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Original Article

Lack of Immune Resilience Negatively Affects Physical Resilience: Results From the InCHIANTI Follow-Up Study

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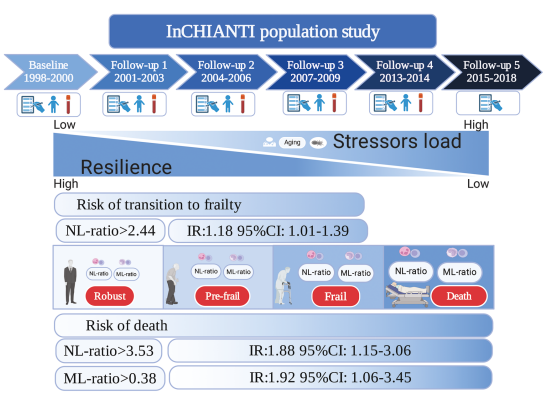
Abstract

There is consistent evidence that immune response declines with aging, with wide interindividual variability and a still unclear relationship with the development of frailty. To address this question, we assessed the role of immune resilience (capacity to restore immune functions), operationalized as the neutrophil-to-lymphocytes ratio (NL-ratio) and monocytes-to-lymphocytes ratio (ML-ratio), in the pathway that from robust status shifts to pre-frailty and frailty, and finally to death. The InCHIANTI study enrolled representative samples from the registry lists of 2 towns in Tuscany, Italy. Baseline data were collected in 1998, with follow-up visits every 3 years. The 1 453 participants enrolled were assessed and followed for lifestyle, clinical condition, physical performance, clinical, and physiological measures. For the purpose of this analysis, we used only 1 022 subjects aged 65 or older at baseline. Participants in the 3 highest deciles of distribution for NL-ratio (>2.44) were more likely to experience a transition from robust to pre-frail, and to overt frailty status. Moreover, NL-ratio (tenth decile > 3.53) and ML-ratio (tenth decile > 2.02) were both predictors of mortality. These results were independent of chronological age, sex, comorbidities, and chronic low-grade inflammation assessed by high sensitivity C-reactive protein measurement. The 2 leucocytes-derived ratios, NL-ratio and ML-ratio, represent markers of immune resilience and predict changes in physical resilience and mortality. These biomarkers are inexpensive because they are based on data routinely collected in clinical practice and can be used to assess the risk of frailty progression and mortality.

Clinical Trials Registration Number: NCT01331512

Keywords: Frailty, Biology of aging, Immunosenescence, Mortality

Graphical Abstract



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Aging is often accompanied by time-dependent nonlinear functional decline of organ function, with a wide interindividual heterogeneity (1). These changes are the expression of cellular damage accumulation, a common denominator and hallmark of the aging process (2). Classic examples of age-related changes are the progressive impairment of immunological response to infections, and recovery from diseases, although the effect of aging is very heterogeneous across individuals. Recently, Ahuja et al. suggested that while some people remain healthier throughout stressors and life, others experience rapid decline of health and function; they hypothesized that this advantage is afforded by their level of "optimal immune resilience" (IR) (3). IR has been previously defined as the capacity to preserve and/or rapidly restore immune functions after a stressor event, and it could be viewed as the operational force of immunocompetence (4,5). Working on a large data set, the authors proposed 2 distinct metrics: the balance between CD8+ and CD4+ T-cell levels and a longevity associated gene expression pattern representing immunocompetence (3).

Recently, we studied the neutrophils-to-lymphocytes ratio (NL-ratio) and monocytes-to-lymphocytes ratio (ML-ratio) as markers of change in the innate and adaptive immunity during aging in the InCHIANTI study (6) and replicated these findings in the Baltimore Longitudinal Study of Aging (7). NL-ratio increases with age and predicts a higher risk of mortality, while a lower NL-ratio is inversely related to comorbidities (6,7). Moreover, changes in NL-ratio (inversely) and ML-ratio (directly) were associated over time with muscle area, muscle density, and handgrip strength test (6), which are considered features of the Frailty syndrome (8). The mechanistic link of NL-ratio, ML-ratio, and frailty involves the intricate interplay between immune resilience, immunosenescence, inflammation, and musculoskeletal health (7,9). Altogether these data suggested that leukocytes-derived ratios could be considered reliable proxy markers of biological age, better if compared to leukocytes absolute count and to circulating serum levels of cytokines or C-reactive protein (CRP), markers of inflammation that undergo extreme fluctuation with time within the same individuals (10). Both NL-ratio and ML-ratio are considered markers of a clinical or subclinical inflammatory state, in keeping with the accepted theory of inflammaging (11). We hypothesize that in addition to be biomarkers of inflammation, leukocytes-derived ratios may also convey information on the causal pathway leading to the development of frailty, defined as a state of decreased homeostatic ability to physiological stressors, and impaired adaptive capability (12) that results from cumulative declines of physical reserves in multiple subsystems, and causes increased vulnerability to adverse outcomes (13). According to this definition, the frailty phenotype may be considered a crude indicator of what Whitson et al. indicate as a collapse of physical resilience, and a decrease in resilience often precedes overt frailty (14). Physical resilience is a personal trait that influences the ability to resist a functional decline or to recover physical health following a stressor event (15) and is further affected by intrinsic as well as extrinsic genetic and environmental factors (14). Resilience has been regarded as the opposite of frailty, and robustness descriptors often rely on steady resilience (14), although recent evidence suggests that resilience is still present in frailty and modulates its clinical consequences (16,17). The lack of age-related resilience as a link between immune function and frailty was previously suggested (18), but this has not been experimentally studied. We hypothesize that changes in immune resilience constitute a characteristic trait of aging (3), which progresses in parallel with frailty and can influence its phenotypic expression. To address this hypothesis, we tested whether the NL-ratio and ML-ratio as markers of immune resilience, reflect the probability of transition in a multistep pathway from robust status to pre-frailty and then to frailty and death.

Method

Sample Description

The InCHIANTI study protocol has been described in detail elsewhere (19). Briefly, 1 453 participants aged 21–102 were recruited from 2 cities in Tuscany for a baseline visit conducted between 1998 and 2000. The participants were followed every 3 years with follow-up visits. The last follow-up with complete assessment and clinical visit was the FU-4 (2013–2015), but at that time some biological markers (CRP) could not be measured; therefore, in these analyses data up to FU-3 (2007–2009) were included and, accordingly, mortality was assessed till 2018 (end date of FU5). The study protocol was approved by the Italian National Institute of Research and in the United States, the protocol was given an exemption status by the National Institutes of Health Intramural Research Program Institutional Review Board (Exemption #11976) and informed consent was obtained from participants at each visit.

Laboratory Assay

Differential white blood cells count was assessed through an automated system at the Laboratory of Clinical Chemistry and Microbiological Assays, SS. Annunziata Hospital, Azienda Sanitaria 10, Florence, Italy, using at baseline a Hematology SE 9000 Autoanalyzer (Sysmex, Kobe, Japan, provided by DASIT, Milano, Italy); at follow-up 1, a Coulter LH 750 Hematology Autoanalyzer (Beckman Coulter Inc., Brea, CA); at follow-up 2 and follow-up 3, a Sysmex XE 2100 (DASIT—Milano-Italy). Neutrophil-to-lymphocyte ratio (NL-ratio) and monocytes-to-lymphocyte ratio (ML-ratio) were derived from leukocytes absolute number count (20), and deciles of distribution for both ratios were also analyzed. Serum high-sensitivity CRP was measured by immunonephelometric assay and monoclonal antibodies in duplicate with the Dade Behring BN II Nephelometer (Dade Behring Inc., Deerfield, IL).

Diseases and Comorbidities

The diagnosis of major medical conditions was ascertained according to preestablished criteria that combine reported doctor diagnosis, eventually supported by medical records, physical examination, blood tests, and drugs prescription (21). Comorbidities score was calculated summing the number of diseases reported at baseline and all follow-up visits (angina, cancer, hepatic diseases, acute myocardial infarction, congestive heart failure, stroke, Parkinson disease, peripheric arterial disease, diabetes, chronic obstructive pulmonary disease [COPD], asthma, and osteoarthritis).

Frailty Phenotype

Frailty phenotype was defined according to Fried definition, and assessed in every time of the study, as the presence of at least 3 of the following domains (12): (1) unintentional weight loss, a reduction in weight > 4.5 kg in the past 12 months; (2) exhaustion, defined as a feeling of needing an

effort to do everything for more than 2 times in the week before interview; (3) reduced physical activity, defined as having performed less than 2–4 hours of light exercise per week; (4) walking speed, defined as time needed to walk at usual pace for 4 m in the upper quintile; and (5) grip strength in the gender-specific bottom quintile.

Mortality

Data on all causes mortality up to 20 years following the start of the study were evaluated through municipal general registry from 1998 to 2018.

Statistical Analysis

Baseline characteristics were compared among frailty phenotype (robust, pre-frail, and frail) for the variables of interest, and differences among groups were evaluated using analysis of variance for continuous variables. We used generalized linear mixed models (GLMMs) to predict frailty phenotype progression that incorporated 4 repeated measurements; we specified a Poisson distribution, and 2 separate models analyze, respectively, NL-ratio and ML-ratio deciles of distribution. Whereas to assess the mortality risk was also used GLMMs, but with a binomial distribution, also in this analysis 2 different models were used to assess the predictive risk for NL-ratio and ML-ratio deciles of distribution.

The covariates of interest included in all the models predicting frailty phenotypes are: age, sex, comorbidities number, and PCR, whereas for all causes 20-year mortality risk, in addition to previous was included also Frailty phenotype.

SAS version 9.4 for Windows (SAS Institute, Inc., Cary, NC, USA) was used for all data processing and statistical analyses. We set the level of statistical significance at $p < .05$ (2-sided).

Results

The total population enrolled in this study consider 1 022 subjects at baseline, aged 65 or more, with a total of 3 106 follow-up assessment visits. Overall, 1 338 subject visits were classified as robust, of which 503 (37.59%) at baseline, 377 (28.18%) at first follow-up, 276 (20.63%) at second follow-up, and 182 (13.60%) at third follow-up (Table 1 and Supplementary Figure 1). According to Fried's frailty criteria, 3 106

Table 1. Descriptive of the Study Population According to Frailty Status

		Robust	Pre-Frail	Frail	*p* Value
		1 338	1 450	318	
Enrolled at baseline		503 (37.59)	438 (30.21)	81 (25.47)	<.001
Follow-up 1		377 (28.18)	342 (23.59)	78 (24.53)	
Follow-up 2		276 (20.63)	372 (25.66)	85 (26.73)	
Follow-up 3		182 (13.60)	298 (20.55)	74 (23.27)	
Chronological age (y)		74.53 ± 5.46	79.01 ± 7.17	83.11 ± 6.60	<.001
Sex female *n* (%)		632 (47.23)	913 (62.97)	207 (65.09)	<.001
Multimorbidity		1.65 ± 1.43	2.41 ± 1.73	3.20 ± 1.85	<.001
NL-ratio		2.07 ± 0.96	2.30 ± 1.22	2.43 ± 1.21	<.001
>90° percentile	>3.53	97 (4.25)	170 (11.72)	42 (13.21)	<.001
80–90° percentile	3.53–2.82	110 (8.22)	157 (10.83)	44 (13.84)	
70–80° percentile	2.82–2.44	115 (8.59)	154 (10.62)	43 (13.52)	
60–70° percentile	2.44–2.17	134 (10.01)	144 (9.93)	31 (9.75)	
50–60° percentile	2.17–1.97	143 (10.69)	132 (9.10)	32 (10.06)	
40–50° percentile	1.97–1.77	140 (10.46)	155 (10.69)	23 (7.23)	
30–40° percentile	1.77–1.62	147 (10.99)	137 (9.45)	26 (8.18)	
20–30° percentile	1.62–1.43	135 (10.09)	141 (9.72)	33 (10.38)	
10–20° percentile	1.43–1.19	157 (11.73)	128 (8.83)	23 (7.23)	
<10° percentile	<1.19	160 (11.96)	132 (9.10)	21 (6.60)	
ML-ratio		0.22 ± 0.10	0.25 ± 0.13	0.27 ± 0.12	<.001
>90° percentile	>0.38	80 (5.98)	180 (12.41)	46 (14.47)	<.001
80–90° percentile	0.30–0.38	131 (9.79)	159 (10.97)	52 (16.35)	
70–80° percentile	0.27–0.30	131 (9.79)	165 (11.38)	32 (10.06)	
60–70° percentile	0.23–0.27	132 (9.87)	139 (9.59)	44 (13.84)	
50–60° percentile	0.21–0.23	117 (8.74)	138 (9.52)	28 (8.81)	
40–50° percentile	0.19–0.21	144 (10.76)	135 (9.31)	27 (8.49)	
30–40° percentile	0.17–0.19	132 (9.87)	140 (9.66)	34 (10.69)	
20–30° percentile	0.15–0.17	142 (10.61)	142 (9.79)	20 (6.29)	
10–20° percentile	0.12–0.15	171 (12.78)	131 (9.03)	15 (4.72)	
<10° percentile	<0.12	158 (11.81)	121 (8.34)	20 (6.29)	
C-reactive protein		3.78 ± 7.26	5.62 ± 9.95	7.75 ± 15.13	<.001

Notes: NL-ratio = neutrophils-to-lymphocytes ratio; ML-ratio = monocytes-to-lymphocytes ratio.

follow-up assessment visits could be classified as pre-frail 1 450 (1–2 criteria), and 318 as frail (>3). The frail group was older (for-trend p value < .001), included more women (for-trend p value < .001), reported more diseases (for-trend p value < .001), and had higher level of inflammation (CRP for-trend p value < .001), compared to robust and pre-frail subjects (Table 1). Frail participants also had higher levels of both the NL-ratio and the ML-ratio when these variables were expressed as continuous variables (for-trend p value < .001) and deciles (for-trend p value < .001).

Tables 2 and 3 show the results of GLMMs, predicting the risk of transition during study follow-up through the Fried's criteria, from robust to pre-frail to frail. In the model that considers NL-ratio, the incidence rate for the increase in the number of frailty criteria was 1.18 (95% CI: 1.01–1.39, p value = .03) for NL-ratio > 3.53 (tenth decile of distribution), 1.28 (95% CI: 1.05–1.55, p value = .01) for NL-ratio between 2.82 and 3.53 (ninth decile of distribution), and 1.31 (95% CI: 1.28–1.59, p value = .007) for NL-ratio between 2.44 and 2.82 (eighth decile of distribution), compared to those subjects with NL-ratio < 1.19 (first decile of distribution). No differences in the risk of becoming frail were found for the other NL-ratio deciles of distribution compared to first decile of distribution (Table 2). Males were less prone to become frail compared to females (p value < .001), whereas chronological age (IR: 1.05; 95% CI: 1.04–1.05; p value < .001), number of diseases (IR: 1.12; 95% CI: 1.09–1.15; p value < .001), and CRP (IR: 1.01; 95% CI: 1.00–1.02; p value .04) were all independent risk factors for transition to frailty over the follow-up. No effect for the interaction between variables considered in the model could be found.

In the model that considers ML-ratio, no differences in the risk of becoming frail were found for the ML-ratio deciles of distribution (Supplementary Table 1). Males were less prone to become frail compared to females (OR: 0.76; 95% CI: 0.70–0.83; p value < .001), whereas chronological age (p value < .001), number of diseases (p value < .001), and CRP (p value = .03) were all independent risk factors for transition to frailty during the follow-up. No second–order effect for the interaction between variables considered in the model could be detected (Supplementary Table 1).

Mortality Risk

During the follow-up, 428/1 022 subjects (41.87%) died, with a very similar percentage distribution in the different follow-ups, although the mortality between follow-up 3 and follow-up 4 was slightly higher. Subjects who died were: older (p value > .001), more likely to be male (p value = .02), had higher inflammatory markers (p value < .001), and had higher number of diseases (Table 3). Both NL-ratio and ML-ratio were significantly higher in those who died compared to those who survived. NL-ratio and ML-ratio expressed as ordinal variable (quantiles) were also significantly associated with mortality (for-trend p value < .001; Table 3).

In the model that considers NL-ratio as a mortality risk factor, the highest deciles of distribution (NL-ratio > 3.53) showed a significantly higher risk of death compared to lowest decile of distribution (NL-ratio < 1.19); no differences in the risk of death were found for the other NL-ratio deciles of distribution (Table 4). Males were more prone to show a higher mortality compared to females (p value < .001), whereas chronological age (OR: 1.16; 95% CI: 1.14–1.18; p value < .001), multimorbidity (OR: 1.32; 95% CI: 1.24–1.4; p value < .001), and CRP (OR: 1.01; 95% CI: 1.00–1.02; p value = .02) were independent risk factors for death during the follow-up. No second-order effect for the interaction between variables considered in the model could be found.

In the model that considers ML-ratio as a mortality risk factor, the highest deciles of distribution (ML-ratio > 0.38) showed a statistically significant higher risk of death compared to the lowest decile (ML-ratio < 0.12; Table 5).

Table 2. GLMM, Factors Predicting Changes in Frailty Status, According to NL-Ratio Decile of Distribution, Adjusting for Chronological Age, Sex, Number of Comorbidities, and C-Reactive Protein

		IR	95% CI	p Value
NL-ratio	range			
>90° percentile	>3.53	1.18	1.01–1.39	.03
80–90° percentile	3.53–2.82	1.28	1.05–1.55	.01
70–80° percentile	2.82–2.44	1.31	1.08–1.59	.007
60–70° percentile	2.44–2.17	1.15	0.94–1.41	.17
50–60° percentile	2.17–1.97	1.08	0.89–1.33	.43
40–50° percentile	1.97–1.77	1.13	0.93–1.39	.23
30–40° percentile	1.77–1.62	1.09	0.89–1.34	.39
20–30° percentile	1.62–1.43	1.19	0.97–1.45	.10
10–20° percentile	1.43–1.19	1.04	0.84–1.29	.70
<10° percentile	<1.19		reference	
Chronological age		1.05	1.04–1.05	<.001
Male sex		0.77	0.70–0.84	<.001
Female sex			Reference	
Multimorbidity		1.12	1.09–1.15	<.001
C-reactive protein		1.01	1.00–1.02	.04

Notes: IR = incidence rate; NL-ratio = neutrophils-to-lymphocytes ratio; 95% CI = 95% confidence interval.

Table 3. Descriptive of the Study Population According to Mortality for All Causes

		Alive	Death	*p* Value
		2 678	428	
Died between baseline and follow-up 1		923 (34.47)	99 (23.13)	<.001
Died between follow-up 1 and 2		698 (26.06)	99 (23.13)	
Died between follow-up 2 and 3		633 (23.64)	100 (23.36)	
Died between follow-up 3 and 4		424 (15.83)	130 (30.37)	
Chronological age (y)		76.47 ± 6.41	83.96 ± 7.27	<.001
Sex female *n* (%)		1 530 (57.13)	222 (51.87)	.02
Multimorbidity		2.00 ± 1.61	3.20 ± 1.84	<.001
NL-ratio		2.15 ± 1.04	2.62 ± 1.48	<.001
>90° percentile	>3.53	220 (8.22)	89 (20.79)	<.001
80–90° percentile	3.53–2.82	263 (9.82)	48 (11.21)	
70–80° percentile	2.82–2.44	275 (10.27)	37 (8.64)	
60–70° percentile	2.44–2.17	256 (9.56)	53 (12.38)	
50–60° percentile	2.17–1.97	265 (9.90)	42 (9.81)	
40–50° percentile	1.97–1.77	288 (10.75)	30 (7.01)	
30–40° percentile	1.77–1.62	276 (10.31)	34 (7.94)	
20–30° percentile	1.62–1.43	279 (10.42)	30 (7.01)	
10–20° percentile	1.43–1.19	278 (10.38)	30 (7.01)	
<10° percentile	<1.19	278 (10.38)	35 (8.18)	
ML-ratio		0.23 ± 0.11	0.29 ± 0.16	<.001
>90° percentile	>0.38	221 (8.25)	85 (19.86)	<.001
80–90° percentile	0.30–0.38	278 (10.38)	64 (14.95)	
70–80° percentile	0.27–0.30	285 (10.64)	43 (10.05)	
60–70° percentile	0.23–0.27	271 (10.12)	44 (10.28)	
50–60° percentile	0.21–0.23	240 (8.96)	43 (10.05)	
40–50° percentile	0.19–0.21	265 (9.90)	41 (9.58)	
30–40° percentile	0.17–0.19	267 (9.97)	39 (9.11)	
20–30° percentile	0.15–0.17	274 (10.23)	30 (7.01)	
10–20° percentile	0.12–0.15	297 (11.09)	20 (4.67)	
<10° percentile	<0.12	280 (10.46)	19 (4.44)	
C-reactive protein		4.48 ± 8.23	8.56 ± 15.58	<.001
Frailty index				<.001
Robust		1 283 (47.91)	55 (12.85)	
Pre-frail		1 189 (44.40)	261 (60.98)	
Frail		206 (7.69)	112 (26.17)	

Notes: ML-ratio = monocytes-to-lymphocytes ratio; NL-ratio = neutrophils-to-lymphocytes ratio.

Males experienced higher mortality compared to females (p value < .001), whereas chronological age (p value < .001), number of diseases (p value < .001), and CRP (p value = .002) were all independent risk factors for death during the follow-up. No second-order effect for the interaction between variables considered in the model could be found.

Discussion

The most important result of this study was that that participants in the 3 highest deciles of NL-ratio (NL-ratio > 2.44) were at higher risk of change in physical resilience status, from robust to pre-frail, to overt status of frailty compared to the other participants. Moreover, the highest decile of distribution was a predictor of mortality for both NL- and ML-ratio. In either case, the results were independent of chronological age, sex, comorbidities, and chronic low-grade inflammation. To the best of our knowledge, this is the first time that the NL-ratio has been shown to predict physical resilience, in a free-living population.

In a study of 5 106 older adult cancer patients, the Nutrition Status and Clinical Outcome of Common Cancers (INSCOC) previously found that a NL-ratio greater than 3 was an independent risk factor for frailty, assessed according to Fried's criteria (22). This study has many substantial differences from our InCHIANTI-cohort. First, the patients enrolled in the INSCOC had a clinical diagnosis of cancer, whereas the InCHIANTI-population is a free-living representative population. Cancer patients tend to have high level of inflammation. Moreover, cancer is often associated with cachexia and not sarcopenia, which is considered a hallmark of frailty (12). In the INSCOC, the NL-ratio was assessed only at enrollment. Therefore, the INSCOC study could only assess NL-ratio predictive role for frailty but not the

Table 4. GLMM, Factors Predicting All Causes Mortality, According to NL-Ratio Decile of Distribution, Adjusting for Chronological Age, Sex, Number of Comorbidities, and C-Reactive Protein

		IR	95% CI	*p* Value
NL-ratio	range			
>90° percentile	>3.53	1.88	1.15–3.06	.01
80–90° percentile	3.53–2.82	0.95	0.56–1.61	.86
70–80° percentile	2.82–2.44	0.81	0.47–1.40	.46
60–70° percentile	2.44–2.17	1.55	0.91–2.64	.10
50–60° percentile	2.17–1.97	0.95	0.56–1.61	.85
40–50° percentile	1.97–1.77	0.73	0.41–1.28	.28
30–40° percentile	1.77–1.62	0.89	0.50–1.57	.67
20–30° percentile	1.62–1.43	0.76	0.43–1.33	.33
10–20° percentile	1.43–1.19	0.91	0.50–1.57	.76
<10° percentile	<1.19		reference	
Chronological age		1.14	1.12–1.16	<.001
Male sex		1.79	1.41–2.26	<.001
Female sex			Reference	
Multimorbidity		1.26	1.19–1.33	<.001
C-reactive protein		1.01	1.00-1.02	.04
Frailty index				
Robust	0	0.24	0.16–0.36	<.001
Pre-frail	1–2	0.64	0.48–0.86	.004
Frail	≥3		Reference	

Notes: IR = incidence rate; NL-ratio = neutrophils-to-lymphocytes ratio; 95% CI = 95% confidence interval.

Table 5. GLMM, Factors Predicting All Causes Mortality, According to ML-Ratio Decile of Distribution, Adjusting For Chronological Age, Sex, Number of Comorbidities, and C-Reactive Protein

		IR	95% CI	*p* Value
ML-ratio	range			
>90° percentile	>0.38	1.92	1.06–3.45	.03
80–90° percentile	0.30–0.38	1.57	0.85–2.88	.14
70–80° percentile	0.27–0.30	1.19	0.63–2.22	.60
60–70° percentile	0.23–0.27	1.31	0.70–2.45	.40
50–60° percentile	0.21–0.23	1.77	0.94–3.31	.11
40–50° percentile	0.19–0.21	1.90	0.99–3.62	.10
30–40° percentile	0.17–0.19	1.45	0.77–2.71	.25
20–30° percentile	0.15–0.17	1.25	0.64–2.43	.52
10–20° percentile	0.12–0.15	0.98	0.48–1.98	.97
<10° percentile	<0.12		reference	
Chronological age		1.13	1.11–1.15	<.001
Male sex		1.73	1.37–2.19	<.001
Female sex			Reference	
Multimorbidity		1.26	1.19–1.33	<.001
C-reactive protein		1.01	1.00–1.02	.01
Frailty index				
Robust	0	0.25	0.17–0.37	<.001
Pre-frail	1–2	0.66	0.49–0.89	.007
Frail	≥3		Reference	

Notes: IR = incidence rate; ML-ratio = monocytes-to-lymphocytes ratio; 95% CI = 95% confidence interval.

change over time of the ratio. In a similar study that enrolled 581 women with breast cancer undergoing chemotherapy, the authors found that the white blood cells-derived ratios (namely NL-ratio and ML-ratio) were associated with frailty, but each study time was analyzed separately with different linear regression analysis. In that study, to counteract the effects

of chemotherapy on white blood cells, growth hormone was prescribed to restore the leukocytes number and function (23). NL-ratio, platelet-to-lymphocyte ratio, and systemic immune-inflammation index were assessed in the Rugao Longitudinal Aging Study, and related to the risk of developing frailty. The authors found that increased NL-ratio carried an increased risk of incident frailty (24). In this case too, the NL-ratio was measured only at enrollment, thus considered not susceptible to modification. The NL-ratio was reported to be associated with frailty in a cross-sectional analysis of a hospitalized population of 565 patients, reporting several comorbidities and showing an inverse ratio of frailty to robust distribution at enrollment, compared to the general population (25), with almost half the cases being classified as frail (26).

There are sparse reports on the predictive role of the ML-ratio: the Singapore Longitudinal Aging Study showed the ML-ratio as an indicator of the onset and progression of comorbidity, with impact on the mortality (27). Another study found the ML-ratio to be a marker associated with disease progression, interacting with disability and frailty (28).

Altogether, our data demonstrate that NL-ratio predicts frailty, independently of major confounders such as age, sex, multimorbidity, and inflammation. Our results add to our understanding of the role of leukocytes-derived ratio in the aging process and in age-associated diseases. More in detail, the dynamic variation of the ratio during the follow-up of the study, represents the immune resilience state of the older adult, the ability to respond to damage induced by aging induced stressors, and to restore immunocompetence (18). Similarly, frailty can vary with time and could be considered as a marker of physical resilience (14). The 2 ratios were associated with physical resilience, and independent of chronological age, meaning that immune resilience, as also physical resilience, is surely age-related (29) but does not represent the "collateral effect" of chronological age itself, rather it results from an interaction between hereditary and acquired factors (29).

Of note, in our study, women showed a higher propensity to transit and to progress into different levels of frailty, whereas men showed significantly higher mortality. Those are not a new notions, for example, in the Berlin Initiative Study (BIS), in line with our results, 22% of women and 14% of men improved their frailty status, whereas 27% of women and 33% of men worsened their frailty status (30). Similar results were also obtained from the Multidomain Alzheimer Preventive Trial, with no change in frailty status in 68%, a worsening in 19%, and an improvement in frailty status in the remaining 13% (31). A hypothesis that could explain those differences could link the immunological gender dimorphism (3,32) to the male–female health-survival paradox (33), implying that women have higher immunocompetence, and longevity, but lower physical reserve.

The ML-ratio and NL-ratio were independent from comorbidities in predicting death from all causes, meaning that diseases could influence the value of the ratios, as previously demonstrated in the same population study (6), but the recovery of the immunological response is modulated at an individual level. In other words, the immunological and physical responses to diseases and comorbidities had different phenotypic expressions.

Finally, NL-ratio and ML-ratio have been considered as markers of inflammation and their role was studied mainly as indicators of evolution and prognosis of diseases, such as in congestive heart failure (20), cancer (34), and neurological diseases (35,36). In our study, the associations between the ratios and frailty status as well as mortality, were independent of the CRP levels, an established marker of inflammation. Therefore, the 2 ratios represent more than another measure of age-related chronic low-grade inflammation (inflammaging) (37). In our opinion, the NL- and ML-ratio represent a 2-side connector (immunological and physical) of the same process, that is, resilience.

Limitations

The most important limitation of this study concerns the accuracy to identify when a stressful event occurred, to assess its severity, and more importantly how much it may have affected the immune and physical homeostatic capacities of the enrolled subjects. In fact, with the data available from the InCHIANTI study, we could only observe changes in the subjects' immune status and in the level of physical "robustness," but we could not identify the stressor that might have determined them. This will probably be possible using other studies such as the "Study of Physical Resilience and Aging" (SPRING) (38). Moreover, it would have been very interesting to test whether a lag-time between increased ratios and mortality could be detected in the population. However, because of the elapsed time, nearly 3 years between follow-ups, and the limited follow-up time, this hypothesis could not be tested in the current study.

Another limitation to consider is the use of CRP as indirect marker of inflammaging instead of IL-6. IL-6 probably could better represent inflammaging, but we used CRP because we wanted to adopt a routinely available, low-cost marker that can be useful in clinical practice.

Conclusion

The 2 leucocytes-derived ratios, NL-ratio, and ML-ratio, represent markers of immune resilience and predict changes in physical resilience and mortality. Such inexpensive and easy-to-obtain markers could also be used in daily practice to assess the individual immune resilience and to estimate the risk of frailty progression. Longitudinal studies encompassing different race and geography are needed to confirm the usefulness of these markers in geriatric clinical practice.

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

The authors (Ra.P., Ro.P., A.D.I., S.B., C.M., E.S., S.V., T.T., and L.F.) declare no conflict of interest. S.V. and L.F. are

members of the Editorial Board of *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences*.

Data Availability

The data sets used and/or analyzed during the current study are available from the responsible authors for the InCHIANTI study (Luigi Ferrucci) on reasonable request. Data of the InCHIANTI study are available to all researchers upon justified request using the proposal form available on the InChianti website (https://www.nia.nih.gov/inchianti-study, accessed on April 13, 2023).

Author Contributions

Ra.P.: conceptualization, interpretation of data, and drafted the work. Ro.P.: conceptualization, interpretation of data, drafted, and revised the work. A.D.I: conceptualization, acquisition, analysis, and drafted the work. S.B.: design of the work, interpretation of data, and revised the work. C.M.: analysis, interpretation of data, and drafted the work. S.V.: analysis, interpretation of data, and drafted the work. E.S.: acquisition, analysis, interpretation of data, and drafted the work. T.T.: acquisition, analysis, interpretation of data, and revised the text. L.F.: design, acquisition, analysis, interpretation of data, and revised the text. All authors have read and agreed to the present version of the manuscript.

Ethics Approval

The InCHIANTI study baseline was approved by the Ethical committee at INRCA, Ancona (protocol 14/CE, February 28, 2000) as the FU1 (protocol 45/01, January 16, 2001). InCHIANTI study, FU-2 and FU-3 were approved by the Local Ethical Committee at Azienda Sanitaria Firenze (protocol 5/04, May 12, 2004). The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of INRCA in Ancona (Italy). Clinical Trial Registration: NCT01331512.

Consent to Participate

Written informed consent was obtained from the subjects to participate in the Study at each time (baseline visit and follow-ups).

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