CLINICAL INVESTIGATION

Combined Inflammation and Metabolism Biomarker Indices of Robust and Impaired Physical Function in Older Adults

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OBJECTIVES: To determine whether combinations of inflammatory markers are related to physical function.
DESIGN AND SUBJECTS: secondary analysis of baseline of three observational studies of community-dwelling older adults
MEASUREMENTS: The baseline data from 3 cohorts of older adults with different health and disease status were employed. Twenty markers of inflammation and metabolism were individually assessed for correlation with usual gait speed and were separated into robust and impairment quartiles. For the robustness and impairment indices, individual markers were selected using step-wise regression over bootstrapping iterations, and regression coefficients were estimated for the markers individually and collectively as an additive score.
RESULTS: We developed a robustness index involving 6 markers and an impairment index involving 8 markers corresponding positively and negatively with gait speed. Two markers, glycine and tumor necrosis factor receptor 1 (TNFR1), appeared only in the robustness index, and TNFR2; regulated on activation, normal T-cell expressed and secreted; the amino acid factor; and matrix metallopeptidase 3; appeared only in the impairment index.
CONCLUSION: Indices of biomarkers were associated with robust and impaired physical performance but differ, in composition suggesting potential biological differences that may contribute to robustness and impairment. **J Am Geriatr Soc 66:1353–1359, 2018.**

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Chronological age does not fully reflect biological or functional age.[1] Functional aging has been associated with physiological perturbations, such as inflammation, mitochondrial dysfunction, and loss of proteostasis,[2] with many biochemical intermediates involved. Attempts to better characterize functional aging have used clinical and laboratory parameters;[3,4] studies of circulating biomarkers of inflammation, coagulation, and endothelial function have generally indicated various perturbations of the inflammatory and coagulant systems associated with functional aging.[5,6]

Several circulating biomarkers in older adults have been associated with function, as reflected by physical performance measures such as gait speed.[7,8] We observed that 6 circulating inflammatory markers were negatively associated with gait speed.[7] We also reported that an acylcarnitine factor was inversely associated with the Short Physical Performance Battery score,[8] but no single marker was a particularly strong predictor, suggesting that combining individual metabolic and inflammatory markers to form indices might better predict physical performance. In addition, although the physically robust elderly population is attracting attention,[9] it has not been as well molecularly characterized as the frail older adult population. It is clinically important to molecularly characterize cohorts that perform well in aging as well as those that do not.

In this study, we assessed the association between the combination of multiple inflammatory, coagulant, and metabolic biomarkers and good or poor physical performance and derived robustness and impairment indices to characterize these relationships. We hypothesized that these indices would differ significantly in molecular composition, perhaps reflecting differing biological etiologies of robustness and impairment.

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MATERIAL AND METHODS

Contributing Studies

This study used the baseline data from 3 longitudinal cohorts: the Veterans Learning to Improve Fitness and Function in Elders (LIFE) Study (2004–2007) compared older veterans receiving home-based physical activity counseling with those receiving usual primary care and studied their changes in physical activity and function (N=74, aged 79.2±4.8);[10] Prediction of Osteoarthritis Progression (POP; 2003–2008) evaluated predictors of osteoarthritis progression in older men and women (N=137, aged 65.9±11.6);[11] and the Carolina Region Interaction of Aging Genes and Environment (CARRIAGE Family Study, 2002, 2004, 2006) followed a large extended family made up of mainly African and Native American members for traits associated with cardiovascular disease and arthritis (N=30, aged 71.6±5.5)[12]. All 3 studies were conducted at Duke University or the Durham Veterans Affairs Medical Center, received annual approval from their institutional human studies review boards, and were participating external studies of the Duke Older American Independence Center analysis focused on understanding functional decline in older adults. Demographic characteristics of the volunteers in the cohorts are described in Appendix Table S1.

Biomarkers

The biomarkers included metabolites and 17 inflammation-related markers selected for previous associations with function.[7] Blood was collected using venipuncture, separated to yield plasma and serum, and immediately stored at −80°C. POP samples were all obtained 2 hours postprandially; sampling of blood was not standardized for LIFE or CARRIAGE. The metabolites included an amino acid (AA) factor derived from principal component analysis (PCA) of 15 amino acids and validated in the 3 studies used here;[13] glycine, representing a second AA PCA factor;[13] and an acylcarnitine PCA factor derived from 45 acylcarnitines.[8,13] AAs and acylcarnitines were measured using tandem mass spectrometry, as previously described.[14–18] Ten of the inflammatory markers were measured on a multiplex bead panel (Luminex, Invitrogen, Carlsbad, CA): granulocyte colony-stimulating factor (GCSF); interleukin (IL)-1 receptor antagonist (IL-1RA); IL-2; IL-8; monocyte chemotactic protein-1; regulated on activation, normal T-cell expressed and secreted (RANTES); tumor necrosis factor alpha (TNFα); TNF receptor 1 (TNFR1); TNFR2; and vascular endothelial growth factor. Five other markers were measured using enzyme-linked immunosorbent assay: IL-6 (MSD Ultrasensitive Assay, Meso Scale Discovery, Gaithersburg, MD), matrix metalloproteinase 3 (MMP-3; BioSource, Camarillo, CA), TNF-related apoptosis-inducing ligand (BioSource), leptin (Millipore, Billerica, MA), and vascular cell adhesion molecule (R&D Systems, Minneapolis, MN). Paraoxonase was quantified according to organophosphatase-specific activity of paraoxonase (Invitrogen, Eugene, OR). D-dimer was measured according to immunoassay (American Diagnostica, Stamford, CT). All markers were measured in serum, except for D-dimer, which was measured in plasma.[7] These conditions and tests had been determined to provide best reproducibility and sensitivity.[19] Intra- and interday coefficients of variation (CVs) of targeted acylcarnitine and AA assays in longitudinal, quality-control sera were less than 15%. For inflammatory markers, all samples were analyzed in duplicate, and analyses were repeated for out-of-range high values and for any duplicates with CVs greater than 10%. These CVs are within the generally accepted range of the field (<15%).[20,21] Individuals with an undetectable level of a marker, being informative for distinguishing quartiles, were assigned a value of half the lower level of detection of the assay. Large neutral AAs, including the essential branched-chain AAs isoleucine, leucine, and valine; the sulfur-containing AA methionine; and the aromatic AAs phenylalanine and tyrosine highly weighted the AA factor.[13] Medium- to long-chain acylcarnitines with acyl side chains of 10 to 18 carbons highly weighted the acylcarnitine factor; there was a small contribution from odd chain species, largely derived from catabolism of AAs.[8,13]

Physical Performance

All three studies used usual gait speed as a measure of physical performance, measured using 1 trial (CARRIAGE) or the faster of 2 trials (LIFE, POP) of a 10-m usual-pace walk[10] Gait speed is a common measurement for gauging physical performance in older adults and is closely related to mortality, health, and independence.[22,23]

Statistical Analysis

The independent variables included 17 inflammatory markers, the acylcarnitine factor, the AA factor, and glycine; the dependent variable was usual gait speed. Biomarkers were coded as 0 or 1, using cut-points for the highest and lowest quartiles of the markers correlated in a positive (robustness) or negative (impairment) direction with gait speed determined using the weighted Spearman correlation coefficients (Appendix Table S2).[7]

To create the robustness score, biomarker values in the robustness quartile were assigned a value of 1; values in all other quartiles were assigned a value of 0. Similarly, to create the impairment score, values in the impairment quartile were assigned a value of 1. For IL-2, TNFα, and vascular endothelial growth factor, the percentage of values below the lower level of detection exceeded 25%. All subjects in this group were assigned to the lower quartile, resulting in group size differences.

To determine the markers to be used for the robustness index, the 0 or 1 indicators for each biomarker in the panel were entered into a stepwise regression of gait speed. We retained only significant predictors as determined using 1,000 bootstrapped models with the entire panel—adjusting for age, sex, race, and body mass index (BMI)—to address possible model instability. Variables retained in 50% or more of the bootstrapped models were included in the final index (Appendix Table S3). The same procedure was performed independently to determine the list of markers to be included in the impairment index.

To evaluate the robustness and impairment indices, each individual's score was defined as the proportion of the 1's observed for the biomarkers in the respective models, and linear regression was performed of gait speed on the index score. Multivariable regression was performed to assess the relative contribution of each biomarker in the models. Participants with missing biomarkers (n=7) were excluded from the indices. The indices were evaluated in each cohort individually, and their homogeneity was assessed. The correlation of the impairment and robustness indices was assessed.

RESULTS

Biomarker Robustness Index

Six markers qualified for this index: glycine, IL-6, TNFR1, D-dimer, IL-2, and paraoxonase, of which glycine is a metabolic marker; IL-6, IL-2, TNFR1, and paraoxonase are inflammatory markers; and D-dimer is an inflammatory and coagulant marker. Table 1 displays the cut-off values; lower values of 4 markers (IL-6, TNFR1, D-dimer, glycine) and higher values of 2 markers (IL-2, paraoxonase) correlated positively with gait speed. The relative contribution of each biomarker, as assessed using standardized multivariable regression, is also shown in Table 1. Figure 1 shows and Appendix Table S4 details predicted gait speed based on robustness score for each cohort, with and without adjustments for age, race, sex, and BMI. The adjustments slightly weakened the correlation between the index and gait speed. Linear models were chosen to demonstrate the relationships between gait speed and robustness score; removing 1 outlier did not alter this correlation in the LIFE Study (Appendix Table S5). Testing homogeneity of effect sizes indicated nonhomogenous effects across studies (Q-statistic=8.15, degrees of freedom (df)=2, p=.02), but because 2 of the 3 studies showed Positive correlations between gait speed and robustness index and the other, smallest study (CARRIAGE), was inconclusive regarding trend, the index could still reasonably be considered a marker of robustness.

Biomarker Impairment Index

Eight markers were retained for the impairment index. Table 2 displays the cut-points for the impairment index model; higher values of 5 markers (IL-6, TNFR2, the AA factor, D-dimer, RANTES) and lower values of 3 markers (paraoxonase, MMP-3, IL-2) correlated with the lowest quartile of gait speed. Of the 8 markers, AA factor is a metabolic marker; IL-6, IL-2, TNFR2, paraoxonase, MMP-3, and RANTES are inflammatory markers; and D-dimer is an inflammatory and coagulant marker. The relative contribution of each biomarker, as assessed using standardized multivariable regression, is also shown in Table 2. Similar to the robustness index, linear models were shown to represent the correlations in Figure 2 and detailed with and without adjustments in Appendix Table S4. Again, the adjustments slightly weakened the correlation between the index and gait speed. The 3 cohorts all showed negative correlations between gait speed and the impairment index score. Testing homogeneity of effect sizes indicated homogenous effects across studies (Q-statistic=0.98, df=2, p=.61), allowing for an interpretable overall weighted summary effect. Fixed-effects meta-analysis indicated a decrease of 0.045 m/s (95% confidence interval=–0.063 to –0.027) in gait speed per 0.1-point increase in impairment index score.

Correlation between indices

The two indices have a correlation of –0.353 in CARRIAGE, –0.380 in POP, and –0.334 in LIFE, indicating that markers of fast and slow gait speed are sufficiently different from each other and that our indices are not simply 2 extremes of a single index. Two markers, glycine and TNFR1, appeared only in the robustness index, and TNFR2, RANTES, the AA factor, and MMP-3 appeared only in the impairment index, suggesting that certain markers and their associated physiological processes may be more significant in people performing at one end of the spectrum of gait speed but not the other. Four other markers, IL-6, IL-2, D-dimer, and paraoxonase, appeared in both indices, suggesting an association with fast and slow gait speed.

Table 1. Cut-Points and Weights for Biomarker Robustness Index Model for Each Cohort Using Multivariable Regression

Biomarker	Cutoff	Veterans Learning to Improve Fitness and Function in Elders Study, N = 73[a]	CARolinas Region Interaction of Aging, Genes and Environment, N = 30[b]	Prediction of Osteoarthritis Progression, N = 135[c]
		Beta (Standard Error) P-Value		
IL-6	<0.67	0.136 (0.094) .15	–0.029 (0.092) .76	0.027 (0.050) .59
Glycine	<249,279.41	0.074 (0.063) .24	0.239 (0.159) .15	0.055 (0.048) .26
Tumor necrosis factor receptor 1	<1,870.10	0.154 (0.075) .04		–0.004 (0.078) .96
D-dimer	<333.26	0.109 (0.102) .29	–0.171 (0.132) .21	0.074 (0.046) .12
Paxillin	≥7.37	0.187 (0.196) .34	–0.007 (0.097) .94	0.085 (0.046) .06
IL-2	≥47.27	0.125 (0.080) .12	–0.128 (0.106) .24	0.034 (0.044) .45

[a]F(9,63) = 4.067, p > F = < .001, adjusted for age, body mass index (BMI), sex, and race; adjusted coefficient of determination (R^2) = 0.277; root mean square error (RMSE) = 0.247.
[b]F(8,21) = 2.278, p > F = .06, adjusted for age, BMI, sex, and race; adjusted R^2 = 0.261; RMSE = 0.194.
[c]F(10,124) = 5.053, p > F = < .001, adjusted for age, BMI, sex, and race; adjusted R^2 = 0.232; RMSE = 0.219.
IL = interleukin.

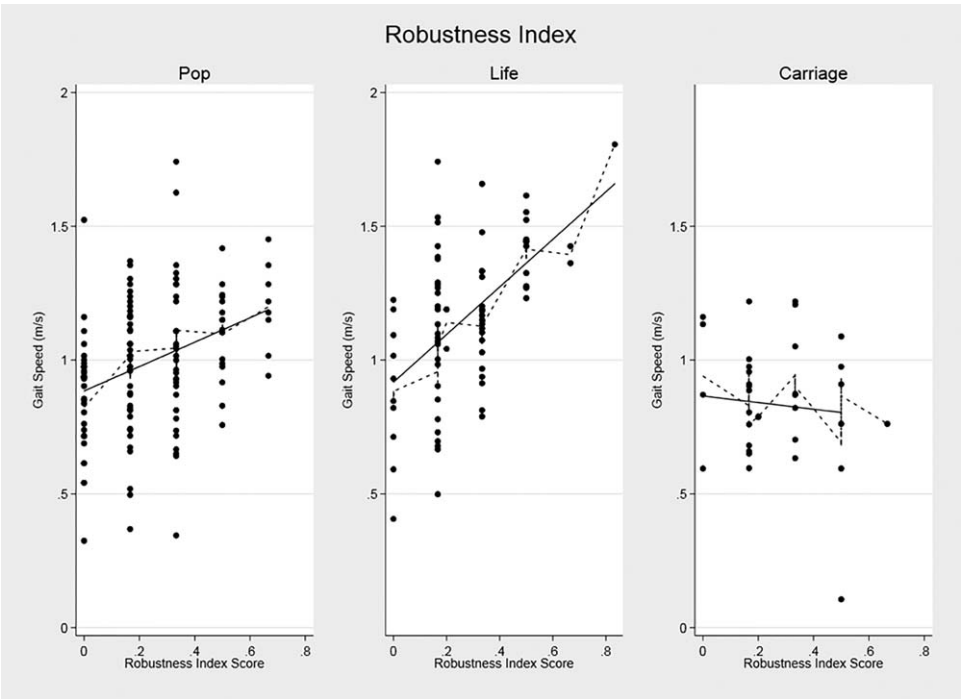

Figure 1. Gait speed predicted according to robustness index score according to cohort.

The correlation between biomarkers was mostly nonsignificant or weak, with few exceptions (e.g., TNFα with GCSF, TNFα with IL-1RA, GCSF with IL-1RA) (Appendix Table S6).

DISCUSSION

Robustness and impairment indices, derived from a combination of inflammatory and metabolic markers, had a statistically significant but moderate correlation with gait speed. Adjusting for age, race, sex, and BMI appeared to decrease the strength of the correlations, showing that the correlation of the indices with gait speed depends on demographic variables and may differ for each subgroup. Individually, some of the markers we studied have been significantly associated with gait speed[7], but our study is the first to demonstrate that indices using levels of circulating small molecules involved in inflammatory, coagulant, and metabolic pathways in combination correlated better with a quantifiable physical performance measure

Table 2. Cut-Points and Weights for Biomarker Impairment Index Model for Each Cohort According to Multivariable Regression

Biomarker	Cutoff	Veterans Learning to Improve Fitness and Function in Elders Study, N = 73[a]	CARolinas Region Interaction of Aging, Genes and Environment, N = 29[b]	Prediction of Osteoarthritis Progression, N = 135[c]
		Beta (Standard Error) P-Value		
IL-6	≥1.75	–0.226 (0.067) .001	–0.119 (0.148) .43	–0.012 (0.047) .79
Regulated on activation, normal T-cell expressed and secreted	≥19,549.44	0.069 (0.068) .32	–0.203 (0.264) .45	–0.009 (0.067) .89
Tumor necrosis factor receptor 2	≥3,038.65	–0.080 (0.081) .32	–0.023 (0.095) .81	–0.109 (0.049) .03
Amino acid factor	≥0.87	0.058 (0.087) .51	–0.057 (0.087) .52	–0.107 (0.044) .02
D-dimer	≥824.50	–0.049 (0.070) .48	–0.120 (0.107) .28	–0.093 (0.056) .10
Matrix metallopeptidase 3	<4.28		–0.026 (0.129) .84	–0.055 (0.043) .20
IL-2	<12.65	–0.072 (0.068) .29	0.023 (0.091) .81	–0.035 (0.040) .39
Paxillin	<4.54	–0.084 (0.064) .20	–0.090 (0.156) .57	–0.088 (0.061) .15

[a]$F(10,62) = 4.143$, $p > F = < .001$, adjusted for age, BMI, sex, and race; adjusted coefficient of determination (R^2) = 0.304; root mean squared error (RMSE) = 0.243.
[b]$F(11,17) = 1.074$, $p > F = .43$, adjusted for age, BMI, sex, and race; adjusted $R^2 = 0.028$; RMSE = 0.179.
[c]$F(12,122) = 5.169$, $p > F = < .001$, adjusted for age, BMI, sex, and race; adjusted $R^2 = 0.272$; RMSE = 0.213.
IL = interleukin.

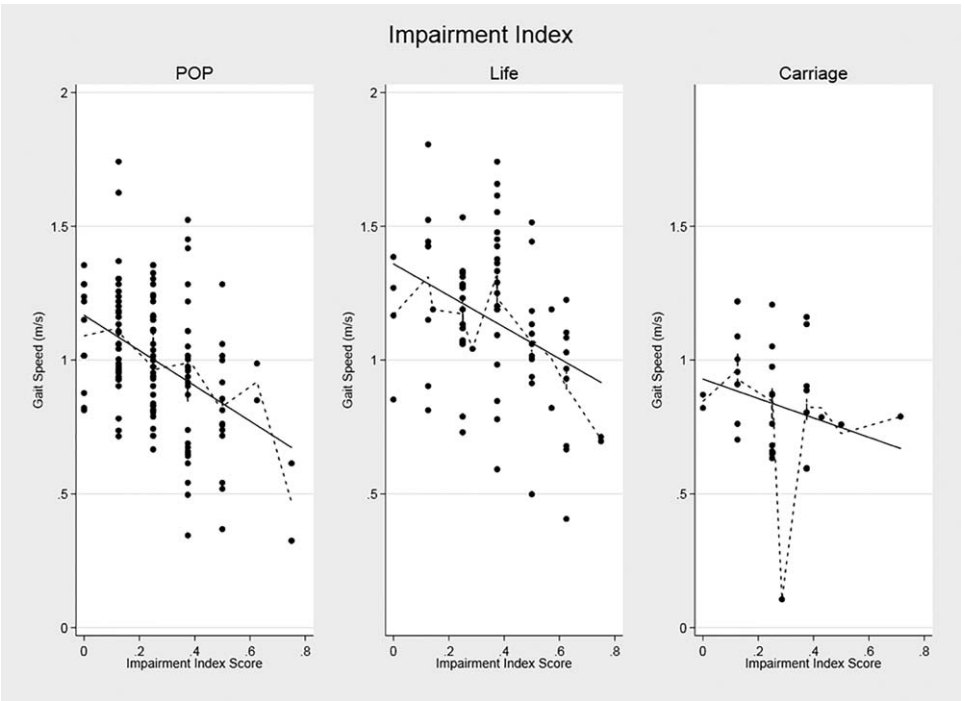

Figure 2. Gait speed predicted according to impairment index score according to cohort.

than using inflammatory or metabolic markers individually. Although small variations in circulating concentrations of each molecule were not strongly associated individually with gait speed[7], the cumulative effect of multiple such alterations appeared to have a statistically significant ability to predict physical function, similar to the cumulative effect of combining deficits from a wide range of laboratory tests and clinical examinations.[3,4,24] This appeared to be effective even if the markers of inflammation, metabolism, and coagulation were not indicators of a single pathway but contributed something individually to systems underlying robustness or impairment.

The robustness index is composed of a different combination of biomarkers than the impairment index. In our robustness index, a score above 2/6 corresponds to a predicted gait speed faster than 1 m/s. As others have described, a gait speed faster than 1 m/s suggests healthy aging and better than average life expectancy,[25,26] although it is unclear whether there is added robustness for gait speeds substantially faster than 1 m/s. Furthermore, although there are individuals with very low gait speeds in the impairment index (e.g., <0.5 m/s), because the mean gait speed values corresponding to the highest impairment index score of 0.8 can be as high as 0.9 m/s (LIFE), we are limited in our ability to apply the index to individuals with very slow gait speed, so these indices require further validation in bigger datasets before they can be considered clinically meaningful.

The markers present in the robustness index model are not identical to those of the impairment index. In almost all previous studies involving functional performance in aging, markers were selected based on correlation with poor outcomes such as frailty or mortality.[3,4,27] Given previous genomic studies of healthy aging,[9] it is worth considering that the high and poor performers are different phenotypes that correspond to different molecular characterizations; our results also suggest this. The differences in biomarkers associated with fast and slow gait speed hint at differences in biological processes underlying good and poor functioning. Although we have not studied the specific recovery responses of these individuals to a stressor (indicative of resilience), it is possible that individuals scoring high on the robustness index may be more resilient. This is an area for future study.

Although the relationship between individual markers and physical performance was not the objective of this study, there are mechanisms by which the biological functions that these markers represent might contribute. Some markers, such as D-dimer and IL-6, have been modestly associated with mortality and poor physical performance.[7,28,29] They were present in the robustness and impairment indices, suggesting that their relationship with a spectrum of function may be more linear than other markers that preferentially predict only good or poor function. Others such as high TNFR1 and TNFR2 have been correlated with poor physical performance.[7,30] Here, high TNFR2 was included as a predictor of slow gait speed and low TNFR1 of fast gait speed, suggesting that these markers predict good performance, as well. The use of decreasing TNFR1\TNFR2 as a sign of improvement after exercise therapies for patients with osteoarthritis and chronic heart disease supports this.[31,32] RANTES, a proinflammatory cytokine, is high in frail, older adults[33] and increases with aging.[34] It is not clear how high RANTES is related to slow gait speed.

The AA factor has been associated with metabolic disease[35,36] and with older age and greater BMI.[13] Branched chain AAs—the major components of the AA factor—have also been associated with development of insulin resistance and type 2 diabetes mellitus.[37] This could

explain our finding of the association between high AA factor and slow gait speed after controlling for BMI. Another finding of interest was the association between low circulating glycine concentrations and robustness. Low glycine levels are associated with obesity and insulin resistance,[35,38,39] and high glycine appears to protect against regulated cell death.[40] Most of the glycine studies were conducted in middle-aged populations,[35,38,39] so this relationship may be different in older adults.

Low MMP-3, paraoxonase, and IL-2 levels were associated with slower gait speed. MMP-3 is involved in proteolytic pathways that are altered in aging[41] and with faster extracellular matrix degradation and greater MMP-3 concentrations in osteoarthritis.[42] Paraoxonase is a component of extracellular matrices. Low MMP-3 and paraoxonase may be related to a decline in activity of the matrix repair processes. It was surprising that low IL-2 appeared in the impairment index. Released by T-cells, IL-2 is expected to rise with other inflammatory markers during decline in physical performance. A potential explanation may lie in T-cell exhaustion, a phenomenon associated with chronic infection and malignancy,[43] in which IL-2 is the first cytokine to decrease.[44] It is possible that our finding is related to the sentinel event of a similar exhaustion process associated with age-related inflammation.

Our study has limitations. The sample size was limited relative to the number of biomarkers. The lack of longitudinal data does not allow any conclusions regarding causation or predictions of functional trajectories. The sample may not be representative of the general aging population. The indices are limited in their ability to distinguish between individuals with faster and slower gait speeds. Also, our findings require replication in other cohorts. Thus, our results should be considered hypothesis generating.

In conclusion, we have developed biomarker robustness and impairment indices that correlate with gait speed and suggest that, regardless of the effect of individual markers of inflammatory, coagulant, and metabolic pathways, the cumulative effect may be important and of use for assessing robustness, which may be related to resilience, and impairment, which may be related to frailty. This study is the first report on a cumulative index focused on robust aging as opposed to frailty. Although our indices correlated with statistically significant differences in gait speed, the clinical significance of these findings awaits further studies of larger and longitudinal cohorts.

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Author Contributions: Zuo, Luciano, Pieper, Cohen: Concept and design, acquisition of laboratory test - based data, selection of laboratory test - based variables and providing information on them. Zuo, Luciano and Pieper: Statistical analyses. All authors: data interpretation, preparation of manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1. Demographics and average gait speed by study.

Table S2. Correlation of the individual biomarkers with gait speed

Table S3. Percentage of stepwise models where candidate biomarker included, of 1000 repetitions drawn over whole dataset. Retained variables have been highlighted (>50%).

Table S4. Robustness and impairment index models by mean score

Table S5. Robustness index model by mean robustness score for LIFE, after removing one outlier

Table S6. Pairwise correlation matrix between all biomarkers in this study. Significant and strong correlations have been highlighted

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