and function of the heart. It has become essential in hospital care, from diagnosis and treatment evaluation to post-treatment follow-up.

On the other hand, BNP provides less information than echocardiography. However, it is not only a straightforward test that requires only a blood sample but can also provide information during exercise. Usually, it is technically challenging to take echocardiography other than at rest. Furthermore, because the BNP test does not require special equipment or techniques, unlike echocardiography, it has the advantage of being easily performed in health check-ups in the community.

Thomas J, et al. stated that BNP is an independent variable from variables measured by echocardiography, such as LV mass, LA diameter, LVSD, LVEF, and others. They suggested that BNP may be a predictor of future atrial fibrillation, heart failure, and stroke [11].

Remember that echocardiography and BNP have complementary aspects and that the information from both tests can give us a more abundant contribution. Jasmine Grewal et al. also reported that BNP can predict diastolic dysfunction in heart failure with preserved ejection fraction (HfpEF) [12]. Although echocardiography is excellent for assessing cardiac function, learning the echocardiographic technique is time-consuming, and installing the equipment is somewhat cost-expensive. In such limited clinical settings, BNP is an excellent objective, reproducible, standardized alternative test for echocardiography in diagnosing heart failure. BNP measurement is helpful in situations when echocardiography is not available.

However, BNP is not always a substitute for echocardiography. We should use both tests to compensate for each other's deficiencies. We identified BNP as a significant variable in predicting mortality from the present study in follow-up of residents' health check-ups. BNP may predict the early detection of heart failure and the development of subsequent cardiovascular events by predicting asymptomatic cardiovascular disorders.

## Diabetes Biomarker HbA1c and Inflammation Marker FLCs

Immunoglobulin FLCs, extracted as a mortality-related factor, are widely known to increase monoclonal FLC production in myeloma and amyloidosis; since FLC measurement techniques were established, the diagnosis and treatment of these related diseases have advanced dramatically [13,14].

We focus on increasing polyclonal FLCs in inflammatory diseases. FLCs have been reported to be helpful as a nonspecific marker of inflammation of the vascular endothelial system associated with abnormal lipid metabolism and chronic inflammation caused by autoimmune diseases such as rheumatoid arthritis [15-17].

B-lymphocytes produce FLCs during immunoglobulin synthesis and are involved in several critical immune response processes. In some conditions, such as inflammatory bowel disease, it causes mast cell degranulation and stimulates local inflammatory responses by releasing inflammatory mediators. FLCs may also play an important role in renal disease because of their low molecular weight, which may be directly toxic to proximal tubular cells. Thus, elevated FLCs have been reported to cause inflammatory responses and over-activation of the immune system, which may affect life-sustaining physiological processes and increase the risk of impaired health [18]. Dispenzieri A, et al. reported in a study on the life prognosis of the general population in terms of nonclonal serum immunoglobulin-free light chains that high FLCs were not limited to specific causes of death and that the risk of death was significantly higher for most causes of death. Therefore, they concluded that measuring FLCs helps predict the life prognosis of the general population [19].

A study on inflammatory biomarkers of FLCs in diabetes by Matsumori A and Shimada T, co-authors of this study, reported that FLCL is increased in diabetes and is more specific and sensitive than HbA1c for the diagnosis of type 2 diabetes [20,21].

They suggested that the significant decrease in FLCs in diabetes patients (as a result of increased FLCL) may indicate that an essential mechanism of the inflammatory cascade in the pathogenesis of type 2 diabetes is the nuclear factor kappa-light-chain-enhancer of activated B Cells (NF-κB: a factor that regulates the transcription of FLCK in immunoglobulin-producing B cells and plasma cells) and that this activation of NF-κB is associated

with the development of inflammatory and autoimmune diseases [22]. NF-κB can sustain inflammation and cause tissue damage. They have reported that FLCs are a promising inflammation biomarker associated with NF-κB activation [23].

Matsumori et al. reported that a significant decrease in FLCs, i.e., an increase in $FLC_L$, explains chronic inflammation caused by the onset of type 2 diabetes. They also report that FLCs increases in diabetic patients and that $FLC_L$ and $FLC_K$ /L ratio are more specific and sensitive markers in diabetes diagnosis than HbA1c. Therefore, the relationship between HbA1c and FLCs must be very tight from the diabetes perspective. Our study results highlight $FLC_L$ as a diabetes-related variable with a high odds ratio of a diabetes-related marker, HbA1c. This result strongly suggests that $FLC_L$ reflects chronic inflammation in diabetes. The potential of FLCs as a chronic inflammation biomarker in diabetes is an invaluable finding in our research, with practical implications for diagnosis and treatment. FLCs are a promising biomarker that can indicate the presence and progression of chronic inflammation in health check-ups [24].

**BNP and FLCs**

While BNP and FLCs are independent survival/death risk factors, immune and cardiovascular functions interact with each other; FLCs have been reported to be positively correlated with BNP, left ventricular end-diastolic diameter, end-systolic diameter, and pulmonary artery pressure in heart failure patients [23]. Many risk factors for CVD activate NF-κB and promote the production of inflammatory molecules, leading to inflammation. As described for the association between diabetes and inflammation, FLCs have been considered an inflammation biomarker for NF-κB activation. Furthermore, FLCs are associated with poor CVD outcomes as well as an increased risk of total mortality in the general population [24].

Activation of NF-κB in atrial fibrillation suggests a local immune and inflammatory response within the atrium. As mentioned above, FLCs are considered an indicator of NF-κB activation, inflammation, and immune response, and their levels have been reported to increase in myocarditis and heart failure. Previous studies have shown a high frequency of atrial myocarditis in atrial fibrillation without cardiac disease. FLCs are essential early to detect asymptomatic atrial fibrillation.

In isolated atrial fibrillation without cardiac disease, $FLC_{K/L}$ is elevated, and the B lymphocytes and plasma cells that produce them are activated. Therefore, anti-inflammatory therapy suppressing NF-κB, which regulates FLCs, is promising in atrial fibrillation with elevated FLCs [24,25].

**Relevance of FLCs to Cancer**

Chronic inflammation due to cancer may also be a factor in increasing FLCs. Cancer, excluding myeloid tumours, was the most common cause of death during the current observation period, accounting for 14.7% of deaths. Some cancer cells have immune evasion mechanisms to evade immune attack, and the relationship between tumour-derived inflammation and immune response is known. Cancer cells cause inflammation in surrounding tissues and activate the immune system [26-28]. As part of such an immune response, increased production of immunoglobulin FLCs may be involved. Therefore, when FLC values are high, we should pay attention to hematopoietic tumours such as multiple myeloma and other cancers. This fact suggests that FLC measurement is helpful as a clinical screening test in health check-ups.

**Impact of LDL-C on Life Outcomes**

As is well known, hyper LDL-C is a risk factor for CVD. However, in a 10-year follow-up study of 3,684 participants in Japan, LDL-C emerged as a predictor of all-cause mortality: lower LDL-C levels were associated with an increased risk of all-cause mortality and a significantly increased risk among those with LDL-C below 70 mg/dL [29]. A Danish prospective cohort study also found a U-shaped relationship between too high and too low LDL-C levels linked with an increased risk of all-cause mortality; even with low LDL-C, the risk of all-cause mortality was higher; instead, the hazard ratio was higher for low than for high LDL-C levels [30]. The results of both studies are consistent with our findings, which show that low LDL-C levels are associated with an increased risk of all-cause mortality and that the association is significant in models considering age and gender. Cholesterol is an essential component of cell membranes and also closely contributes to the synthesis of bile acids and the absorption of vitamin E. Cholesterol plays a crucial role in sustaining our lives. Cholesterol deficiency decreases the absorption of vitamin E. It reduces protection from oxidative injury in various body organs, including increased lipid peroxide, closely related to atherosclerosis (Niki) [31]. These mechanisms have been reported to weaken cell membranes and vessel walls, making them more susceptible to intracerebral haemorrhage [32,33]. There are also reports that lower LDL level is associated with lower cognitive function [34]. The results of this study reconfirm that LDL-C is an essential nutrient that affects life expectancy and needs to be well managed.

In summary, HbA1c, BNP, FLCs, and LDL-C emerged as mortality-associated variables from our follow-up study of resident health check-ups. We believe these results will help us understand the close relationship among the three variables, in which diabetes leads to various types of inflammation, progressing to CVD [35].

According to the Ministry of Health, Labour, and Welfare, the number of people receiving specific health check-ups in Japan in FY2021 was approximately 30 million, and the health check-ups uptake rate was 56.5%. Those aged 60 or older accounted for half of the examinees. The percentage of those visiting the hospital due to injury or illness has increased sharply from 42% for those in their 50s to 59% for those in their 60s and 73% for those aged 80 or older. Under these circumstances, we expect biomarkers to play a vital role in identifying undiscovered diseases without symptoms and finding out high-risk patients who need treatment by utilizing biomarkers in residents' health check-ups and other regular health check-ups. In the future, we would like to actively work on introducing prospective measurements of BNP and FLCs, focusing on predicting undiscovered diseases and HbA1c.

## Limitations

We regret that we were unable to collect detailed information on the occurrence of events on the survival curves. We also regret that we had to exclude the participants for whom we could not confirm the date of death or survival at the 11th-year survey from the survival analysis; we would like to review our research plan to make a reliable communication network to confirm the death or survival of the participants.

## Conclusions

This study identified BNP, a heart failure biomarker, $FLC_L$, related to immune function, and HbA1c, a diabetes-related factor, as significant risk factors in predicting survival or death. The study also emphasizes that LDL-C is pivotal in achieving healthy longevity. The potent roles of these biomarkers in early detection and disease prevention are significantly promising. These biomarker measurements could greatly enhance future health check-ups in quality, aiding in detecting unwellness and correcting high risks. Avoiding or improving poor lifestyle habits may enable us to live long and happily.

## Author Information

All authors declare that they have no conflicts of interest regarding the publication of this paper.

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