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Research Article

Blood-Based Biomarkers and Long-term Risk of Frailty—Experience From the Swedish AMORIS Cohort

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Abstract

Background: Frailty is associated with reduced quality of life, poor health outcomes, and death. Past studies have investigated how specific biomarkers are associated with frailty but understanding biomarkers in concert with each other and the associated risk of frailty is critical for clinical application.

Methods: Using a sample aged ≥59 years at baseline from the Swedish AMORIS (Apolipoprotein MOrtality RISk) cohort (n = 19 341), with biomarkers measured at baseline (1985–1996), we conducted latent class analysis with 18 biomarkers and used Cox models to determine the association between class and frailty and all-cause mortality.

Results: Four classes were identified. Compared to the largest class, the Reference class (81.7%), all other classes were associated with increased risk of both frailty and mortality. The Anemia class (5.8%), characterized by comparatively lower iron markers and higher inflammatory markers, had hazard ratio (HR) = 1.54, 95% confidence interval (CI) 1.38, 1.73 for frailty and HR = 1.76, 95% CI 1.65, 1.87 for mortality. The Diabetes class (6.5%) was characterized by higher glucose and fructosamine, and had HR = 1.59, 95% CI 1.43, 1.77 for frailty and HR = 1.74, 95% CI 1.64, 1.85 for mortality. Finally, the Liver class (6.0%), characterized by higher liver enzyme levels, had HR = 1.15, 95% CI 1.01, 1.30 for frailty and HR = 1.40, 95% CI 1.31, 1.50 for mortality. Sex-stratified analyses did not show any substantial differences between men and women.

Conclusions: Distinct sets of commonly available biomarkers were associated with development of frailty and monitoring these biomarkers in patients may allow for earlier detection and possible prevention of frailty, with the potential for improved quality of life.

Keywords: Death, Frail, Longitudinal, Population-based

Frailty is a clinical condition described as being unable to respond adequately to acute and chronic stressors and it makes an individual more vulnerable to these stressors and related outcomes. Among older adults, frailty prevalence estimates range from 5.8% to 27.3% across 10 European countries, depending on age and country (1). Frailty is defined by reduced strength, endurance, and physical function and it is associated with poor health outcomes, including loss of independence, reduced quality of life, longer hospital stays, hospital readmission, cognitive impairment and delirium, and death (2). However, frailty progression can be slowed or reversed, and it may also be preventable, if identified early enough (3). Therefore, developing tools to determine who is at risk of becoming frail would have significant implications for improving quality of life for many older adults (4) and reducing health care costs (5).

Research into blood-based biomarkers as predictors of geriatric conditions and diseases has increased in recent years. Because frailty is a condition with such amorphous criteria, identifying predictive factors or biomarkers to determine who is at risk has proven challenging. While previous studies have shown that certain blood-based biomarkers are associated with risk of frailty, most studies have examined markers independently of each other. Studies have shown that inflammatory markers (eg, tumor necrosis factor-alpha, C-reactive protein [CRP], interleukin 6), hormones (eg, testosterone, insulin-like growth factor 1, dehydroepiandrosterone), metabolic

markers (eg, glucose, glycated hemoglobin), and other clinical markers (eg, hemoglobin, albumin) are all independently associated with risk of frailty, though with mixed results (6). A drawback with this piecemeal approach—examining biomarkers independently of each other—is that changes in these markers are prevalent in healthy aging (7), and biomarkers often operate in relation to each other rather than separately, so the information they may provide analyzed in isolation is limited. Therefore, combining markers, to create a panel, may provide more information about how they tend to cluster and, also, how they are associated to the risk of frailty.

To that end, we have utilized the Swedish AMORIS (Apolipoprotein MOrtality RISk) cohort to investigate the association of 18 serum biomarkers with longitudinal risk of frailty, assessed with the Hospital Frailty Risk Score (HFRS), as well as mortality. Using a data-driven approach to try to capture biological processes, we aimed to determine and describe patterns of baseline biomarkers, in concert with each other, in the population to provide more information about future risk of frailty and mortality in the different groups. We additionally a priori chose to conduct sex-stratified analyses, because of the well-known gender paradox in frailty research (8).

Method

Study Design and Participants

AMORIS is a large cohort with extensive information on biomarkers from health examinations of individuals residing in the greater Stockholm area during the period 1985–1996. The cohort has been described in detail elsewhere (9,10). Participants were either healthy individuals referred for clinical laboratory testing as part of a routine health checkup in the occupational setting or were outpatients. Using the 10-digit Swedish personal identification number, the AMORIS cohort has been linked to multiple Swedish national registers to enable longitudinal follow-up of the participants. Among the linked registers are the Patient Register, the Cause of Death Register, the National Cancer Register, the Swedish Censuses from 1970 to 1990, and the National Population Register. These registries provide national and essentially complete data on demographics, socioeconomic status, vital status and cause-specific mortality, emigration, hospital and outpatient specialty care diagnoses, and incident cases of cancer. The current study is part of a research program that is performed in concordance with the Declaration of Helsinki and was approved by the ethics board of Karolinska Institutet.

A total of 25 080 AMORIS participants aged ≥59 (born between 1893 and 1927) at the time of blood measurement were eligible for inclusion in the current study. We excluded 5394 people who did not have complete measurements on the included lipid biomarkers and 345 people who experienced frailty or death within the first year of follow-up, leaving 19 341 participants in the analytical sample. Included participants had complete measurements at the baseline health examination for the following 18 blood-based biomarkers: total cholesterol, glucose, creatinine, fructosamine, CRP, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, platelets, alkaline phosphatase, iron, hemoglobin, mean corpuscular volume, total iron-binding capacity, lactate dehydrogenase, B leucocytes, and uric acid. These markers can be grossly divided into 4 categories—metabolic, liver and kidney enzymes, iron status, and inflammatory markers—and have been documented to be associated with aging-related conditions and outcomes (7). Additionally, they are standard serum biomarkers available in routine health checkups. All biomarkers were measured on the same day, using fully automated methods with automatic calibration performed on fresh blood samples at the CALAB (Central Automation Laboratory) (11). These blood-based biomarkers were the primary independent variables included in the analysis.

Frailty Index and Mortality

We measured frailty using the HFRS, which calculates the sum of 109 International Classification of Diseases (ICD)-10 hospital codes weighted from 0.1 to 7.1 (12). The HFRS is an established frailty assessment designed for use in administrative registry data of hospital admissions. We translated the codes from the international version of the ICD-10 to the Swedish version of the ICD-9 and ICD-10 because the baseline health examinations of the AMORIS cohort took place prior to the implementation of ICD-10 in Sweden (1997) (Supplementary Table 1). Hospitalizations were ascertained using the National Patient Register nationally from 1987 and regionally from 1964 until December 31, 2011. Specialized outpatient care was assessed using the same register from 2001. Individuals with a score of greater than 5 were considered frail (12). We summed primary and secondary codes for everyone annually. If an individual did not seek inpatient or outpatient care for a given year, they were assigned a score of "0" on the HFRS.

We additionally examined all-cause death as an outcome. Death and date of death were determined by record linkage to the National Cause of Death Register until December 31, 2011 (end of follow-up).

Statistical Analysis

Participant baseline characteristics by follow-up frailty status were described and compared using chi-squared tests and *t* tests for dichotomous and continuous variables, respectively. Then, we examined biomarkers to create a more parsimonious model to satisfy the principle of local independence of latent class models. The estimated latent variables from the latent class analysis (LCA) measured the underlying "health profile" of individuals. We calculated Pearson correlation coefficients between the biomarkers included in the study. These analyses showed, for example, and as expected, strong correlations between Apolipoprotein A (Apo-A) and Apo-B and total cholesterol (rho 0.81–0.93). Subsequently, we used regression and the variance inflation factor in postestimation to evaluate multicollinearity. These methods paired with evidence from the literature drove our model-building approach.

LCA is a cluster-based model that reduces the dimension of the data by clustering covariates into latent classes. LCA uses measured variables (eg, blood-based biomarkers) to identify unmeasured class membership (eg, underlying health status). LCA relies on dichotomous predictor variables. Therefore, we dichotomized the biomarkers based on sample-specific cut-points (Figure 1) to include them in the LCA models. We chose to use sample-specific cut-points because current clinical cut-points are not always relevant for biomarker levels measured in the 1980s. For example, the mean lipid levels in the population are generally lower now than they were in the 1980s, in part because although statins were available in 1987, they were not widely used until the late 1990s (13). We determined sample-specific risk cut-points, informed by the current clinical cut-points, previous studies in the literature, and the scatterplot of each biomarker and with the frailty score (Supplementary Material).

Using these dichotomized variables, latent, or "unmeasured," classes were created. We used PROC LCA in SAS software v9.4 (SAS Institute Inc., Cary, NC) to fit latent classes using 18 biomarkers. Individual participant class membership was then

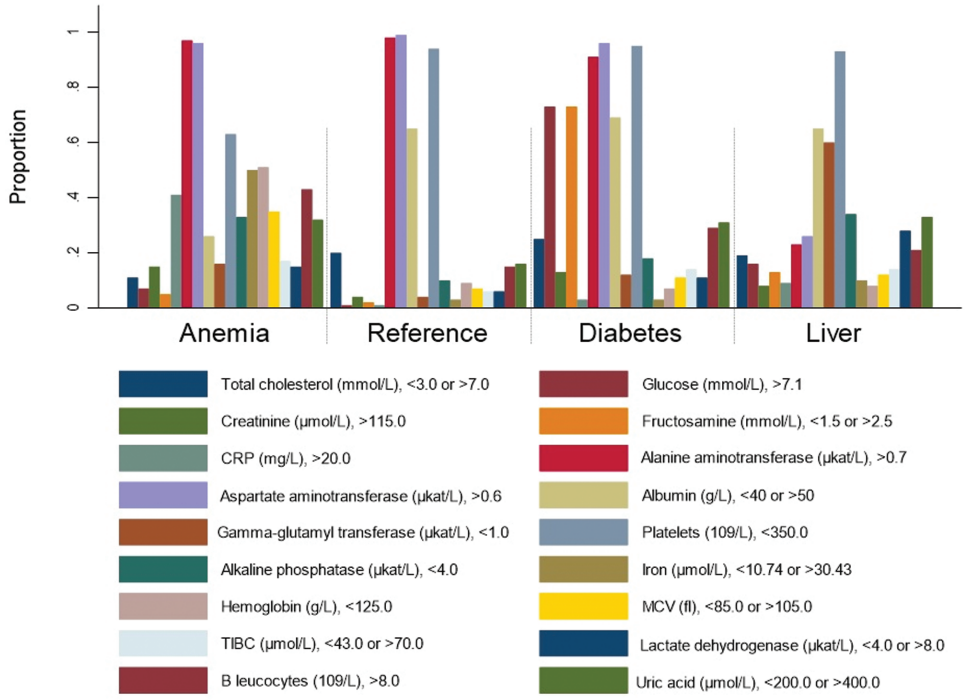

Figure 1. Class proportions and class-specific probabilities of being within a given range for each of the biomarker from a 4-class model. Full color version is available within the online issue.

determined based on highest, or best, class membership probability. We determined the optimal number of classes by fitting models using a stepwise method with different number of classes (ie, 3, 4, 5). We used corrected Akaike Information Criterion and adjusted Bayesian Information Criterion to determine the best fitting model and optimal number of classes, considering that any class should have at least 5% of the sample (14,15). We subsequently *z*-scored the biomarkers to compare them between classes, which allowed us to more easily interpret the patterns of biomarkers clustering in the classes.

We subsequently fit Cox proportional hazard models, adjusted for sex and baseline age, to determine the associated relative risk of frailty for each class compared to a Reference class. We additionally used Cox proportional hazard models to examine the association between latent classes and mortality, adjusted for sex and baseline age. We plotted survival curves using Kaplan–Meier plots. To reduce the risk of capturing reverse causality, we censored events (frailty and deaths) that occurred during the first year of follow-up. In sensitivity analysis, we stratified the models by sex, to determine whether it is an effect modifier. Cox proportional hazard models were fit using Stata version 16.1 (StataCorp LLC, College Station, TX).

Results

Participants included in this analysis were aged 59 years or older at baseline and had complete data for all biomarkers included in the model at the baseline health examination (N = 19 341). Baseline characteristics of all included individuals, by follow-up frailty status, are presented in Table 1. Continuous variables are presented as mean and standard deviation (*SD*) and dichotomous variables are presented as number and percentage. By the end of the follow-up period (mean [*SD*] 12.2 [6.5]), there were 14 193 (73.3%) nonfrail and 5148 (26.6%) frail participants. Frail and nonfrail individuals had statistically significant different values on several biomarker measures at baseline.

Based on corrected Akaike Information Criterion and adjusted Bayesian Information Criterion estimates and class proportion distribution, the best fit was found at a 4-class model. Goodness-of-fit statistics are presented in Supplementary Table 2. Class 2 accounted for 81.7% (N = 15 804) of the sample and was the Reference class, with *z*-scored biomarkers that were all near the null; thus, we called this class "Reference" (Figure 2). Class 1 accounted for 5.8% (N = 1123) of the sample and was characterized by lower levels of iron, hemoglobin, and albumin, which are associated with anemia, and higher levels of CRP and platelets, which are associated with an inflammatory response (16). We called Class 1 "Anemia." Class 3, which accounted for 6.5% (N = 1262) of the sample, was primarily characterized by higher levels of glucose and fructosamine, biomarkers associated with prediabetes and diabetes, so we called it "Diabetes." Indeed, the mean glucose level was 9.65 (Supplementary Table 3), indicating that type 2 diabetes is likely common among these individuals. Class 4, which accounted for 6.0% (N = 1152) of the sample, was primarily characterized by higher levels of liver enzymes, including aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, and alkaline phosphatase, so we called it "Liver." Biomarker proportions and *z*-scored biomarker means, to make the biomarkers more comparable, are shown by class in Figures 1 and 2, respectively. The actual means of the biomarkers by class are show in Supplementary Table 3.

In Cox proportional hazard models, the Anemia, Diabetes, and Liver classes were associated with risk of frailty, as compared to the Reference class (Figure 3). The Diabetes class had the highest hazard ratio (HR = 1.59, 95% confidence interval [CI] 1.43, 1.77), followed by the Anemia (HR = 1.54, 95% CI 1.38, 1.73) and Liver (HR = 1.15, 95% CI 1.01, 1.30) classes. Kaplan–Meier survival curves for these analyses are presented in Supplementary Figure 1.

Table 1. Participant Characteristics and Mean Values of the Biomarkers at Baseline by Follow-up Frailty Status, Mean (*SD*) or *N* (%), *N* = 20 109

	All (*n* = 19 341)	Became Frail (*n* = 5148)	Stayed Nonfrail (*n* = 14 193)	*p* Value
Female	11900 (61.5)	3359 (65.2)	8541 (60.2)	<.001
Age	72.2 (6.6)	73.1 (6.7)	71.9 (6.5)	<.001
Follow-up time (years)	12.2 (6.5)	11.2 (6.6)	12.6 (6.4)	<.001
Total cholesterol (mmol/L)	6.1 (1.1)	6.1 (1.1)	6.1 (1.1)	.905
Glucose (mmol/L)	5.5 (1.8)	5.4 (1.7)	5.5 (1.8)	.464
Creatinine (μmol/L)	85.1 (22.7)	85.1 (22.2)	85.0 (22.9)	.788
Fructosamine (mmol/L)	2.1 (0.3)	2.1 (0.3)	2.1 (0.3)	.059
CRP (mg/L)	6.8 (15.2)	6.9 (13.1)	6.8 (15.9)	.873
Alanine aminotransferase (μkat/L)	0.40 (0.39)	0.38 (0.37)	0.40 (0.40)	.004
Aspartate aminotransferase (μkat/L)	0.39 (0.26)	0.38 (0.23)	0.39 (0.27)	.004
Albumin (g/L)	41.3 (2.7)	41.2 (2.7)	41.3 (2.6)	.155
Gamma-glutamyl transferase (μkat/L)	0.54 (0.81)	0.52 (0.73)	0.55 (0.84)	.020
Platelets (10^9/L)	256.1 (70.1)	258.7 (73.5)	255.2 (69.2)	.003
Alkaline phosphatase (μkat/L)	3.1 (1.5)	3.1 (1.3)	3.1 (1.5)	.844
Iron (μmol/L)	17.3 (5.1)	17.1 (5.1)	17.3 (5.1)	.019
Hemoglobin (g/L)	139.4 (12.6)	138.7 (12.6)	139.6 (12.6)	<.001
MCV (fl)	92.2 (5.4)	92.2 (5.5)	92.2 (5.4)	.666
TIBC (μmol/L)	58.1 (7.7)	58.3 (7.7)	58.0 (7.7)	.082
Lactate dehydrogenase (μkat/L)	6.3 (1.3)	6.3 (1.2)	6.3 (1.4)	.782
B leucocytes (10^9/L)	6.7 (2.8)	6.8 (3.3)	6.7 (2.6)	.260
Uric acid (μmol/L)	307.0 (81.8)	306.2 (83.6)	307.3 (81.2)	.398

Notes: CRP = C-reactive protein; MCV = mean corpuscular volume; *SD* = standard deviation; TIBC = total iron-binding capacity.

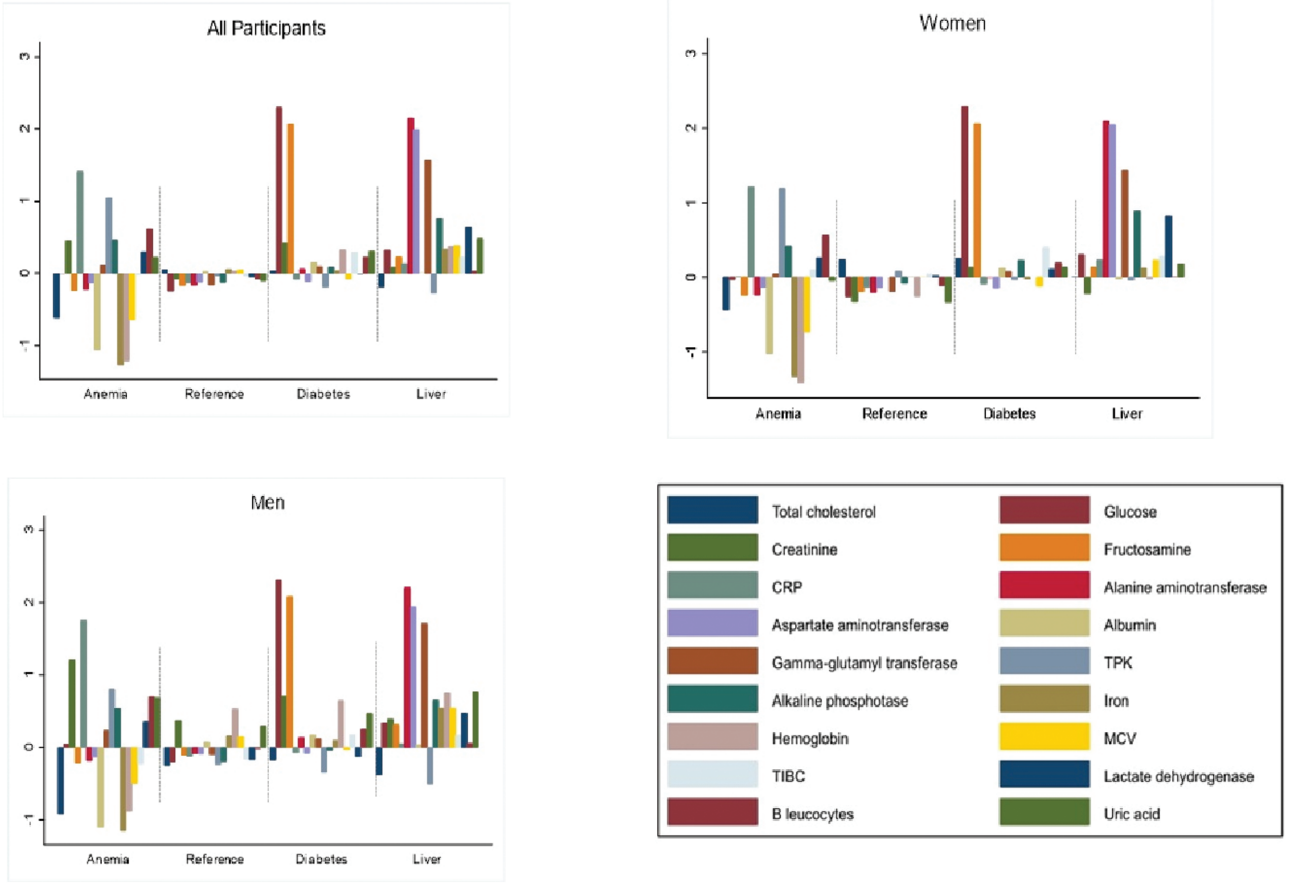

Figure 2. Levels of biomarkers, *z*-scored for easier comparison, in each class shown among all participants and for men and women, separately. Full color version is available within the online issue.

In sex-stratified analyses, men showed marginally higher associated risk of frailty than women. Among men, the Anemia (HR = 1.75, 95% CI 1.44, 2.13), Diabetes (HR = 1.61, 95% CI 1.37, 1.90), and Liver (HR = 1.20, 95% CI 0.99, 1.45), albeit nonsignificantly, classes had increased risk, compared to the Reference class. Similarly, among women, the Diabetes risk of frailty (HR = 1.57, 95% CI 1.36, 1.83) and Anemia (HR = 1.45, 95% CI 1.26, 1.67) were associated with significantly greater risk of frailty, while the Liver class (HR = 1.11, 95% CI 0.94, 1.31) had a slightly lower HR.

In analyses examining mortality, the Anemia (HR = 1.76, 95% CI 1.65, 1.87) and Diabetes (HR = 1.74, 95% CI 1.64, 1.85) classes had similar HRs, and the Liver class was associated with a somewhat lower risk (HR = 1.40, 95% CI 1.35, 1.63) (Figure 4). The Kaplan–Meier survival curves for these analyses are presented in Supplementary Figure 1. In sex-stratified analyses, men had moderately higher morality than women. The Anemia class was associated with double the mortality risk (HR = 1.83, 95% CI 1.64, 2.03) compared to the Reference class, while the Diabetes (HR=1.72, 95% CI 1.57, 1.87) and Liver (HR = 1.48, 95% CI 1.35, 1.63) classes were associated with slightly lower increased mortality risk. Among women, compared to the Reference class, the Anemia (HR = 1.72, 95% CI 1.58, 1.87), Diabetes (HR = 1.76, 95% CI 1.61, 1.92), and Liver (HR = 1.31, 95% CI 1.19, 1.45) classes were all statistically significantly associated with increased morality.

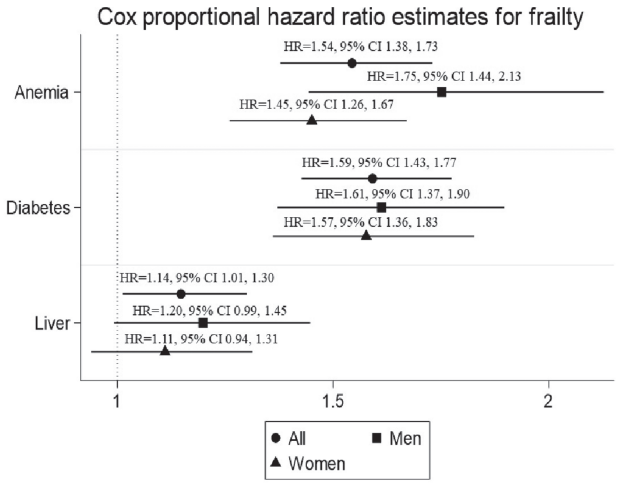

Figure 3. Cox proportional hazard models for the association between class membership probability and frailty, adjusted for baseline age and sex (where appropriate). Normal class as reference group; all participants: *n* failures = 5148, years at risk = 236 371; men: *n* failures = 1789, years at risk = 87 446; women: *n* failures = 3359, time at risk = 148 925.

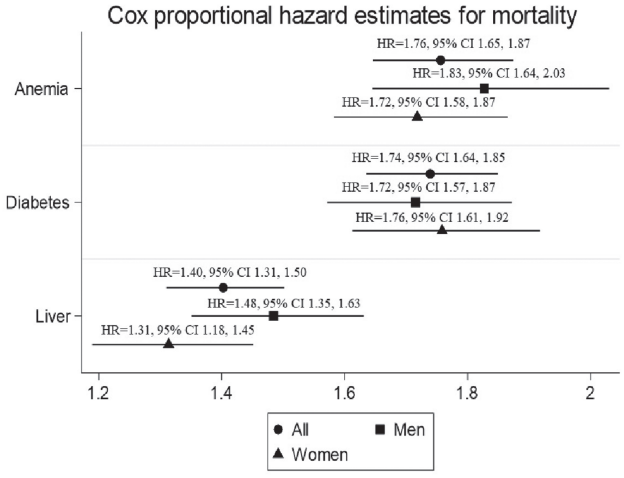

Figure 4. Cox proportional hazard models for the association between class membership probability and mortality, adjusted for baseline age and sex (where appropriate). Normal class as reference group; all: *n* of failures = 14 447, time at risk = 250 201; men: *n* failures = 5844, time at risk = 91 563; women: *n* failures = 8603, time at risk = 158 638.

Discussion

In this large cohort study, we used LCA to identify 3 classes, using 18 biomarkers, measured at ages 59 and older, and examined the association with later-life frailty and all-cause mortality. This study used biomarkers that are commonly measured in clinic and investigated them in concert with each other, thus eliminating the need to complete different, potentially expensive assays, but still providing new information about risk of frailty and mortality based on biomarker combinations. The Anemia class, associated with a high risk of mortality, as well as frailty, was characterized by higher levels of inflammatory markers and lower levels of iron, hemoglobin, and albumin. The Diabetes class, characterized by higher levels of glucose and fructosamine, was associated with the highest risk of frailty, as well as mortality. The Liver class, characterized by higher levels of liver enzymes, was associated with both frailty and mortality. Each of these classes accounted for approximately 6% of the sample, while the Reference class accounted for approximately 80% of the sample and did not have particularly high or low levels of any of the biomarkers. Models examining frailty and mortality outcomes were similar. Although the Diabetes class was associated with highest HR for frailty and the Anemia class with highest mortality, the frank differences in HRs were small and CIs were overlapping. This suggests that the classes, and the biomarkers that characterize them, are warning signs for both frailty and mortality.

We a priori had decided to additionally conduct sex-stratified analyses, because of the well-documented gender paradox observed in frailty research—women are more likely to live to older ages than men but are also more likely to become frail (8). In this study, men had slightly higher risk of frailty compared to women, though CIs overlapped. The patterns of mortality were similar among men and women and matched that of the overall sample. However, interestingly, the statistical association between class membership and both frailty and mortality tended to be higher among men than women. Although past studies have shown sex differences in the association between CRP levels and muscle strength (17), it was in the opposite direction—being more robust in women. These findings suggest that more research investigating how the association between biomarkers and frailty differs in men and women is needed.

In this study, we defined 3 risk classes associated with frailty and mortality. The Anemia class was termed such because it was characterized by biomarker levels suggesting anemia and inflammation processes (16). Anemia is common among older adults, with prevalence estimates ranging from 20% to 30% and increasing with age (18,19). It is a risk factor for falls, frailty, and early mortality, and there is an increased prevalence of anemia among frail individuals (20–22). Additionally, low hemoglobin, even within the normal range, have been associated with frailty. One study found that individuals with lower hemoglobin levels had a 6 times greater risk of frailty, compared to those with

higher levels (23). Similarly, inflammation, particularly chronic, low-grade inflammation, which is a pervasive process known as "inflammaging," is associated with frailty (24). Although iron status indicators are associated with inflammatory markers, like CRP (25), it also seems that chronic inflammation may lead to a decrease in hemoglobin levels, thus leading to anemia. Via this pathway, both chronic inflammation and anemia could contribute to the development of frailty (26,27). Because our measurement of all biomarkers occurred only at baseline, it is unknown whether the increased risk of frailty we saw associated with this class began with inflammation or anemia, but this study shows that the interplay of the two is important for frailty development. Critically, we saw higher levels of inflammatory markers in this class, but not in the other risk classes, thus suggesting that the relationship between anemia and inflammation is more strongly associated with frailty than the combination of inflammatory markers with the biomarkers of the Diabetes or Liver classes.

For example, although diabetes is linked with inflammation (28), the Diabetes class was characterized by higher levels of glucose and fructosamine but not inflammatory markers. Diabetes is highly prevalent among older adults (29), and frailty prevalence is higher among diabetics and diabetes is more prevalent among frail individuals (30,31). Among type 2 diabetics, higher glucose, glycated hemoglobin, and insulin levels were associated with lower muscle strength (32). Moreover, higher insulin levels in nondiabetics are associated with lower lean muscle mass and reduced gait speed and grip strength (33). Again, because biomarker levels were assessed at only one time point in this study, it is possible that higher glucose and fructosamine levels led to greater inflammation, muscle protein breakdown (34), and eventual frailty. However, diabetic markers are also linked to frailty through vascular dysfunction and hormonal imbalance (35). Diabetes is associated with cardiovascular disease and microvascular problems, and while the exact mechanisms are still unclear, these conditions are in turn associated with frailty (36,37). Diabetes is also linked to frailty through hormones, suggesting that the association differs by sex. Specifically, it has been shown that men with diabetes have lower testosterone levels, while diabetic women have higher levels (38,39), and lower levels of testosterone can also lead to a decline in muscle protein synthesis (40). These 3 hypothesized pathways—inflammatory, vascular, and hormonal—need to be further elucidated, to fully understand the risk between diabetes-related biomarkers and risk of frailty.

The third risk class in this study, the Liver class, was characterized by higher levels alanine aminotransferase and aspartate aminotransferase, which are well-known markers of chronic liver disease and liver injury and have been associated with increased risk of mortality (41), though not consistently (42). Elevated liver enzymes have been associated with use of common medications (eg, nonsteroidal anti-inflammatory drugs, statins) and certain conditions (eg, alcohol abuse, heart failure), though more recent data have shown that moderately high levels occur in diabetes, nonalcoholic fatty liver disease, obesity, and hyperlipidemia (43). In this study, we saw a separation between the Diabetes class and the Liver class. However, the Liver class also had slightly higher glucose levels, perhaps suggesting higher liver enzyme levels were driven by metabolic dysfunction. Age (44,45) and sex (46), although adjusted for here, have also been shown to affect alanine aminotransferase levels. Though, notably, further exploration of the data showed that this class, as in the other classes, had a near equal division between men (51%) and women (49%). Additionally, chronological age seems not to be a contributing factor, because this class had the youngest mean age (71 years) at baseline. We may, instead, be observing a reverse association. Studies have shown that alanine aminotransferase levels may be influenced by accelerated aging and frailty, independent of its role in liver function (44,45). The Liver class may additionally be characterized by accelerated aging, or a more advanced biological age (47), increasing liver enzyme levels and risk of frailty. Previous work has suggested that older adults with lower hemoglobin and liver enzyme levels should be examined regularly for frailty (48,49). However, overall, there may be a U-shaped, time-dependent relationship between frailty and liver enzymes, similar to the relationship between liver enzymes and mortality (50).

Strengths and Limitations

This study has multiple strengths, including a large sample size, a longitudinal design with more or less complete follow-up in registry data, and the inclusion of several commonly assessed biomarkers, investigated in tandem, measured in one lab with consistently implemented standardized methodology. However, the findings must also be considered within the context of the study's limitations. First, although use of registry data minimizes attrition, there were some AMORIS participants who did not have complete baseline measures for all 18 biomarkers included in this study. This may have introduced some selection, though it is unlikely to have affected the results substantially. Indeed, in post hoc age-adjusted Cox proportional hazard models specifying inclusion/exclusion as the independent variable, we did not find a substantial difference between risk of frailty and morality. Second, the biomarkers were measured between 1985 and 1996, and at least some of the mean biomarker levels in the population were different than compared to current mean levels. This necessitated the use of sample-specific cut-points in a few instances, which do not necessarily exactly match current clinical cutoffs. However, we believe that the general patterns of the biomarkers that define the 3 risk classes are of interest both in research and the clinic, and the findings can be applied to current biomarker measures that are commonly measured in general clinical practice. Third, frailty is a difficult syndrome to define and diagnose. We used a validated score that has good overlap with established frailty measures and can be applied to registry data (12), which allowed for a larger sample size, but may have led to a misestimation of frailty, because it is not as sensitive as some other commonly-used frailty measures. Finally, findings from this study may not be directly generalizable to more diverse populations, for which biomarker levels and associations with frailty and mortality may differ (42). Future research exploring any differences by ethnicity or race and sex would benefit this work and the findings we presented here.

Conclusions

Evidence from this study suggests that several commonly available biomarkers could be monitored in patients at risk for frailty. Our findings suggest extending existing recommendations to include participants with elevated liver enzyme levels, inflammatory marker levels, and diabetes-related markers, respectively. More work exploring groups and patterns of biomarkers—and how demographic variables modify them—and risk of frailty is needed. This work becomes more critical as the aging population grows along with multimorbidity and the burden of frailty.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None declared.

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References

1. Santos-Eggimann B, Cuénoud P, Spagnoli J, Junod J. Prevalence of frailty in middle-aged and older community-dwelling Europeans living in 10 countries. *J Gerontol A Biol Sci Med Sci*. 2009;64:675–681. doi:10.1093/gerona/glp012
2. Morley JE, Vellas B, van Kan GA, et al. Frailty consensus: a call to action. *J Am Med Dir Assoc*. 2013;14:392–397. doi:10.1016/j.jamda.2013.03.022
3. Puts MTE, Toubasi S, Andrew MK, et al. Interventions to prevent or reduce the level of frailty in community-dwelling older adults: a scoping review of the literature and international policies. *Age Ageing*. 2017;46:383–392. doi:10.1093/ageing/afw247
4. Kwon J, Yoshida Y, Yoshida H, Kim H, Suzuki T, Lee Y. Effects of a combined physical training and nutrition intervention on physical performance and health-related quality of life in prefrail older women living in the community: a randomized controlled trial. *J Am Med Dir Assoc*. 2015;16:263.e1–263.e8. doi:10.1016/j.jamda.2014.12.005
5. Yamada M, Arai H, Sonoda T, Aoyama T. Community-based exercise program is cost-effective by preventing care and disability in Japanese frail older adults. *J Am Med Dir Assoc*. 2012;13:507–511. doi:10.1016/j.jamda.2012.04.001
6. Saedi AA, Feehan J, Phu S, Duque G. Current and emerging biomarkers of frailty in the elderly. *Clin Interv Aging*. 2019;14:389–398. doi:10.2147/CIA.S168687
7. Mitnitski A, Collerton J, Martin-Ruiz C, et al. Age-related frailty and its association with biological markers of ageing. *BMC Med*. 2015;13:161. doi:10.1186/s12916-015-0400-x
8. Arber S, Cooper H. Gender differences in health in later life: the new paradox? *Soc Sci Med*. 1999;48:61–76. doi:10.1016/s0277-9536(98)00289-5
9. Van Hemelrijck M, Harari D, Garmo H, et al. Biomarker-based score to predict mortality in persons aged 50 years and older: a new approach in the Swedish AMORIS study. *Int J Mol Epidemiol Genet*. 2012;3:66–76.
10. Walldius G, Malmström H, Jungner I, et al. Cohort profile: the AMORIS cohort. *Int J Epidemiol*. 2017;46:1103–1103i. doi:10.1093/ije/dyw333
11. Jungner I, Marcovina SM, Walldius G, Holme I, Kolar W, Steiner E. Apolipoprotein B and A-I values in 147576 Swedish males and females, standardized according to the World Health Organization-International Federation of Clinical Chemistry First International Reference Materials. *Clin Chem*. 1998;44:1641–1649.
12. Gilbert T, Neuburger J, Kraindler J, et al. Development and validation of a Hospital Frailty Risk Score focusing on older people in acute care settings using electronic hospital records: an observational study. *Lancet*. 2018;391:1775–1782. doi:10.1016/S0140-6736(18)30668-8
13. Tobert JA. Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. *Nat Rev Drug Discov*. 2003;2:517–526. doi:10.1038/nrd1112
14. Nylund K, Asparouhov T, Muthén B. Deciding on the number of classes in latent class analysis and growth mixture modeling: a Monte Carlo simulation study. *Struct Equ Modeling*. 2007;14:535–569. doi:10.1080/10705510701575396
15. Nasserinejad K, van Rosmalen J, de Kort W, Lesaffre E. Comparison of criteria for choosing the number of classes in Bayesian finite mixture models. *PLoS One*. 2017;12:e0168838. doi:10.1371/journal.pone.0168838
16. Chen Y, Zhong H, Zhao Y, Luo X, Gao W. Role of platelet biomarkers in inflammatory response. *Biomark Res*. 2020;8:28. doi:10.1186/s40364-020-00207-2
17. Canon ME, Crimmins EM. Sex differences in the association between muscle quality, inflammatory markers, and cognitive decline. *J Nutr Health Aging*. 2011;15:695–698. doi:10.1007/s12603-011-0340-x
18. Bach V, Schruckmayer G, Sam I, Kemmler G, Stauder R. Prevalence and possible causes of anemia in the elderly: a cross-sectional analysis of a large European university hospital cohort. *Clin Interv Aging*. 2014;9:1187–1196. doi:10.2147/CIA.S61125
19. Bosco Rde M, Assis EP, Pinheiro RR, Queiroz LC, Pereira LS, Antunes CM. Anemia and functional capacity in elderly Brazilian hospitalized patients. *Cad Saude Publica*. 2013;29:1322–1332. doi:10.1590/s0102-311x2013000700007
20. Chaves PH, Semba RD, Leng SX, et al. Impact of anemia and cardiovascular disease on frailty status of community-dwelling older women: the Women's Health and Aging Studies I and II. *J Gerontol A Biol Sci Med Sci*. 2005;60:729–735. doi:10.1093/gerona/60.6.729
21. Penninx BW, Pluijm SM, Lips P, et al. Late-life anemia is associated with increased risk of recurrent falls. *J Am Geriatr Soc*. 2005;53:2106–2111. doi:10.1111/j.1532-5415.2005.00491.x
22. Zakai NA, Katz R, Hirsch C, et al. A prospective study of anemia status, hemoglobin concentration, and mortality in an elderly cohort: the Cardiovascular Health Study. *Arch Intern Med*. 2005;165:2214–2220. doi:10.1001/archinte.165.19.2214
23. Juárez-Cedillo T, Basurto-Acevedo L, Vega-García S, et al. Prevalence of anemia and its impact on the state of frailty in elderly people living in the community: SADEM study. *Ann Hematol*. 2014;93:2057–2062. doi:10.1007/s00277-014-2155-4
24. Franceschi C, Bonafè M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci*. 2000;908:244–254. doi:10.1111/j.1749-6632.2000.tb06651.x
25. McSorley ST, Jones I, McMillan DC, Talwar D. Quantitative data on the magnitude of the systemic inflammatory response and its relationship with serum measures of iron status. *Transl Res*. 2016;176:119–126. doi:10.1016/j.trsl.2016.05.004
26. Leng S, Chaves P, Koenig K, Walston J. Serum interleukin-6 and hemoglobin as physiological correlates in the geriatric syndrome of frailty: a pilot study. *J Am Geriatr Soc*. 2002;50:1268–1271. doi:10.1046/j.1532-5415.2002.50315.x
27. Leng SX, Hung W, Cappola AR, Yu Q, Xue QL, Fried LP. White blood cell counts, insulin-like growth factor-1 levels, and frailty in community-dwelling older women. *J Gerontol A Biol Sci Med Sci*. 2009;64:499–502. doi:10.1093/gerona/gln047
28. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest*. 2005;115:1111–1119. doi:10.1172/JCI25102
29. Ogurtsova K, da Rocha Fernandes JD, Huang Y, et al. IDF Diabetes Atlas: global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract*. 2017;128:40–50. doi:10.1016/j.diabres.2017.03.024
30. Castro-Rodríguez M, Carnicero JA, Garcia-Garcia FJ, et al. Frailty as a major factor in the increased risk of death and disability in older people with diabetes. *J Am Med Dir Assoc*. 2016;17:949–955. doi:10.1016/j.jamda.2016.07.013
31. Thein FS, Li Y, Nyunt MSZ, Gao Q, Wee SL, Ng TP. Physical frailty and cognitive impairment is associated with diabetes and adversely impact functional status and mortality. *Postgrad Med*. 2018;130:561–567. doi:10.1080/00325481.2018.1491779
32. Lee MR, Jung SM, Bang H, Kim HS, Kim YB. Association between muscle strength and type 2 diabetes mellitus in adults in Korea: data from the Korea National Health and Nutrition Examination Survey

(KNHANES) VI. *Medicine (Baltimore)*. 2018;97:e10984. doi:10.1097/md.0000000000010984
33. Yang CW, Li CI, Li TC, et al. The joint association of insulin sensitivity and physical activity on the skeletal muscle mass and performance in community-dwelling older adults. *Exp Gerontol*. 2017;95:34–38. doi:10.1016/j.exger.2017.05.006
34. Perkisas S, Vandewoude M. Where frailty meets diabetes. *Diabetes Metab Res Rev*. 2016;32(suppl 1):261–267. doi:10.1002/dmrr.2743
35. Assar ME, Laosa O, Rodríguez Mañas L. Diabetes and frailty. *Curr Opin Clin Nutr Metab Care*. 2019;22:52–57. doi:10.1097/MCO.0000000000000535
36. Angulo J, El Assar M, Rodríguez-Mañas L. Frailty and sarcopenia as the basis for the phenotypic manifestation of chronic diseases in older adults. *Mol Aspects Med*. 2016;50:1–32. doi:10.1016/j.mam.2016.06.001
37. Kirkman MS, Briscoe VJ, Clark N, et al; Consensus Development Conference on Diabetes and Older Adults. Diabetes in older adults: a consensus report. *J Am Geriatr Soc*. 2012;60:2342–2356. doi:10.1111/jgs.12035
38. Needham BL, Kim C, Mukherjee B, Bagchi P, Stanczyk FZ, Kanaya AM. Endogenous sex steroid hormones and glucose in a South-Asian population without diabetes: the Metabolic Syndrome and Atherosclerosis in South-Asians Living in America pilot study. *Diabet Med*. 2015;32:1193–1200. doi:10.1111/dme.12642
39. Strollo F, Strollo G, Morè M, et al. Low-intermediate dose testosterone replacement therapy by different pharmaceutical preparations improves frailty score in elderly hypogonadal hyperglycaemic patients. *Aging Male*. 2013;16:33–37. doi:10.3109/13685538.2013.773305
40. Morley JE. Androgens and aging. *Maturitas*. 2001;38:61–71; discussion 71. doi:10.1016/s0378-5122(00)00192-4
41. Yokoyama M, Watanabe T, Otaki Y, et al. Association of the aspartate aminotransferase to alanine aminotransferase ratio with BNP level and cardiovascular mortality in the general population: the Yamagata Study 10-year follow-up. *Dis Markers*. 2016;2016:4857917. doi:10.1155/2016/4857917
42. Schneider AL, Lazo M, Ndumele CE, et al. Liver enzymes, race, gender and diabetes risk: the Atherosclerosis Risk in Communities (ARIC) Study. *Diabet Med*. 2013;30:926–933. doi:10.1111/dme.12187
43. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol*. 2003;98:960–967. doi:10.1111/j.1572-0241.2003.07486.x
44. Dong MH, Bettencourt R, Barrett-Connor E, Loomba R. Alanine aminotransferase decreases with age: the Rancho Bernardo Study. *PLoS One*. 2010;5:e14254. doi:10.1371/journal.pone.0014254
45. Dong MH, Bettencourt R, Brenner DA, Barrett-Connor E, Loomba R. Serum levels of alanine aminotransferase decrease with age in longitudinal analysis. *Clin Gastroenterol Hepatol*. 2012;10:285–290.e1. doi:10.1016/j.cgh.2011.10.014
46. Mijovic V, Contreras M, Barbara J. Serum alanine aminotransferase (ALT) and gamma-glutamyltransferase (gamma-GT) activities in north London blood donors. *J Clin Pathol*. 1987;40:1340–1344. doi:10.1136/jcp.40.11.1340
47. Jylhävä J, Pedersen NL, Hägg S. Biological age predictors. *EBioMedicine*. 2017;21:29–36. doi:10.1016/j.ebiom.2017.03.046
48. Wawer AA, Jennings A, Fairweather-Tait SJ. Iron status in the elderly: a review of recent evidence. *Mech Ageing Dev*. 2018;175:55–73. doi:10.1016/j.mad.2018.07.003
49. Liu Z, Que S, Xu J, Peng T. Alanine aminotransferase-old biomarker and new concept: a review. *Int J Med Sci*. 2014;11:925–935. doi:10.7150/ijms.8951
50. Kim KN, Joo J, Sung HK, Kim CH, Kim H, Kwon YJ. Associations of serum liver enzyme levels and their changes over time with all-cause and cause-specific mortality in the general population: a large-scale national health screening cohort study. *BMJ Open*. 2019;9:e026965. doi:10.1136/bmjopen-2018-026965