ORIGINAL ARTICLE

Evaluation of Biomarkers from Routine Laboratory Indicators for Establishing Molecular Age Score with Considering Survival Probability

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SUMMARY

Background: The goal was to establish a system for assessing molecular age (MA) based on common biomarkers of aging, disease, and end-of-life processes for assessing the development of chronic diseases at the molecular level. Methods: Routine clinical laboratory indexes, including biochemical lab tests and complete blood count, were used as common biomarkers in end-of-life patients, who underwent treatment at the intensive care unit (ICU) of a hospital and died within one month. Biomarkers that were significantly different both between the patients and the controls and between the young and elderly groups could be used for the establishment of a MA index at the molecular level. Results: Only albumin (ALB) was suitable as an index of MA. MA score could be obtained by using survival probability as dependent variable and using age and Alb as independent variable. MA score was 0.02Alb - 0.01age + 0.45. MA score was presented as the value of survival probability. MA score was < 0.5 in 94.3% of the ICU patients with chronic disease. For normal individuals an MA score < 0.5 was found in 5.1%. The percentage of patients an MA score < 0.50 was considerably higher in cancer, COPD, and cardiocerebrovascular diseases groups than in the elderly group, although the chronological age of elderly group was similar with the diseases groups. Conclusions: When considering death, the MA score is suitable for assessing the pre-chronic disease and health status at the molecular level and could provide a simple and effective tool for the early diagnosis and management of chronic conditions.


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KEY WORDS
biological age, biomarkers, chronic disease, risk assessment, clinical chemistry

LIST OF ABBREVIATIONS

ALB - albumin
ALT - alanine aminotransferase
AST - aspartate aminotransferase
AUC - area under the curve
BA - biological age
Chol - cholesterol
COPD - chronic obstructive pulmonary disease
CRE - creatinine
DB - direct bilirubin
Glu - glucose
GGT - γ-glutamyl transpeptidase
Hb - hemoglobin
HDL - high-density lipoprotein
ICU - intensive care unit
LDL - low-density lipoprotein
Lym - lymphocyte
Neut - neutrophil count
MA - molecular age
PCDS - pre-chronic disease stage
Plt - platelet count
RBC - red blood cell
ROC - receiver operating characteristic
TB - total bilirubin
TP - total protein
TRIG - triglyceride
UA - uric acid
WBC - white blood cell

INTRODUCTION

Most aged people have chronic diseases that eventually lead to death; therefore, it is important to establish a method for evaluating aging by using biomarkers. This is also a challenge for laboratory medicine. Biological age (BA) is a composite index of physical conditions. It is an objective expression of aging and can be determined using a variety of models [1-3]. BA is based on an assessment of the degree of weakening with age and the amount of lifetime left [4-6]. BA is determined from physiological indexes, such as the blood pressure [7], pulmonary function [8,9], physical frailty [10-12], heart rate [13], and from psychological variables [14-16], which are known to change with time. However, these indexes do not necessarily reflect the internal physiological conditions, and they may not be directly associated with the development and progression of death. Therefore, a BA assessment system based on the above indexes may not be sensitive for determining the risk of death or chronic diseases. There are differences in the molecular mechanisms associated with aging, disease, and death; therefore, the biomarkers for aging, disease, and death may also be different. Nevertheless, there are intrinsic connections between these three processes, so a biomarker that is common to these processes could be used as an indicator of the biological process of aging and predisposition to disease. Next, selection criteria of biomarkers should be applied as follows: (1) measurement reliability and feasibility; (2) biomarker could reflect an aging process; (3) the biomarker could predict mortality or disease event better than chronological age, and (4) responsiveness to intervention [17]. Because most aged people also have chronic diseases that eventually lead to death, the aged group that we observed were mostly in the early stages of chronic diseases or had better health. Actual data related to aging cannot be obtained from the aged group. In previous studies, evaluation of biomarkers did not consider dead individuals from diseases and only considered survival individuals, who were usually in the early stages of chronic diseases or had better health, resulting in the bias. Therefore, this paper uses end-of-life patients to adjust observed data and to construct a molecular age score for grading from health, disease to end-of-life. So far, a combination of the above three processes (i.e., aging, disease, and death) has not been used for the evaluation of biomarkers and determination of biological age at the molecular level (molecular age, MA) in most previously reported studies, although clinical laboratory indexes and molecular biological biomarkers have been used for evaluating BA [18-20] and general health conditions [21-23]. The pre-chronic disease stage (PCDS) is characterized by molecular-level changes that occur before organic impairment and are associated with chronic disease (Figure 1) [24]. Because aging plays a major role in the development of chronic diseases [25,26], aging at the molecular level should be the first indicator of PCDS. Thus, a MA assessment system based on biomarkers common to aging, disease and death may be able to reflect aging changes early and predict the occurrence of chronic diseases during old age. More importantly, MA-based biomarkers may also be able to reflect antiaging intervention [27]. Our previous study found that several commonly used clinical laboratory indexes, including blood biochemistry and blood cell count can be used as end-of-life markers, which could predict survival time in terminally ill patients [4-6], it may be possible to establish a MA assessment system for evaluating the risk of age-related diseases (pre-chronic disease status). In this study, we evaluated MA by using biomarkers sensitive to both aging and end-of-life processes in order to evaluate the risk of age-related diseases or pre-chronic diseases.

MATERIALS AND METHODS

The basic principle of establishing a system for assessing molecular age (MA) at the molecular level is to comprehensively consider levels of biochemical markers from survival part and dead part at an age and calculate survival probability from biochemical markers as molecular age score for assessing the development of chronic diseases as shown in Figure 2.

Patient and public involvement

All procedures involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Institutional Ethics Committee of Dalian Medical University approved the study and waived the need for written informed consent from the participants due to the observational nature of the study. About 2,000 subjects were screened for enrollment in this study.
Younger group
The younger group (control group) consisted of 197 individuals (95 males and 102 females) between 20 and 30 years of age, who had visited the hospital for routine health check-up examinations and not for specific medical treatment for their disease, and individuals who did not have a history of long-term medication. The exclusion criteria were the presence of chronic diseases including coronary and cerebrovascular disease, COPD and cancer, according to the medical records. Individuals with hepatitis were also excluded.

Elderly group
This group consisted of 198 individuals (105 males and 93 females). The group included individuals aged over 80 years who had visited the hospital for routine health check-up examinations and not specific medical treatment for their disease. The other inclusion and exclusion criteria were the same as those for the elder group.

ICU patients
The intensive care unit (ICU) group consisted of 106 patients (61 males and 45 females), who had a mean age (± SD) of 75.6 ± 8.1 years. This group included critically ill patients who underwent treatment at the ICU of the Second Affiliated Hospital of Dalian Medical University. The inclusion criteria were the presence of multiple chronic diseases [mainly those with coronary and cerebrovascular disease, cancer and chronic obstructive pulmonary disease (COPD)] and death within one month (mean survival time: 7.6 days) according to the medical records. The exclusion criteria were accidental injuries and no record of the time of death.

Health groups
These groups consisted of individuals between 55 and 75 years of age for establishing the model. Each subgroup consisted of 10 males and 10 females. The other inclusion and exclusion criteria were the same as those for the elderly group.

Disease group and control group
The disease group included three subgroups: cancer, coronary heart disease, and COPD. The cancer group included 274 randomly selected preoperative tumor patients (183 men and 91 women, 63.2 ± 8.1 years), mainly those with gastrointestinal tumor (excluding liver cancer) and lung cancer (as evidenced by the surgical intervention and/or pathologic findings recorded). Only the preoperative and pretreatment data for these patients were used. The coronary and cerebrovascular group included 144 patients (57 men and 87 women, 64.9 ± 8.4 years) with coronary heart disease (as evidenced by the coronary angiography and/or coronary CT findings), cerebral thrombosis, and cerebral hemorrhage (as evidenced by the MRI and/or CT findings). All the patients were first-onset patients who had not undergone any specific medical treatment for their cardiocerebrovascular condition and who did not have a history of long-term medication. The COPD group was composed of 165 inpatients (55 men and 110 women, 67.6 ± 13.7 years). We included individuals diagnosed with stage II to IV COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria [28]. The control group consisted of 721 individuals (405 males and 307 females) between 50 and 80 years of age, who visited the hospital for regular medical check-ups. Their mean age (± SD) was 65.0 ± 10.3 years. The inclusion and exclusion criteria were the same as those for the health group as mentioned above.

Blood analyses
Routine biochemical lab tests and complete blood count were selected in clinical laboratory. Blood samples (3.5 mL) were drawn from the antecubital vein from each group after overnight fasting between 8:00 - 9:00 in the morning. The collected blood was put in the separation gel coagulation accelerator tube and centrifuged to separate serum. Enzyme levels, liver synthesis function, liver metabolic function, blood lipid levels, blood glucose level, renal function were assessed. Alanine aminotransferase (ALT, 9 - 50 U/L), aspartate aminotransferase (AST, 15 - 40 U/L), γ-glutamyl transpeptidase (GGT, 10 - 60 U/L), urea (2.8 - 7.1 mmol/L), creatinine (CRE, 50 - 120 μmol/L), uric acid (UA, 208 - 428 μmol/L), total bilirubin (TB, 2 - 20 μmol/L), and direct bilirubin (DB, 0 - 6 μmol/L) were measured with a Hitachi automatic biochemistry analyzer. Total protein (TP, 65 - 85 g/L), albumin (ALB, 40 - 55 g/L), cholesterol (Chol, 2.80 - 5.20 mmol/L), high-density lipoprotein (HDL, 0.8 - 3.26 mmol/L), low-density lipoprotein (LDL, < 3.12 mmol/L), triglyceride (TRIG, 0.22 - 2.29 mmol/L), and glucose (Glu, 3.60 - 6.10 mmol/L) were measured with a Hitachi 7170s automatic biochemistry analyzer. Blood samples of the participants were also drawn from the antecubital vein and anticoagulated with ethylenediaminetetraacetic acid (EDTA) for the complete blood count. The complete blood count parameters including red blood cell (RBC, 3.80 ~ 5.10 10^{12}/L), hemoglobin (Hb, 115.00 ~ 150.00 g/L), white blood cell (WBC, 3.50 ~ 9.50 10^{9}/L), neutrophil count (Neut, 1.80 ~ 6.30 10^{9}/L), lymphocyte (Lym, 1.10 ~ 3.20 10^{9}/L), platelet count (Plt, 125.00 ~ 350.00 10^{9}/L) were measured with an automated hematology analyzer (SF-3000; Sysmex, Tokyo, Japan). The investigations were carried out in the clinical laboratory of our university hospital using standard commercial reagent kits. The coefficient of intra-assay variation was lower than 5% for each item assayed.

Indicator selection
ROC analysis was used for screening the indexes. An area under the curve (AUC) of 0.5 indicated that the index was not significant. When the ROC-AUC value was < 0.5, it was subtracted from 1 (1 - AUC) to facilitate...
comparison. Indexes with ROC-AUC values > 0.75 or ROC-AUC values < 0.25 were considered significant. Both aging-sensitive and end-of-life-sensitive indexes were selected as candidates.

**Biological variation**

Biological variation was considered as evaluation parameter for valid biomarkers. A total of 5 female and 5 male young adult volunteers were recruited as subjects (20 ~ 23 years old). Nutritionally balanced meals were provided throughout the protocol. Subjects have normal sleep during the night, and if a subject was unable to stay asleep for more than 7 hours, the data were discarded.

Subjects were investigated from 07:00 on day 1 until 07:00 on day 2; blood samples were drawn on day 1 and day 2 at 07:00. According to the protocol, all data were valid. The biological variation was calculated as follow [29]:

\[ BV = \sqrt{\frac{\sum (D1 - D2)^2}{n}} / \frac{\sum D1}{n} \]

where BV represents biological variation; D1 and D2 represents data from day 1 and day 2, respectively; n represents sample counts. A biomarker with BV < 0.1 was considered a valid biomarker for MA.

**Establishing molecular age score**

Health and ICU groups as mentioned above were used to establish molecular age score. The mean of significant MA biomarkers selected according to the protocol should be calculated in each group. It is assumed that the people belong to the group of 75 years for their life expectancy [30], the survival probability of this group is 0.50, and that data from ICU group could represent death data; thus, the actual mean of significant MA biomarker at 75 years should be mean between mean of 75 years and ICU group. Mortality should increase mark communicable disease [25], therefore, it is also assumed that the survival probability of 55 years is 0.95. Thus, regression equation of MA score could be obtained by using survival probability as dependent variable and using age and mean of biomarker at 55, 75, and 95 (ICU group) as independent variables. That MA score < 0.50 was suggested as a PCDS.

**Statistical analyses**

The significance of the differences between the groups was determined using one-way ANOVA for continuous data and using a chi-squared test for binary data. Analyses were undertaken using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). A difference was considered statistically significant when the p-value was less than 0.05 (by a two-tailed test).

**RESULTS**

The findings from receiver operating characteristic (ROC) analysis for the biochemical indexes and the complete blood count for the elderly, younger, and ICU groups were shown in Figure 3 and 4 and in Table 1 and 2. The two indicators showed correlation with aging and end-of-life (ICU group); therefore, they were suitable as indexes of MA.

The findings from BV of candidate biomarkers for one day were shown in Table 3. The urea with BV was > 0.1 and Alb with BV was < 0.1. Therefore, Alb was suitable as index of MA.

The relation between Alb and survival probability at key age groups was summarized in Table 4. MA score could be obtained by using survival probability as dependent variable and using age and Alb as independent variables.

\[ MA = 0.02Alb - 0.01age + 0.45 \]

The relationships between different age groups and MA scores were shown in Figure 5. The MA score decreased and presented a linear quantitative relationship with age increase (r = 0.996, p = 0.004).

MA scores in different chronic disease groups were shown in Table 5. There were significant differences in MA score between disease and control groups (p < 0.05). An MA score < 0.50 was suggested as a PCDS. The percentage of patients in the PCDS (MA score < 0.50) was considerably lower in each of the disease groups than in the control group (p < 0.05). The data were also shown in detail in Table 5.

**DISCUSSION**

Although aging is one of the most important risk factors for most chronic diseases, we believe that aging process and the occurrence of chronic diseases may be independent of each other [31]. Therefore, adopting general aging indexes such as skin wrinkling or graying of the hair to predict disease occurrence may not be ideal. In this study, end-stage patients with various diseases were enrolled to select and establish indexes for MA. MA score was established on the basis of survival probability rather than calendar age. With the help of biomarkers and calendar age, MA score at the molecular level should be more accurate than aging phenotypes and calendar age.

Biomarkers generally are non-linear in aging, diseases, and death [32]. Two observed indexes in our study were found to be suitable as both end-of-life and aging markers and suitable as MA on the basis of population data. However, the stability in an individual, such as BV, is one important reason for MA score working at the molecular level, we therefore finally selected Alb as an index of MA or PCDS.

Routine biochemical lab tests were used in this study as in other reports [33,34]. Quality control is considered to be important in the clinical context, and close attention
Table 1. Original data of ROC analysis of biochemical indexes in the elderly, younger, and end-of-life groups.

<table>
<thead>
<tr>
<th>Indexes</th>
<th>Quartiles (25th, 50th, 75th)</th>
<th>ROC-AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Elderly (1)</td>
<td>Younger (2)</td>
</tr>
<tr>
<td>ALT</td>
<td>13.97, 19.00, 23.59</td>
<td>12.00, 16.00, 26.00</td>
</tr>
<tr>
<td>AST</td>
<td>21.00, 25.40, 30.00</td>
<td>18.00, 21.00, 25.00</td>
</tr>
<tr>
<td>GGT</td>
<td>14.50, 19.21, 26.00</td>
<td>13.00, 16.64, 25.00</td>
</tr>
<tr>
<td>TP</td>
<td>68.48, 71.10, 74.50</td>
<td>70.30, 73.2, 75.97</td>
</tr>
<tr>
<td>TP</td>
<td>68.48, 71.10, 74.50</td>
<td>70.30, 73.2, 75.97</td>
</tr>
<tr>
<td>ALP</td>
<td>41.34, 42.96, 44.33</td>
<td>45.10, 46.40, 48.28</td>
</tr>
<tr>
<td>TB</td>
<td>11.15, 13.15, 15.93</td>
<td>10.49, 13.50, 17.39</td>
</tr>
<tr>
<td>DB</td>
<td>3.10, 4.00, 5.33</td>
<td>3.53, 4.53, 5.86</td>
</tr>
<tr>
<td>UREA</td>
<td>5.15, 6.07, 7.09</td>
<td>3.66, 4.23, 5.2</td>
</tr>
<tr>
<td>CREA</td>
<td>66.77, 76.00, 90.00</td>
<td>57.77, 67.77, 80.88</td>
</tr>
<tr>
<td>UA</td>
<td>302.06, 349.00, 407.50</td>
<td>267.50, 332.00, 408.42</td>
</tr>
<tr>
<td>GLU</td>
<td>5.33, 5.77, 6.75</td>
<td>4.92, 5.19, 5.49</td>
</tr>
<tr>
<td>CHOL</td>
<td>4.22, 4.80, 5.69</td>
<td>3.93, 4.50, 4.93</td>
</tr>
<tr>
<td>TRIG</td>
<td>1.01, 1.41, 1.86</td>
<td>0.73, 1.05, 1.44</td>
</tr>
<tr>
<td>HDL</td>
<td>1.04, 1.20, 1.44</td>
<td>1.06, 1.25, 1.45</td>
</tr>
<tr>
<td>LDL</td>
<td>2.29, 2.83, 3.51</td>
<td>2.18, 2.67, 3.07</td>
</tr>
</tbody>
</table>

Boldface: candidate biomarker.

Table 2. Original data of ROC analysis of complete blood count in the elderly and younger groups.

<table>
<thead>
<tr>
<th>Indexes</th>
<th>Quartiles (25th, 50th, 75th)</th>
<th>ROC-AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Elderly (1)</td>
<td>Younger (2)</td>
</tr>
<tr>
<td>WBC</td>
<td>5.15, 5.98, 7.03</td>
<td>5.14, 5.97, 7.20</td>
</tr>
<tr>
<td>Neut</td>
<td>2.84, 3.50, 4.29</td>
<td>2.61, 3.31, 4.05</td>
</tr>
<tr>
<td>Lym</td>
<td>1.47, 1.89, 2.29</td>
<td>1.79, 2.20, 2.61</td>
</tr>
<tr>
<td>RBC</td>
<td>4.22, 4.45, 4.78</td>
<td>4.48, 4.81, 5.13</td>
</tr>
<tr>
<td>Hb</td>
<td>129.00, 137.00, 146.00</td>
<td>134.00, 145.00, 156.00</td>
</tr>
<tr>
<td>Plt</td>
<td>160.00, 191.00, 219.25</td>
<td>203.00, 231.00, 266.00</td>
</tr>
</tbody>
</table>

Table 3. The original data and biological variation of candidate biomarkers for one day.

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Mean ± SD</th>
<th>Biological variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>Alb</td>
<td>47.040 ± 2.082</td>
<td>47.540 ± 1.218</td>
</tr>
<tr>
<td>Urea</td>
<td>3.936 ± 0.734</td>
<td>4.132 ± 0.477</td>
</tr>
</tbody>
</table>
Table 4. The relationship between albumin and survival probability at key age groups.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Survival probability</th>
<th>Albumin Average</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>0.95</td>
<td>44.32 ± 1.93</td>
<td>44.32</td>
</tr>
<tr>
<td>75</td>
<td>0.50</td>
<td>43.23 ± 1.60</td>
<td>(43.23 + 29.44) / 2 = 36.34</td>
</tr>
<tr>
<td>95</td>
<td>0.05</td>
<td>29.44 ± 5.62 *</td>
<td>29.44</td>
</tr>
</tbody>
</table>

* Albumin in ICU group.

Table 5. Percentage of patients in the pre-chronic disease state (i.e., MA score < 0.50) in each group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age</th>
<th>Number of patients</th>
<th>MA score (25th, 50th, 75th)</th>
<th>Patients with MA score &lt; 0.5 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary and cerebrovascular</td>
<td>64.9 ± 8.4</td>
<td>144</td>
<td>0.54, 0.63, 0.71</td>
<td>17.4</td>
</tr>
<tr>
<td>disease patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer patients</td>
<td>63.2 ± 8.1</td>
<td>274</td>
<td>0.52, 0.63, 0.73</td>
<td>21.5</td>
</tr>
<tr>
<td>COPD patients</td>
<td>67.9 ± 13.7</td>
<td>165</td>
<td>0.47, 0.60, 0.74</td>
<td>30.9</td>
</tr>
<tr>
<td>Control</td>
<td>65.0 ± 10.3</td>
<td>712</td>
<td>0.58, 0.66, 0.80</td>
<td>5.1</td>
</tr>
<tr>
<td>ICU patients</td>
<td>75.6 ± 8.1</td>
<td>106</td>
<td>0.19, 0.29, 0.39</td>
<td>94.3</td>
</tr>
</tbody>
</table>

| p-value                             | < 0.001       | < 0.001            |                             |                                 |

Figure 1. Pre-chronic disease status and its association with life processes.
Figure 2. Evaluation of molecular age with considering survival probability, illustrated by albumin (ALB).

Figure 3. ROC curve for the albumin between the elderly and younger groups (A) and between the elderly and end-of-life groups (B).
Figure 4. ROC curve for the urea between the elderly and younger groups (A) and between the elderly and end-of-life groups (B).

Figure 5. The relationship between different age groups and MA score.
is paid to it in each clinical laboratory. It is beneficial for comparing measured results between different times and laboratories. ALB is synthesized by the liver. Its synthesis is reduced and consumption is increased during aging and end-of-life processes, which are therefore associated with lower serum ALB levels [35-37]. Therefore, based on their biological mechanism, ALB is an independent indicator and suitable as a variable for constructing a model to determine MA.

Typically, BA is determined with the help of a set of age-dependent variables that are used as independent variables and chronological age as a dependent variable [38,39]. Values obtained from an algorithm, such as multiple linear regression, can be interpreted as BA [39, 40]. Unfortunately, there have not been any generally accepted algorithms until now [41,42]. We screened the biomarker with end-of-life patients, elderly, and young groups to construct a MA for grading from health, disease to end-of-life. Ideally, health status or aging process should be assessed based on the MA rather than calendar age; therefore, using the MA may be more appropriate than the common method.

We trust MA has translational significance as it is, to our knowledge, the first report of the concept of MA with considering death (survival probability), which could make medical professionals more aware of the repercussions of their performance on medical decisions at the molecular level in early stage of disease occurrence rather than that stage of histopathological impairments. The MA could also be used as an independent dimension to describe chronic diseases at the molecular level.

If the life expectancy is presumed to age 75, its survival probability of this age group after birth should be 50%. Basing the calculation on this, the actual mean of ALB at 75 years should be the mean between the mean of 75 years and of end-of-life (ICU) groups. Mortality should increase markedly when a population exceeds the age of 55 for non-communicable disease [25]; therefore, it is also assumed that the survival probability of 55 years is 0.95. Thus, the regression equation of MA score could be obtained by using survival probability as the dependent variable and using age and mean of biomarker at 55, 75, and 95 (ICU group) as independent variables. We found that the MA score presented a linear quantitative relationship with age increase, implying that the adopted survival probability as MA score for the construction was valid.

MA score was presented as the value of survival probability in this study. MA score < 0.50 was considered to be PCDS at the molecular level, but not pathomorphologic disease stage. MA score was < 0.50 in approximately 95% of the ICU patients with chronic disease; however, this value was only found in 5.1% of the healthy elderly group. Further to verify MA, sample sizes in the cancer, coronary heart disease, and COPD groups were over 100 for each. Results showed that MA score was significantly lower in each disease group than that in the control group. The results could be accepted due to relatively large sample size, and it implied that MA score was reasonable.

MA score indicated that PCDS was lower in the disease group than in the ICU group. This is probably because most of the specimens were collected from individuals with mild disease, as patients with more severe disease had either entered the end-of-life stage or died. At the end of the disease, the percentage of patients in the PCDS should be 100%; this is in keeping with our interpretation, to some extent. Therefore, MA score < 0.50 in normal individuals without pathomorphologic features implies a higher risk of chronic disease.

Cancer, COPD, and cardiocerebrovascular diseases are responsible for more than 80% of disease-related deaths in China [43]. Our findings indicate that the percentage of patients in PCDS (MA score < 0.50) was considerably higher in the above three disease groups than in the elderly group, although the chronological age of elderly group was similar with disease groups. Therefore, our findings indicate that the MA evaluation system used here may be beneficial for describing PCDS at the molecular level. In our opinion, during the PCDS that precedes pathological damage caused by the disease, appropriate interventions are likely to postpone and reverse the pre-chronic diseases status. Evidently, a MA system may be useful for early diagnosis. Unlike pathologic alterations, changes in molecular levels (description with MA) are largely reversible [44]; therefore, MA could reflect the clinical importance of the diagnosis of PCDS and management of chronic conditions.

One of the main limitations of this study is that only Alb was selected for assessing PCDS; therefore, the study can be considered to be preliminary. However, we believe that one highly-effective biomarker is better than a combination of more less effective biomarkers. Our findings still suggest that (1) biological age at the molecular level can provide a better understanding of PCDS, and (2) the available method with considering death has been developed to assess pre-chronic disease. It should be pointed out that MA score cannot be considered to represent the actual survival probability, and it is therefore designated as the MA score.

In conclusion, biomarkers that were significantly different both between the end-of-life patients and the elderly groups and between the young and elderly groups were chosen to establish a MA at the molecular level. Thus, the aging process could be assessed based on MA rather than calendar age. Results showed that the established MA system comprising ALB provides a simple and effective method for evaluating the health status and PCDS at the molecular level. The MA construct established in this study can also be used as an independent dimension to describe the characteristics of chronic disease at the molecular level, and combined with clinical manifestations, MA can be used to comprehensively assess diseases from new systems. Further studies are warranted to develop a more feasible and measurable marker than ALB, such as endocrine parameters, for accurate prediction of the degree of aging and PCDS.
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Authors’ Contributions:
Liu Hui and Yang Guang conceived the analysis and wrote the final version of the manuscript. Li Yuzhong provided technical support on the method.

Ethics Approval:
The Institutional Ethics Committee of Dalian Medical University approved the study and waived the need for written informed consent from the participants due to the observational nature of the study (2019-3-5).

Declaration of Interest:
None declared

References:


