# BIOLOGICAL AGE ESTIMATION OF THE PARTICIPANTS IN THE 32-ND BULGARIAN ANTARCTIC EXPEDITION

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**Abstract:** It is assumed that Biological Age better evaluates the physiological deterioration of the organism than chronological age. Specific blood biomarkers have shown the ability to detect differences in Biological Age, even in young and healthy individuals, before the development of disease or phenotypic manifestations of accelerated aging. This work aimed to calculate the Biological Age of the participants in the 32nd Bulgarian Antarctic Expedition, assessing their objective physiological state and formatting recommendations in case of increased levels of health risk. Traveling to Antarctica and staying there is a prerequisite for a rise of the participants' stress levels, related to logistics, the change of the social environment, and work in adverse climatic conditions. Data indicated that exposure to stress increased the Biological Age in humans, but it decreased after the stress resolved. This research involved 28 individuals, 22 men, and 6 women, at a mean age of 43.04 ± 8.19 years (between 26 and 56 years old). All participants resided at the Bulgarian Antarctic base "St. Kliment Ohridski" at Livingstone Island for 30 days. The journey to the base involved several connecting flights passing through different time zones, and finally, with the Bulgarian naval research vessel "St. Cyril and Methodius" (RSV 421) across the Drake Passage and in the reverse order when returning. Blood samples were taken from the participants twice (before and after the expedition). Sera was obtained after natural coagulation and centrifugation at 3000 rpm per 20 min. and the following markers were measured in it: White Blood Cells (WBC), Mean Corpuscular Volume (MCV), Red Cell Distribution Width (RDW), Lymphocytes% (LYM%), Glucose (Glu), Creatinine (Crt), Albumin (Alb), Alkaline Phosphatase (ALP), and C-Reactive Protein (CRP). Biological Age and the 10-year Mortality Risk were calculated according to the formula of Levine et al., (2018) with the correction made by Liu et al. (2018). The results showed a variation of the Biological Age values between 10.3 years younger and 8.4 years older (mean 3.52 ± 4.06 years younger) than the chronological age of the individuals before the expedition and 15.7 years younger and 1.3 years older (mean 6.02 ± 4.05 years younger) than the chronological age of the individuals after returning. Concerning the 10-year Mortality Risk, the results showed a variation between 0.26% and 5.49% (mean 2.05 ± 1.44%) before the expedition and between 0.24% and 4.33% (mean 1.73 ± 1.14%) after the expedition. Both indicators decreased statistically significantly after the expedition, with about 2.5 years for the Biological Age and about 0.3% for the 10-year Mortality Risk. In conclusion, the obtained results indicated that participation in the Bulgarian Antarctic expeditions does not lead to an increase in Biological Age and 10-year Mortality Risk. In this study, the Biological Age of participants in an Antarctic expedition was calculated for the first time. Likely, the results for Biological Age and 10-year Mortality Risk in other more severe conditions at the Antarctic mainland closer to the Pole may differ significantly. It can be recommended that blood samples for calculating the Biological Age be also taken during the stay at the polar base, which will allow a complete picture of the stressful conditions of polar expeditions to be obtained.
**Keywords:** Biological Age, blood markers, Bulgarian Antarctic Expedition, Mortality Risk.

## 1. INTRODUCTION

It is assumed that Biological Age better evaluates the physiological deterioration of the organism than chronological age (Bortz et al., 2023). Various methods have been proposed for estimating Biological Age: telomere length, DNA-methylation, proteomics, metabolomics, glycomics, wearable sensor data, and blood-based clinical biomarkers (Jylhävä et al., 2023; Horvath & Raj, 2018; Frenck et al., 1998; Macdonald-Dunlop et al. 2022; Krištić et al. 2014; Pyrkov et al., 2021). Epigenetic clocks, especially Horvath's clock and its variants, are considered the most accurate and cutting-edge methods for determining Biological Age. However, blood biomarker-based methods are more readily available and inexpensive, making them extremely practical for continuous monitoring (Bortz et al., 2023). Specific blood biomarkers have shown the ability to detect differences in Biological Age, even in young and healthy individuals, before the development of disease or phenotypic manifestations of accelerated aging (Jia et al., 2017). The method of Levine et al. (2018), based on the multiple correlation of several biochemical and hematological indicators with the Biological Age determined by DNA methylation, has gained wide application in recent years. Some clinical laboratories even offer online calculators for computing Biological Age using this method. The Biological Age is not a constant. Data have shown that Biological Age undergoes a rapid increase in response to diverse forms of stress, which is reversed following recovery from stress (Poganik et al., 2023). The elevation of Biological Age by stress may be quantifiable, which allows various therapeutic substances and strategies to be evaluated to improve people's healthspan (Moqri et al., 2023). Traveling to Antarctica and staying there is a prerequisite for a rise of the participants' stress levels related to logistics, change of the social environment, and work in adverse climatic conditions. Thus, the present work aimed to calculate the Biological Age of the participants in the 32nd Bulgarian Antarctic Expedition, assessing their objective physiological state and formatting recommendations in case of increased levels of health risk.

## 2. MATERIALS AND METHODS

**Participants**

In the present work took part 28 individuals, 22 men, and 6 women, at a mean age of 43.04 ± 8.19 years (between 26 and 56 years). All participants have travelled from Bulgaria by plane to South America, and from there by ship crossing the Drake Passage to Livingston Island (62°36′S 60°30′W 62.600°S 60.500°W) part of the South Shetlands Archipelago, Antarctica, resided at the Bulgarian Antarctic base "St. Kliment Ohridski" at Livingstone Island for 30 days and went back to Bulgaria. All participants were informed of the objectives of the study and signed a Consent for voluntary participation in it, according to the Helsinki Declaration for Ethical Principles for Medical Research involving Human Subjects.

**Hematological analyses**

Blood samples were taken from the participants twice (before and after the expedition). Blood was drawn by a laboratory technician. Sera was obtained after natural coagulation of the obtained blood and centrifugation at 3000 rpm per 20 min. In the sera were measured the following markers: White Blood Cells (WBC), Mean Corpuscular Volume (MCV), Red Cell Distribution Width (RDW), Lymphocytes% (LYM%), Glucose (Glu), Creatinine (Crt), Albumin (Alb), Alkaline Phosphatase (ALP), and C-Reactive Protein (CRP) in licensed Laboratory.

**Biological Age and the 10-year Mortality Risk calculation**

Biological Age and the 10-year Mortality Risk were calculated according to the formula of Levine et al. (2018) with the correction made by Liu et al. (2018). This calculation involved three steps. First, an auxiliary index "xb" was calculated by a linear equation with a constant term (-19.907) and multiplication of the measured values of the blood indicators with specific coefficients (Equation 1). Second, the obtained "xb" was used in Equation 2 to calculate the 10-year Mortality Risk. Third, the Biological Age was calculated using Equation 3.

Equation 1:

$$\begin{aligned} xb = -19.907 &- 0.0336 \times \mathit{Albumin} + 0.0095 \times \mathit{Creatinine} + 0.1953 \times \mathit{Glucose} \\ &+ 0.0954 \times \ln(\mathit{CRP}) - 0.0120 \times \mathit{Lymphocyte\ Percent} \\ &+ 0.0268 \times \mathit{Mean\ Cell\ Volume} + 0.3306 \times \mathit{Red\ Cell\ Distribution\ Width} \\ &+ 0.00188 \times \mathit{Alkaline\ Phosphatase} + 0.0554 \times \mathit{White\ Blood\ Cell\ Count} \\ &+ 0.0804 \times \mathit{Chronological\ Age} \end{aligned}$$

Equation 2:

$$\text{10-year Mortality Rate} = 1 - \exp\left(\frac{-1.51714 \times \exp(\text{xb})}{0.0076927}\right)$$

Equation 3:

$$\text{Biological Age} = 141.50 + \frac{\ln[-0.00553 \text{ x } \ln(1\text{-10-year Mortality Rate})]}{0.09165}$$

**Statistics**

The obtained results were processed with the statistical package SPSS 26 (IBM, USA). Descriptive statistics were performed, and the minimum, maximum, and mean values and their standard deviation were presented. The distribution normality was checked with the Shapiro-Wilk test and, according to the result for each parameter, was checked the significance of the differences in the mean values by either the parametric Student's test for dependent samples or the non-parametric Wilcoxon test for dependent samples.

## 3. RESULTS

Table 1 presents the values of biochemical parameters measured in blood serum, as well as the Biological Age and 10-year Mortality Risk calculated on their basis. RDW, LYM%, Crt, ALP, and calculated Biological Age showed a normal distribution according to the Shapiro-Wilk test. The LYM%, ALB, and the calculated indicators Biological Age, 10-year Mortality Risk, and the average difference between chronological and Biological Age were statistically significant.

***Table 1. Biochemical indicators determined the Biological Age and 10-year Mortality Risk measured before and after the expedition.***

| | Before expedition | | | After expedition | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Min** | **Max** | **Mean ± SD** | **Min** | **Max** | **Mean ± SD** | **Distribution** | **Significance** |
| **WBC [G/L]** | 3.8 | 20.3 | 7.0 ± 3.1 | 3.9 | 13.3 | 7.01 ± 2.11 | | 0.611 |
| **MCV [fL]** | 78.4 | 107.3 | 88.7 ± 5.52 | 77.9 | 103.7 | 89.09 ± 4.86 | | 0.649 |
| **RDW [%]** | 11.5 | 14.7 | 12.7 ± 0.76 | 11.4 | 13.7 | 12.69 ± 0.49 | Normal | 0.935 |
| **LYM% [%]** | 21.3 | 50.4 | 33.96 ± 6.95 | 22.1 | 50.2 | 34.2 ± 6.7 | Normal | **0.038** |
| **Glu [mmol/L]** | 4.5 | 7.3 | 5.38 ± 0.68 | 4.4 | 7.7 | 5.3 ± 0.75 | | 0.586 |
| **Crt [umol/L]** | 58.0 | 100.0 | 82.18 ± 11.94 | 62.0 | 103.0 | 84.89 ± 10.85 | Normal | 0.081 |
| **Alb [g/L]** | 43.0 | 51.7 | 47.97 ± 2.38 | 39.8 | 52.0 | 44.63 ± 2.55 | | **0.001** |
| **ALP [U/L]** | 48.0 | 103.0 | 72.32 ± 16.29 | 45.0 | 120.0 | 73.54 ± 16.64 | Normal | 0.513 |
| **CRP [mg/L]** | 0.2 | 71.9 | 5.01 ± 13.31 | 0.3 | 13.9 | 2.94 ± 3.48 | | 0.495 |
| **Mortality [%]** | 0.26% | 5.49% | 2.05 ± 1.44% | 0.24% | 4.33% | 1.73 ± 1.14% | | **0.020** |
| **BioAge [Years]** | 19.8 | 53.4 | 39.52 ± 8.8 | 16.8 | 49.3 | 37.02 ± 8.55 | Normal | **0.001** |
| **Delta* [Years]** | -10.3 | 8.4 | -3.52 ± 4.06 | -15.7 | 1.3 | -6.02 ± 4.05 | Normal | **0.001** |

Delta* – the difference between chronological and calculated Biological Age

The results showed that before the expedition, the Biological Age values varied between -10.3 years and +8.4 years from the chronological age. Thus, according to the calculated mean value of the Biological Age, the participants were younger, by 3.52 ± 4.06 years, than their chronological age. After the expedition, the Biological Age values varied from -15.7 to +1.3 years in comparison to the chronological age of the individuals. Therefore, after returning, the mean value of this difference almost doubled (-6.02 ± 4.05 years younger) compared to the values before the expedition (-3.52 ± 4.06 years younger). Concerning the 10-year Mortality Risk, the results showed a variation between 0.26% and 5.49% (mean 2.05 ± 1.44%) before the expedition and between 0.24% and 4.33% (mean 1.73 ± 1.14%) after the expedition. Both indicators decreased statistically significantly after the expedition, with about 2.5 years for the Biological Age and about 0.3% for the 10-year Mortality Risk.

## 4. DISCUSSIONS

In this study, the Biological Age of participants in an Antarctic expedition was calculated for the first time. It has been suggested that the Biological Age may reflect the effect of different stressful factors and evaluate better the physiological deterioration of the organism than the chronological age. Conducting research and scientific work in the harsh conditions of Antarctica is associated with physical and psychological stress for researchers on this continent. Several studies have shown the presence of signs of psychological stress in polar explorers, incl. among the Bulgarian Antarctic expeditions participants, especially at the beginning of the expedition and in people with little expedition experience (Domuschieva-Rogleva et al., 2017; Iancheva et al., 2023). Stress can also be caused by hard physical work and severe weather conditions. Articles reflecting on the effects of extreme conditions on the health of Antarctic researchers examine stressors such as the effects of low temperatures, ultraviolet light, high-altitude hypoxia, which can lead to sleep disturbances, changes in endocrine secretion, immune function, circadian rhythms, mood, etc. Evidence for these processes has been reported primarily in crews of Antarctic wintering expeditions (Palinkas et al., 2008; Nicolas et al., 2022; Sandal et al., 2018). Most of these changes, although a result of adaptation to the extreme conditions of Antarctica, can have an impact on human health, especially in more extreme conditions and longer residence in unfavorable conditions. Levels of the adrenal steroid hormone cortisol are a widely accepted marker of stress because they rise significantly after physical and emotional stress. Various studies have reported variations in cortisol levels in Antarctic expedition participants depending on the specific conditions. Like our results, studies at the German station Neumayer III (at sea level) found that despite stressful conditions, cortisol levels remained stable in expedition participants (Strewe et al., 2018). This may be due to the way stress interacts with other hormonal and neuroendocrine responses in different environments. Kern et al. (2019) found that cortisol showed a moderate increase from before to after the Antarctic expedition and concluded that the effect of explorers' stress was moderate. The other study showed a modest reduction in cortisol (Johnsen et al., 2020; 2023). The study by Zabara et al. (2021) found that there were no significant health complications during the expeditions. Neither reactivated nor primary viral infections, as well as clinical autoimmune, were registered. The polar environment induced increased stress but did not involve either clinical manifestations or elements of immunosuppression (Zabara et al., 2021). In the present study, although it was statistically insignificant, we found almost two-fold lower mean CRP values (Table 1), suggesting a reduction in the inflammatory state. Similar results for the reduction of pro-inflammatory markers have been reported by other authors (Del Coco et al., 2023).

Furthermore, studies have reported a salutogenic effect of participation in Antarctic expeditions (Palinkas & Suedfeld, 2008; Sandal et al., 2006; Ráčková et al., 2024). General emotional stability of individuals (Peri et al., 2000; Sandal et al., 2006), predominance of positive over negative emotions (Sandal et al., 2006; Sandal et al., 2018; Pattyn et al., 2017), reducing depression, anxiety, fatigue, and confusion (Palinkas et al., 1995) have been observed. In addition, accumulated experience from participating in previous expeditions is an essential factor for adaptability to a challenging environment (Bartone et al., 2018; Ráčková et al., 2024).

Our results showed small statistically insignificant variations in the parameters studied, except for LYM% and ALB. These, as well as all the parameters studied herein, change naturally with advancing age. In addition to age, lymphocytes are also affected by inflammatory responses (Lin et al., 2016). Albumin is an indicator known for its regular changes with age, too (Gom et al. 2007). In the present study, despite the very small changes in the individual age-correlated blood indicators, the integral indicator - 10-year Mortality Risk and Biological Age reliably decreased after the expedition, which shows that the synchronous change in the studied parameters gives a more adequate assessment of the general state of the organism and suggests the benefit of their use in order to obtain a complete picture of the biological effects on the organism of the participants.

## 5. CONCLUSIONS

In this study, the Biological Age of participants in an Antarctic expedition was calculated for the first time. The main conclusions of the study are that the calculation of Biological Age based on a complex of scientifically based indicators can provide an integral assessment of the impact of polar expedition conditions on the human organism, identify individual participants who are particularly adversely affected, and provide guidelines for reduction of undesirable effects on the polar explorers. Our research suggested that the integral Biological Age indicator may show reliable changes even when the biochemical markers fluctuated insignificantly.

According to our results, participation in the Bulgarian Antarctic expeditions did not lead to an increase in Biological Age and even resulted in a slight reduction. Likely, the results for Biological Age and 10-year Mortality Risk in other more severe conditions at the Antarctic mainland closer to the Pole may differ significantly. Based on the obtained results, it can be recommended that blood samples for calculating the Biological Age also be taken during the stay at the polar base, which will allow a complete picture of the stressful conditions of polar expeditions to be obtained. This method can also be applied to participants in high-altitude and space expeditions, oil platform workers, and others.

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