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Research Article

The Role of Soluble Costimulatory Molecules as the Biomarkers for Aging Predictors

Peran Molekul Kostimulator Terlarut sebagai Biomarker untuk Prediktor Penuaan

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ABSTRACT

This study aimed to determine the role of the soluble costimulatory molecules in aging and the association with the presence of comorbid in aged individuals. Thirty-two elderly and twenty healthy subjects were included in this study. The soluble costimulatory molecules sCD28, sCD80, sCD86, sCD163, and sCTLA4 were measured using ELISA. The presence of comorbid was documented from medical records. Charlson Comorbidity Index (CCI) was measured to evaluate the survival/mortality risk for the subjects. The levels of the majority of soluble costimulatory molecules significantly increased in the elderly participants, while the level of sCD86 was comparable. There were weak positive correlations between the subject's age and levels of sCD28 (R=0.214, p=0.048), sCTLA4 (R=0.238, p=0.041), and sCD80 (R=0.317, p=0.012). sCD80 were discovered to be the best to predict immune aging in the elderly with AUC 0.71 [0.57-0.86], sensitivity 53,1%, specificity 80.0%, and cut off 129ng/ml. Most of the elderly participants had at least one comorbid, in which approximately 25.0% and 3.1% of the subjects were classified as mild and moderate CCI. Multivariate analysis showed that comorbidities in elderly individuals have been associated with levels of sCTLA4 ≥26.5ng/ml and sCD80 ≥129.0ng/ml. Furthermore, subjects with comorbid (CCI ≥1) were associated with sCD80 ≥129.0ng/ml (OR 12.44 [95% CI 1.32–117.03], p=0.027). Considering these results, sCD28, sCTLA4, and sCD80 can be developed as biomarkers for predicting immune aging and elderly comorbidities, respectively.

Keywords: *Comorbidities, elderly, immune aging, soluble costimulatory molecules*

ABSTRAK

Penelitian ini bertujuan untuk mengetahui peran molekul kostimulatori terlarut dalam penuaan dan hubungannya dengan adanya komorbiditas pada individu lanjut usia. Tiga puluh dua individu lanjut usia dan dua puluh subjek sehat dilibatkan dalam penelitian ini. Molekul kostimulatori terlarut sCD28, sCD80, sCD86, sCD163 dan sCTLA4 diukur menggunakan ELISA. Adanya komorbiditas didokumentasikan dari catatan medis. *Charlson Comorbidity Index (CCI)* diukur untuk mengevaluasi risiko kelangsungan hidup/kematian subjek. Sebagian besar kadar molekul kostimulatori terlarut didapatkan mengalami peningkatan kadar yang signifikan pada individu lanjut usia, sedangkan kadar dari sCD86 sebanding. Terdapat korelasi positif yang lemah antara usia dan kadar sCD28 (R=0.214, p=0.048), sCTLA4 (R=0.238, p=0.041), dan sCD80 (R=0.317, p=0.012). Pada penelitian ini didapatkan bahwa sCD80 merupakan penanda terbaik untuk memprediksi penuaan sistem imun pada orang tua dengan AUC 0,71 [0,57-0,86], sensitivitas 53,1%, spesifisitas 80,0%, dan cut off 129ng/ml. Sebagian besar individu lanjut tua memiliki setidaknya satu komorbiditas, di mana sekitar 25,0% dan 3,1% dari subyek diklasifikasikan sebagai CCI ringan dan sedang. Analisis multivariat menunjukkan bahwa komorbiditas pada individu lanjut usia berhubungan dengan kadar sCTLA4 26,5ng/ml dan sCD80 129,0ng/ml. Selanjutnya, subjek dengan komorbiditas (CCI) dikaitkan dengan sCD80 129,0ng/ml (OR 12,44 [95% CI 1,32–117,03], p=0,027). Mempertimbangkan hasil ini, sCD28, sCTLA4, dan sCD80 masing-masing dapat dikembangkan sebagai biomarker untuk memprediksi penuaan sistem imun dan komorbiditas pada lansia.

Kata Kunci: Lanjut usia, molekul kostimulatori terlarut, penuaan imun, penyakit penyerta

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INTRODUCTION

Aging is a complex and multifactorial process characterized by a decrease in the body's physiological functions (1). This process contributes to the increase of chronic deseases, causing people to live their lives with health problems and disabilities. Furthermore, aging significantly increases the healthcare and social financing burden (2). In the sixth decade of life, the immune system undergoes age-related changes and dysregulation due to defects in the initiation and resolution of immune responses. These are widely referred to as immune aging or immunosenescence, characterized by multiple alterations in the phenotypes and functions of the innate and adaptive immune system (3,4).

According to the United Nations Secretariat, there is currently a trend of progressive aging of the immune system in the world. The latest world population prospective report showed there were 962 million people in the world aged > 60 years, it is estimated to increase to 1.4 billion in 2030 and 2.1 billion in 2050. The aging trend and the disease burden causes a distruption in public health care and social health systems, creating social and economic burdens, which threatening the political stability (3,5). Additionally, the alteration leads to a low-grade inflammation called inflammaging. A previous study discovered that inflammaging originates from many elderly comorbidities and age-related chronic diseases, such as atherosclerosis, obesity, diabetes, and neurodegenerative disorders (3). Therefore, it can be concluded that immune aging is a risk factor for the development of many chronic diseases. It also causes increased susceptibility to various infections and contributes to the development of cancer, cardiovascular, and autoimmune disease (5).

Immune aging is a natural process in the elderly and individuals with autoimmune diseases, such as SLE (systemic lupus erythematosus)(6,7). This is evidenced by the discovery of the Immune Risk Profile (IRP) marker, a biomarker for aging detection (5). The detection of immune aging is a significant problem since IRP is the only marker that can be used as its predictor. This marker can only be detected by measuring the percentage of costimulatory markers on the surface of immune cells using the flow cytometry method, which tends to be difficult due to a lack of advanced technologies, takes a long time, and special expertise. Therefore, this study aims to discover new markers of immune aging using the soluble form of costimulatory molecules as candidate biomarkers.

Several costimulatory molecules, such as CD28, CD80, CD86, CD163, and CTLA-4, have been investigated for their essential role in the immune aging process. Previous research reported that these molecules have a soluble form of sCD28, sCD80, sCD86, sCD163, and sCTLA-4, which circulate freely and have been studied for their role in various autoimmune diseases (8). The increased sCD28 levels indicate a regulatory mechanism to compensate for T cell activation. However, this tends to be higher in cases of immune aging (9). The soluble costimulatory molecules sCD80 and sCD86 were suggested to play essential roles in the aging immune system (10). The increased levels of sCD80 are closely related to dysregulation of T cell activation, which is associated with immune aging (11). The sCD86 markers also play an essential role in immune system regulation by binding to the CD28 and CTLA-4 molecules, interfering with their binding mechanism. Its levels increased in various immune-aging-related diseases (12). sCD163 plays a role in the acceleration of T cell aging and immunosenescence, which could occur due to chronic immune activation (13). Meanwhile, it was discovered that sCTLA-4 levels are lower in patients with autoimmune diseases and are associated with immune aging (9).

The advantage of using the soluble costimulatory markers as the receptor is their ability to be detected in serum using the ELISA method, which is easier to perform than flow cytometry (14). This study aims to determine the role of soluble costimulatory molecules as predictors of immune aging biomarkers. Therefore, it can be developed to detect immune aging and can predict comorbidities in the elderly more efficiently and quickly in the future.

METHODS

Study Design

This study design used an observational analysis with a case-control approach. Therefore, this study aimed to determine the levels of sCD28, sCD80, sCD86, sCD163, and sCTLA-4 and their role as predictive biomarkers for immune aging.

Study Subjects

The participants were elders within the range of 60-85 years old from the Junrejo Batu Health Center, Malang, Indonesia. Those that are not communicative and cannot provide clear information are excluded. Thirty-two old subjects were recruited in this study. Finally, twenty healthy individuals between 20 and 28 years were included as controls, because young individual have better immunity function and not yet undergo an immunosenescence. The Ethics Committee of Saiful Anwar Hospital, Malang, Indonesia, approved this study. This is evidenced by the Ethical Clearance Sheet No. 400/085/K.3/302/2020, dated March 23, 2020. The presence of comorbidities was documented from the medical records of the participants. The Charlson comorbidity index (CCI) is a method for predicting mortality by classifying comorbid conditions and has been widely utilized to measure the burden of disease. Comorbidity or disease burden in geriatric patients is calculated by CCI which consists of 19 disease conditions and age categories with each score.

Table 1. The CCI: the 19 item-version

Assigned weights for each condition	Conditions
1	Myocardial infarct
1	Congestive heart failure
1	Cerebrovascular disease
1	Dementia
1	Chronic pulmonary disease
1	Connective tissue disease
1	Ulcer disease
1	Mild liver disease
1	Diabetes
2	Hemiplegia

Table 1. The CCI: the 19 item-version (Cont.)

Assigned weights for each condition	Conditions
2	Moderate or severe renal disease
2	Diabetes with end organ damage
2	Any tumor without metastasis
2	Leukemia
2	Lymphoma
3	Moderate or severe liver disease
6	Metastatic solid tumor
6	AIDS

The total score of the CCI consists of a simple sum of the weights, with higher scores indicating not only a greater mortality risk but also more severe comorbid conditions. The sum of the weights of comorbid diseases that the patient has can then be classified as no comorbid if the total weight is 0. If the total weight is 1-2 then it can be categorized as mild. If the total weight is 3-4 then it can be categorized as moderate. When the total weight is 5, it can be categorized as severe.

Peripheral Venous Blood Sampling

The Doctors sampled 2.5 mL of the subject venous blood at The Saiful Anwar Hospital Rheumatology Polyclinic Malang and Junrejo Batu Health Center. The samples were stored into vacutainer EDTA (BD vacutainer Catalog no. 367955). Furthermore, they were used to measure biomarkers in dissolved form, such as sCD28, sCD80, sCD86, sCD163, and sCTLA-4.

Serum Preparation

A sample of venous blood in a vacutainer EDTA is left at room temperature for 15-30 minutes, then centrifuged at a speed of 3,000 rpm for 20 minutes. The serums left on the table were slowly picked using micropipettes. Afterward, they were transferred into the Eppendorf tube to be labeled and stored at temperatures of -20°C until the ELISA analysis was completed.

Measurement of Soluble Costimulatory Molecules using ELISA

The serum concentration of soluble costimulatory molecules in the elderly and the control group was detected using ELISA Kit: Human Soluble CD28 (sCD28) ELISA Kit Mybiosource catalog number: MBS3801986, HSCD 80 (sCD80) ELISA Kit MBS catalog number: MBS705535, HSCD 86 (sCD86) ELISA Kit MBS catalog number: MBS702416, HSCD163 (sCD163) ELISA Kit MBS catalog number: MBS284938, and Human Soluble Cytotoxic T-Lymphocyte Associated Antigen 4 (Sctla4) ELISA Kit MBS catalog number: MBS030414.

The serum prepared for the ELISA test was transferred into the Eppendorf tube. The reagents, samples, and standards were prepared according to factory instructions and placed at room temperature. Furthermore, the assay was conducted according to the manufacturer's protocol. Optical Density (O.D.) was determined within 5 minutes at 450 nm using a Microelisa Stripplate reader.

Statistics

Statistical analysis was conducted using the Mann-Whitney comparison test. The correlation between each biomarker was determined with the Spearman correlation test. Furthermore, the diagnostic performances of each biomarker were assessed using the receiver operating characteristic (ROC) curve. The researcher conducted a multivariate analysis of the Logistics Regression test to analyze the external variables affecting the results. Statistical analysis was performed using IBM SPSS Statistics version 23. Therefore, a p-value less than 0.05 was considered statistically significant.

RESULTS

Characteristics of the Subjects

Twenty and thirty-two young and old subjects, respectively, were recruited for this study. Table 1 shows the characteristics of the participants. The young and old subjects are between 20–28 and 60–85 years. The age comparison between groups was statistically significant (p=0.000). Furthermore, the sex distribution between groups has a similar proportion of males and females (p=0.404), as shown in Table 2.

Table 2. Characteristics of subject

Characteristics	Young (n=20)	Old (n=32)	p
Age (years old)			
- Mean ± SD	23.6±2.2	70.3±7.9	0.000*
- Range	20–28	60–85	
Gender (n [%])			
- Male	5(25.0)	5(15.6)	0.404
- Female	15(75.0)	27(84.4)	
Comorbidities, (n [%])			
- Asthma	-	2(6.25)	-
- Diabetes	-	7(21.8)	
- Hypertension	-	6(18.8)	
- Dyslipidemia	-	5(15.6)	
- Hyperuricemia	-	3(9.4)	
- Peptic ulcer disease	-	2(6.3)	
- No comorbidities	-	11(34.4)	
Charlson comorbidity index (CCI), n (%)			
- None (0)	-	23(71.9)	-
- Mild (1 – 2)	-	8(25.0)	
- Moderate (3 – 4)	-	1(3.1)	
- Severe (≥5)	-	0(0)	

Note: *Showed statistically significant, with p<0.05

Most older participants had at least one comorbid on their medical history, while only 11 subjects (34.4%) had none. The most common type was diabetes (21.8%) and hypertension (18.8%). According to the CCI from the participants, 25.0% of subjects were classified as mild, while 1 participant (3.1%) was classified as moderate.

Comparison of the Soluble Costimulatory Molecule Levels According to the Age

Table 3 compares the soluble costimulatory markers between young and older participants. Several of them, including sCD28 (p=0.038), sCTLA4 (p=0.025), sCD163 (p=0.047), and sCD80 (p=0.010) are significantly higher in older individuals. However, the sCD86 level was found increased among young and old participants.

Table 3. Comparison of the soluble costimulatory markers levels between groups

Markers	Young (n = 20)	Old (n = 32)	P
sCD28 (ng/ml)	15.4(6.2–23.2)	24.7(14.8–31.9)	0.038*
sCTLA4 (ng/ml)	19.6 (13.0–31.6)	29.4(22.2–44.3)	0.025*
sCD86 (ng/ml)	0.3(0.3–0.7)	0.3(0.3–0.8)	0.853
sCD163 (ng/ml)	0.0(0.0–0.1)	0.1(0.0–0.1)	0.047*
sCD80 (ng/ml)	127.0 (125.0–129.0)	130.0 (128.5–7892.5)	0.010*

Note: *Showed statistically significant, with p<0.05

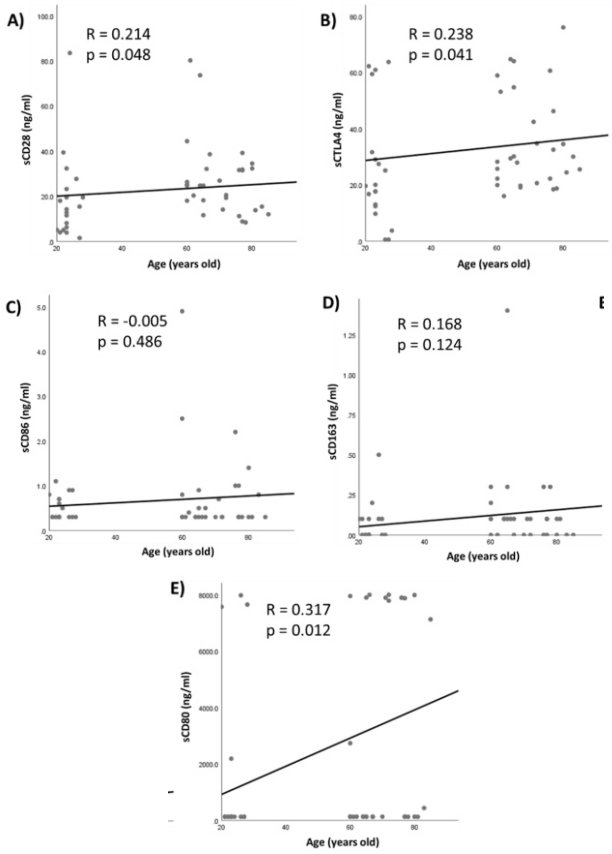

Figure 1. Correlation of the soluble costimulatory markers with the age of subjects

Figure 1 presents the correlation analyses between the markers with age. The sCD28 (R=0.214, p=0.048), sCTLA4 (R=0.238, p=0.041), and sCD80 (R=0.317, p=0.012) levels have a weak positive correlation with the age of the subjects, while sCD86 and sCD163 show no correlation.

Performance of the Soluble Costimulatory Markers in Predicting the Old Age

Receiver operating characteristics (ROC) curve analysis was performed to observe the predictive ability of the markers in determining older age.

Table 4 shows the optimal cut-off value, AUC, sensitivity, and specificity of the markers associated with aging. sCD80 with an AUC of 0.71 was the best predictor of older age, followed by sCTLA4 and sCD28 with AUC of 0.70 and 0.67, respectively. This occurred at an optimal cut-off value, sensitivity, and specificity of 129.0 ng/ml, 53.1%, and 80.0%.

Association of the Soluble Costimulatory Markers with the Presence of Comorbid

According to the cut-off value from the previous analysis, the association of these markers with the presence and severity of the comorbid based on the CCI score was determined. This was performed to confirm the role of these markers in aging.

Table 5 showed the association of soluble costimulatory markers with the presence and severity of comorbidities among older individuals. sCD28 levels ≥19.9ng/ml did not have any relationship with the presence or severity of comorbid among older people. However, it was associated with levels of sCTLA4 ≥26.5ng/ml and sCD80 ≥129.0ng/ml. According to the CCI score, more severe comorbidities (CCI ≥1) were only related to sCD80 ≥129.0ng/ml.

DISCUSSION

Immune aging is changing the compartment and function of innate and adaptive immune system compartments and function, associated with changes in response to infection, pro-inflammatory properties, and increased risk for developing autoimmune and many chronic diseases (15). The adaptive immune system's main compartments that change due to the aging process are T lymphocyte cells responsible for protection against intracellular pathogens or damaged cells, such as cancer (16). Furthermore, immune aging can be characterized by the appearance of immune risk profile (IRP) markers, such as an inverse of CD4+/CD8+ ratio, accumulation of CD8+ memory T lymphocyte cells (CD8+CD45RO+ T lymphocyte cells), decreased T CD8+ naïve lymphocyte cell, loss or decrease in CD27, CD28, CD86, CD80 T lymphocyte cell expression, and increased expression of CD57 and CTLA-4 costimulatory molecules (17).

CD28 is a costimulatory molecule that provides signals for T-cell activation and survival. The decreased CD28 expression is one of the markers of the immune aging process (18). CD163 is a marker of macrophage-specific proteins, and the regulated expression of these receptors indicated a significant change in inflammation (19). High expression of CD163 in macrophages showed a significant

Table 4. Performance of the soluble costimulatory markers for the prediction of aging

Marker	AUC	95% CI	p	Cut-off	Sensitivity	Specificity
sCD28	0.67	0.51–0.84	0.039*	19.9	63.3%	70.0%
sCTLA4	0.70	0.53–0.86	0.020*	26.5	58.6%	63.2%
sCD86	0.51	0.35–0.68	0.868	0.3	40.6%	57.9%
sCD163	0.66	0.49–0.81	0.067	0.1	19.4%	89.5%
sCD80	0.71	0.57–0.86	0.010*	129.0	53.1%	80.0%

Note: *Showed statistically significant, with p<0.05

Table 5. Association of the soluble costimulatory markers with the presence of comorbid among older individuals

Marker	Comorbid Present			CCI Score ≥1		
	OR	95% CI	p	OR	95% CI	p
sCD28 ≥19.9 ng/ml	1.60	0.32–7.90	0.564	2.63	0.44–15.78	0.292
sCTLA4 ≥26.5 ng/ml	5.33	1.07–26.62	0.041	4.55	0.77–26.83	0.094
sCD80 ≥129.0 ng/ml	11.34	1.86–68.11	0.008*	12.44	1.32–117.03	0.027*

response to inflammation, indicating an aging immune system condition. CD80 and CD86 are transmembrane costimulatory ligand molecules expressed by APCs that can bind to CD28 and CTLA-4. These molecules bind to the CTLA-4 expressed by the APC after T-cell activation. The expression of CTLA-4 prevents the binding of CD28 to CD80 and CD86, thereby inhibiting the formation of costimulatory signals and blocking the T-cell activation pathway (20). However, these molecules significantly decrease with age while CTLA-4 increases (21).

Detection of immune aging is currently a significant problem, which can only be detected from IRP (5). However, IRP can only be observed using flow cytometry by measuring the percentage of costimulatory markers on the surface of immune cells, which tends to be difficult, requires sophisticated tools, takes a long time, and special skills. Previous studies showed that CD28, CD80, CD86, CD163, and CTLA-4 have a soluble form (sCD28, sCD80, sCD86, sCD163 and sCTLA-4), which circulate freely. Furthermore, there were investigated for their role in various autoimmune diseases (8). These dissolved markers were formed due to the release of the membrane cell or alternative production during the mRNA splicing process (8). Therefore, this study was conducted to detect immune aging using a soluble costimulatory marker.

The advantage of using soluble costimulatory markers is their ability to be detected in serum with a simpler method. Previous studies showed that molecules such as CD28, CD80, CD86, CD163, and CTLA-4 play an essential role in the immune aging process. Furthermore, they have soluble forms (sCD28, sCD80, sCD86, sCD163, and sCTLA-4) that circulate freely and are investigated for their role in various autoimmune diseases(8).

This study showed that the soluble costimulatory markers were higher in the elderly. sCD28 (R=0.214, p=0.048), sCTLA4 (R=0.238, p=0.041), and sCD80 (R=0.317, p=0.012) have a weak positive correlation with the age of the subjects. The results also stated that the best soluble costimulatory markers for predicting older age were sCD80 with an AUC of 0.71, then sCTLA4 and sCD28 with an AUC of 0.70 and 0.67, respectively. This study was in line with the research by Sakthivel, stating that the elderly with an average age of 70 years have high levels of sCD28, sCTLA4, and sCD80. Additionally, the level of soluble costimulatory markers was positively associated with pro-inflammatory cytokines such as TNF-, IL-6, IL-1α, IL-1β, and IFN-γ as well as IL-8, which indicates the presence of a chronic inflammatory process that occurs in elderly (22,23). Jubel *et al*., also showed that sCTLA4 levels were high in elderly patients (mean age 71 years) with periprosthetic joint infectious disease (24). This soluble marker is released by Treg cells, monocytes, and immature DCs (25). Blocking sCTLA-4 leads to increased levels of cytokines, especially IFN-γ (12). However, another study discovered that sCTLA-4 inhibited the inhibitory effect of CTLA-4 on T cells (26).

A possible explanation for this result is that the effect of sCTLA-4 is dependent on the activation status of the cells involved. Meanwhile, this soluble marker could inhibit CD80-CD28 interaction in resting cells and the CD80-CTLA-4 interaction on activated T cells (26). Several studies have also suggested that prolonged T-cell activation in elderly patients with myasthenia gravis can release sCD28 early in the immune response (27). Additionally, an increase in the soluble costimulatory molecule sCD80 is closely related to dysregulation of T cell activation in the elderly (11).

Immune aging is also characterized by an increased chance of developing autoimmune disorders. The deficiency of CD28 in T cells is associated with increased production of pro-inflammatory cytokines, namely Tumor Necrosis Factor (TNF), Interleukin-6 (IL-6), C-reactive protein (CRP), and others. The pro-inflammatory environment and the development of self-reactive T and B cells promote persistent systemic inflammation. Furthermore, it can induce immune aging and the development of morbidities, such as cardiovascular disease and cachexia (28).

Previous study found a decreased levels of mCD28 and increased levels of sCD28 in elderly and healthy individuals with NSCLC. This study concluded that with increasing age and the aging process, there is a downregulation of mCD28 expression and an upregulation of sCD28 levels. Levels of soluble costimulatory molecules correlate positively with increased levels of pro-inflammatory cytokines, such as TNF-α, IL-6, IL-1α, IL-1β, and IFN-γ and IL-8 (30). Hamzaoui *et al.,* also showed that the increased expression of sCD28 reflects an inflammatory condition in patients with Behçet's disease. This study hypothesized that this inflammatory condition might lead to the release of the CD28 membrane form, which in turn explains the lack of the CD28 marker on CD8+ T lymphocytes (31).

Immune aging is characterized by multiple alterations in the phenotypes and functions of the innate and adaptive immune system, which lead to a lowgrade inflammation called inflammaging (3,4). A previous study discovered that inflammaging is the origin of many eldery comorbidties and age-realted chronic diseases, such as atherosclerosis, obesity, diabetes, osteoporotic, and neurodegenerative diseases (3,28). In each disease, a DAMP triggers intetaction with recognition receptors in a different pattern. Oxidized lipoproteins can be the initiator and glycated end product in atherosclerosis and type 2 diabetes. Meanwhile, neurodegenerative processes can be a viral product or misfolded protein (3). These show the importance of the inflammatory process as the primary factor influencing the occurrence of chronic diseases.

Inflammatory pathways play an essential role in the development and maintenance of the health and plasticity of the central nervous system (CNS). Physiologically, immune-associated receptors such as MHC-I, TNFR1, and IL-1R participate in long-term potentiation (LTP) and modulate learning and memory formation. Furthermore, excessive neuroinflammation induces direct impairment

of cognitive function (31). Immune aging also refers to the gradual decline in the system that correlates with an increase in the frequency and severity of cancer. Monoclonal antibodies targeting the CTLA-4, PD-1 or PD-L1 checkpoint molecules are promising anticancer therapies that produce an outstanding and long-lasting clinical response (32).

This study was the first to examine the possibility of several soluble costimulatory molecules as predictive biomarkers of the presence and severity of comorbidities in the elderly. It showed that sCTLA4 ≥26.5ng/ml and sCD80 ≥129.0ng/ml were statistically associated with comorbidities in the elderly. The most common comorbid were diabetes (21.8%) and hypertension (18.8%). The more severe comorbidities (CCI ≥1) were associated with sCD80 ≥129.0ng/ml levels, which means the more comorbid diseases suffered, the marker of aging will increase.

Previously the elevated levels of sCTLA4 were associated with several autoimmune diseases, such as autoimmune thyroid, type 1 diabetes, diffuse cutaneous systemic sclerosis, systemic lupus erythematosus, and myasthenia gravis (20,33). Higher levels of this soluble maker could interfere with the binding of the membrane form of CTLA-4 with CD80/CD86, resulting in reduced inhibitory signaling (33). Aberrations in the expression of sCD80 were reported in the serum of patients with systemic lupus erythematosus. Furthermore, its increased levels are closely related to dysregulation of T cell activation and could reflect autoimmune disease (11). These autoimmune diseases are closely related to the incidence of inflammation, which is the sorting pathway for the development of most comorbidities and age-related disorders.

A previous study discovered that inflammaging is the origin of many elderly comorbidities and age-related chronic diseases, such as atherosclerosis, obesity, diabetes, and neurodegenerative disorders (3). In type II diabetes mellitus, this inflammatory reaction depends on the help of T cells cytokines and costimulatory signals, one of which is the interaction of CD28 and B7 release (CD80 dan CD86) (34). Wong *et al.,* showed a decrease and increase in the sCTLA4 levels and sCD28 levels of type II diabetes patients, which were in contrast with the current study (34). This research showed that sCTLA-4 levels significantly increased in the elderly with diabetes as their comorbid disease. However, until now, there have been no studies on sCD80 levels in comorbid diseases, such as diabetes and hypertension.

From the explanation above, it can be concluded that soluble costimulatory modules, such as sCD80, sCTLA4, and sCD28, can be developed as promising predictive biomarkers of immune aging in the future. Additionally, sCD80 and sCTLA4 can be developed to predict the presence of comorbidities in the elderly. This study was constrained by the small number of samples collected; it is believed that a larger sample size will more precisely define the concentrations of dissolved costimulatory molecules.

CONFLICTS OF INTEREST

None

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