Am J Transl Res 2025;17(10):7927-7941
www.ajtr.org /ISSN:1943-8141/AJTR0165912

Original Article

Effects of aging on immune function and inflammatory biomarkers

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Received May 9, 2025; Accepted October 13, 2025; Epub October 15, 2025; Published October 30, 2025

Abstract: Objective: To explore the clinical significance of multimodal plasma biomarkers and the alterations in their interrelationships within the immune microenvironment during aging. Methods: A total of 83 elderly participants were included, all of whom were free from serious illnesses, fever, mental disorders, and severe hearing, speech, or comprehension impairments in the past two weeks. Based on the definition of "elder" by the World Health Organization (WHO) and the United Nations (UN), participants in this study were divided into three groups: the "Light-Old" (LO group, under 70 years), the "Moderate-Old" (MO group, 70-79 years), and the "Heavy-Old" (HO group, 80 years and above). This stratification aimed to explore the effect of different aging degrees. The samples were analyzed for coagulation markers, nerve damage markers, and metabolic markers. Basic demographic data, including height, weight, age, and gender, were also recorded. Results: Statistical analysis revealed significant differences among the 3 age groups in phosphorylated tau (P-Tau181, F=5.214, P=0.007) and white blood cells (WBC, F=3.278, P=0.044). Furthermore, interleukin-6, thrombin-antithrombin complex, thrombomodulin (TM), plasminogen-plasmin α1 complex (PIC), WBC, blood glucose (GLU), and P-Tau181 all showed an increasing trend with age. Gender-based analysis revealed significant differences in high-density lipoprotein (t=5.738, P<0.001), total cholesterol (t=2.530, P=0.013), and GLU (t=2.840, P=0.006). Spearman correlation analysis indicated a strong positive correlation between PIC and TM (correlation coefficient =0.65). Conclusion: Aging significantly influences the clinical relevance of biomarkers, particularly for coagulation, inflammatory, and immune mechanisms. The reference ranges for various biomarkers in the elderly should be further refined to reflect their unique physiologic conditions.

Keywords: Mild cognitive impairment, elderly population, aging, physiologic changes, blood biomarkers, combined detection

Introduction

With the accelerating aging of the global population, the health management of the elderly has become a critical public health issue to be addressed [1]. However, current health assessment systems fail to fully consider the effects of multi-system physiologic decline during aging, particularly in the field of blood biomarker detection. The potential interference of aging on biomarker levels is often overlooked, leading to systematic biases in evaluation results [2, 3]. Blood biomarkers, as convenient, safe, and sensitive diagnostic tools, are widely used for clinical diagnosis and health monitoring, playing a significant role in the health management of the elderly [4, 5]. Through the combined analysis of multidimensional biomarkers, a comprehensive assessment of an individual's physiologic and pathologic state can be achieved, providing more precise evidence for health management [6]. Nevertheless, current blood biomarker detection systems often fail to consider the technical interference of aging in the

https://doi.org/10.62347/JZXF4972

detection process, and lack specific evaluation standards tailored to the elderly population. Notably, the physiologic state of the elderly differs significantly from that of younger individuals. Aging is associated with pathologic and physiologic changes, such as immune system decline, metabolic disorders, vascular endothelial damage, and chronic low-grade inflammation, all of which can directly affect blood biomarker expression and detection accuracy, hindering clinical interpretation [7].

In the immune microenvironment, endothelial tissues, coagulation functions, inflammatory mechanisms, and metabolic functions interact through highly complex molecular and cellular signaling networks, forming a dynamically balanced system. Following endothelial injury, the exposure of vascular endothelium activates the extrinsic coagulation pathway, upregulating tissue factor (TF) expression and initiating the coagulation cascade [8]. This process generates thrombin, which catalyzes the conversion of fibrinogen into fibrin, ultimately forming a thrombus to prevent bleeding. Simultaneously, thrombin activates endothelial and immune cells through protease-activated receptor (PAR) signaling pathways, promoting the release of inflammatory mediators and recruiting neutrophils and monocytes to the injury site, initiating an inflammatory response to clear pathogens and necrotic tissue. Moreover, inflammatory mediators enhance the glycolytic capacity of immune cells by metabolic reprogramming, providing energy and biosynthetic precursors for the inflammatory response and tissue repair. Metabolic intermediates (e.g., succinate) amplify the inflammatory response by activating the inflammasome (NLRP3), while lactate regulates gene expression by inhibiting histone deacetylases (HDACs), influencing immune cell function and tissue repair processes.

Aging is a universal biological phenomenon that inevitably affects the human immune system. The immune system, a key defense against pathogens, interacts with the nervous, circulatory, and other systems [9-11]. Aging leads to immunosenescence, a decline in immune system function, which affects the composition, number, and function of immune organs, cells, and cytokines [12]. In the context of aging, this complex immune network becomes significantly disrupted [13]. Immunosenescence is marked by the decline of both innate and adaptive immune functions and a chronic low-grade inflammatory state, known as “inflammaging”, characterized by persistent release of pro-inflammatory cytokines (e.g., IL-6, TNF-α) and weakened anti-inflammatory mechanisms. This chronic inflammation exacerbates tissue damage and repair dysfunction, promoting fibrosis and abnormal tissue remodeling [14]. Aging-related metabolic disorders (e.g., insulin resistance, mitochondrial dysfunction, and decreased NAD+ levels) lead to energy metabolism imbalances, further impairing immune and tissue repair cell functions [15, 16]. The coagulation system also undergoes significant changes with aging, characterized by increased coagulation factor activity, weakened anticoagulant mechanisms, and reduced fibrinolytic function, resulting in a hypercoagulable state and an increased risk of thrombosis [17]. These age-related pathophysiologic changes contribute to a decline in tissue repair capacity, increased susceptibility to chronic diseases (e.g., atherosclerosis, diabetes, neurodegenerative diseases), and accelerated functional decline and pathologic progression [18].

While immune senescence was historically considered detrimental, recent studies have revised this view, emphasizing that immunosenescence is a multifactorial, dynamic, and complex phenomenon regulated throughout the human lifespan [19, 20].

Therefore, the detection of plasma biomarkers not only reflects changes in the immune microenvironment but also reveals how aging alters the clinical significance and systemic correlations of these biomarkers [21, 22]. For instance, the expression levels and interrelationships of tissue injury biomarkers (e.g., thrombomodulin, tPAIC, Aβ, P-Tau181), coagulation biomarkers, metabolic biomarkers, and inflammatory biomarkers are significantly altered in aging individuals. These changes not only provide potential targets for early diagnosis and intervention of aging-related diseases but also offer important evidence for optimizing the health assessment system for the elderly. Future research should further investigate the molecular mechanisms of multi-system interactions in aging to enhance the application of precision medicine in elderly health management.

Materials and methods

Patient selection

This study employed a cross-sectional retrospective design with 600 elderly individuals who underwent health check-ups at the Affiliated Hospital of Hubei Provincial Government between September and November 2023.

The inclusion criteria were as follows: (1) age > 60 years; (2) no severe infections or inflammatory conditions in the past three months; (3) absence of severe underlying diseases or well-controlled chronic conditions; (4) no mental disorders; no severe hearing, speech, or comprehension impairments; (5) complete clinical files. The exclusion criteria included: (1) use of immunosuppressants or anticoagulants within the past week; (2) presence of severe metabolic or endocrine disorders; (3) major surgery or trauma within the past three months; (4) language, severe visual, or hearing impairments hindering cooperation with researchers; evident mental or emotional abnormalities; (5) incomplete clinical files.

The World Health Organization (WHO) and the United Nations (UN) define the elderly as individuals aged 60 years and above. However, significant differences in physiologic, psychological, and social functions exist among individuals within this broad age group. Therefore, in geriatric medical research, further subdivision of the elderly population is a common practice. Typically, individuals aged 60-70 years are referred to as "young-old", who generally maintain relatively intact physical function and have lower incidences of chronic diseases and cognitive impairment. Those aged 70-80 years are categorized as "middle-old", characterized by a significantly increased risk of chronic diseases and cognitive decline. Individuals aged 80 years and above are classified as "oldest-old", who often experience marked physical deterioration and a substantially higher prevalence of cognitive impairment and multiple chronic conditions. This classification is consistent with the elderly definition standards of WHO and UN while capturing the significant differences in health status and functional capacity across these age subgroups, thereby enabling more precise data analysis and interpretation in research. Based on these criteria, 83 participants were selected. Using this rationale, participants in this study were divided into three groups: the "Light-Old" (LO group, under 70 years), the "Moderate-Old" (MO group, 70-79 years), and the "Heavy-Old" (HO group, 80 years and above). This stratification aimed to explore the impact of different aging degrees. Blood samples were collected following ethical approval, and the study protocol was approved by the Ethics Committee of The Affiliated Hospital of Hubei Provincial Government.

Data extraction

Participant general information, including age, gender, weight, height, and body mass index (BMI), were collected from the electronic medical records. Cognitive function was assessed using a Neuropsychological Test Battery combined with years of education, providing baseline data for the study. Laboratory indices, including thrombin-antithrombin complex (TAT), plasminogen-plasmin α1 complex (PIC, μg/mL), thrombomodulin (TM), tissue-type plasminogen activator-plasminogen inhibitor-1 complex (tPAIC), C-reactive protein (CRP), and interleukin-6 (IL-6), were measured using magnetic microparticle chemiluminescence assays. Beta-amyloid 42 (Aβ42) and phosphorylated tau protein 181 (P-Tau181) were detected using single-molecule fluorescence array technology (Roche Diagnostics, Mannheim). White blood cell count (WBC) was measured by flow cytometry. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), blood uric acid (UA), and blood glucose (GLU) were analyzed using colorimetric and turbidimetric methods on an automatic biochemical analyzer. All assays were performed on the LX20 autoanalyzer (Beckman-Coulter, Woerden, The Netherlands).

Sample collection and examination

Participants visited the hospital for health check-ups between 7:00 and 9:00 am. Basic information was recorded, and BMI was calculated. Blood samples were collected by antecubital vein puncture after an overnight fast. A total of 4 mL of blood was collected: 2 mL in a sodium citrate anticoagulation tube and 2 mL in an EDTA-K2 anticoagulation tube. Except for Aβ42 and P-Tau181, all other samples were

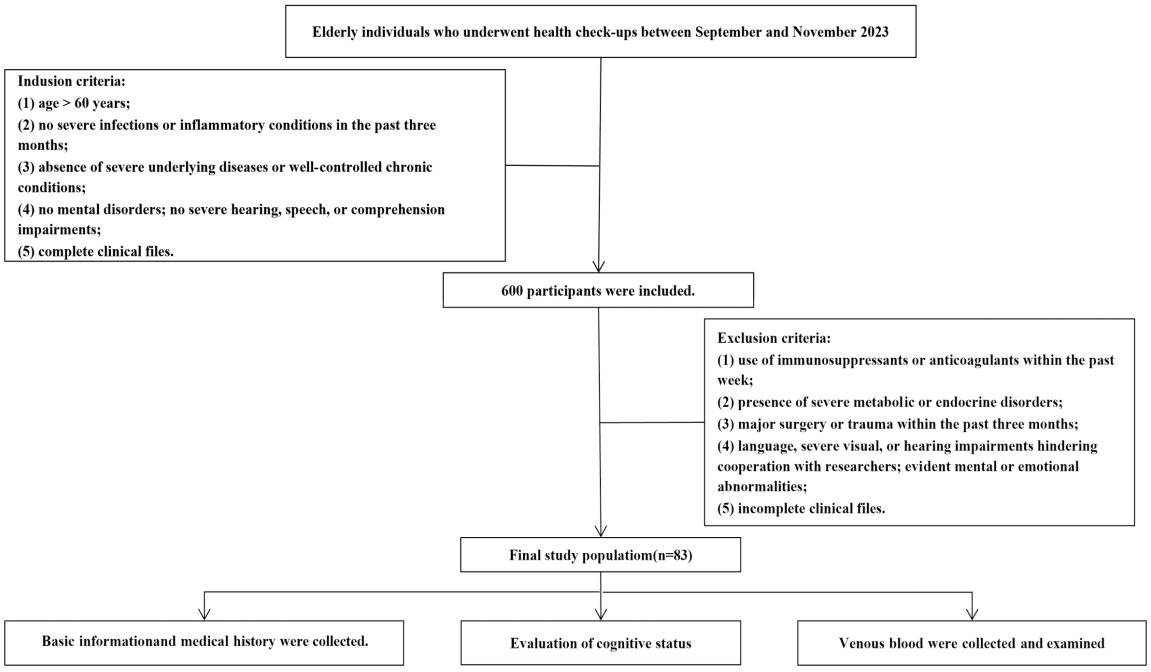

Figure 1. Sample selection and experimental process.

immediately sent to the laboratory for testing and stored at -21°C after separation. After approximately 7 months, EDTA-K2 plasma samples were retrieved, and Aβ42 and P-Tau181 levels were measured using a single-molecule fluorescence array instrument. The levels of serum IgG, IgA, and IgM were determined by immunoturbidimetry, using kits purchased from Sigma-Aldrich (St. Louis, MO, USA), as shown in **Figure 1**.

Statistical analysis

Data analysis was conducted using SPSS statistical software (Version 27.0). Normality of the data was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using the Levene test. For data that were normally distributed with homogeneous variance, independent samples t-tests were used for comparisons between two groups, and one-way analysis of variance (ANOVA) was used for comparisons among more than two groups, followed by Tukey test. For normally distributed data with unequal variance, Welch's t-test or Welch's ANOVA was applied. Non-normally distributed data with homogeneous variance were analyzed using the Mann-Whitney U test for two-group comparisons, and the Kruskal-Wallis H test for more than two groups. All statistical tests were two-tailed, with a significance level set at $P < 0.05$.

Correlation analysis was performed using Spearman's rank correlation coefficient, with values ranging from -1 to 1: a value of 0 indicated no correlation, values closer to 1 indicated a stronger positive correlation, and values closer to -1 indicated a stronger negative correlation. Multiple logistic regression was used to calculate the odds ratio for aging indicators. Receiver operating characteristic (ROC) curves were used to evaluate the prediction value of these indicators. Descriptive statistics for continuous variables were expressed as mean ± standard deviation (Mean ± SD), while categorical variables were presented as frequencies and percentages. The sample was stratified based on cognitive impairment status (MCI and HC) for basic characteristic analysis.

Results

Comparison of basic information

Significant differences in age were observed among the groups ($P < 0.001$). However, no significant differences were found in BMI ($P=0.735$) or gender distribution ($P=0.101$), as shown in **Table 1**.

Table 1. Comparison of baseline characteristics

	Total	Light-old (n=20)	Moderate-old (n=50)	Heavy-old (n=13)	F	P
Age (years old)	73.80±6.09	66.75±2.36	73.86±2.74	84.38±3.07	167.0	<0.001
Gender (Male/Female)	31/52	5/15	18/32	8/5	4.593	0.101
BMI (kg/m^2)	24.59±3.98	24.56±4.28	24.80±4.12	23.82±2.97	0.309	0.735
Hypertension	31	8	18	5	0.106	0.948
Hyperlipidemia	30	7	17	6	0.733	0.693
Diabetes	22	5	13	4	0.151	0.927

Notes: BMI, body mass index.

Table 2. Comparison of different aging levels

	Light-old (n=20)	Moderate-old (n=50)	Heavy-old (n=13)	F	P
WBC (10^9/L)	5.07±0.87	5.48±1.10	6.17±0.88*,#	3.278	0.041
HDL (mmol/L)	1.50±0.37	1.40±0.38	1.33±0.26	1.070	0.348
TC (mmol/L)	5.46±0.91	4.89±1.22	5.20±0.86	2.048	0.136
TAT (ng/mL)	12.68±7.53	13.77±8.70	16.94±7.96	1.082	0.344
TM (U/mL)	13.82±12.94	17.13±14.79	20.73±15.50	0.912	0.406
tPAIC (ng/mL)	4.01±1.52	5.30±8.66	4.57±1.93	0.269	0.765
PIC (μg/mL)	1.50±0.65	1.72±0.95	2.24±1.83	1.901	0.156
TG (mmol/L)	1.52±0.80	1.60±0.82	1.58±0.19	0.080	0.923
LDL (mmol/L)	2.87±0.68	2.51±0.85	2.89±0.62	2.153	0.123
IL-6 (pg/mL)	24.13±34.27	37.22±74.31	44.21±79.08	0.404	0.669
CRP (mg/L)	1.81±3.58	1.24±1.83	1.21±0.92	0.480	0.620
Aβ42 (ng/L)	4.81±2.02	5.25±3.52	5.02±3.02	0.146	0.865
P-Tau181 (pg/mL)	0.71±0.36	0.78±0.49	1.22±0.59*,#	5.214	0.007

Notes: *P<0.05 vs light-old group; #P<0.05 vs moderate-old group. WBC, White blood cell count; HDL, high-density lipoprotein; TC, Total cholesterol; TAT, thrombin-antithrombin complex; TM, thrombomodulin; tPAIC, tissue-type plasminogen activator-plasminogen inhibitor-1 complex; PIC, plasminogen-plasmin α1 complex; TG, triglycerides; LDL, low-density lipoprotein; IL-6, interleukin-6; CRP, C-reactive protein; Aβ42, Beta-amyloid 42; P-Tau181, phosphorylated tau protein 181.

Comparison of different aging levels

Kruskal-Wallis test results showed significant differences in P-Tau181 (P=0.007), and WBC count (P=0.044) am-ong the three groups (**Table 2**). Further analysis using the Mann-Whitney U test with Benjamini-Hochberg correction revealed that P-Tau181 levels in the HO group were significantly higher than those in both the LO group (P=0.004) and MO group (P=0.007). Similarly, WBC counts in the HO group were significantly higher than those in the LO group (P=0.009). Box plots and group mean trend lines further demonstrated that P-Tau181 levels and WBC counts showed an increasing trend with advancing age across the LO, MO, and HO groups. Although no statistically significant differences were observed in IL-6, PIC, TAT, or TM among the three groups, these indicators also exhibited a gradual upward trend with increasing age (**Figure 2**).

Comparison of different genders

Participants were further stratified by gender to investigate the effect of gender on the diagnostic significance of biomarkers in the context of aging.

Statistical analysis showed significant differences in HDL (P < 0.001) and TC (P=0.013) between the two groups, with females having markedly higher HDL and TC levels than males (both P<0.05, **Table 3**).

Comparison of levels of blood uric acid (UA) and glucose (GLU)

As shown in **Tables 4** and **5**, the levels of UA and GLU were found to increase progressively with age. However, there was no significant difference in GLU levels among aging groups (P=0.077). In terms of gender differences, no

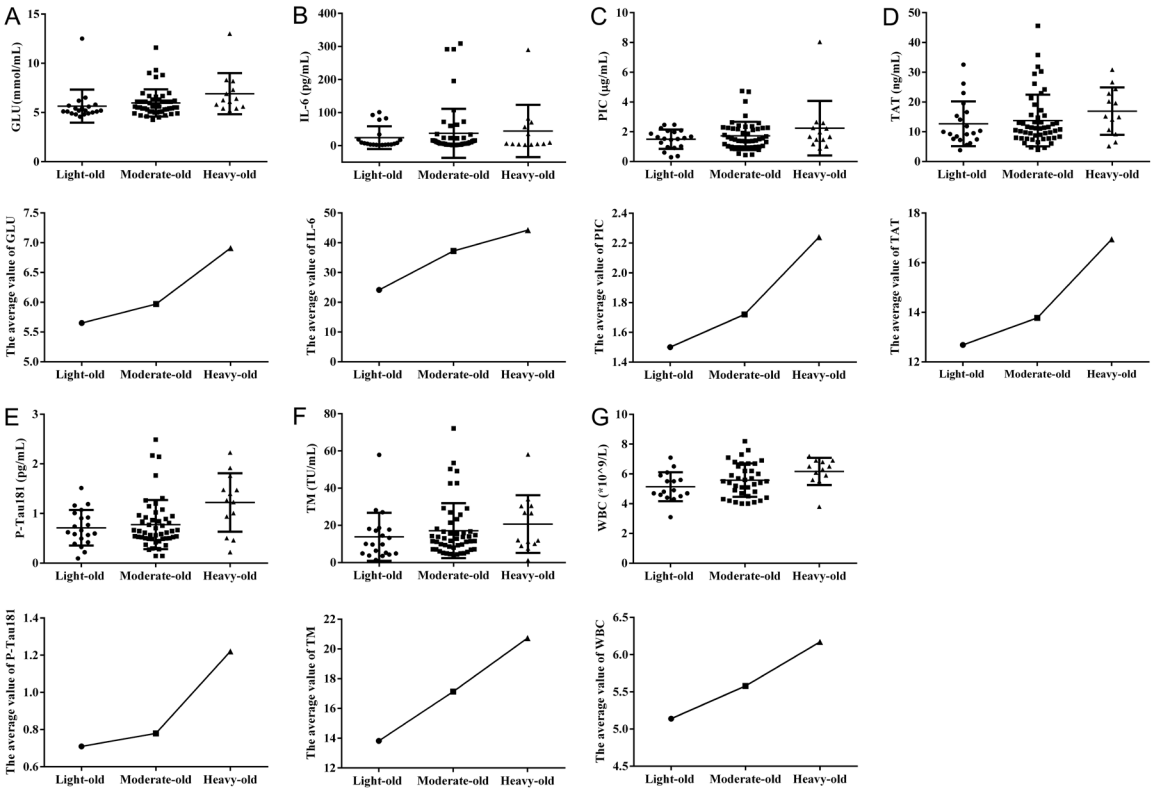

Figure 2. Trends of biomarkers in across aging groups. A: Box plot and average line graph of blood glucose (GLU); B: Box plot and average line graph of interleukin 6 (IL-6); C: Box plot and average line graph of plasminogen-plasmin α1 complex (PIC); D: Box plot and average line graph of thrombin-antithrombin complex (TAT); E: Box plot and average line graph of phosphorylated tau protein 181 (P-Tau181); F: Box plot and average line graph of thrombomodulin (TM); G: Box plot and average line graph of white blood cell (WBC).

Table 3. Comparison of both genders

	Male (n=31)	Female (n=52)	t	P
Age	75.45±6.30	72.81±5.79	1.947	0.055
BMI	23.07±3.08	25.45±6.05	0.333	0.740
WBC (10^9/L)	5.54±1.08	5.60±1.11	0.199	0.843
HDL (mmol/L)	1.16±0.29	1.56±0.32	5.738	<0.001
TC (mmol/L)	4.69±1.02	5.31±1.11	2.530	0.013
TAT (ng/mL)	14.62±9.25	13.63±7.81	0.520	0.604
TM (U/mL)	20.21±17.00	14.92±12.49	1.628	0.108
tPAIC (ng/mL)	4.41±2.77	5.15±8.34	0.481	0.632
PIC (μg/mL)	1.95±1.47	1.63±0.76	1.292	0.200
TG (mmol/L)	1.73±0.85	1.49±0.67	1.402	0.165
LDL (mmol/L)	2.50±0.71	2.75±0.83	1.382	0.171
IL-6 (pg/mL)	51.83±84.90	25.22±52.70	1.764	0.081
CRP (mg/L)	1.34±2.10	1.39±2.39	0.969	0.923
Aβ42 (ng/L)	5.06±2.23	5.14±3.56	0.118	0.906
P-Tau181 (pg/mL)	0.97±0.44	0.75±0.53	1.932	0.057

Notes: BMI, body mass index; WBC, White blood cell count; HDL, high-density lipoprotein; TC, Total cholesterol; TAT, thrombin-antithrombin complex; TM, thrombomodulin; tPAIC, tissue-type plasminogen activator-plasminogen inhibitor-1 complex; PIC, plasminogen-plasmin α1 complex; TG, triglycerides; LDL, low-density lipoprotein; IL-6, interleukin-6; CRP, C-reactive protein; Aβ42, Beta-amyloid 42; P-Tau181, phosphorylated tau protein 181.

Table 4. Comparison of blood uric acid and blood glucose across aging groups

	Light-old (n=20)	Moderate-old (n=50)	Heavy-old (n=13)	F	P
UA (μg/L)	313.26±33.30	351.00±36.04*	381.55±40.93*,#	0.286	<0.001
GLU (mmol/L)	5.65±1.68	5.97±1.38	6.91±2.09	2.652	0.077

Notes: *P<0.05 vs light-old group; #P<0.05 vs moderate-old group. UA, uric acid; GLU, blood glucose.

Table 5. Comparison of blood uric acid and blood glucose between Male and Female group

	Male (n=31)	Female (n=52)	t	P
UA (μg/mL)	351.07±40.81	344.08±42.69	0.733	0.465
GLU (mmol/L)	6.66±2.22	5.67±0.95	2.840	0.006

Notes: UA, uric acid; GLU, blood glucose.

Table 6. Comparison of immunoglobulin levels across Aging Groups

	Light-old (n=20)	Moderate-old (n=50)	Heavy-old (n=13)	F	P
IgG (g/L)	12.13±1.15	11.37±1.34*	10.26±1.00*	0.181	<0.001
IgA (g/L)	2.73±0.53	2.32±0.36*	2.14±0.47*	0.190	<0.001
IgM (g/L)	1.40±0.51	1.32±0.50	1.25±0.39	0.009	0.678

Notes: *P<0.05 vs Light old group; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M.

Table 7. Comparison of immunoglobulin levels between Male and Female group

	Male (n=31)	Female (n=52)	t	P
IgG (g/L)	11.35±1.39	11.39±1.36	0.128	0.898
IgA (g/L)	2.38±0.46	2.40±0.47	0.189	0.851
IgM (g/L)	1.28±0.50	1.35±0.48	0.633	0.529

Notes: IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M.

significant difference was observed in UA levels between males and females (P=0.465), while males had higher GLU levels than females (P=0.006).

Comparison of immunoglobulin levels

As shown in **Tables 6** and **7**, the levels of IgG, IgA, and IgM were found to decrease progressively with age, there were statistical differences in IgA and IgG between the three groups (both P<0.001). Additionally, gender difference analysis showed no significant differences in IgG, IgA, or IgM levels between males and females (all P>0.05).

Multiple logistic regression analysis for aging

Multiple logistic regression analysis was performed on indicators with significant differences across multiple groups. Results showed that when the light-old group was used as the reference, moderate-old (MO) individuals had significant differences in UA (odds ratio [OR]=1.034, P=0.003) and IgA (OR=0.096, P=0.006) (**Table 8**). For the heavy-old group, significant differences were observed in WBC (OR=3.269, P=0.040), UA (OR=1.054, P=0.001), IgG (OR=0.309, P=0.007), and IgA (OR=0.024, P=0.005) (**Table 9**). Furthermore, when the MO group was used as the reference, the HO group had a significant difference in IgG (OR=0.488, P=0.032) (**Table 10**).

Comparison with the general reference ranges

Currently, clinical reference ranges for conventional blood biomarkers do not account for the effect of aging on biomarker detection. This effect stems not only from physiological changes in subjects but also from age-related alterations in blood composition, which may further reduce the accuracy of detection technologies. On one hand, aging is accompanied by complex physiologic changes, such as decreased metabolic function, increased inflammatory levels,

Table 8. Multivariate logistic regression analysis for moderate-old (light-old as reference)

	β	Standard Error	Wald	P	Odd ratio	95% CI
Intercept	0.404	4.725	0.007	0.932	1.498	-
UA (continuous variable)	0.034	0.011	8.933	0.003	1.034	1.012-1.058
IgG (continuous variable)	-0.414	0.263	2.472	0.116	0.661	0.395-1.107
IgA (continuous variable)	-2.339	0.844	7.682	0.006	0.096	0.018-0.504

Notes: UA, uric acid; IgG, immunoglobulin G; IgA, immunoglobulin A.

Table 9. Multivariate logistic regression analysis for heavy-old (light-old as reference)

	β	Standard Error	Wald	P	Odd ratio	95% CI
Intercept	-2.823	8.259	0.117	0.732	-	-
WBC (continuous variable)	1.184	0.576	4.228	0.040	3.269	1.057-10.109
P-tau181 (continuous variable)	1.701	1.167	2.124	0.145	5.478	0.556-53.949
UA (continuous variable)	0.052	0.016	10.394	0.001	1.054	1.021-1.087
IgG (continuous variable)	-1.174	0.433	7.362	0.007	0.309	0.132-0.722
IgA (continuous variable)	-3.747	1.345	7.767	0.005	0.024	0.002-0.329
IgM (continuous variable)	-1.069	1.192	0.804	0.370	0.343	0.033-3.554

Notes: UA, uric acid; WBC, white blood cell; P-Tau181, phosphorylated tau protein 181; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M.

Table 10. Multivariate logistic regression analysis for heavy-old (moderate-old as reference)

	β	Standard Error	Wald	P	Odd ratio	95% CI
Intercept	-2.244	6.331	0.126	0.723	0.106	-
WBC (continuous variable)	0.523	0.424	1.523	0.217	1.687	0.735-3.869
P-tau181 (continuous variable)	1.142	0.681	2.815	0.093	3.132	0.825-11.889
UA (continuous variable)	0.020	0.011	3.098	0.078	1.020	0.998-1.042
IgG (continuous variable)	-0.717	0.334	4.618	0.032	0.488	0.254-0.939
IgA (continuous variable)	-1.205	1.015	1.410	0.235	0.300	0.041-2.190

Notes: UA, uric acid; WBC, white blood cell; P-Tau181, phosphorylated tau protein 181; IgG, immunoglobulin G; IgA, immunoglobulin A.

and declining organ function - all of which may lead to significant changes in blood composition, including fluctuations in the levels of proteins, lipids, and inflammatory factors. On the other hand, aging may affect the accuracy of detection technologies; for example, increased blood viscosity or changes in cellular components may interfere with the performance of detection devices, resulting in biased results.

Existing clinical reference ranges for blood biomarkers are typically based on data from young or middle-aged populations and may not accurately reflect the physiologic status of the elderly. Therefore, establishing independent reference ranges for blood biomarkers in the elderly and optimizing detection technologies to adapt to age-related physiologic changes are crucial for improving diagnostic accuracy. Given that participants in this study were either healthy elderly individuals or those with well-controlled underlying diseases, we statistically analyzed the detection results of all participants and compared them with currently commonly used clinical reference ranges.

By reviewing the literature and collecting commonly used reference ranges in hospitals, this study finally determined the most frequently used normal reference ranges for biomarkers and physiologic indicators (including BMI). Subsequently, statistical analysis was performed on all samples to obtain the mean and median values of each indicator. The results showed that TAT (mean: 14.00, exceeding the upper reference limit of 10.00; median: 11.20, exceeding the upper reference limit of 7.20), TM (mean: 16.90, exceeding the upper refer-

Table 11. Comparison of various indicators and their corresponding reference ranges

	Reference range	Average	Median	Average vs Reference	Median vs Reference
WBC	4-10*10^9/L	5.56*10^9	5.50*10^9	Normal	Normal
HDL	0.93-1.81 mmol/L	1.41	1.41	Normal	Normal
TC	2.85-5.70 mmol/L	5.08	5.04	Normal	Normal
TAT	0-4 ng/mL	14.00	11.2	High by 10.00	High by 7.20
TM	3.8-13.8 TU/mL	16.90	11.91	High by 11.80	High by 6.81
tPAIC					
Male	<17 ng/mL	4.41	3.92	Normal	Normal
Female	<10.5 ng/mL	5.15	3.90	Normal	Normal
PIC	0-0.8 μg/mL				
GLU	3.9-6.1 mmol/L	1.75	1.54	High by 0.95	High by 0.74
TG	0.45-1.70 mmol/L	1.58	1.46	Normal	Normal
LDL	2.67-3.03 mmol/L	2.66	2.6	Normal	Normal
IL-6	<7 pg/mL	35.16	7.89	High by 28.16	High by 0.89
CRP	<10 mg/L	1.37	0.68	Normal	Normal

Notes: WBC, White blood cell count; HDL, high-density lipoprotein; TC, Total cholesterol; TAT, thrombin-antithrombin complex; TM, thrombomodulin; tPAIC, tissue-type plasminogen activator-plasminogen inhibitor-1 complex; PIC, plasminogen-plasmin α1 complex; TG, triglycerides; LDL, low-density lipoprotein; IL-6, interleukin-6; CRP, C-reactive protein; Aβ42, Beta-amyloid 42; P-Tau181, phosphorylated tau protein 181.

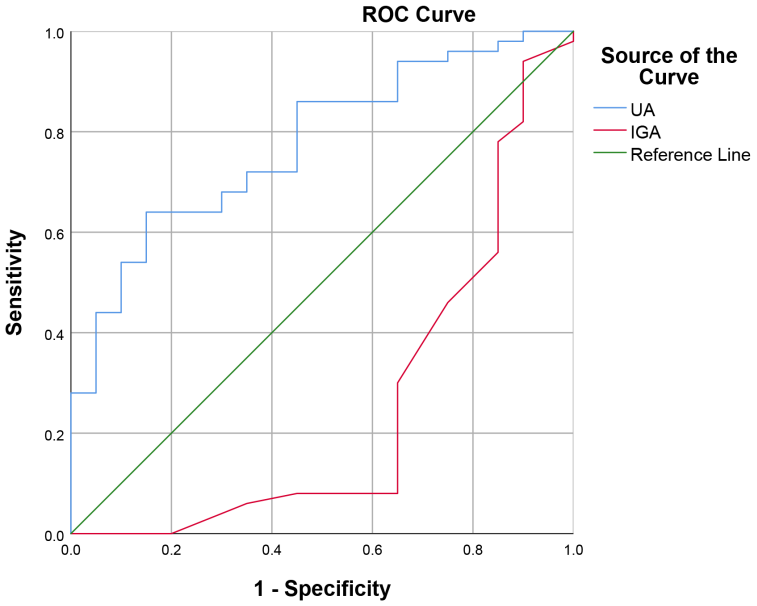

Figure 3. ROC for indicators in predict moderate-old (light-old as reference).

Table 12. ROC for indicators in predict moderate-old (light-old as reference)

	Area under curve	Standard error	P	95% CI
UA	0.776	0.058	0.000	0.663-0.889
IgA	0.253	0.076	0001	0.104-0.401

Notes: UA, uric acid; IgA, immunoglobulin A.

ence limit of 11.80; median: 11.91, exceeding the upper reference limit of 6.81), PIC (mean: 1.75, exceeding the upper reference limit of 0.95; median: 1.54, exceeding the upper reference limit of 0.74), and IL-6 (mean: 35.16, exceeding the upper reference limit of 28.16; median: 7.89, exceeding the upper reference limit of 0.89) all exceeded their respective upper reference limits. Other indicators fell within the reference ranges (**Table 11**).

Receiver operator characteristic curve analysis of indicators

ROC analysis was performed on variables with significant differences by the multiple regression analysis. Results showed that when the LO group was used as the reference, UA was a significant predictor of the MO group, with an AUC of 0.776 (**Figure 3**; **Table 12**). For the HO group, WBC, UA, IgG, and IgA were significant predictors, among which WBC and UA had higher AUC values and certain predictive value (**Figure 4**; **Table 13**). When the

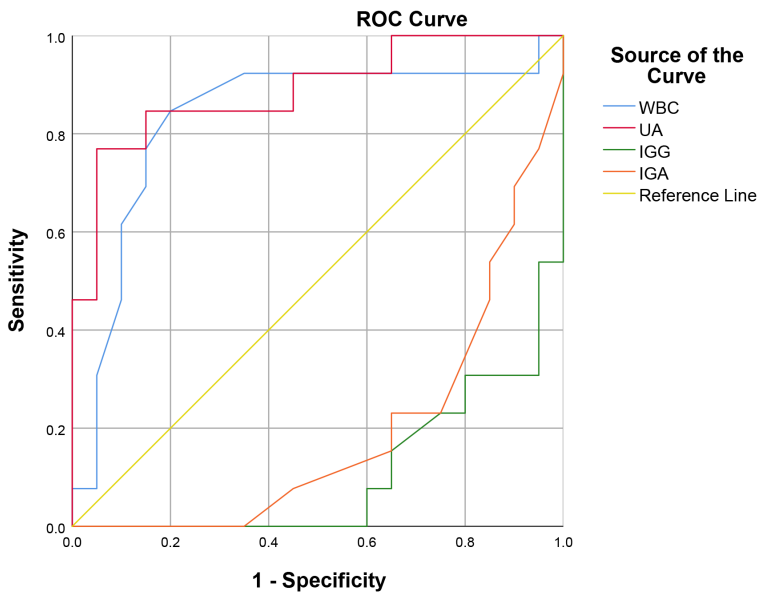

Figure 4. ROC for indicators in predict heavy-old (light-old as reference).

Table 13. ROC for indicators in predict heavy-old (light-old as reference)

	Area under curve	Standard error	P	95% CI
WBC	0.833	0.082	0.001	0.671-0.994
UA	0.888	0.062	0.000	0.766-1.000
IgG	0.108	0.055	0.000	0.001-0.215
IgA	0.192	0.075	0.003	0.045-0.340

Notes: UA, uric acid; WBC, white blood cell; IgG, immunoglobulin G; IgA, immunoglobulin A.

MO group was used as the reference, IgG was a significant predictor for the HO group; however, its AUC value was relatively low (AUC=0.255), indicating limited diagnostic value (**Figure 5**; **Table 14**).

Correlation between various biomarkers, age, and BMI

Blood biomarkers, as sensitive indicators of internal homeostasis, can systematically reflect metabolic and functional changes in the body under physiologic or pathologic conditions. In the immune microenvironment, biological processes such as inflammation, coagulation, lipid metabolism, and neural injury are tightly regulated through complex molecular networks. Studies have shown that inflammatory factors not only activate the coagulation cascade but also disrupt lipid metabolism by affecting the expression of genes related to hepatic lipid metabolism. Meanwhile, coagulation byproducts (e.g., thrombin and fibrin) exhibit neurotoxicity, directly contributing to neural injury. Furthermore, oxidized low-density lipoprotein (oxidized LDL) derived from abnormal lipid metabolism can exacerbate inflammatory responses and endothelial damage, forming a vicious cycle. These molecular interaction mechanisms are supported by extensive clinical and experimental data.

In geriatric medicine, the correlations among these biomarkers are particularly prominent and clinically significant, since aging is often accompanied by chronic low-grade inflammation and immune senescence - both of which are closely associated with coagulation dysfunction, metabolic disorders, and neurodegeneration. Given that the biomarkers in this study were primarily selected based on the coagulation system, inflammatory mechanisms, lipid metabolism, and neural injury, correlation analysis of all biomarkers was conducted to explore how their interrelationships are affected by aging.

Correlation analysis and heatmap visualization (**Figure 6**) showed that biomarkers with a correlation coefficient absolute value exceeding 0.5 included TAT and TM (moderate positive correlation, r=0.57), PIC and TM (strong positive correlation, r=0.65), and LDL and TC (very strong positive correlation, r=0.94) - the latter being the most significant correlation among all biomarkers. No highly significant correlations were observed for other biomarkers.

Discussion

Aging primarily affects the immune system through “immunosenescence” and “chronic low-grade inflammation” [11]. Immunosenescence refers to the age-related decline in immune function, leading to reduced efficiency and sensitivity of immune responses [20]. Chronic low-grade inflammation, a consequ-

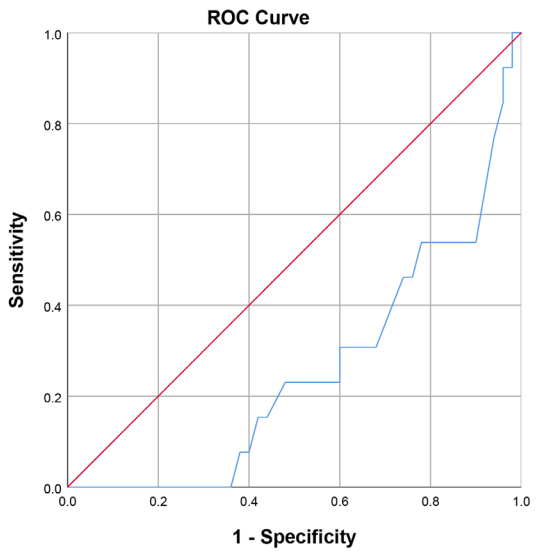

Figure 5. ROC for indicators in predict heavy-old (moderate-old as reference).

Table 14. ROC for indicators in predict heavy-old (moderate-old as reference)

	Area under curve	Standard error	P	95% CI
IgG	0.255	0.071	0.007	0.116-0.395

Notes: IgG, immunoglobulin G.

ence of immunosenescence, is further exacerbated by factors such as vascular aging, mitochondrial dysfunction, changes in adipose tissue, gut microbiota imbalance, and hormonal fluctuations [21, 22]. These factors contribute to the accumulation of inflammatory markers like IL-6 and CRP in older adults [23]. Due to immunosenescence, the body struggles to clear sources of chronic inflammation, such as adipose tissue, pathogens, or senescent cells, leading to persistently elevated IL-6 and CRP levels. However, in the context of immunosenescence, the response to inflammation or infection may be less pronounced compared to younger individuals. Conversely, white blood cell (WBC) count is also influenced by immunosenescence [24]. Older adults experience reduced WBC production and responsiveness due to immune decline and decreased bone marrow hematopoietic function, which may lead to lower or normal WBC counts. However, some studies suggest that older adults may exhibit a higher proportional increase in WBC counts in response to acute inflammation or infection [25].

Our study shows that IL-6 and CRP levels were elevated beyond conventional reference ranges, indicating baseline chronic low-grade inflammation in older adults. In contrast, WBC counts remained within the normal reference range. Previous research suggests that WBC counts in the elderly may not accurately reflect inflammation but could serve as an indicator of cardiovascular function when combined to CRP [26]. Additionally, IL-6 and WBC counts increase progressively with age, whereas CRP does not follow this trend. This may be due to CRP's nature as an acute-phase reactant. These findings highlight how immunosenescence and chronic low-grade inflammation alter the clinical interpretation of inflammatory markers in older adults. The interpretation of test results should consider individual differences, particularly the degree of aging, and account for baseline elevation of inflammatory factors in the population studied.

Immunoglobulins play a vital role in the B-cell-mediated humoral immune response, providing immune defense, immune surveillance, and maintaining immune homeostasis. IgA is divided into serum and secretory types, with the serum form having antibacterial, antitoxin, and antiviral effects, while the secretory form participates in local immunity to prevent pathogen invasion. IgG is the only immunoglobulin that can cross the placenta, providing innate immunity. IgM is the first immunoglobulin produced in response to antigenic stimulation and is highly effective against bacterial and viral infections. Our study found that IgG, IgM, and IgA levels gradually decreased with age, indicating a decline in the body's immunoglobulin capacity with aging.

We further investigated the interaction between inflammation and coagulation biomarkers in the aging process. Our results showed that the lack of significant correlation between inflammatory markers (IL-6, CRP) and coagulation biomarkers (TAT, TM, PIC, tPAIC) aligns with the hypothesis that aging alters the interaction between inflammation and coagulation systems [22]. Previous studies have shown that inflammatory markers often correlate with coagulation markers, reflecting their interaction at the biomarker level [8, 27]. Inflammation activates the coagulation system, while coagulation factors exacerbate the inflammatory response. Anti-coagulant and anti-inflammatory

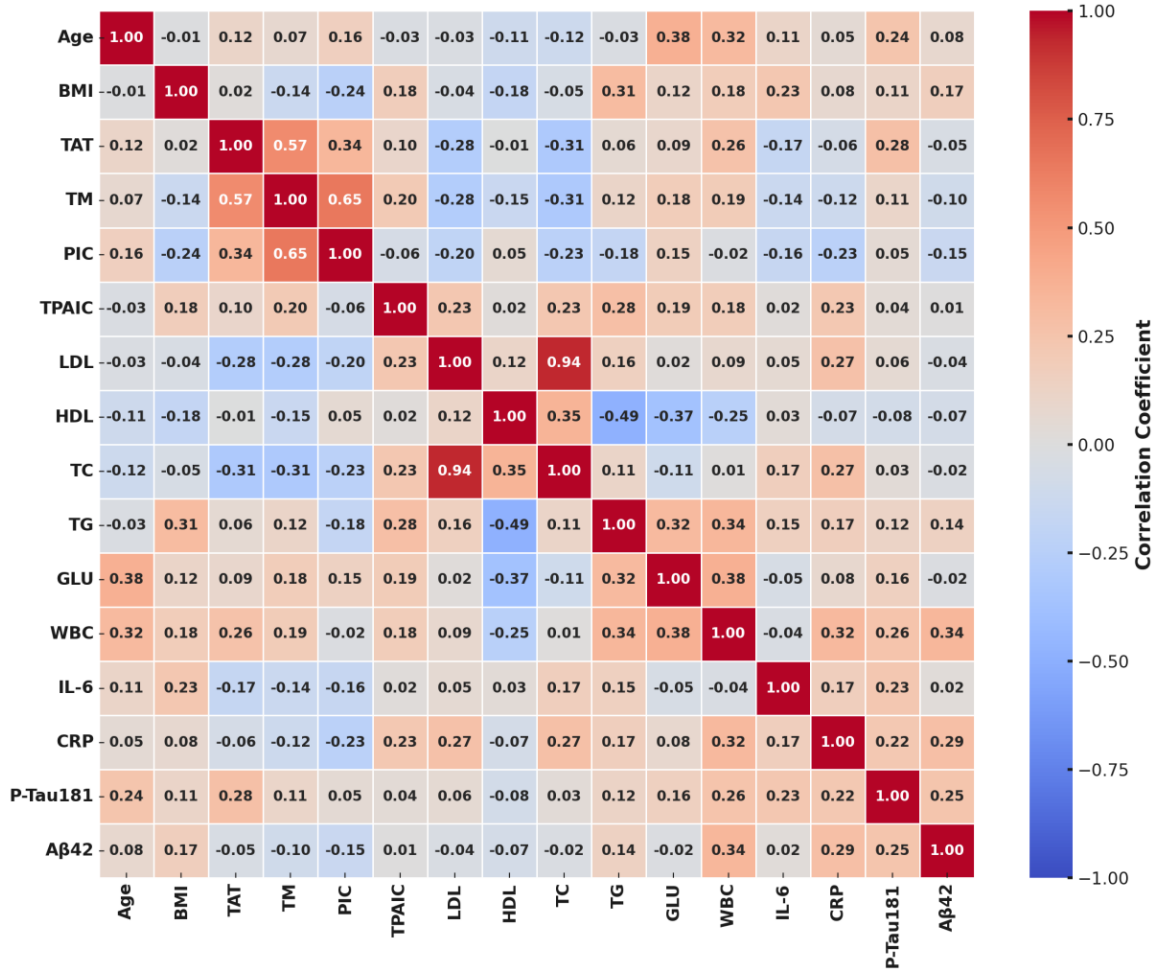

Figure 6. Correlation heatmap between various biomarkers, age, and body mass index (BMI) shows that the closer it is to 1, the stronger the positive correlation; the closer it is to -1, the stronger the negative correlation; and the closer it is to 0, the weaker the correlation. BMI, body mass index; WBC, White blood cell count; HDL, high-density lipoprotein; TC, Total cholesterol; TAT, thrombin-antithrombin complex; TM, thrombomodulin; tPAIC, tissue-type plasminogen activator-plasminogen inhibitor-1 complex; PIC, plasminogen-plasmin α1 complex; TG, triglycerides; LDL, low-density lipoprotein; IL-6, interleukin-6; CRP, C-reactive protein; Aβ42, Beta-amyloid 42; P-Tau181, phosphorylated tau protein 181.

factors can also have reciprocal effects. In our study, IL-6 and CRP indirectly activate the coagulation system by promoting inflammation, leading to increased coagulation markers like TAT, PIC, and tPAIC. Conversely, these coagulation markers also regulate the expression of inflammatory markers like IL-6 and CRP during the inflammation-coagulation interaction. This interaction is particularly pronounced in chronic low-grade inflammation [8]. However, in the elderly, immunosenescence, impaired endothelial function, and weakened fibrinolytic/anticoagulant mechanisms significantly affect the intersection of inflammation and coagulation networks, increasing their complexity [22]. Aging leads to a reduced endothelial cell response to inflammatory markers like IL-6 and CRP [28], causing compensatory increases in IL-6 and CRP while weakening their regulatory effect on coagulation. Additionally, the decline in fibrinolytic and anticoagulant functions with aging enhances the promoting effect of inflammatory markers on coagulation, keeping both coagulation and inflammatory biomarkers elevated in the elderly population. Despite this, due to immunosenescence, the impact of these elevated biomarkers on coagulation and inflammation is likely less pronounced than in younger individuals. Stratifying the elderly into different age groups showed that TAT, PIC, and TM

exhibited a gradual upward trend with age, although differences between groups did not reach statistical significance. This suggests that endothelial cell damage worsens with aging, dysregulating coagulation and fibrinolysis. Additionally, IL-6, WBC, and P-tau181 levels increased with age, reflecting intensifying chronic inflammation and neural damage. The upward trend in GLU may indicate worsening metabolic dysfunction with aging. Moreover, the comparison of the three age groups showed that P-tau181 and WBC counts increased more significantly in the advanced age group. Recent studies suggest that blood p-tau181 is a candidate diagnostic marker for Alzheimer's disease [29, 30]. Elevated plasma p-tau181 levels have been observed prior to the onset of symptoms in Alzheimer's patients [31], and aging is the greatest risk factor for late-onset Alzheimer's disease (LOAD) [32].

Our study found that gender differences in blood biomarkers became more pronounced with aging. The interpretation of blood biomarkers in aging should consider gender differences [33]. In our gender-stratified analysis, HDL levels were significantly higher in females than in males. This aligns with studies showing that estrogen contributes to higher HDL levels in women, even postmenopause, when estrogen fluctuations occur [34]. Although older women tend to have higher HDL levels, they also exhibit higher LDL and TC levels, which are influenced by estrogen levels and fat distribution changes in postmenopausal women [35-38]. This may lead to elevated TC and other lipid markers, contributing to cardiovascular risk. In contrast, P-tau181 levels were significantly higher in males than in females, both before and after propensity score matching. While there is no consensus on P-tau181 accumulation in older men and women, studies suggest that women are more prone to neurodegenerative diseases like Alzheimer's, with earlier and faster tau phosphorylation [39-42]. However, in healthy older adults, men tend to show higher P-tau181 levels than women, particularly with age [43].

This study did not exclude participants based on medication use, as older adults are often advised to take health supplements or medications (e.g., statins, calcium channel blockers) even without symptoms [44, 45]. Our aim was to explore the effect of medication use on biomarkers. Research has shown that TC, TG, and LDL are positively correlated with aging [46], but in our study, these markers showed a negative correlation with age. This suggests that long-term medication use, such as statins, may have influenced the results. However, group comparisons revealed that TC, TG, and LDL were not significantly affected by medication use, consistent with prior studies. Although some participants were on antihypertensive and antiplatelet medications, no significant effect on inflammatory and coagulation biomarkers was observed. None of the participants were taking anticoagulant medications.

This study has several limitations. The small sample size reduces statistical power and increases the risk of false-negative results. Although trends were observed, the small sample size limits the ability to draw definitive conclusions. Additionally, the sample size prevented stratified analysis of participants taking medications, making it difficult to fully explore the effect of medications on biomarkers. As a retrospective study, the quality of data sources may vary, and the completeness and timeliness of data were difficult to control. Furthermore, the inability to control for confounding variables may affect causal inferences. The study also focused on a limited number of biomarkers and did not deeply explore their interrelationships in the context of aging. Although aging is a major risk factor for chronic diseases, biomarkers of biological aging can predict outcomes and serve as surrogate endpoints for evaluating interventions promoting healthy aging. Future large-sample prospective studies are needed to explore quantifiable biomarkers of aging and their diagnostic value.

In conclusion, our research revealed that aging significantly affects the clinical relevance of biomarkers, particularly in coagulation, inflammation, and immune mechanisms. Reference ranges for various biomarkers in the elderly should be further refined to reflect the unique physiologic conditions of older adults.

Acknowledgements

This work was supported by Hubei Provincial Science and Technology Department's "Jiebang System" Technology Project (KJJB-2023000058).

Disclosure of conflict of interest

None.

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