Original Article

Correlation analyses between age and indices in routine blood laboratory tests suggest potential aging biomarkers

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Background: Aging, especially its related immune senescence, is an important risk factor for many diseases, including neurodegenerative diseases, cardiovascular diseases and tumors. One major hurdle to study aging is the lack of a comprehensive set of biomarkers to evaluate and predict the progress of aging. The biomarkers for immune senescence are crucial for effective vaccination and optimal immunotherapy.

Methods: In this study, to identify potential biomarkers linked to aging from routine blood laboratory tests, we analyzed the correlations between aging and indices in peripheral blood cell population, cytokines, Complete Blood Count and Blood Chemistry panel. Furthermore, we analyzed the differences of immune cells and inflammatory cytokines among different age groups. In addition, differential gene expression was evaluated by using whole transcriptome data of 258 samples, which was subsequently used for pathway analysis.

Results: Our results showed significant correlations between some of the indices with aging, and some were also associated with genders and ethnics. Transitional B cells were negatively correlated with aging, and several cytokines, including CCL22, CXCL9, IL21, IL23A and IL31, were related to aging as well. In addition, we found several genes associated with aging, including *SERPINA1*, *ORM2*, *MS4A1*, *ETS1*, *CD27* and *IL7R*.

Conclusions: Our study demonstrated that changes of a couple of indices from routine blood laboratory tests and gene expression had correlation to aging, and these could potentially be used as biomarkers of aging.

Keywords: Aging; biomarkers; routine blood laboratory tests

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Introduction

Population aging is a worldwide socioeconomic issue and has a great impact on the health care system. From a biological perspective, aging is a natural, multi-factorial phenomenon characterized by the accumulation of degradation processes, which in turn are supported by multiple changes and damages within molecular pathways (1). Such changes and damages will eventually impair the functions of cells and tissues. Therefore, aging is the most serious risk factor for almost all noncommunicable diseases,

including cardiovascular diseases, tumors, diabetes and neurological diseases.

With the aggravation of population aging, there is a great interest in determining the predictors, that is, biomarkers of population aging. The study on biomarkers, which refers to the measurements of cellular, biochemical, or molecular changes, has shown significant value in health prediction in humans. Several aging clocks have been proposed that use various biomarkers to predict the actual physiological age of humans (2). Currently, the most accurate markers reported are methylated markers used in prediction models for muscle aging (3), while the transcription and metabolomics are not (4). Although many theories have been put forward to explain the phenomenon of aging, the lack of sensitive and specific biomarkers has been a great hurdle to predicting human aging (5). Recent attempts to use techniques such as principal component analysis to statistically link multiple biomarkers running in different physiological networks have led to a more comprehensive understanding of age-related health (6). The transition from a simple method based on one biomarker at a time to a systematic analysis method that integrates multiple biomarkers simultaneously provides an opportunity to identify the comprehensive biomarkers specific to aging (7). Therefore, discovering biomarkers that can predict disease occurrence remains an important goal.

The decline of the immune system, known as immune senescence, includes a series of changes in the innate and adaptive immune systems, and is accompanied with human aging. These result in increased susceptibility to infections, reduce the efficacy of vaccination, and increase the incidence of tumors, neurodegenerative diseases and metabolic imbalances in the elderly (8). In order to meet the health-related needs of the growing elderly population, the study on age-related immune aging needs to be developed rapidly. In addition, in order to develop appropriate preventive and therapeutic strategies, we must understand the potential biological mechanisms that lead to age-related pathogenesis (9). In this study, the differences of indices from routine blood laboratory tests, including Complete Blood Count, blood biochemistry panel were compared among different age groups. Furthermore, we analyzed the differences of immune cells and inflammatory cytokines, as well as the differences in gene expression levels among different age groups. Our results showed significant correlations between some indices with aging, and some might even be associated with genders and ethnics. Our work provides clues to look for potential biomarkers of aging in the future. We present the following article in accordance with the MDAR reporting checklist (available at https://aob.amegroups.com/article/view/10.21037/aob-20-79/rc).

Methods

Data sources

Data sources for this article are available at http://10kimmunomes.org/. Here, we proposed the 10,000 immune bodies project (10KIP), which was a framework to develop multiple human immune system references from the ImmPort, a publicly available source of immune system data. Although some measurement types were sparse in the existing immune port databases, the existing data allowed multiple robustness comparisons. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

Data management and statistical analysis were performed by using IBM SPSS version 25 (IBM, Armonk, NY, USA). Spearman rank correlation was used to calculate the correlation between biomarkers and age, sex and race. Nonparametric test was used to calculate the differences among different age groups. Graphic representation was performed by using GraphPad Prism 7.0.

Differentially expressed genes (DEGs) analysis

Statistical analyses were carried out by using R3.6.1 and Bioconductor (http://www.bioconductor.org/). Selection criteria were $|\log_2 FC|>0.5$ and $P<0.001$.

Functional analysis of DEGs

Using DAVID (https:/ david.ncifcrf.gov/) to analyze DEGs. The five most significant functions of DEG enrichment were selected for analysis.

Protein-protein interaction networks

In this study, the interaction network diagram of target genes coding proteins was drawn by STRING database in order to understand the mutual regulation relationship between DEGs.

Table 1 The correlation analysis between aging and cell population indices

Cell population	All (n=562)	Young (n=193)	Middle-aged (n=113)	Elder (n=256)	P value
Age, years	55.81 (30.35, 77.25)	26.5 (22.4, 30.7)	52.3 (49.0, 55.0)	79.1 (69.0, 85.0)	<0.001
Gender, male/female	236/326	77/116	47/66	112/144	0.712
Race, White/Black/Asian/Other	404/11/84/63	109/1/46/37	70/8/24/11	255/2/14/15	<0.001
B cells	4.32 (6.74, 9.85)	8.09 (5.82, 11.35)	7.21 (5.04, 9.69)	5.72 (3.56, 8.74)	<0.001
CD16 neg monocytes	6.23 (9.51, 13.63)	8.03 (5.31, 12.10)	8.15 (5.75, 11.38)	11.34 (7.85, 15.87)	<0.001
CD16 pos monocytes	0.28 (0.57, 1.04)	0.40 (0.11, 0.94)	0.47 (0.25, 0.73)	0.74 (0.42, 1.33)	<0.001
CD4 T cells	30.79 (23.87, 38.93)	28.78 (23.21, 36.82)	30.35 (24.32, 38.44)	32.27 (24.58, 39.82)	0..097
CD8 T cells	12.69 (8.90, 18.22)	16.13 (11.54, 20.42)	13.27 (9.50, 18.63)	10.74 (7.37, 15.35)	<0.001
Central Memory CD4 T cells	9.02 (5.53, 13.76)	6.21 (3.79, 10.05)	9.27 (6.50, 13.76)	11.41 (7.53, 16.56)	<0.001
Central Memory CD8 T cells	1.71 (1.06, 2.69)	1.34 (0.64, 2.15)	1.87 (1.14, 3.06)	1.81 (1.17, 2.89)	<0.001
Effector CD4 T cells	1.86 (0.85, 3.38)	2.66 (1.58, 4.38)	1.93 (0.88, 3.30)	1.38 (0.60, 2.65)	<0.001
Effector CD8 T cells	2.08 (1.35, 3.36)	1.92 (1.15, 3.33)	2.25 (1.50, 3.54)	2.11 (1.50, 3.34)	0.084
Effector Memory CD4 T cells	6.04 (4.54, 8.21)	5.44 (3.91, 7.29)	6.71 (4.83, 9.29)	6.32 (4.83, 8.45)	<0.001
Effector Memory CD8 T cells	2.08 (1.35, 3.36)	1.92 (1.15, 3.33)	2.25 (1.50, 3.54)	2.11 (1.50, 3.34)	0.036
Gamma Delta T cells	4.44 (3.35, 6.46)	4.76 (3.36, 7.42)	4.31 (3.23, 5.88)	4.39 (3.38, 6.02)	0.155
Lymphocytes	81.26 (76.67, 85.91)	83.39 (78.39, 86.64)	79.04, 87.42)	79.42 (73.55, 83.71)	<0.001
Memory B cells	0.68 (0.44, 1.15)	0.87 (0.47, 1.25)	0.72 (0.47, 1.15)	0.56 (0.37, 0.99)	0.004
Monocytes	10.19 (6.92, 14.95)	8.61 (5.87, 12.79)	8.89 (6.16, 11.91)	12.51 (8.47, 17.13)	<0.001
Naive B cells	1.70 (0.96, 2.84)	1.82 (1.23, 2.96)	1.97 (1.05, 2.94)	1.37 (0.70, 2.59)	<0.001
Naive CD4 T cells	10.67 (6.16, 16.92)	13.79 (8.85, 18.50)	10.05 (6.27, 15.54)	8.69 (4.71, 15.44)	<0.001
Naive CD8 T cells	5.32 (3.21, 9.21)	9.67 (6.66, 12.42)	5.91 (3.79, 8.10)	3.54 (2.18, 5.18)	<0.001
NK cells	4.41 (2.46, 7.08)	3.12 (1.55, 5.29)	4.23 (2.62, 7.21)	5.38 (3.05, 8.02)	<0.001
NKT cells	4.54 (2.97, 6.69)	4.25 (2.44, 6.59)	4.55 (3.12, 6.69)	4.78 (3.23, 6.81)	<0.001
Plasmablasts	1.17 (0.77, 1.78)	1.30 (0.83, 1.90)	1.11 (0.81, 1.84)	1.08 (0.76, 1.66)	0.063
T cells	51.65 (43.21, 59.21)	54.75 (46.73, 59.93)	51.12 (41.72, 59.46)	49.70 (42.00, 57.29)	0.001
Transitional B cells	0.63 (0.43, 1.05)	0.74 (0.48, 1.41)	0.74 (0.44, 1.31)	0.54 (0.34, 0.84)	<0.001
Tregs	3.10 (2.17, 4.70)	2.74 (1.96, 4.15)	2.84 (2.06, 4.09)	3.71 (2.44, 5.55)	<0.001

Data presented as median (P25, P75).

Results

Correlations between aging and indices in cell population and blood tests

To identify potential biomarkers related to aging, the correlations between aging and different indices were analyzed, including 24 cell population indices from 562 persons, 78 cytokines from 952 persons, 19 Complete Blood Count indices from 1,155 persons, 7 blood lipids and 18 other indices in blood chemistry panel from 664 persons (*Tables 1-5*). Coefficients of Spearman Rank Correlation were calculated and the results were shown in *Figure 1*. Among cell populations, Naive CD8 T cells, Naive CD4 T cells, Naive B cells, B cells and Transitional B cells showed negative correlations to aging, while NK cells, Central Memory CD4 T cells, Central Memory CD8 T cells and Monocytes

Table 2 The correlation analysis between aging and cytokines

Cytokines	All (n=952)	Young (n=344)	Middle-aged (n=132)	Elder (n=476)	P value
Age, years	59.89 (29.19, 76.62)	26.81 (23.02, 30.71)	52.00 (48.42, 55.00)	76.62 (68.49, 84.99)	<0.001
Gender, male/female	406/546	155/189	55/77	196/280	0.525
Race, White/Black/Asian/Other	738/20/106/88	220/7/61/56	83/10/26/13	435/20/106/88	<0.001
BDNF	3535.73 (244.86, 474.62)	369.95 (295.18, 428.90)		351.45 (213.40, 508.08)	0.739
CCL1	39.45 (27.30, 63.36)	45.52 (22.41, 63.36)		38.89 (29.48, 55.70)	1.000
CCL11	52.20 (37.55, 68.02)	47.33 (33.31, 66.66)	54.55 (40.61, 70.89)	53.40 (39.37, 68.79)	0.001
CCL13	29.66 (28.03, 31.29)	28.03 (26.51, 28.60)		30.72 (29.64, 31.33)	0.242
CCL17	12.11 (8.98, 15.23)	8.98 (8.98, 9.98)		15.23 (15.23, 15.23)	0.317
CCL2	65.19 (46.47, 92.62)	64.23 (48.02, 90.23)	59.75 (41.59, 93.47)	67.69 (47.03, 94.80)	0.161
CCL21	44.36 (28.55, 54.01)	42.84 (27.11, 58.20)		44.36 (29.77, 48.33)	0.834
CCL22	242.78 (194.88, 296.41)	256.96 (195.33, 315.82)	254.79 (235.28, 296.41)	163.46 (109.72, 226.70)	0.011
CCL24	136.74 (100.98, 164.41)	110.16 (83.62, 170.76)		140.72 (130.23, 155.79)	0.345
CCL27	34.36 (27.74, 39.30)	45.48 (14.22, 54.28)		33.38 (27.87, 37.10)	0.439
CCL3	59.78 (32.38, 121.61)	63.38 (33.76, 116.09)	54.54 (29.39, 104.99)	58.22 (31.84, 147.49)	0.450
CCL4	170.31 (74.88, 373.42)	147.35 (58.87, 320.20)	158.71 (69.54, 351.19)	208.62 (89.54, 418.99)	<0.001
CCL5	1,185.13 (833.26, 1,579.79)	1,242.71 (875.98, 1,659.50)	1,140.99 (543.69, 1,591.10)	1,122.02 (836.60, 1,539.60)	0.025
CCL7	54.37 (37.83, 75.91)	55.17 (37.20, 75.94)	55.00 (38.39, 74.08)	53.72 (37.87, 75.91)	0.971
CCL8	40.76 (22.80, 48.05)	41.52 (26.89, 62.63)		33.21 (22.19, 47.26)	0.345
CD40LG	263.58 (123.34, 825.38)	358.14 (143.05, 893.76)	167.75 (95.91, 645.53)	241.19 (127.69, 823.92)	0.002
CSF1	8.29 (4.19, 13.81)	7.00 (4.45, 14.36)	10.99 (4.16, 19.07)	8.47 (3.39, 12.80)	0.023
CSF2	101.07 (60.56, 161.15)	105.06 (63.03, 164.06)	106.96 (57.54, 153.83)	95.96 (61.18, 164.15)	0.548
CSF3	30.46 (16.30, 57.49)	38.12 (22.45, 66.38)	24.58 (14.14, 54.39)	25.78 (15.95, 51.01)	<0.001
CX3CL1	118.61 (80.61, 188.60)	141.19 (80.89, 191.78)	83.45 (54.64, 112.25)	114.29 (83.79, 164.45)	0.311
CXCL1	34.12 (21.01, 61.64)	39.93 (21.99, 66.53)	28.92 (14.56, 60.77)	31.02 (21.62, 59.38)	0.036
CXCL10	85.80 (64.64, 111.72)	83.21 (61.91, 110.49)	84.68 (62.59, 118.26)	87.22 (67.51, 111.87)	0.396
CXCL12	74.47 (59.60, 112.61)	82.86 (59.75, 119.99)		73.18 (59.33, 111.78)	0.684
CXCL13	34.43 (27.24, 44.29)	40.68 (27.24, 44.57)		35.44 (26.67, 38.17)	0.475
CXCL5	104.31 (42.39, 209.94)	129.24 (48.34, 273.15)	90.25 (34.44, 206.45)	94.11 (43.52, 184.36)	0.017
CXCL8	30.02 (13.69, 72.21)	22.32 (9.24, 38.77)	33.68 (14.52, 120.40)	40.95 (16.16, 93.53)	<0.001
CXCL9	115.97 (69.54, 193.35)	85.41 (49.80, 128.23)	102.97 (64.50, 170.43)	150.65 (94.60, 243.76)	<0.001
EGF	75.10 (51.09, 118.37)	77.07 (51.93, 115.94)	59.58 (30.49, 88.67)	75.02 (35.14, 121.78)	0.562
FASLG	32.21 (20.41, 49.69)	35.41 (22.30, 54.34)	33.38 (19.20, 49.45)	30.01 (19.83, 49.10)	0.007
FGF2	21.99 (9.84, 43.82)	25.08 (11.44, 43.98)	15.64 (563, 43.19)	22.35 (9.90, 43.98)	0.003

Table 2 (*continued*)

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Cytokines	All (n=952)	Young (n=344)	Middle-aged (n=132)	Elder (n=476)	P value
FIGF	24.58 (17.34, 33.38)	27.25 (16.35, 41.72)		24.32 (17.58, 32.61)	0.429
FLT3	29.31 (19.78, 37.79)	31.24 (20.21, 41.38)		28.18 (19.78, 34.34)	0.600
FLT3LG	29.22 (19.12, 59.14)	29.22 (19.68, 37.92)	41.06 (12.00, 70.11)		0.734
HGF	77.46 (56.39, 105.49)	72.24 (51.02, 96.23)	81.30 (53.62, 108.17)	81.48 (58.82, 108.91)	0.003
ICAM1	7,397.69 (3,437.47, 14,493.99)	6,137.93 (3,159.17, 11,332.64)	6,817.91 (2,305.43, 14,932.88)	8,441.99 (4,250.30, 16,074.77)	0.001
IFN1	5.24 (3.72, 12.42)	5.10 (3.98, 8.18)	3.86 (3.65, 11.85)	5.41 (3.73, 13.68)	0.003
IFNA2	55.97 (37.03, 82.82)	59.77 (38.11, 85.73)	41.40 (37.03, 45.77)	51.15 (37.84, 61.07)	0.395
IFNB1	9.70 (5.64, 14.65)	10.51 (6.37, 19.51)	9.70 (4.82, 14.60)	9.01 (5.47, 13.78)	0.013
IFNG	27.49 (11.55, 48.80)	28.46 (13.76, 56.66)	30.63 (8.02, 56.48)	26.77 (11.10, 43.83)	0.034
IL10	10.79 (5.51, 20.52)	8.23 (4.38, 18.33)	15.01 (4.37, 35.82)	12.23 (6.55, 19.73)	0.001
IL12B	36.83 (21.06, 55.41)	32.94 (19.25, 55.36)	40.32 (24.33, 53.26)	37.38 (22.42, 56.24)	0.330
IL13	31.71 (21.01, 44.06)	34.57 (23.68, 48.12)	32.44 (23.14, 44.25)	29.21 (17.30, 42.07)	<0.001
IL15	44.18 (22.19, 73.01)	49.73 (25.56, 84.08)	46.61 (19.62, 84.47)	41.14 (20.27, 65.28)	0.005
IL16	57.44 (39.71, 81.02)	81.02 (51.52, 86.65)		47.48 (39.01, 57.44)	0.059
IL17A	11.48 (5.26, 21.95)	12.63 (5.76, 24.16)	11.54 (4.38, 20.15)	11.27 (5.50, 19.99)	0.080
IL17F	5.25 (3.96, 9.70)	5.84 (4.25, 12.18)	6.56 (3.98, 11.62)	4.99 (3.74, 7.79)	<0.001
IL18	45.07 (29.82, 66.90)	45.38 (22.92, 76.55)		44.44 (29.82, 66.90)	0.926
IL1A	32.00 (17.15, 52.19)	32.57 (17.21, 51.14)	32.98 (17.34, 57.12)	31.46 (17.15, 51.97)	0.810
IL1B	9.57 (5.38, 17.58)	10.51 (5.86, 18.78)	10.01 (4.37, 17.72)	8.95 (5.31, 15.47)	0.519
IL1R1	257.35 (75.84, 956.86)	146.93 (57.09, 996.39)	269.09 (141.08, 1,156.05)	305.58 (117.88, 931.47)	<0.001
IL2	71.01 (40.00, 127.03)	82.54 (47.09, 135.86)	59.42 (33.12, 144.48)	66.10 (38.87, 114.32)	0.005
IL20	58.35 (51.42, 60.79)	60.23 (55.54, 60.86)		58.03 (39.34, 58.35)	0.327
IL21	54.10 (35.23, 74.71)	56.58 (56.09, 81.56)		35.81 (35.14, 69.84)	0.001
IL22	70.57 (32.68, 110.09)	79.54 (52.40, 127.96)		68.02 (31.14, 109.91)	0.559
IL23A	15.43 (15.12, 16.30)	16.35 (16.07, 16.42)		15.26 (15.07, 15.50)	<0.001
IL27	17.77 (13.44, 65.71)	13.96 (13.93, 54.42)		24.26 (13.28, 68.53)	0.575
IL2RA	80.02 (51.49, 93.44)	80.49 (52.76, 94.13)	51.67 (27.55, 75.78)		0.240
IL3	42.21 (35.26, 53.09)	42.21 (35.26, 53.09)			
IL31	50.67 (50.55, 50.98)	51.58 (46.62, 51.65)		50.64 (50.59, 50.85)	0.093
IL4	20.76 (13.59, 29.39)	19.10 (13.60, 30.64)	20.92 (11.76, 30.42)	20.84 (14.57, 28.14)	0.575
IL5	105.79 (63.84, 175.78)	129.32 (72.69, 207.79)	103.35 (63.37, 164.80)	95.86 (61.19, 152.80)	<0.001
IL6	16.95 (10.67, 39.13)	13.19 (10.78, 24.34)	22.18 (7.82, 160.10)	20.45 (10.97, 52.57)	0.014
IL7	151.26 (107.56, 214.44)	166.74 (115.55, 243.33)	136.40 (93.49, 218.07)	145.12 (105.40, 197.04)	<0.001
IL9	45.82 (13.76, 46.42)	14.29 (13.86, 33.43)		46.28 (13.65, 46.50)	0.168

Table 2 (*continued*)

Table 2 (*continued*)

Cytokines	All (n=952)	Young (n=344)	Middle-aged (n=132)	Elder (n=476)	P value
KITLG	57.72 (32.57, 95.47)	57.72 (32.54, 91.12)	60.64 (33.09, 95.40)	56.74 (32.42, 97.76)	0.829
LEP	1,598.66 (551.99, 3,541.07)	1,425.99 (458.78, 3,307.60)	1,309.77 (416.44, 3,077.36)	1,736.76 (770.53, 3,664.17)	0.012
LIF	18.84 (12.92, 28.11)	20.83 (13.09, 32.80)	21.07 (14.27, 30.44)	17.64 (12.56, 24.95)	<0.001
LTA	6.30 (4.92, 10.53)	6.21 (3.88, 10.89)	7.07 (3.66, 12.61)	6.33 (5.52, 9.44)	0.136
NGF	65.37 (39.60, 90.64)	72.43 (42.49, 109.25)	59.82 (36.40, 87.96)	64.39 (39.76, 83.20)	0.002
PIGF	23.12 (11.16, 57.82)	37.17 (11.33, 72.61)		17.14 (11.13, 57.82)	0.437
RETN	721.79 (394.87, 1,166.55)	744.64 (426.01, 1,166.65)	675.70 (246.63, 1,017.99)	732.57 (392.27, 1,188.87)	0.093
SERPINE1	1,737.60 (1,179.47, 2,690.94)	1,994.88 (1,455.91, 2,916.09)	1,404.73 (643.76, 1,835.73)	1,697.76 (1,141.84, 2,608.45)	<0.001
TGFA	17.45 (9.64, 27.21)	16.98 (8.64, 27.14)	20.32 (14.57, 31.78)	16.97 (8.55, 24.85)	0.024
TGFB1	19.76 (12.90, 29.66)	21.02 (14.30, 33.99)	18.61 (11.85, 30.52)	18.85 (12.38, 26.68)	0.002
TNF	23.29 (11.48, 50.59)	23.83 (11.56, 50.02)	23.82 (12.01, 59.85)	22.90 (11.48, 49.78)	0.630
TNFSF10	73.59 (50.01, 105.06)	77.87 (50.16, 107.92)	70.27 (47.01, 100.03)	71.70 (50.93, 105.68)	0.661
VCAM1	3,160.31 (1,105.48, 6,676.43)	4,044.48 (1,802.02, 7,496.88)	3,136.24 (320.89, 5,695.18)	2,795.18 (943.31, 6,574.75)	<0.001
VEGFA	36.61 (21.32, 62.71)	37.45 (22.34, 64.01)	32.10 (18.75, 61.17)	37.08 (21.33, 62.54)	0.598

Data presented as median (P25, P75).

showed positive correlations to aging (*Figure 1A*). Meanwhile, cytokines CCL22 and IL21 showed negative correlations to aging, while CXCL8 and CXCL9 showed positive correlations to aging (*Figure 1B*). As seen in *Figure 1C*, mean corpuscular hemoglobin concentration (MCHC) (r=–0.044, P=0.138) and platelet count (r=–0.143, P<0.001) showed negative correlations to aging. In terms of blood chemistry panel, total cholesterol and non-high-density lipoprotein (HDL) cholesterol calculation showed negative correlations to aging (*Figure 1D*), while urea nitrogen and albumin showed positive correlations to aging (*Figure 1E*).

Correlation of indices to aging differed in genders

Coefficients of Spearman Rank Correlation were calculated between same index and aging in different genders, and the results indicated that some indices had different correlations to aging in different genders.

(I) In terms of cell population, Effector CD8 T cells and Effector Memory CD8 T cells showed positive correlations to aging in males, and Gamma Delta T cells and Plasmablasts showed negative correlations to aging in males. However, none of these showed correlations to aging in females. Meanwhile, CD4 T cells and Tregs showed positive correlations, and Memory B cells showed a negative correlation to aging only in females (*Table 6*, *Figure 2A*).

(II) In terms of cytokines, IFN1, LEP and RETN showed positive correlations to aging in males, and NGF, SERPINE1 and TGFB1 showed negative correlations to aging in males. Meanwhile, IL1R1 had a positive correlation to aging, while CXCL1, CXCL5, IL23A and FASLG showed negative correlations in females (*Table 7*, *Figure 2B*).

(III) In terms of complete blood count, neutrophil, red cell distribution width (RDW) and WBC showed positive correlations to aging, while HCT, HGB and RBC showed negative correlations to aging in males. Meanwhile, basophil and monocyte percentages showed positive correlations to aging, while lymphocyte count showed a negative correlation to aging in females (*Table 8*, *Figure 2C*).

(IV) In terms of indices of blood lipids, Cholesterol to HDL ratio, low-density lipoprotein (LDL) cholesterol, LDL to HDL ratio, non-HDL cholesterol calculation, total cholesterol and Triglyceride showed negative correlations to aging in males (*Table 9*, *Figure 2D*).

Table 3 The correlation analysis between aging and complete blood count indices

Blood Count indices	All (n=1,155)	Young (n=244)	Middle-aged (n=369)	Elder (n=542)	P value
Age, years	58.26 (48.00, 71.00)	30.26 (25.72, 37.28)	54.00 (50.04, 57.00)	72.00 (65.51, 78.00)	<0.001
Gender, male/female	431/724	101/143	250/331	211/331	0.041
Race, White/Black/ Asian/Other	928/42/103/82	192/19/10/23	262/16/55/36	474/7/38/23	<0.001
Basophil count (K/μL)	3.12 (3.09, 3.13)	3.09 (3.04, 3.15)	3.12 (3.10, 3.13)	3.12 (3.11, 3.13)	<0.001
Basophil (%)	0.39 (0.24, 0.67)	0.39 (0.19, 0.67)	0.40 (0.29, 0.69)	0.39 (0.21, 0.59)	0.076
Eosinophil count (K/μL)	3.13 (3.07, 3.22)	3.11 (3.02, 3.22)	3.13 (3.08, 3.21)	3.14 (3.08, 3.22)	0.002
Eosinophil (%)	2.17 (1.49, 2.95)	2.20 (1.59, 2.71)	2.17 (1.47, 3.11)	2.10 (1.51, 2.97)	0.938
HCT (%)	43.25 (40.55, 45, 89)	43.47 (40.50, 45.99)	43.13 (40.49, 45.85)	43.25 (40.69, 45.95)	0.875
HGB (g/dL)	14.71 (13.80, 15.70)	14.81 (13.78, 16.01)	14.70 (13.80, 15.60)	14.70 (13.80, 15.60)	0.230
Lymphocyte count (K/μL)	3.81 (3.49, 4.21)	3.92 (3.45, 4.46)	3.84 (3.51, 4.21)	3.75 (3.46, 4.12)	0.013
Lymphocyte (%)	31.76 (28.76, 34.66)	31.87 (30.00, 33.57)	32.06 (29.66, 35.96)	31.45 (27.66, 34.26)	0.023
MCH (pg)	30.76 (29.62, 31.86)	30.66 (29.44, 31.76)	30.62 (29.52, 31.72)	31.02 (29.82, 32.02)	0.002
MCHC (g/dL)	34.21 (33.71, 34.81)	34.59 (34.00, 35.36)	34.21 (33.71, 34.61)	34.11 (33.61, 34.61)	<0.001
MCV (fL)	90.11 (87.01, 92.86)	88.79 (85.16, 91.00)	89.91 (86.51, 92.11)	91.11 (88.11, 93.31)	<0.001
Monocyte count (K/μL)	3.34 (3.24, 3.45)	3.30 (3.19, 3.49)	3.33 (3.24, 3.42)	3.36 (3.27, 3.46)	<0.001
Monocyte (%)	8.27 (7.45, 9.23)	8.31 (7.46, 9.06)	8.25 (7.30, 9.10)	8.26 (7.59, 9.35)	0.246
Neutrophil count (K/μL)	4.59 (3.89, 5.45)	4.75 (3.79, 5.69)	4.53 (3.86, 5.34)	4.58 (3.94, 5.43)	0.368
Neutrophil (%)	56.48 (53.45, 60.25)	56.26 (54.51, 58.68)	56.48 (51.95, 60.65)	56.55 (53.25, 60.65)	0.754
Platelet count (K/μL)	269.20 (236.20, 305.20)	278.59 (241.00, 324.01)	271.64 (241.20, 303.51)	264.20 (231.20, 299.20)	0.001
RBC (M/μL)	4.96 (4.60, 5.26)	4.88 (4.48, 5.21)	5.00 (4.66, 5.28)	4.97 (4.62, 5.27)	0.026
RDW (%)	12.39 (11.84, 13.19)	12.10 (11.44, 13.03)	12.44 (11.89, 13.12)	12.49 (11.89, 13.29)	0.003
WBC (K/μL)	5.60 (4.66, 6.66)	5.71 (4.63, 6.87)	5.56 (4.66, 6.56)	5.56 (4.76, 6.63)	0.428

Data presented as median (P25, P75). HCT, hematocrit; HGB, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; RDW, red blood cell distribution width; WBC, white blood cells.

(V) In terms of indices in blood chemistry panel, alanine transaminase (ALT) and serum glutamic-pyruvic transaminase (SGPT) showed negative correlations to aging in males. Meanwhile, glucose and urea nitrogen showed positive correlations to aging, and calcium and total protein showed negative correlations to aging in females (*Table 10*, *Figure 2E*).

Correlation of index to aging differed in races

Coefficients of Spearman Rank Correlation were calculated between the same index and age among different races, including Asian, Black, White and other races, and the results indicated that some indices had different correlations to aging among races.

(I) In terms of cell population, B cells, Central Memory CD4 T cells, Effector CD4 T cells, Naive CD4 T cells, Naive CD8 T cells and T cells showed correlations to aging in White, Asian and other races groups, but not in Black group. Among them, Central Memory CD4 T cells showed a positive correlation to aging, while other cells showed negative correlations. In White and other races groups, CD16+ monocytes and CD16– monocytes

Table 4 Correlation analysis between aging and blood lipid indices

Blood lipid	All (n=664)	Young (n=30)	Middle-aged (n=250)	Elder (n=384)	P value
Age, years	63.00 (54.00, 74.00)	43.00 (42.00, 44.00)	54.00 (50.00, 56.00)	72.00 (66.50, 77.00)	<0.001
Gender, male/female	239/425	9/21	72/178	158/226	0.005
Race, White/Black/Asian/Other	507/10/99/48	16/0/9/5	164/8/54/24	327/2/36/19	<0.001
Cholesterol/HDL (%)	2.90 (2.40, 3.70)	3.10 (2.20, 3.40)	3.05 (2.50, 3.70)	2.90 (2.40, 3.70)	0.137
HDL cholesterol (mg/dL)	64.00 (52.00, 79.00)	60.50 (50.00, 73.00)	64.00 (54.00, 79.00)	63.00 (51.00, 79.00)	0.409
LDL cholesterol (mg/dL)	111.00 (89.00, 132.00)	102.00 (83.00, 128.00)	115.00 (96.00, 138.00)	109.00 (86.00, 127.00)	0.002
LDL/HDL (%)	1.70 (1.30, 2.20)	1.85 (1.20, 2.20)	1.80 (1.40, 2.20)	1.65 (1.25, 2.20)	0.118
Non-HDL cholesterol Calc (mg/dL)	128.00 (104.00, 149.00)	118.00 (94.00, 145.00)	132.50 (112.00, 154.00)	124.00 (100.00, 146.00)	0.004
Total cholesterol (mg/dL)	195.00 (172.00, 218.00)	177.50 (158.00, 201.00)	199.00 (178.00, 225.00)	192.00 (169.00, 216.00)	<0.001
Triglyceride (mg/dL)	71.00 (50.00, 102.00)	62.50 (47.00, 90.00)	74.00 (52.00, 106.00)	70.00 (50.00, 102.00)	0.237

Data presented as median (P25, P75). HDL, high-density lipoprotein; LDL, low-density lipoprotein.

showed positive correlations to aging, while lymphocytes showed a negative correlation. CD8 T cells and Transitional B cells showed negative correlations in Asian and White. Meanwhile, Central Memory CD8 T cells, Effector Memory CD4 T cells, Effector Memory CD8 T cells, Naive B cells, NKT cells and Tregs showed correlations to aging only in White, among which Naive B cells showed a negative correlation while all others showed positive correlations. Furthermore, Memory B cells showed a negative correlation to aging in Asian and Black groups. NK cells showed a positive correlation to aging in Asian, Black and White, while Plasmablasts showed a negative correlation only in other races (*Table 11*, *Figure 3A*).

(II) In terms of cytokines, CCL11 and IL1R1 showed positive correlations, while FASLG and IL17F showed negative correlations to aging in Asian and White groups. CSF1, FGF2, IL12B, IL17A and TNFSF10 showed positive correlations only in Black, and CCL5 showed a negative correlation only in Asian. IL6 showed a positive correlation, while CSF3 and SERPINE1 showed negative correlations in Asian and other races groups. Furthermore, CXCL10, HGF, ICAM1, IFN1, IL4, LEP, LTA and VEGFA showed positive correlations, while CXCL5, IL13 and IL7 showed negative correlations only in White. CXCL9 and IL5 showed correlations to aging in White and other races, with CXCL9 being positive and IL5 being negative. IFNG and IL10 showed correlations in Asian and White, with IL10 being positive and IFNG being negative. IL1A and IL2 showed correlations in Black and White, with IL1A being positive in both groups. Interestingly, IL2 showed a positive correlation in Black, while showing a negative correlation in White. Lastly, IFNB1, LIF and NGF showed negative correlations in other races (*Table 12*, *Figure 3B*).

(III) In terms of Complete Blood Count test, basophil count showed a positive correlation to aging in both Black and White. eosinophil percentage and RBC showed positive correlations in Black. In White and other races, RDW percentage showed a positive correlation, while lymphocyte count showed a negative correlation. monocyte percentage showed a positive correlation in Black and other races. Some indices showed correlations only in White, in which eosinophil count, MCH, MCV and monocyte showed positive correlations, while HGB, lymphocyte percentage, MCHC and platelet count showed negative correlations (*Table 13*, *Figure 3C*).

(IV) In terms of indices of blood lipids, cholesterol to HDL ratio showed a negative correlation only in Asian, while LDL cholesterol and non-HDL cholesterol Calc showed negative correlations only in White. Triglyceride showed a negative

Table 5 Correlation analysis between aging and indices in blood chemistry panel

Blood chemistry panel	All (n=664)	Young (n=30)	Middle-aged (n=250)	Elder (n=384)	P value
Age, years	63.00 (54.00, 74.00)	43.00 (42.00, 44.00)	54.00 (50.00, 56.00)	72.00 (66.50, 77.00)	<0.001
Gender, male/female	239/425	9/21	72/178	158/226	0.005
Race, White/Black/Asian/Other	507/10/99/48	16/0/9/5	164/8/54/24	327/2/36/19	<0.001
Albumin total (g/dL)	3.90 (3.70, 4.10)	3.95 (3.80, 4.20)	3.90 (3.70, 4.10)	3.90 (3.70, 4.00)	0.101
Alkaline phosphatase total (U/L)	85.00 (72.50, 99.00)	80.50 (61.00, 97.00)	84.00 (70.00, 96.00)	86.00 (74.50, 101.00)	0.042
ALT SGPT (U/L)	35.00 (31.00, 41.00)	36.00 (32.00, 40.00)	36.00 (31.00, 41.00)	34.00 (31.00, 41.00)	0.261
Anion gap (mmol/L)	7.00 (6.00, 8.00)	6.50 (5.00, 8.00)	7.00 (5.00, 8.00)	7.00 (6.00, 9.00)	0.574
AST SGOT (U/L)	22.00 (18.00, 26.00)	20.50 (18.00, 23.00)	21.50 (18.00, 26.00)	22.00 (18.00, 26.00)	0.400
C reactive protein (mg/dL)	0.40 (0.30, 0.80)	0.30 (0.20, 0.60)	0.40 (0.30, 0.80)	0.40 (0.30, 0.80)	0.683
Calcium (mg/dL)	9.00 (8.80, 9.30)	9.00 (8.80, 9.20)	9.10 (8.80, 9.30)	9.00 (8.80, 9.30)	0.641
Chloride (mmol/L)	103.00 (101.00, 104.00)	103.00 (102.00, 104.00)	103.00 (101.00, 104.00)	103.00 (101.00, 105.00)	0.766
CO_2 (mmol/L)	29.00 (27.00, 30.00)	29.00 (28.00, 30.00)	29.00 (28.00, 31.00)	29.00 (27.00, 30.00)	0.390
Creatinine (mg/dL)	0.90 (0.70, 1.00)	0.80 (0.70, 0.90)	0.85 (0.70, 1.00)	0.90 (0.80, 1.00)	0.012
ESR (mm/hr)	8.50 (3.75, 17.50)		4.00 (3.50, 15.00)	9.00 (8.00, 20.00)	0.514
Globulin (g/dL)	3.40 (3.20, 3.70)	3.30 (3.10, 3.70)	3.50 (3.20, 3.70)	3.40 (3.20, 3.70)	0.055
Glucose (mg/dL)	86.00 (79.00, 93.00)	81.50 (73.00, 89.00)	86.00 (79.00, 92.00)	86.00 (80.00, 94.00)	0.039
Potassium (mmol/L)	4.00 (3.80, 4.20)	3.95 (3.70, 4.30)	3.90 (3.70, 4.20)	4.00 (3.80, 4.20)	0.160
Protein total (g/dL)	7.30 (7.10, 7.60)	7.30 (7.00, 7.60)	7.40 (7.20, 7.70)	7.30 (7.00, 7.60)	0.014
Sodium (mmol/L)	139.00 (137.00, 140.00)	139.00 (137.00, 140.00)	139.00 (137.00, 140.00)	139.00 (137.00, 140.00)	0.988
Total bilirubin (mg/dL)	0.60 (0.50, 0.80)	0.60 (0.50, 0.60)	0.60 (0.50, 0.80)	0.60 (0.50, 0.80)	0.324
Urea nitrogen (mg/dL)	16.00 (13.00, 19.00)	14.00 (13.00, 17.00)	15.00 (13.00, 19.00)	16.50 (14.00, 19.50)	0.001

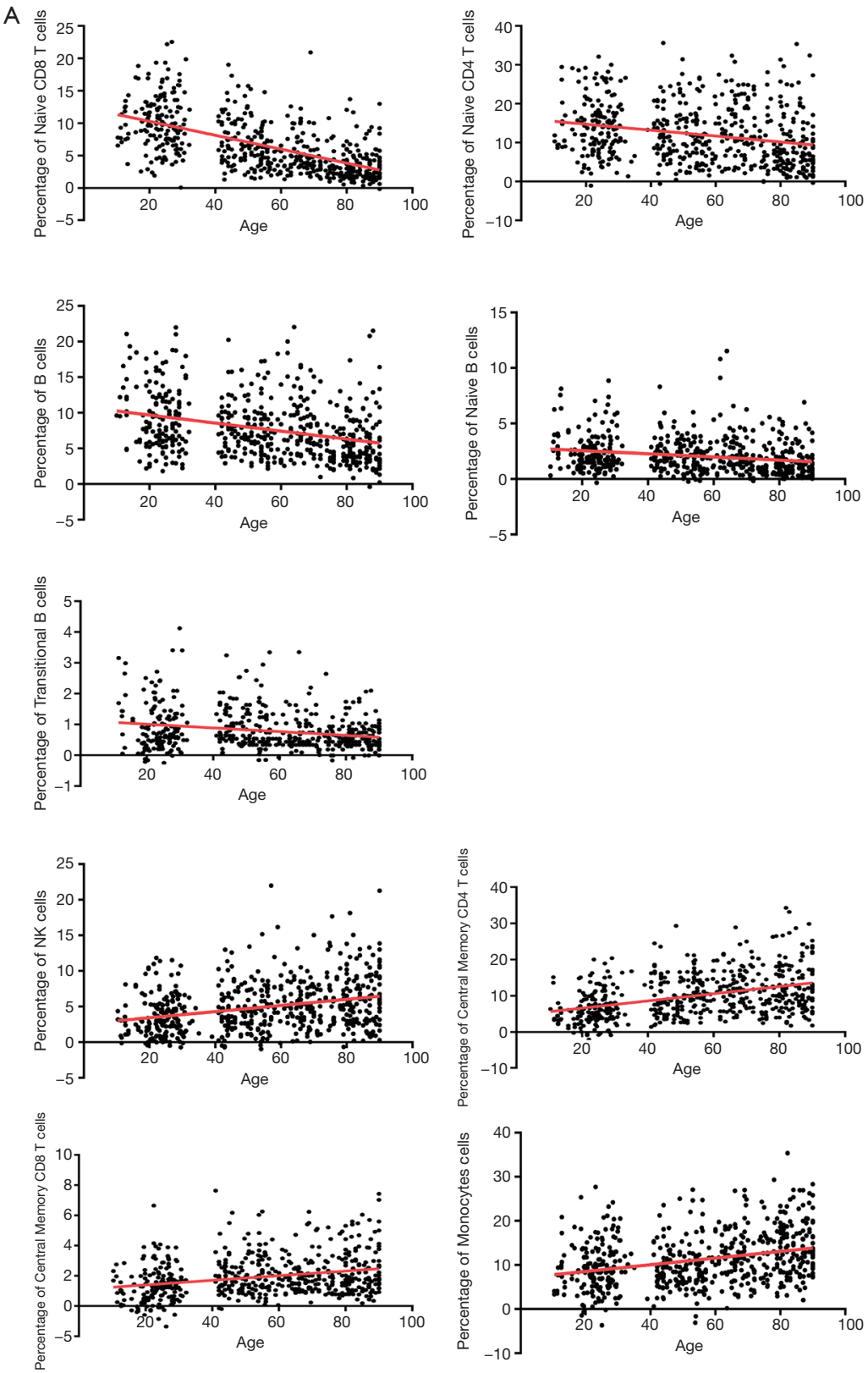
Data presented as median (P25, P75). ALT, alanine transaminase; SGPT, serum glutamic-pyruvic transaminase; AST, aspartate transaminase; SGOT, serum glutamic-oxaloacetic transaminase; ESR, erythrocyte sedimentation rate.

correlation only in Black. Furthermore, LDL to HDL ratio showed a negative correlation in Asian and White, while total cholesterol showed a negative correlation in White and other races (*Table 14*, *Figure 3D*).

(V) In terms of indices in blood chemistry panel, albumin total and calcium showed correlations to aging only in White, with albumin total being negative and calcium being positive. Meanwhile, creatinine, glucose and urea nitrogen showed positive correlations in Asian and White, while globulin showed a negative correlation only in Asian (*Table 15*, *Figure 3E*).

Screening of crucial genes closely related to aging and biological functions of DEGs enrichment

To screen potential biomarkers related to aging, gene expression data were analyzed. Samples were divided into two groups based on age, with >60 being Old group, and <60 being Young group. There were totally 101 differential genes identified. Compared to the Young group, 63 genes were up-regulated and 38 genes were down-regulated in the Old group (*Figure 4A*), and a heat map was constructed (*Figure 4B*). We then performed GO enrichment analysis by using DAVID (https://david.ncifcrf.gov/) with keywords: immune response, cell surface receptor, signaling pathway, cell chemotaxis, B cell proliferation and platelet



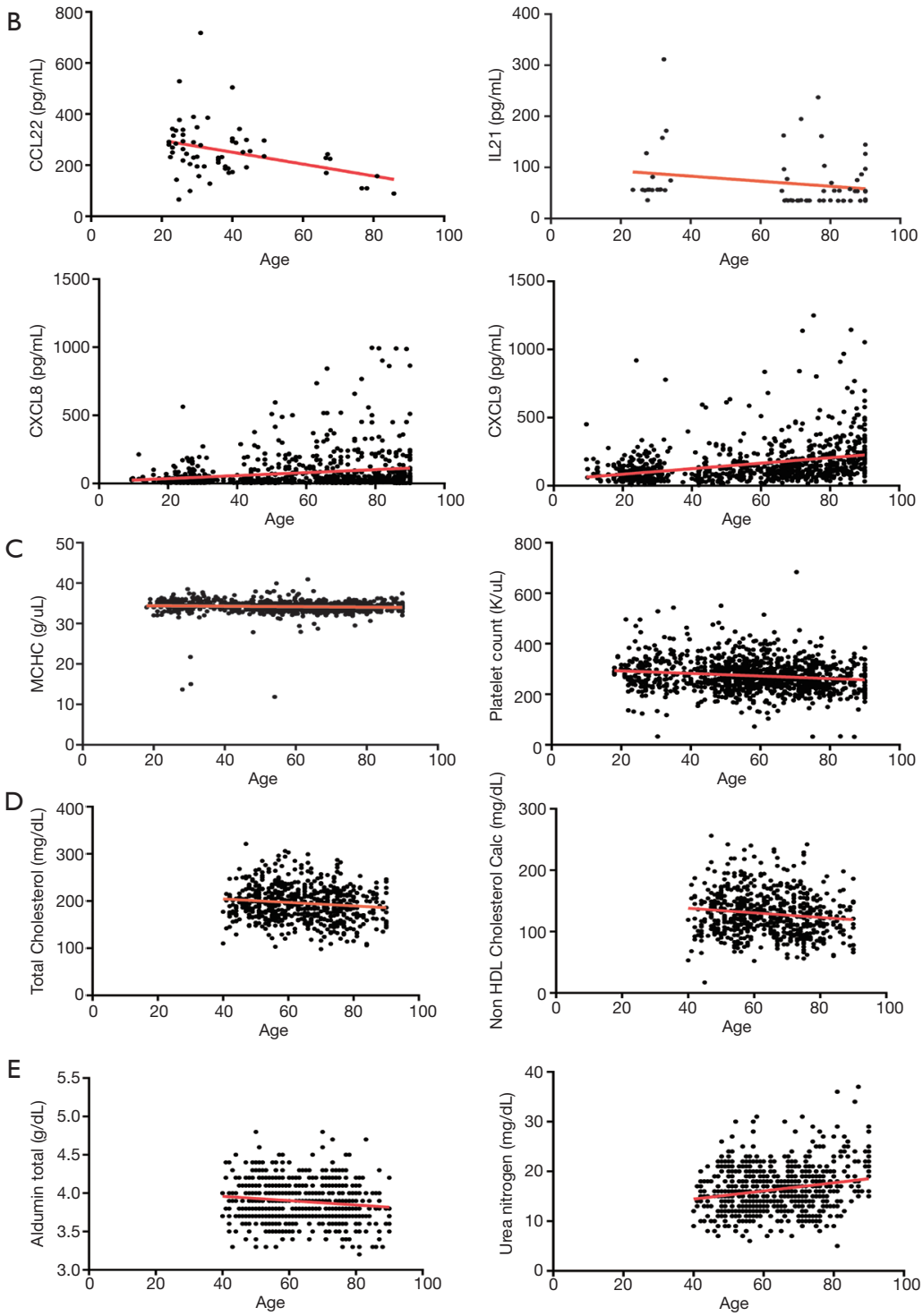
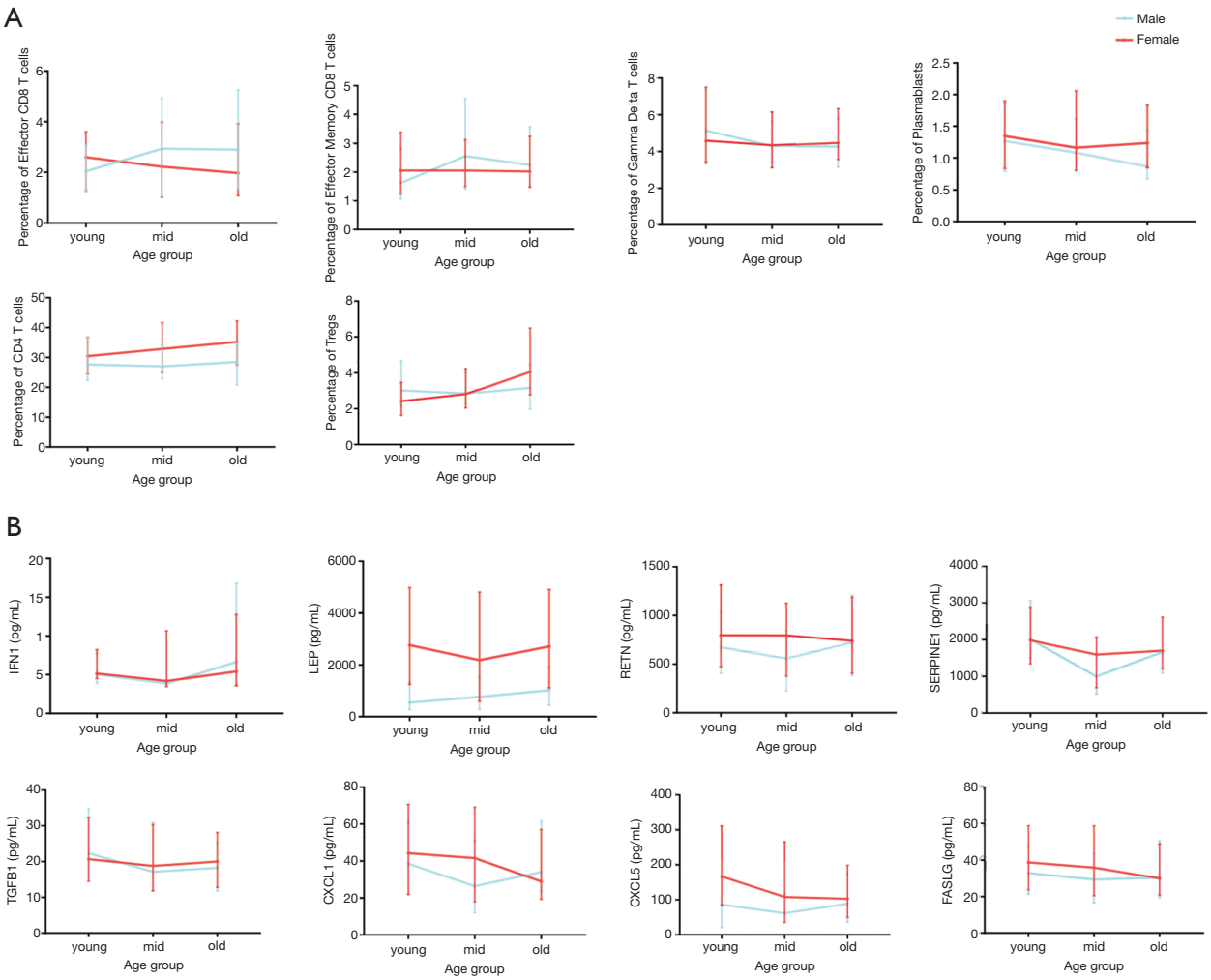


Figure 1 Correlation analyses between various laboratory indices with age. Quantitative changes of indices, including cells (A), cytokines (B), blood count (C), lipid profile (D) and metabolic panel (E) were analyzed for the relationship with age. The cell populations and cytokines with r_s (Spelman rank correlation coefficient) >0.2 and the values of blood count, lipid profile and metabolic panel with r_s >0.1 were graphically represented. Where rs is positive, indicating that biomarkers are positively correlated with age, and negative values indicate that biomarkers are negatively correlated with age, and the greater the absolute value, the greater the correlation. MCHC, mean corpuscular hemoglobin concentration; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 6 Correlation of cell population to aging differed in genders

Cell Population	Female	Male
CD4 T cells	0.144**	–0.043
Effector CD8 T cells	–0.029	0.164*
Effector Memory CD8 T cells	0.052	0.164*
Gamma Delta T cells	–0.009	–0.129*
Memory B cells	–0.168**	–0.076
Plasmablasts	–0.004	–0.229**
Tregs	0.323**	–0.074

*, P<0.05; **, P<0.01.



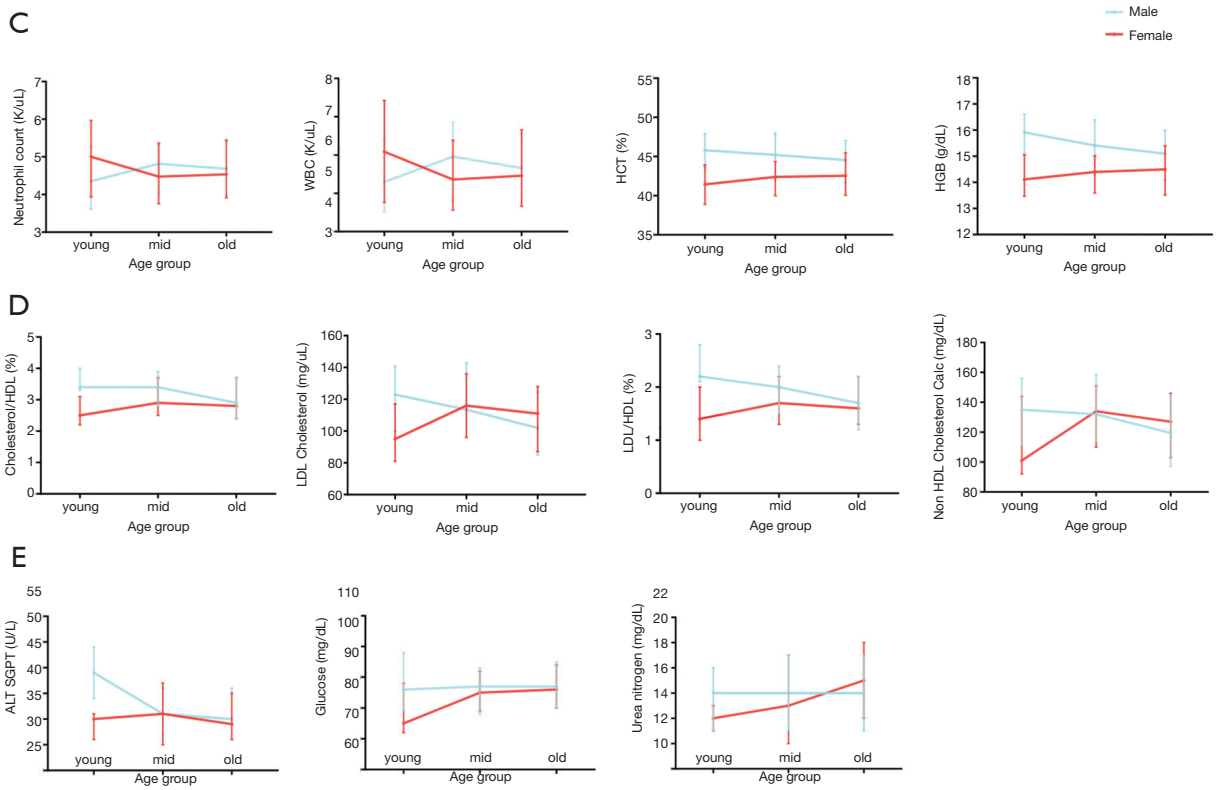


Figure 2 Correlation analyses between particular indices and age in different gender groups. Quantitative changes of the same index, including immune cells (A), cytokines (B), blood cells (C), lipid products (D) and metabolites (E) were analyzed for the relationship with age in different gender groups. WBC, white blood cells; HCT, hematocrit; volume; HGB, hemoglobin; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ALT, alanine transaminase; SGPT, serum glutamic-pyruvic transaminase.

Table 7 Correlation of cytokines to aging differed in genders

Cytokines	Female	Male
CXCL1	–0.119**	–0.032
CXCL5	–0.185**	–0.029
FASLG	–0.133**	–0.098
TGFB1	–0.004	–0.145**
SERPINE1	–0.060	–0.109*
IFN1	0.040	0.115*
IL1R1	0.141**	0.069
LEP	0.057	0.292**
NGF	–0.046	–0.102*
RETN	0.022	0.103*

*, P<0.05; **, P<0.01.

Table 8 Correlation of complete blood count indices to aging differed in genders

Blood count indices	Female	Male
HCT (%)	0.041	–0.164**
RDW (%)	0.060	0.144**
Basophil count (K/μL)	0.107**	0.087
HGB (g/dL)	0.011	–0.239**
Lymphocyte count (K/μL)	–0.101**	–0.093
Monocyte (%)	0.160**	–0.025
Neutrophil Count (K/μL)	–0.029	0.120*
RBC (M/μL)	0.058	–0.102*
WBC (K/μL)	–0.052	0.104*

*, P<0.05; **, P<0.01. HCT, hematocrit; volume; RDW, red blood cell distribution width; HGB, hemoglobin; RBC, red blood cell; WBC, white blood cells.

Table 9 Correlation of blood lipids to aging differed in genders

Blood lipids	Female	Male
Cholesterol/HDL (%)	–0.038	–0.249**
LDL cholesterol (mg/dL)	–0.095	–0.237**
LDL/HDL (%)	–0.052	–0.248**
Non-HDL cholesterol Calc (mg/dL)	–0.076	–0.242**
Total cholesterol (mg/dL)	–0.062	–0.169**
Triglyceride (mg/dL)	0.059	–0.134*

*, P<0.05; **, P<0.01. LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 10 Correlation of blood chemistry panel indices to aging differed in genders

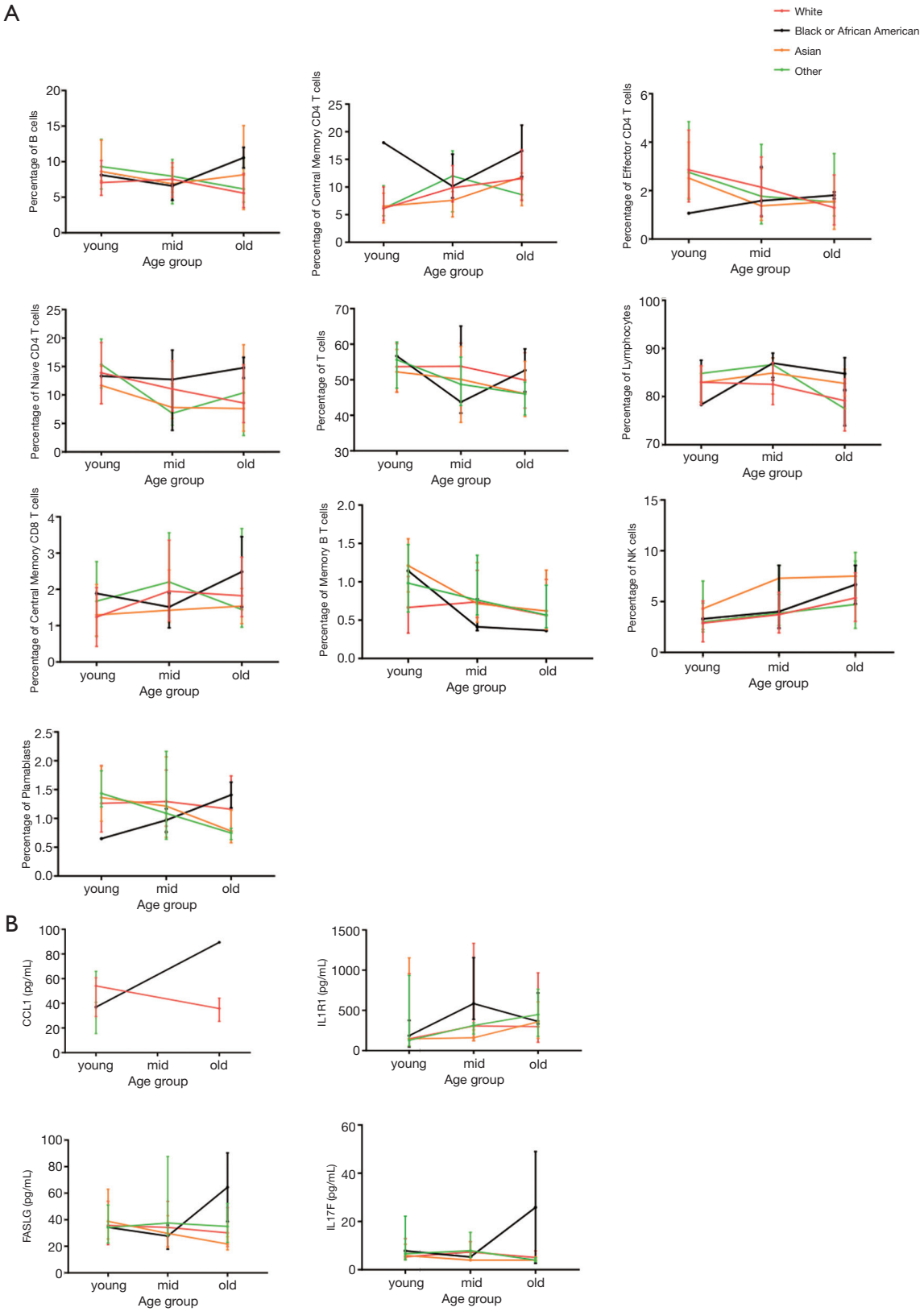
Blood chemistry panel indices	Female	Male
Calcium	0.099*	–0.038
Urea nitrogen	0.231**	0.105
ALT SGPT	–0.048	–0.0173**
Glucose	0.165**	0.098
Protein total	–0.108*	–0.088

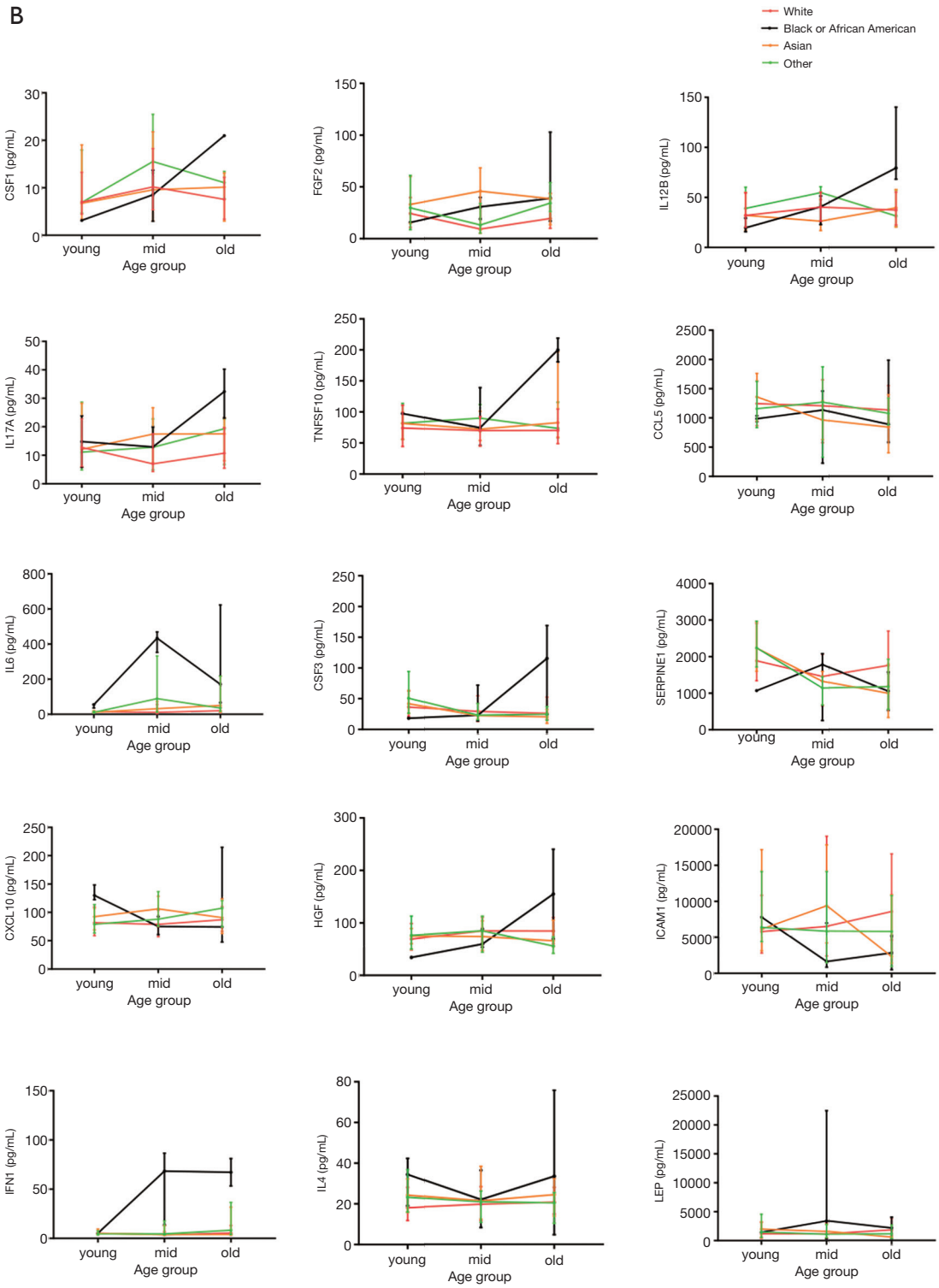
*, P<0.05; **, P<0.01. ALT, alanine transaminase; SGPT, serum glutamic-pyruvic transaminase.

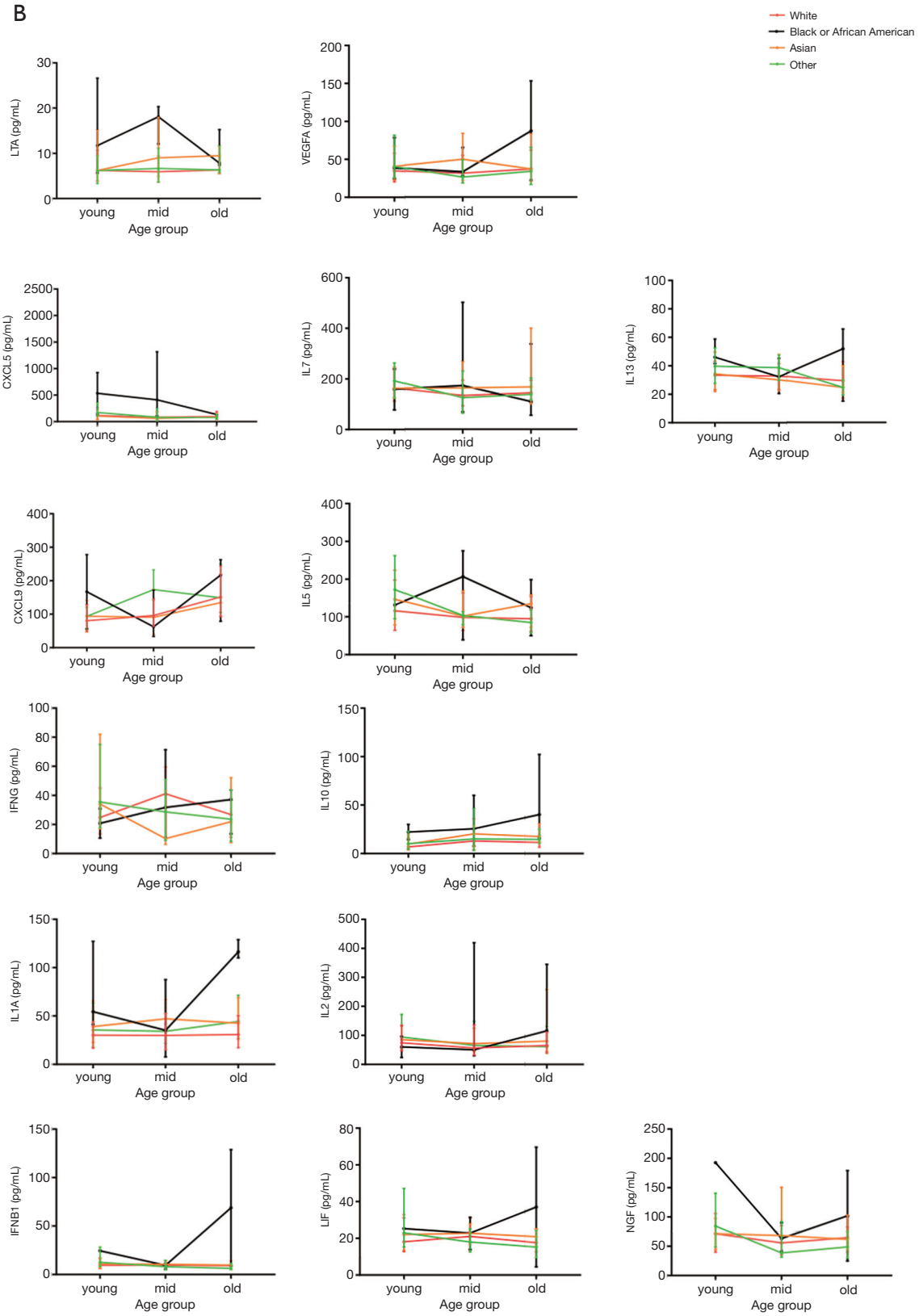
Table 11 Correlation of cell population to aging differed in races

Cell population	Asian	Black or African American	Other	White
B cells	–0.326**	0.137	–0.289*	–0.318**
Monocytes	0.127	0.000	0.263*	0.304**
CD8 T cells	–0.332**	–0.319	–0.173	–0.240**
Central Memory CD8 T cells	0.197	0.059	0.194	0.241**
Central Memory CD4 T cells	0.337**	0.396	0.392**	0.341**
Effector CD4 T cells	–0.370**	–0.214	–0.394**	–0.374**
Naive CD4 T cells	–0.231*	0.296	–0.331**	–0.293**
Naive CD8 T cells	–0.598**	–0.150	–0.545**	–0.582**
Tcells	–0.225*	0.141	–0.0261*	–0.187**
Transitional B cells	–0.346**	–0.405	–0.180	–0.158**
CD16 neg monocytes	0.167	0.000	0.276*	0.290**
CD16 pos monocytes	0.139	0.319	0.288*	0.290**
Effector Memory CD8 T cells	0.008	–0.018	0.189	0.175**
Effector Memory CD4 T cells	0.111	–0.287	0.204	0.174**
Lymphocytes	0.004	–0.005	–0.311**	–0.295**
Memory B cells	–0.399**	–0.770**	–0.175	–0.044
NK cells	0.326**	0.733*	0.141	0.325**
Plasmablasts	–0.158	0.542	–0.396**	–0.052
Naive B cells	–0.007	0.346	–0.052	–0.257**
NKT cells	0.128	–0.146	0.216	0.177**
Tregs	0.169	0.096	0.109	0.118*

*, P<0.05; **, P<0.01.







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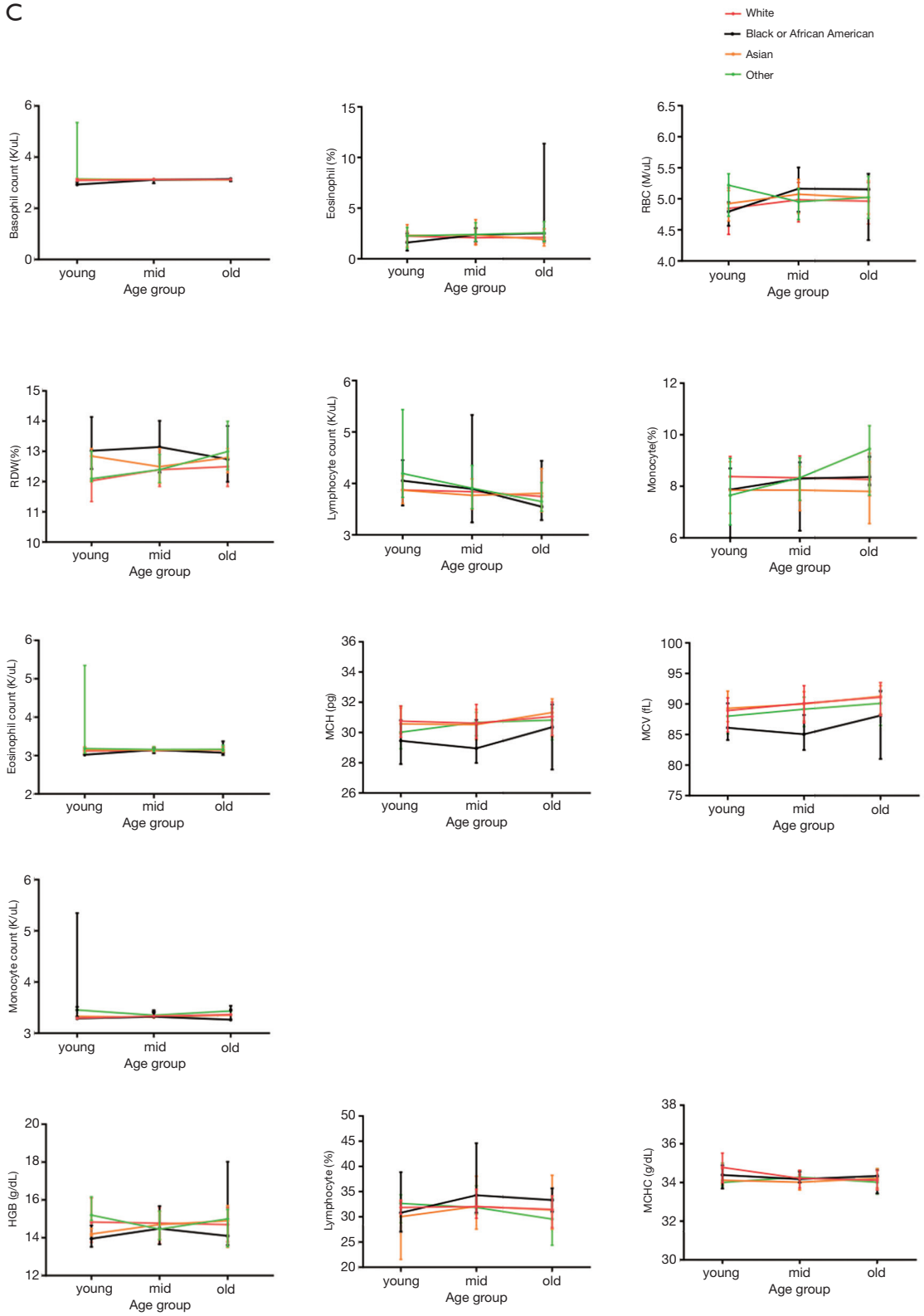

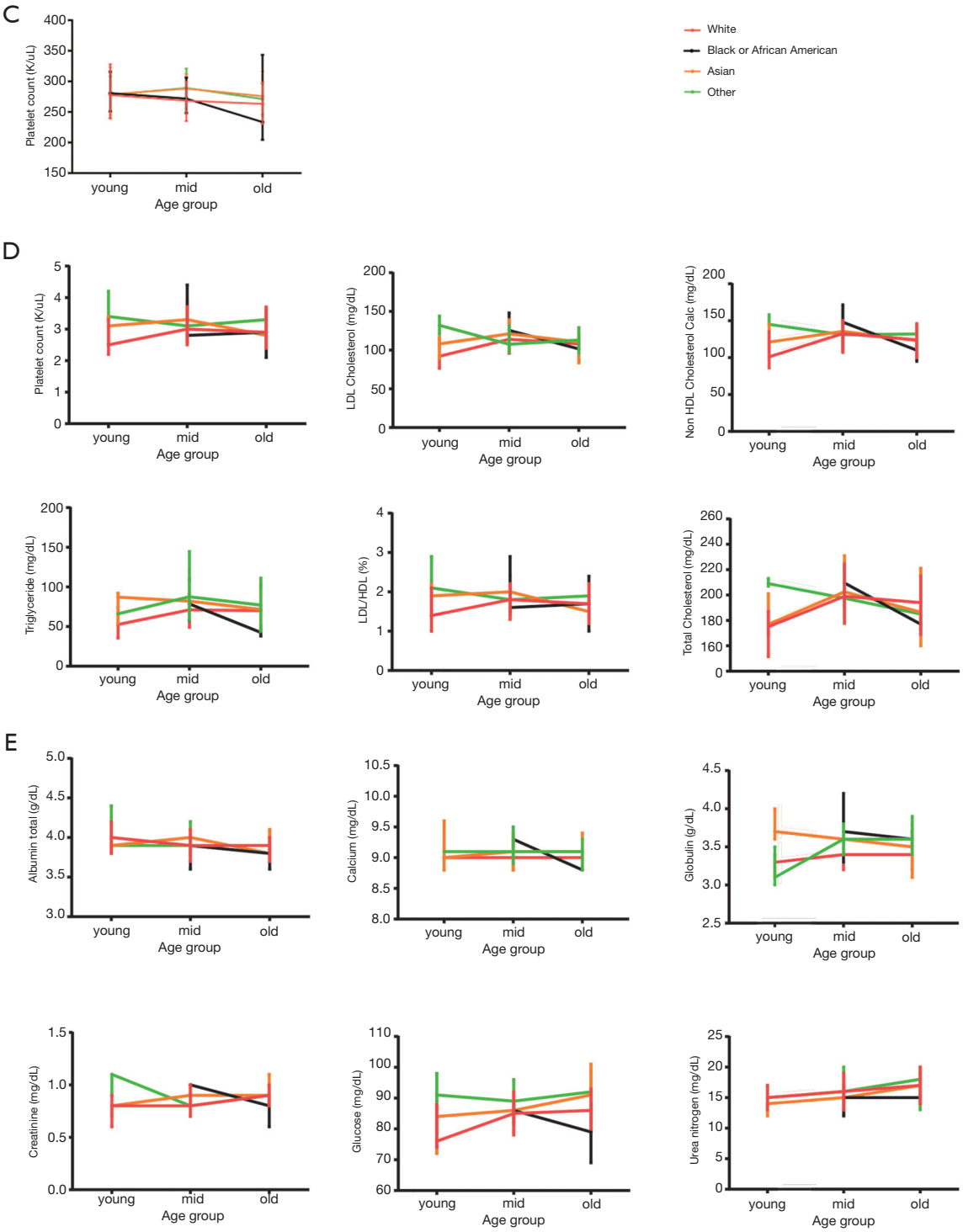

Figure 3 Correlation analyses between particular indices and age in different race groups. Quantitative changes of the same index, including immune cells (A), cytokines (B), blood cells (C), lipid products (D) and metabolites (E) were analyzed for relationship with age in different race groups. RBC, red blood cell; RDW, red blood cell distribution width; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; HGB, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 12 Correlation of cytokines to aging differed in races

Cytokines	Asian	Black or African American	Other	White
CCL5	–0.269**	–0.307	–0.013	–0.014
CSF3	–0.239*	0.155	–0.286**	–0.063
CXCL10	0.147	–0.245	0.151	0.155**
CXCL5	–0.066	–0.363	–0.205	–0.088*
CXCL9	0.154	0.449	0.437**	0.461**
FASLG	–0.300**	0.349	0.149	–0.115**
FGF2	0.033	0.583*	0.016	0.064
HGF	–0.068	0.165	–0.099	0.153**
ICAM1	–0.109	–0.319	–0.063	0.221**
IFN1	0.014	0.633	0.095	0.095*
IFNB1	0.019	0.080	–0.266*	–0.004
IFNG	–0.272**	0.617*	–0.181	–0.095*
IL10	0.344**	0.615*	0.174	0.144**
IL13	–0.095	–0.035	–0.208	–0.109**
IL17F	–0.266*	–0.437	–0.207	–0.159**
IL1R1	0.246*	0.210	0.026	0.105**
IL4	0.083	0.156	–0.087	0.145**
IL5	–0.133	0.248	–0.344**	–0.102**
IL6	0.420**	0.600	0.477**	0.023
IL7	0.071	0.202	–0.200	–0.105**
LEP	–0.147	–0.451	–0.006	0.199**
LIF	0.078	0.019	–0.251*	–0.069
LTA	0.100	–0.055	–0.052	0.175**
NGF	0.089	–0.080	–0.263*	–0.013

*, P<0.05; **, P<0.01.

degranulation (*Figure 4C*).

Protein-Protein Interaction Network analysis

The protein-protein interaction network of DEGs was constructed by using STRING database, and 61 proteins were shown associated (*Figure 5*). A protein interaction network of differential gene expression is shown in *Figure 5A*, and the number of core gene nodes selected from the interaction network is shown in *Figure 5B*, with the node number of 61 genes being ranked from small to large. Genes within the top 10% and enriched in the first five functions were selected, including CCL5, SERPINA1, MS4A1, ORM2, ETS1, CD27 and IL7R.

Discussion

Aging and its related immunodeficiency are associated with increased systemic inflammation, and are also main risk factors for many diseases, including neurodegenerative diseases, cardiovascular diseases and tumors. The biomarkers for the aging of immune system are crucial to evaluate host immune status and develop optimal immunotherapeutic regimens. In this study, we explored the correlation between

Table 13 Correlation of complete blood count indices to aging differed in races

Blood Count indices	Asian	Black or African American	Other	White
MCH	0.193	0.027	0.002	0.092**
MCHC	0.103	–0.097	–0.042	–0.0188**
Basophil count	0.016	0.663**	–0.203	0.100**
RDW percent	0.098	–0.133	0.275*	0.113**
Eosinophil count	–0.002	0.304	–0.035	0.109**
Eosinophil percent	–0.065	0.472**	0.093	0.016
Monocyte count	0.140	–0.077	–0.045	0.162**
Monocyte percent	0.047	0.314*	0.328**	0.028
RBC	–0.046	0.316*	–0.065	0.001
HGB	0.056	0.173	–0.172	–0.105**
Lymphocyte count	–0.024	–0.083	–0.288**	–0.083*
MCV	0.148	–0.028	0.076	0.213**
Platelet count	0.010	–0.119	–0.043	–0.145**

*, P<0.05; **, P<0.01. MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; RBC, red blood cell; HGB, hemoglobin; MCV, mean corpuscular volume.

Table 14 Correlation of blood lipids to aging differed in races

Blood Lipids	Asian	Black or African American	Other	White
Cholesterol/HDL (%)	–0.207*	–0.292	–0.068	–0.067
LDL/HDL (%)	–0.202*	–0.272	–0.091	–0.094*
Total Cholesterol (mg/dL)	–0.055	–0.146	–0.293*	–0.101*
Triglyceride (mg/dL)	–0.153	–0.736*	–0.079	0.071
LDL cholesterol (mg/dL)	–0.102	0.079	–0.193	–0.153**
Non-HDL cholesterol Calc (mg/dL)	–0.145	–0.158	–0.235	–0.116**

*, P<0.05; **, P<0.01. LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 15 Correlation of Blood Chemistry Panel indices to aging differed in races

Blood chemistry penal indices	Asian	Black or African American	Other	White
Globulin	–0.216*	–0.076	0.281	0.029
Glucose	0.264**	–0.018	0.077	0.167**
Urea nitrogen	0.211*	–0.142	0.060	0.169**
Creatinine	0.225*	–0.222	–0.057	0.192**
Albumin total	–0.028	0.327	–0.178	–0.130**
Calcium	0.054	–0.126	–0.107	0.097*

*, P<0.05; **, P<0.01.

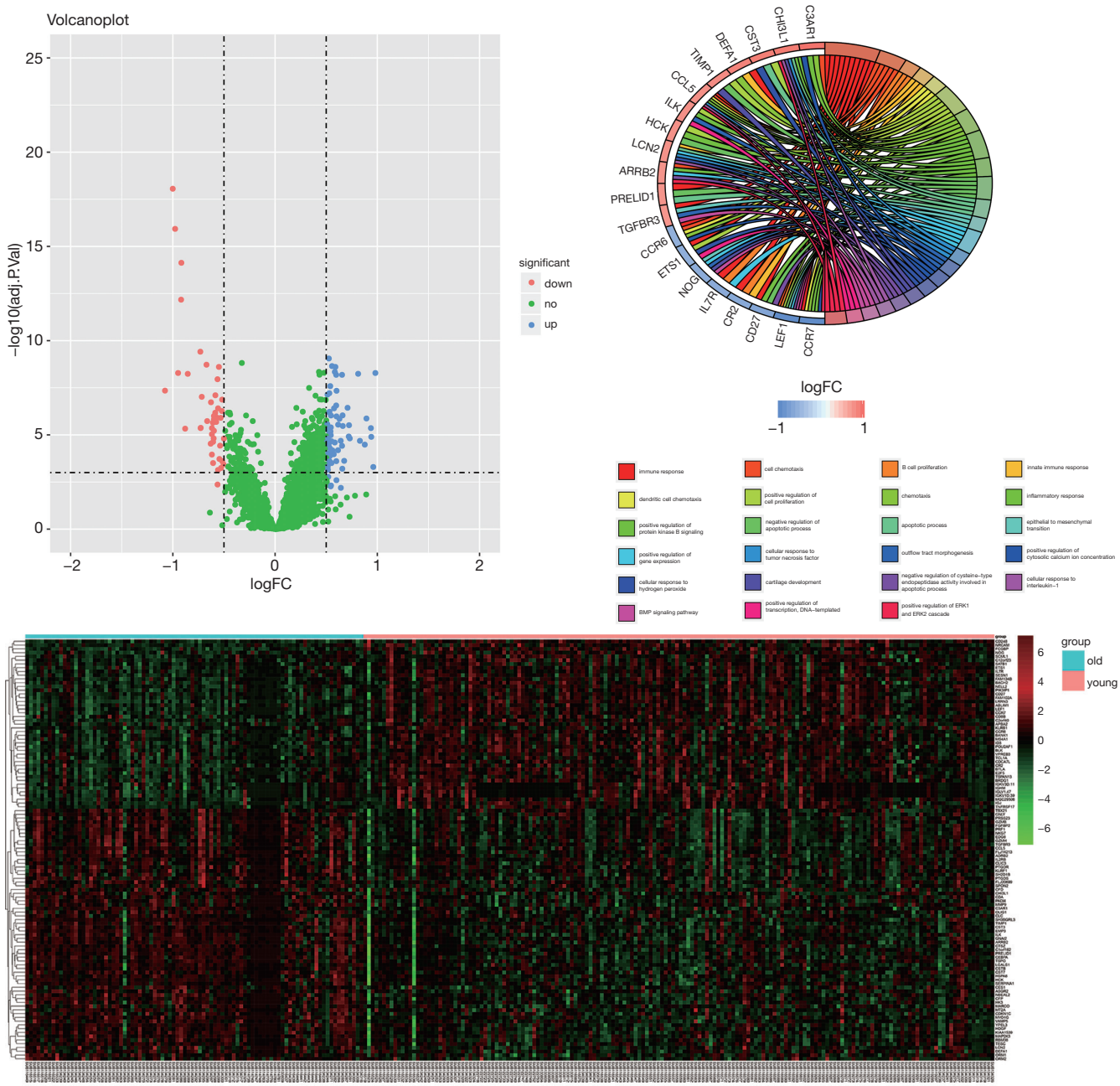

Figure 4 Analyses of gene expression related to age. Volcanic map of DEGs (A). Cluster heat map of DEGs expression in different age groups (B). GO functional enrichment analysis of DEGs (C). DEG, differentially expressed genes; GO, Gene Ontology.

aging and various indices in cell population profiling, Complete Blood Count and blood chemistry panel, in an attempt to identify features which were associated with the aging of immune system and could be used as potential biomarkers.

Our analysis indicated that NK cell number had a positive correlation with aging, which is consistent with a previous study (10). Notably, increase in NK cell number plays a role in the vicious circle of chronic inflammation, which promotes the geriatric disease atherosclerosis (11), and associated with premature biological aging (12). Meanwhile, monocytes increase along aging. Monocytes

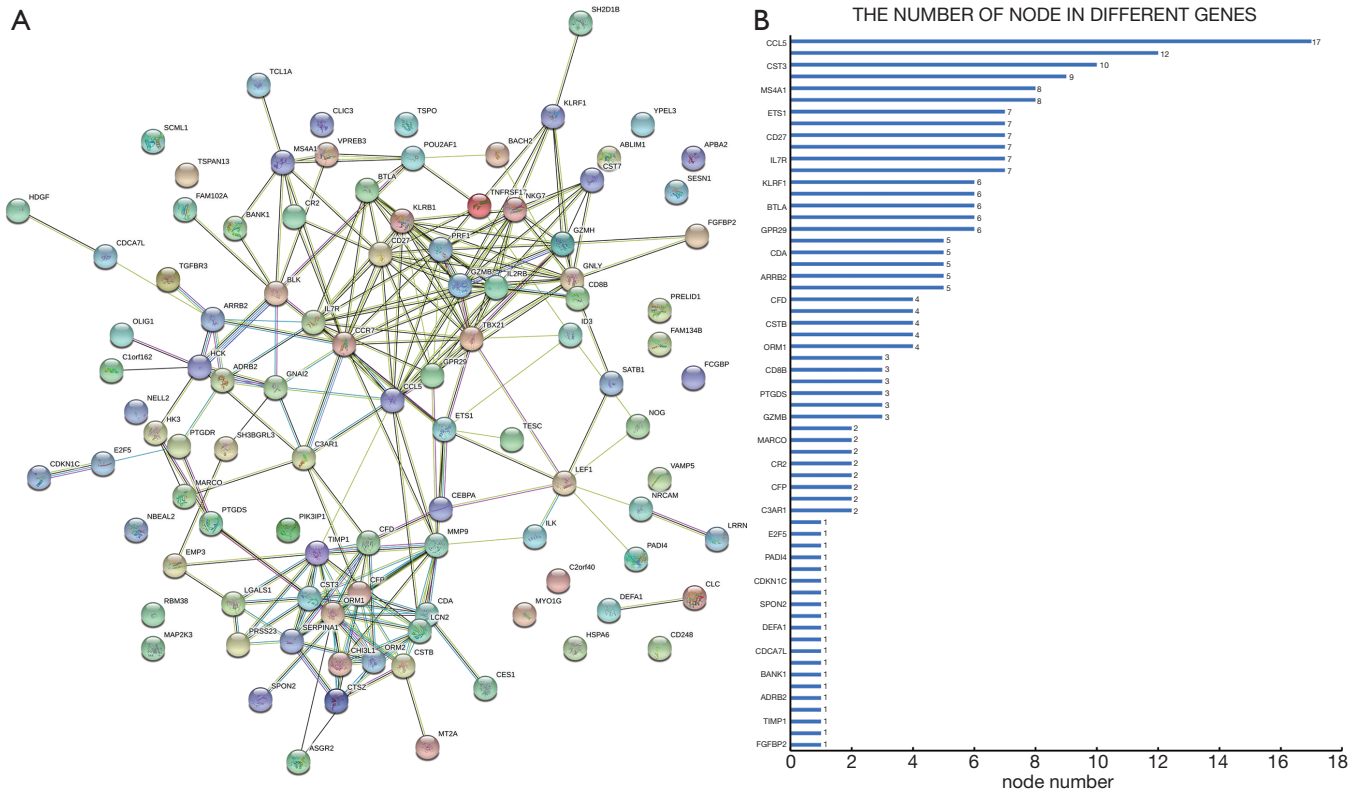

Figure 5 Protein-protein interaction networks. The protein-protein interaction network of DEGs (A). Number of core gene nodes (B). DEG, differentially expressed genes.

are a central driving factor for vascular inflammation in atherosclerosis, involving in the development of the disease, from the earliest asymptomatic atherosclerotic lesions to the final rupture of plaque, resulting in potentially fatal consequences (13). It's also showed that the number of B lymphocytes, including B cells, Naive B cells and transitional B cells, had a negative correlation with aging. B lymphocytes play a pivotal role in humoral immunity and, as powerful APC, are crucial for the development of memory T cells. It's reported that the B cell versatility showed dramatic decrease in some very aged individuals, which was a strong indicator for poor physical condition in the elderly (9). Transitional B cells play an important role in the negative selection of lymphocyte development and the progression of autoimmune diseases (14), thus its reduction could affect the humoral immunity in the aged. In addition, Memory T cells showed a positive correlation, while Naive T cells showed a negative correlation to aging (15). Our study demonstrated that immune cell populations were greatly affected with aging, and Transitional B cells could be used as a biomarker for aging.

By analyzing the changes of blood cytokines in different age groups, we found several cytokines related to aging, including CXCL9, CCL22, IL21, IL23A and IL31. In this study, CXCL9 was the only one showing a positive correlation to aging, while others showed negative correlations. CXCL9 affects the growth, movement, or activation state of the cells, and participates in immune and inflammatory responses. It's chemotactic for activated T cells through binding to CXCR3. It's reported that increased CXCL9 was associated with falls, which might help to correctly understand the mechanism of falls in the elderly (16). CCL22 may play a role in the trafficking of activated/effector T cells to inflammatory sites and other physiological aspects of activated T cells. It's a mild chemoattractant for primary activated T cells and a potent chemoattractant for chronically activated T cells through binding to CCR4 receptor. However, it has no chemoattractant activity for neutrophils, eosinophils, or resting T cells. IL21 is a cytokine with immunoregulatory activity, promoting the transition between innate and adaptive immunity. It has been proved that IL21 could

enhance the thymus production in aged mice (17). IL23A associates with IL12B to form the IL-23 interleukin, a heterodimeric cytokine which functions in innate and adaptive immunity. IL31 associates with OSMR to form the interleukin-31 receptor, which activates STAT3 and STAT1/STAT5 to a lower extent. The relationship between IL23A/IL31 and aging has not been reported in the literature, and our results suggest that they are worthy of further study, and might be used as new biomarkers for aging.

The results of functional gene enrichment and protein interaction analysis showed that differential genes were enriched in pathways related to B cell proliferation and other immuno-signal transduction, indicating that these genes might be highly interacted and play a core role in the process of biological aging. The expression levels of CCL5, SERPINA1 and ORM2 were increased in the aged groups. CCL5 is the chemotaxis of memory T helper cells and eosinophils, and can cause basophils to release histamine and activate eosinophils. SERPINA1 may be associated with Parkinson's disease (18). ORM2 acts as a transport protein in the blood and seems to play a role in regulating the activity of the immune system during the acute response. Meanwhile, the expression levels of MS4A1, ETS1, CD27 and IL7R genes decreased with aging. MS4A1 is a B-lymphocyte-specific membrane protein that plays a role in the regulation of cellular calcium influx necessary for the development, differentiation, and activation of B-lymphocytes. ETS1 can promote apoptosis of endothelial cells (19) and plays a role in maintaining the proliferation of cardiomyocytes. It has been found that the expression of ETS1 is significantly decreased in aging cardiomyocytes (20). CD27 stimulates T cell proliferation and is an initial cell marker (21). It's also down-regulated in the elderly. IL7R has been reported to be lower in the elderly, and some believe that the decreased IL7R may be related to longevity, and the higher expression of IL7R gene is associated with immune-related diseases (22). It has also been reported that IL7R plays a role in age-related neurodegenerative diseases (23). Although its role in aging has not yet been clearly defined, there is no denying that the expression of IL7R does change with aging. Taken together, SERPINA1, ORM2, MS4A1, ETS1, CD27 and IL7R might be used as potential biomarkers of aging.

Analysis on indices in Complete Blood Count test showed that Basophil, MCH and MCV were positively correlated with aging, while MCHC and Platelet were negatively correlated with aging. Meanwhile, blood UREA NITROGEN, CREATININE and GLUCOSE were positively correlated with aging, while ALBUMIN and PROTEIN were negatively correlated with aging. Furthermore, blood cholesterol/HDL ratio, LDL cholesterol, LDL/HDL ratio, non-HDL cholesterol Calc, total cholesterol and Triglyceride were negatively correlated with aging. These are useful indicators for many diseases, however their relationship to age-related diseases is still not clear. Further work will be needed to investigate their potential as biomarkers for aging.

It's interesting that some correlations seemed exclusive to certain genders or races. For example, the significant association between blood lipids and aging was observed only in males. It is well known that abnormalities in blood lipids are risk factors for some diseases. Does this indicate the prevalence of certain diseases in the male population, and is it associated with aging? In addition, we also found that CCL5, Cholesterol/HDL (%) and globulin were significantly associated with aging only in Asian population, and there was no difference among other races group. All these indicate that the changes in the process of aging are affected by multiple factors and more work is needed to elucidate the underlying mechanisms.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://aob.amegroups.com/article/view/10.21037/aob-20-79/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://aob.amegroups.com/article/view/10.21037/aob-20-79/coif). The authors have no conflict of interest to declare.

Ethical Statement: The authors are accountable for all

aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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