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

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Abstract

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.

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Author Contributions

Zuo, Luciano, Pieper, Kraus, 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.

Presentation: Parts relevant to the paper has been presented at the 2017 AGS annual scientific conference and the 2017 IAGG world congress of geriatrics and gerontology

Conflict of Interest

The authors have no conflict of interest to declare.

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 metalloproteinase 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.

Keywords

Inflammation; Metabolism; Robustness; Gait Speed; Deficit Accumulation

Introduction

Chronological age does not fully reflect biological or functional age¹. Functional aging has been associated with physiological perturbations, such as increased inflammation, mitochondrial dysfunction and loss of proteostasis², with many biochemical intermediates involved. Attempts to better characterize functional aging have utilized both 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 six circulating inflammatory markers were negatively associated with gait speed⁷. We also reported that an acylcarnitine (AC) factor was inversely associated with the Short Physical Performance Battery score⁸. However, 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 attention⁹, 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 of the combination of multiple inflammatory, coagulant and metabolic biomarkers with 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.

Material and Methods

Contributing Studies

This study used the baseline data from three longitudinal cohorts: 1) LIFE (Veteran's LIFE study, 2004–2007) followed older veterans with home-based physical activity counseling versus usual primary care and studied their changes in physical activity and function (N=74,

age=79.2±4.8 years)¹⁰; 2) POP (Prediction of Osteoarthritis Progression, 2003–2008) evaluated predictors of osteoarthritis progression in older men and women (N=137, age=65.9±11.6 years)¹¹; 3) the CARRIAGE Family study (Carolina Region Interaction of Aging Genes and Environment, 2002, 2004 and 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, age=71.6±5.5 years)¹². All studies were conducted at Duke University or the Durham Veterans Affairs Medical Centers, received annual approval by their institutional human studies review boards, and were participating external studies of the Duke Older American Independence Center's analysis focused on understanding functional decline in the older adults. Demographics of the volunteers in the cohorts are described in Supplement 1.

Biomarkers

The biomarkers included metabolites and seventeen inflammation-related markers selected for previous associations with function⁷. Blood was collected by venipuncture, separated to yield plasma and serum, and immediately stored at –80°C. POP samples were all obtained 2 hours post-prandially; sampling of blood was not standardized for LIFE or CARRIAGE. The metabolites included: an AA factor derived from principal component analysis (PCA) of 15 amino acids and validated in the three studies used here¹³; glycine, representing a second AA PCA factor¹³; and an AC PCA factor derived from 45 ACs^{8, 13}. AAs and ACs were measured by tandem mass spectrometry as previously described^{14–18}. Ten of the inflammatory markers were measured on the Luminex multiplex bead panel (Invitrogen, Carlsbad, CA): granulocyte colony-stimulating factor (GCSF), IL-1 receptor antagonist (IL-1RA), IL-2, IL-8, monocyte chemotactic protein-1 (MCP-1), RANTES, TNFα, TNFR1, TNFR2, and vascular endothelial growth factor (VEGF). Five other markers were measured by ELISA: IL-6 (the MSD Ultrasensitive Assay, Meso Scale Discovery, Gaithersburg, MD), MMP-3 (BioSource, Camarillo, CA), TNF-related apoptosis-inducing ligand (TRAIL) (BioSource), leptin (Millipore, Billerica, MA) and vascular cell adhesion molecule (VCAM) (R&D Systems, Minneapolis, MN). Paraoxonase (PXN) was quantified by organophosphatase-specific activity of paraoxonase by a kit from Invitrogen (Eugene, OR). D-dimer was measured by immunoassay (American Diagnostica, Stamford, CT). All markers were measured in serum, except for D-dimer in plasma⁷. These conditions and tests had been determined to provide best reproducibility and sensitivity¹⁹. Intra- and inter-day coefficients of variation (CV) of targeted AC and AA assays in longitudinal, quality-control (QC) sera were < 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 coefficients of variation (CVs) above 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 one-half the lower level of detection (LLOD) of the assay. The AA factor was highly weighted by large neutral AAs, including: the essential branched-chain AAs isoleucine, leucine, and valine; the sulfur-containing AA methionine; the aromatic AAs phenylalanine and tyrosine¹³. The AC factor was highly weighted by medium to long-chain ACs with acyl side chains of 10–18 carbons; 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 by one trial (CARRIAGE) or the faster of two trials (LIFE and POP) of a 10-meter walk at usual pace¹⁰. Gait speed is a common measurement for gauging physical performance in the older population and is closely related to mortality, poor health and loss of independence^{22, 23}.

Statistical Analysis

The independent variables included seventeen inflammatory markers, the AC factor, the AA factor, and glycine; the dependent variable was usual gait speed. Biomarkers were coded as 0/1, using cut-points for the highest/lowest quartile of the markers correlated in a positive (Robustness) or negative (Impairment) direction with gait speed determined by the weighted Spearman correlation coefficients (Supplement 2)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, TNF α , and VEGF, the percentage of values below the LLOD 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 0/1 indicators for each biomarker in the panel entered a stepwise regression of gait speed. We retained only significant predictors as determined by 1000 bootstrapped models with the entire panel — adjusting for age, gender, race and BMI — to deal with possible model instability. Variables retained in 50% or more of the bootstrapped models were included in the final index (Supplement 3). 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. In order to assess the relative contribution of each biomarker in the models, multivariable regression was performed. Participants with missing biomarker(s) (seven individuals) were excluded from the indices. 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 PXN, of which glycine is a metabolic marker, IL-6, IL-2, TNFR1 and PXN are inflammatory markers, and D-dimer is both an inflammatory and coagulant marker. Table 1 displays the cut-off values: lower values of 4 markers (IL-6, TNFR1, D-dimer and glycine) and higher values of 2 markers (IL-2 and PXN) correlated positively with gait speed. The relative contribution of each biomarker, as assessed by standardized multivariable regression, is also shown in Table 1. Figure 1 shows, and Supplement 4 details predicted gait speed based on

robustness score for each cohort, adjusted for age, race, gender and BMI. Linear models were chosen to demonstrate the relationships between gait speed and Robustness score, and removing one outlier did not alter this correlation in the LIFE study (Supplement 5). Testing homogeneity of effect sizes indicated non-homogenous effects across studies (Q-statistic = 8.15, df = 2, p-value = 0.02). However, as two of the three studies showed increasing gait speed related to 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 and RANTES) and lower values of 3 markers (PXN, MMP3 and IL-2) correlated with the lowest quartile of gait speed. Of the eight markers, AA factor is a metabolic marker, IL-6, IL-2, TNFR2, PXN, MMP3 and RANTES are inflammatory markers, and D-dimer is both an inflammatory and coagulant marker. The relative contribution of each biomarker, as assessed by standardized multivariable regression, is also shown in Table 2. Similar to the Robustness Index, linear models were chosen in Figure 2 and detailed in Supplement 4. The three cohorts all showed decreasing gait speed related to the Impairment Index score. Testing homogeneity of effect sizes indicated homogenous effects across studies (Q-statistic = 0.98, df = 2, p-value = 0.61), allowing for an interpretable overall weighted summary effect. Fixed-effects meta-analysis indicated a decrease of 0.045 m/s (95% CI: -0.063 to -0.027) in gait speed per 0.1 increase in the Impairment Index score.

Correlation between Indices

The two Indices have a correlation of -0.353, -0.380 and -0.334 in CARRIAGE, POP and LIFE, respectively, indicating that markers of high and low gait speed are sufficiently different, and that our Indices are not simply two extremes of a single index. It is worth noting that two markers, glycine and TNFR1, appeared only in the Robustness Index, while TNFR2, RANTES, the AA factor, and MMP3 appeared uniquely in the Impairment Index. This suggests 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 PXN, appeared in both Indices, suggesting an association with both high and low gait speed.

The correlation between biomarkers was mostly insignificant or weak with few exceptions (e.g., TNF α with GCSF, TNF α with IL-1RA, and GCSF with IL-1RA), shown in Supplement 6.

Discussion

Robustness and Impairment Indices, derived from a combination of inflammatory and metabolic markers, had a statistically significant but moderate correlation with gait speed. Individually, some of the markers we studied have been significantly associated with gait speed⁷. However, our study is the first to demonstrate that, compared to using only

inflammatory or metabolic markers individually, indices using levels of circulating small molecules involved in inflammatory, coagulant and metabolic pathways in combination correlated better with a quantifiable physical performance measure. Although, individually, small variations in the circulating concentrations of each molecule were not strongly associated with gait speed⁷, 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 lab tests and clinical exams^{3, 4, 24}. This appeared effective even if the markers of inflammation, metabolism and coagulation were not indicators of a single pathway, but, individually, contributed something 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 above 1 m/s. As described by others, gait speed above 1 m/s suggest healthier aging and better than average life expectancy^{25, 26}, although it is unclear if there is added robustness for gait speeds substantially greater than 1m/s. Furthermore, while there are individuals with very low gait speeds in the Impairment Index (e.g. <0.5m/s), since 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 of very low gait speed. Therefore, 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 aging⁹, it is worth considering that the high and poor performers are possibly different phenotypes that correspond to different molecular characterizations; our results also suggest this. The differences in biomarkers associated with high and low gait speed hint at differences in biological processes underlying good and poor functioning. Though we have not studied the specific recovery responses of these individuals to a stressor (indicative of resilience), it is possible that those individuals scoring high on the Robustness Index may be more resilient. This is an area for future study.

Although the relationship of individual markers to physical performance was not the objective of this study, there are mechanisms by which the biological functions represented by these markers 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 both the Robustness and Impairment Indices. This suggests that their relationship with a spectrum of function may be linear compared to 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 low gait speed, and low TNFR1 of high gait speed, suggesting that these markers predict good performance, as well. This is supported by the use of decreasing TNFR1/TNFR2 as a sign of improvement after exercise therapies for patients with osteoarthritis and chronic heart disease^{31, 32}. RANTES, a pro-inflammatory cytokine, is

greater in frail, older adults³³ and is elevated with aging³⁴. It is not clear why elevated RANTES relates to low gait speed.

The AA factor has been associated with metabolic disease^{35, 36} and with older age and greater BMI¹³. Branched chain AAs – the major components of the AA factor – have also been associated with development of insulin resistance and type 2 diabetes³⁷. This could explain our finding of an elevated AA factor associated with low gait speed after controlling for BMI. Another finding of interest was the association of low circulating glycine concentrations with robustness. Low levels of glycine are associated with obesity and/or insulin resistance^{35, 38, 39}, and high glycine appears to protect against regulated cell death⁴⁰. Since most of the glycine studies were conducted in middle-aged populations^{35, 38, 39}, it is possible that this relationship may be different in older adults.

Low MMP3, PXN and IL-2, were associated with slower gait speed. MMP3 is involved in proteolytic pathways altered in aging⁴¹, and with increased extracellular matrix degradation and greater MMP3 concentrations in osteoarthritis⁴². PXN is a component of extracellular matrices. Both low MMP3 and PXN may be related to a decrease in 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 the decline of physical performance. A potential explanation may lie in T cell exhaustion, a phenomenon associated with chronic infection and malignancy⁴³, where IL-2 is the first cytokine to decrease⁴⁴. 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 higher and lower 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. This suggests that regardless of the effect of individual markers, the cumulative impact of markers of inflammatory, coagulant and metabolic pathways may be important and of use for assessing both Robustness, which may relate to resilience, and Impairment, which may relate to frailty. This study represents the first report of a cumulative index focused on more 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Sponsor's Role

The sponsor plays no role in the acquisition and analysis of the data.

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Impact Statement:

Physical performance in older adults reflects the functional status of the body and is the result of complex physiological changes. To describe this relationship, we combined biomarkers of inflammation and metabolism and demonstrate that a unique index of robustness and one of impairment could be created. The components of the indices suggest that robustness and impairment may not simply be opposite ends of the spectrum but may relate to somewhat different underlying biology.

Figure 1. Gait speed predicted according to robustness index score according to cohort.
 Details of the model can be found in supplement 4. Lowess curve is an approximation by connecting locally weighted scatterplot means.

----- Lowess curve
 — Fitted curve
 ● Observed individual

Figure 2. Predicted gait speed by Impairment Index scores by cohort.

Details of the model can be found in supplement 4. Lowess curve is an approximation by connecting locally weighted scatterplot means.

----- Lowess curve

— Fitted curve

● Observed individual

Table 1.

Cut points and weights for biomarker Robustness Index model for each cohort by multivariable regression.

Biomarkers	Cutoffs	LIFE ^a			CARRIAGE ^b			POP ^c		
		Beta	SE	p	Beta	SE	p	Beta	SE	p
IL-6 ^d	<0.67	0.136	0.094	0.153	-0.029	0.092	0.756	0.027	0.050	0.590
Glycine	<249279.41	0.074	0.063	0.243	0.239	0.159	0.148	0.055	0.048	0.256
TNFR1 ^e	<1870.10	0.154	0.075	0.044	.	.	.	-0.004	0.078	0.959
D-Dimer	<333.26	0.109	0.102	0.291	-0.171	0.132	0.210	0.074	0.046	0.116
PXN ^f	7.37	0.187	0.196	0.344	-0.007	0.097	0.940	0.085	0.046	0.065
IL-2	47.27	0.125	0.080	0.124	-0.128	0.106	0.241	0.034	0.044	0.447

^aN = 73, F(9,63) = 4.067, p > F = < 0.001, adjusted for age, BMI, gender and race; Adjusted R-squared = 0.277; Root Mean Squared Error (RMSE) = 0.247;

^bCARRIAGE= CARolinas Region Interaction of Aging, Genes and Environment, N = 30, F(8,21) = 2.278, p > F = 0.062, adjusted for age, BMI, gender and race; Adjusted R-squared = 0.261; Root Mean Squared Error (RMSE) = 0.194;

^cPOP= Prediction of Osteoarthritis Progression, N = 135, F(10,124) = 5.053, p > F = < 0.001, adjusted for age, BMI, gender and race; Adjusted R-squared = 0.232; Root Mean Squared Error (RMSE) = 0.219;

^dInterleukin;

^etumor necrosis factor receptor;

^fpaxillin

Table 2.

Cut points and weights for biomarker Impairment Index model for each cohort by multivariable regression.

Biomarkers	Cutoffs	LIFE ^a			CARRIAGE ^b			POP ^c		
		Beta	SE	p	Beta	SE	p	Beta	SE	p
IL-6 ^d	1.75	-0.226	0.067	0.001	-0.119	0.148	0.432	-0.012	0.047	0.795
RANTES ^e	19549.44	0.069	0.068	0.316	-0.203	0.264	0.453	-0.009	0.067	0.889
TNFR2 ^f	3038.65	-0.080	0.081	0.323	-0.023	0.095	0.808	-0.109	0.049	0.028
AA Factor ^g	0.87	0.058	0.087	0.508	-0.057	0.087	0.517	-0.107	0.044	0.016
D-Dimer	824.50	-0.049	0.070	0.485	-0.120	0.107	0.280	-0.093	0.056	0.098
MMP3 ^h	<4.28	.	.	.	-0.026	0.129	0.843	-0.055	0.043	0.202
IL-2	<12.65	-0.072	0.068	0.295	-0.023	0.091	0.806	-0.035	0.040	0.393
PXN ⁱ	<4.54	-0.084	0.064	0.197	-0.090	0.156	0.572	-0.088	0.061	0.153

^aN = 73, F(10,62) = 4.143, p > F = < 0.001, adjusted for age, BMI, gender and race; Adjusted R-squared = 0.304; Root Mean Squared Error (RMSE) = 0.243;

^bCARRIAGE= CARolinas Region Interaction of Aging, Genes and Environment, N = 29, F(11,17) = 1.074, p > F = 0.433, adjusted for age, BMI, gender and race; Adjusted R-squared = 0.028; Root Mean Squared Error (RMSE) = 0.179;

^cPOP= Prediction of Osteoarthritis Progression, N = 135, F(12,122) = 5.169, p > F = < 0.001, adjusted for age, BMI, gender and race; Adjusted R-squared = 0.272; Root Mean Squared Error (RMSE) = 0.213;

^dinterleukin;

^eregulated on activation, normal T cell expressed and secreted;

^ftumor necrosis factor 1;

^gamino acid factor;

^hmatrix metalloproteinase 3;

ⁱpaxillin