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Inflammatory biomarkers, multi-morbidity, and biologic aging

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Abstract

Objectives: To study the association between multi-morbidity percentiles, which is a measure of clinical aging, and interleukin (IL)-6, IL-10, and tumor necrosis factor (TNF)-α.

Methods: Participants 50 to 95 years of age from the Mayo Clinic Study of Aging were assigned age- and sex-specific multi-morbidity percentiles using look-up tables that were reported previously (n = 1646). Percentiles were divided into quintiles for analysis. Plasma IL-6, IL-10, and TNF-α levels were measured in 1595 participants. Median inflammatory marker levels were compared across multi-morbidity quintiles using nonparametric tests.

Results: People with higher multi-morbidity percentiles had significantly higher IL-6 and TNF-α levels compared with those with lower multi-morbidity percentiles. Tests for trend across five multi-morbidity quintiles were significant among women for IL-6 and among participants 70 years of age or older for IL-6 and TNF-α. IL-10 was not associated with multi-morbidity percentiles.

Conclusions: Multi-morbidity percentiles may be a useful clinical index of biological age for future studies, particularly in women and people 70 years of age and older.

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Background

Multi-morbidity increases with aging and is strongly associated with increased health care use, decreased quality of life, and an increased risk of mortality.[1] We and others have proposed that the degree of accumulated multi-morbidity may serve as a simple measure to describe how rapidly people are aging compared with their peers of the same age and sex.[2–5] However, to advance the field of multi-morbidity, it is essential to develop a common phenotype definition that can be easily applied across study populations. The US Department of Health and Human Services (DHHS) has defined multi-morbidity as having two or more chronic conditions among a list of 20 conditions.[6] We have previously used this definition to calculate normative percentiles for multi-morbidity across age, sex, race, and ethnicity.[5] These multi-morbidity percentiles are similar to percentile measures used in childhood growth charts to help pediatricians determine whether a child is growing more or less rapidly than children of the same age and sex. Similarly, our multi-morbidity percentile charts allow investigators to rapidly determine whether a person's number of chronic conditions is similar to that of their peers of the same age and sex. A person with a higher multi-morbidity percentile may, therefore, be aging more rapidly compared with his or her peers, and we have shown that an increase in a person's multi-morbidity percentile is significantly associated with an increase in short-term mortality.[5]

It is important to note that whether a person's multi-morbidity percentile is associated with biomarkers of aging at the cellular or tissue level is unclear. Chronic, low-level inflammation is a hallmark of increasing biological age, and inflammatory marker levels are increased in people with multi-morbidity.[7] People with elevated inflammatory markers are also at an increased risk of disability, hospitalization, and death.[8–10] For this reason, several inflammatory biomarkers have been selected as part of a standard biomarker set for aging that will be used in clinical trials.[11] Defining a clear set of biomarkers that are associated with biologic aging would allow clinicians to better understand which patients are aging more or less rapidly compared with their chronologic peers. Additionally, such biomarker identification will provide insight into the underlying mechanisms of aging, and it may offer new therapeutic targets to delay the biologic aging process. However, such markers must first be shown to be strongly associated with relevant aging outcomes.

Therefore, if multi-morbidity percentiles are a useful clinical measure of biological age to be used in epidemiologic studies and clinical trials, they should also be associated with inflammatory biomarkers of aging. To address this question, we studied the association between multi-morbidity percentiles and plasma interleukin (IL)-6, IL-10, and tumor necrosis factor alpha (TNF-α) levels in a well-characterized, population-based cohort of older adults.

Methods

Study population

This study included a subsample of people participating in the Mayo Clinic Study of Aging (MCSA), a prospective, population-based study of the incidence, prevalence, risk factors, and fluid and imaging biomarkers of mild cognitive impairment among people residing in Olmsted County, MN, USA.[12] Briefly, people ages 70 to 89 years and who resided in Olmsted County in 2004 were identified using the Rochester Epidemiology Project (REP) medical records-linkage system. The REP includes virtually everyone residing in Olmsted County, and thus, it is an ideal population-based sampling frame to obtain study participants who are representative of the general population in this region.[13,14] Potential participants were contacted using an age- and sex-stratified random sampling design and were invited to participate. In 2012, the MCSA study was extended to include Olmsted County, MN residents who were 50 years of age or older. People <50 years or >89 years or those who resided outside of Olmsted County were excluded from the sampling frame. The only additional exclusion criterion was people living in hospice. Characteristics of people who participated were similar to those of people who did not participate, suggesting that the study population was representative of the Olmsted County population aged 50 to 89 years.[15] Our study included all of the people 50 years of age or older from the MCSA who had available plasma measures of IL-6, IL-10, and TNF-α. All participants provided written informed consent, and the MCSA project was approved by the Mayo Clinic and Olmsted Medical Center Institutional Review Boards (Mayo IRB: 14-004401 and OMC IRB: 003-OMC-95).

Inflammatory marker measurements

Blood specimens were obtained from the study participants during a comprehensive in-clinic examination as part of the MCSA. The date of the blood draw was considered the index date for this study. Blood was centrifuged, aliquoted, and stored at $-80°C$. Plasma IL-6, IL-10, and TNF-α were measured using the Simoa HD-1 platform (Quanterix, Lexington, MA, USA) as previously described.[16] The intra-assay coefficient of variation (CV) was 7.1% for IL-6, 4.6% for IL-10, and 4.0% for TNF-α. The inter-assay CV was 5.6% for IL-6, 6.2% for IL-10, and 5.5% for TNF-α. The lower limit of detection was 0.0055 pg/mL for IL-6, 0.0038 pg/mL for IL-10, and 0.016 pg/mL for TNF-α. Analyses included all people with available measures ($n = 1595$).

Multi-morbidity percentiles

We used the DHHS list of 20 chronic conditions and the corresponding International Classification of Diseases (ICD)-9 codes to define multi-morbidity.[6] We used resources from the REP medical records-linkage system to identify the presence of these chronic conditions in our study population.[17] Study participants were considered to have a chronic condition if they had received two codes for that specific condition separated by more than 30 days and within 5 years before the index date. Participant age was calculated on the date that the plasma sample was collected. All participants were then assigned the appropriate age- and sex-specific multi-morbidity percentiles using the look-up tables reported in a previous publication.[5] For example, a 60-year-old woman with only one of the 20 conditions received a percentile score of 29. This percentile indicates that 71% of women aged 60 years have a worse score, placing this woman in the healthier third of the population.

However, a 60-year-old woman with five of the 20 chronic conditions received a percentile score of 93. This percentile indicates that only 7% of women aged 60 years have a worse score, placing this woman in the most affected 10% of the population. Multi-morbidity percentiles were analyzed both as continuous measures and as quintiles. Results were the same regardless of the approach, and the quintile analyses are reported for ease of interpretation.

Statistical analysis

Demographic characteristics and blood levels of each inflammatory biomarker (IL-6, IL-10, and TNF-α) were reported as the number and percent or as the median and inter-quartile range, as appropriate. Because the inflammatory marker distributions were skewed, nonparametric tests were used to compare the median levels of the inflammatory markers across the demographic characteristic strata (Wilcoxon two-sample test and Jonckheere–Terpstra test for trend). Analyses for the association between multi-morbidity percentile quintiles and plasma biomarker levels were also stratified by sex and by age (ages 50–69 years and 70 years and older). Analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), and $p < 0.05$ was considered statistically significant.

Results

Characteristics of the study participants are shown in Table 1. Overall, 46% of the study sample were women, 60% were 70 years of age or older, most were white (98%), and 29% had a high school education or less. Additionally, most participants had two or more chronic conditions (78%).

Men and older people had higher levels of the three inflammatory biomarkers, but race and education were not associated with the biomarker levels (Table 1). People with more chronic conditions had higher IL-6 and TNF-α levels compared with those with fewer chronic conditions ($p < 0.001$ for both), but there was no difference for IL-10 levels. People with higher multi-morbidity percentiles also had higher IL-6 and TNF-α levels compared with those with lower multi-morbidity percentiles, and the tests for trend across the five quintiles were statistically significant in the overall sample ($p = 0.005$ and $p = 0.03$, respectively; Table 1). We note that the multi-morbidity percentiles were age- and sex-specific. Therefore, these measures may be considered age- and sex-adjusted.

Table 2 shows the results stratified by sex. Tests for trend for the increase in biomarker concentration across five quintiles were significant for women for IL-6 ($p = 0.009$) but not for IL-10 or TNF-α. Tests for trend were not significant in men for any of the biomarkers. Table 3 shows analyses stratified by broad age groups. Tests for trend for an increase in biomarker concentration across five quintiles were not significant in the 50–60 years group. Among people 70+ years of age, the tests for trend were significant for IL-6 and TNF-α levels ($p < 0.05$).

Discussion

In this study, we expanded on previous work that described multi-morbidity percentiles and their association with mortality.[5] We found that higher multi-morbidity percentiles were also associated with higher IL-6 and TNF-α levels, and these two pro-inflammatory cytokines were selected as part of a standard set of biomarkers for aging that will be used in clinical trials.[11] Additionally, we found that the associations were statistically significant in women and among people 70 years of age or older. Therefore, our findings suggest that multi-morbidity percentiles may be

Table 1. Characteristics of the study population and distribution of plasma IL-6, IL-10, and TNF-α levels by population characteristics.

Characteristic	N (%)	IL-6 (pg/mL) Median (Q1, Q3)	IL-10 (pg/mL) Median (Q1, Q3)	TNF-α (pg/mL) Median (Q1, Q3)
Sex				
Men	892 (54.2)	2.84 (1.74, 5.80)	0.88 (0.60, 1.86)	4.78 (3.50, 7.74)
Women	754 (45.8)	2.39 (1.44, 4.12)	0.74 (0.52, 1.45)	4.29 (3.25, 6.25)
p[a]		<0.001	<0.001	<0.001
Age (years)				
50–59	225 (13.7)	2.03 (1.26, 3.66)	0.79 (0.49, 1.69)	4.06 (2.87, 6.59)
60–69	436 (26.5)	2.29 (1.46, 3.93)	0.77 (0.54, 1.64)	3.98 (3.16, 6.24)
70–79	582 (35.4)	2.59 (1.62, 4.90)	0.81 (0.57, 1.74)	4.53 (3.45, 7.05)
80+	403 (24.5)	3.40 (2.21, 6.16)	0.90 (0.61, 1.63)	5.50 (4.10, 7.56)
p[b]		<0.001	0.02	<0.001
Race				
White	1616 (98.2)	2.59 (1.60, 4.93)	0.81 (0.56, 1.68)	4.53 (3.38, 6.90)
Non-white	30 (1.8)	2.80 (2.01, 4.85)	0.69 (0.58, 1.06)	4.87 (3.39, 6.19)
p[a]		0.51	0.56	0.81
Education level (years)				
≤12	484 (29.4)	2.75 (1.72, 4.93)	0.81 (0.57, 1.50)	4.74 (3.56, 6.90)
>12–16	786 (47.8)	2.51 (1.58, 4.81)	0.80 (0.55, 1.72)	4.35 (3.29, 6.85)
>16	376 (22.8)	2.59 (1.54, 5.23)	0.87 (0.57, 1.98)	4.52 (3.37, 6.90)
p[b]		0.22	0.33	0.36
Number of chronic conditions				
0–1	354 (21.5)	2.08 (1.33, 4.03)	0.79 (0.53, 1.94)	4.10 (3.05, 7.17)
2	270 (16.4)	2.46 (1.59, 4.80)	0.76 (0.55, 1.67)	4.11 (3.18, 6.11)
3	282 (17.1)	2.42 (1.44, 4.75)	0.79 (0.55, 1.60)	4.53 (3.40, 6.48)
≥4	740 (45.0)	2.97 (1.93, 5.40)	0.83 (0.59, 1.64)	4.98 (3.67, 7.22)
p[b]		<0.001	0.22	<0.001
Multi-morbidity percentile				
Quintile 1	262 (15.9)	2.27 (1.43, 4.26)	0.78 (0.55, 1.60)	4.23 (3.16, 7.27)
Quintile 2	351 (21.3)	2.51 (1.62, 5.46)	0.80 (0.56, 1.74)	4.53 (3.38, 6.82)
Quintile 3	356 (21.6)	2.66 (1.63, 5.02)	0.85 (0.57, 1.63)	4.63 (3.28, 6.69)
Quintile 4	334 (20.3)	2.50 (1.49, 4.66)	0.78 (0.54, 1.56)	4.47 (3.36, 6.59)
Quintile 5	343 (20.8)	2.93 (1.93, 5.14)	0.83 (0.59, 1.72)	4.90 (3.68, 7.08)
p[b]		0.005	0.65	0.03

[a]Wilcoxon two-sample test p value.
[b]Jonckheere–Terpstra test (nonparametric test for trend across levels of ordinal variables, e.g., five quintiles; p value).
IL, interleukin; TNF, tumor necrosis factor.

a useful clinical index of biological age in some groups of older adults.

Altered immune responses are associated with the development and progression of several chronic conditions that are included in multi-morbidity, leading to the concept of "inflammaging" as a key component of the aging process.[4,7,18] In particular, IL-6 and TNF-α are components of the senescent-associated secretory phenotype, and they are markers of other age-related molecular and cellular damage.[19] However, studies that have specifically examined associations between inflammatory markers and general aging outcomes, such as multi-morbidity, are limited. Our study focused

Table 2. Associations between multi-morbidity percentile quintiles and plasma IL-6, IL-10, and TNF-α levels in women and men.

	IL-6 (pg/mL)		IL-10 (pg/mL)		TNF-α (pg/mL)	
Multi-morbidity percentile	Women Median (Q1, Q3)	Men Median (Q1, Q3)	Women Median (Q1, Q3)	Men Median (Q1, Q3)	Women Median (Q1, Q3)	Men Median (Q1, Q3)
Quintile 1	1.89 (1.19, 3.47)	2.49 (1.70, 5.27)	0.67 (0.48, 1.42)	0.90 (0.63, 1.87)	4.08 (2.91, 5.98)	4.39 (3.28, 8.20)
Quintile 2	2.19 (1.44, 4.81)	2.79 (1.78, 5.79)	0.74 (0.52, 1.62)	0.87 (0.58, 1.91)	4.23 (3.16, 6.84)	4.62 (3.46, 6.82)
Quintile 3	2.51 (1.55, 4.18)	2.81 (1.72, 6.11)	0.76 (0.53, 1.43)	0.91 (0.60, 1.86)	4.34 (3.16, 6.25)	4.78 (3.39, 8.28)
Quintile 4	2.14 (1.36, 3.65)	2.80 (1.64, 5.31)	0.78 (0.53, 1.57)	0.78 (0.55, 1.53)	4.29 (3.23, 5.77)	4.68 (3.40, 7.41)
Quintile 5	2.78 (1.90, 4.20)	3.29 (2.00, 6.49)	0.75 (0.55, 1.35)	0.91 (0.64, 2.13)	4.49 (3.58, 6.42)	5.27 (3.87, 8.17)
p[a]	0.009	0.09	0.32	0.87	0.11	0.11

[a]Jonckheere–Terpstra test (nonparametric test for trend across five quintiles; p value).
IL, interleukin; TNF, tumor necrosis factor.

Table 3. Associations between multi-morbidity percentile quintiles and plasma levels of IL-6, IL-10, and TNF-α in people 50–69 years and in people 70+ years.

	IL-6 (pg/mL)		IL-10 (pg/mL)		TNF-α (pg/mL)	
	50–69 years Median (Q1, Q3)	70+ years Median (Q1, Q3)	50–69 years Median (Q1, Q3)	70+ years Median (Q1, Q3)	50–69 years Median (Q1, Q3)	70+ years Median (Q1, Q3)
Quintile 1	2.06 (1.39, 3.69)	2.39 (1.45, 4.88)	0.79 (0.51, 2.08)	0.78 (0.57, 1.49)	3.93 (2.85, 6.35)	4.39 (3.47, 7.94)
Quintile 2	2.06 (1.44, 4.30)	2.79 (1.78, 5.75)	0.74 (0.52, 1.99)	0.82 (0.57, 1.73)	3.79 (3.13, 6.73)	4.89 (3.48, 6.84)
Quintile 3	2.19 (1.36, 3.95)	2.95 (2.00, 5.43)	0.76 (0.54, 1.57)	0.88 (0.60, 1.72)	4.16 (2.96, 6.31)	4.87 (3.59, 7.43)
Quintile 4	2.06 (1.30, 3.21)	2.97 (1.77, 5.18)	0.77 (0.51, 1.51)	0.79 (0.56, 1.56)	3.96 (2.98, 5.73)	4.98 (3.66, 7.14)
Quintile 5	2.56 (1.61, 4.10)	3.28 (2.22, 6.01)	0.79 (0.55, 1.55)	0.87 (0.62, 2.05)	4.24 (3.32, 6.58)	5.34 (3.96, 7.83)
p[a]	0.26	0.003	0.86	0.41	0.35	0.03

[a]Jonckheere–Terpstra test (nonparametric test for trend across five quintiles; p value).
IL, interleukin; TNF, tumor necrosis factor.

on multi-morbidity percentiles, which are a novel age- and sex-adjusted measure of aging. This measure is a refinement of multi-morbidity measures that have been previously used, which focused on simple counts of chronic diseases. Conversely, our multi-morbidity measure takes into account both age and sex and allows for identification of people with a higher burden of multi-morbidity compared with that in peers of the same age and sex. Although the studied outcome is novel, our results are consistent with longitudinal findings from two studies that showed that IL-6 may be an important general marker of biologic aging processes that lead to the progression of multi-morbidity and frailty.[20,21]

Our results are also consistent with findings from Fabbri and colleagues who showed significant cross-sectional associations between multi-morbidity and IL-6 and TNF-α receptor II but not with IL-10.[20] However, our study examined TNF-α levels and not TNF-α receptor II levels. TNF receptors I and II are considered better biomarkers than TNF-α because TNF-α serum levels tend to be low and unstable after storage at −80°C.[11] However, we were limited by the measures available in the MCSA, and only TNF-α has been measured in this population. Although we expected that the problems with accurately measuring TNF-α levels could have biased our results toward no associations, we observed significant associations. Therefore, we predict that the associations between multi-morbidity percentiles and TNF receptor levels may be stronger than the associations that we reported.

Other limitations of this study include the use of cross-sectional data, making it impossible to determine whether an increase in inflammatory biomarkers preceded the development of multi-morbidity, or whether multi-morbidity preceded an increase in inflammatory biomarkers (possible cause–effect inversion). Second, a wide range of aging biomarkers has been identified,[7,8,10,11] and aging and multi-morbidity are the result of many complex biologic mechanisms and not just inflammatory responses. However, we were limited to the data available in the MCSA, and we were not able to examine associations with additional biomarkers. Therefore, further biomarker studies are needed to understand the role of other important biologic processes (e.g., changes in metabolism) in conjunction with inflammation on aging and multi-morbidity. Finally, our study population was limited to people 50 years of age and older who were predominantly white, lived in a single county in Minnesota, USA, and participated in the MCSA. Characteristics of people who participated were similar to those of people who did not participate, suggesting that the study results are likely to be internally valid.[15] However, associations may differ in younger people, and in people of different races or ethnicities. Thus, the generalizability of the results is unclear, and similar studies need to be conducted in populations with different characteristics.

In conclusion, our results indicate that multi-morbidity percentiles were significantly associated with two inflammatory biomarkers of aging in women and in people 70 years of age or older. These data suggest that multi-morbidity percentiles may be a useful clinical index of biological age for future epidemiologic studies and clinical trials in women and older adults.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

1. Marengoni A, Angleman S, Melis R, et al. Aging with multimorbidity: a systematic review of the literature. *Ageing Res Rev* 2011; 10: 430–439.
2. Fabbri E, An Y, Zoli M, et al. Association between accelerated multimorbidity and age-related cognitive decline in older Baltimore longitudinal study of aging participants without dementia. *J Am Geriatr Soc* 2016; 64: 965–972.
3. Vetrano DL, Calderon-Larranaga A, Marengoni A, et al. An international perspective on chronic multimorbidity: approaching the elephant in the room. *J Gerontol A Biol Sci Med Sci* 2018; 73: 1350–1356.
4. Franceschi C, Garagnani P, Morsiani C, et al. The continuum of aging and age-related diseases: common mechanisms but different rates. *Front Med (Lausanne)* 2018; 5: 61.
5. Rocca WA, Grossardt BR, Boyd CM, et al. Multimorbidity, ageing and mortality: normative data and cohort study in an American population. *BMJ Open* 2021; 11: e042633.
6. Goodman RA, Posner SF, Huang ES, et al. Defining and measuring chronic conditions: imperatives for research, policy, program, and practice. *Prev Chronic Dis* 2013; 10: E66.
7. Ferrucci L and Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. *Nat Rev Cardiol* 2018; 15: 505–522.
8. Akbaraly TN, Hamer M, Ferrie JE, et al. Chronic inflammation as a determinant of future aging phenotypes. *CMAJ* 2013; 185: E763–E770.
9. Newman AB, Sachs MC, Arnold AM, et al. Total and cause-specific mortality in the cardiovascular health study. *J Gerontol A Biol Sci Med Sci* 2009; 64: 1251–1261.
10. Cohen HJ, Harris T and Pieper CF. Coagulation and activation of inflammatory pathways in the development of functional decline and mortality in the elderly. *Am J Med* 2003; 114: 180–187.
11. Justice JN, Ferrucci L, Newman AB, et al. A framework for selection of blood-based biomarkers for geroscience-guided clinical trials: report from the TAME Biomarkers Workgroup. *Geroscience* 2018; 40: 419–436.
12. Roberts RO, Geda YE, Knopman DS, et al. The mayo clinic study of aging: design and sampling, participation, baseline measures and sample characteristics. *Neuroepidemiology* 2008; 30: 58–69.
13. St Sauver JL, Grossardt BR, Yawn BP, et al. Data resource profile: the Rochester Epidemiology Project (REP) medical records-linkage system. *Int J Epidemiol* 2012; 41: 1614–1624.
14. St Sauver JL, Grossardt BR, Yawn BP, et al. Use of a medical records linkage system to enumerate a dynamic population over time: the Rochester Epidemiology Project. *Am J Epidemiol* 2011; 173: 1059–1068.
15. Knopman DS, Roberts RO, Pankratz VS, et al. Incidence of dementia among participants and nonparticipants in a longitudinal study of cognitive aging. *Am J Epidemiol* 2014; 180: 414–423.
16. Wennberg AMV, Hagen CE, Machulda MM, et al. The cross-sectional and longitudinal associations between IL-6, IL-10, and TNFα and cognitive outcomes in the mayo clinic study of aging. *J Gerontol A Biol Sci Med Sci* 2019; 74: 1289–1295.
17. Rocca WA, Grossardt BR, Brue SM, et al. Data resource profile: expansion of the Rochester Epidemiology Project medical records-linkage system (E-REP). *Int J Epidemiol* 2018; 47: 368–368j.

18. Franceschi C, Garagnani P, Vitale G, et al. Inflammaging and 'garb-aging'. *Trends Endocrinol Metab* 2017; 28: 199–212.
19. Schafer MJ, Zhang X, Kumar A, et al. The senescence-associated secretome as an indicator of age and medical risk. *JCI Insight* 2020; 5:e133668.
20. Fabbri E, An Y, Zoli M, et al. Aging and the burden of multimorbidity: associations with inflammatory and anabolic hormonal biomarkers. *J Gerontol A Biol Sci Med Sci* 2015; 70: 63–70.
21. Friedman EM, Christ SL and Mroczek DK. Inflammation partially mediates the association of multimorbidity and functional limitations in a national sample of middle-aged and older adults: the MIDUS Study. *J Aging Health* 2015; 27: 843–863.