Brain, Behavior, & Immunity - Health 21 (2022) 100432

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

# Biochemical clusters predict mortality and reported inability to work 10 years later

Nina Bertele [a,c], Alexander Karabatsiakis [b], Anat Talmon [a,d], Claudia Buss [c,e,*]

[a] Psychology Department, Stanford University, Stanford, CA, USA
[b] Institute of Psychology, Department of Clinical Psychology-II, University of Innsbruck, Innsbruck, Austria
[c] Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany
[d] Paul Baerwald School of Social Work and Social Welfare, The Hebrew University of Jerusalem, Mount Scopus, Jerusalem, Israel
[e] Development, Health and Disease Research Program, Department of Pediatrics, University of California Irvine, Irvine, USA

ARTICLE INFO

*Keywords:*
Biomarkers
Risk assessment
Patient stratification
Mortality
Systemic inflammation
High-risk cluster

ABSTRACT

*Background:* Chronic systemic inflammation has been linked to premature mortality and limited somatic as well as mental health with consequences for capability to work and everyday functioning. We recently identified three biochemical clusters of endocrine and immune parameters (C-reactive protein (CRP), interleukin-6 (IL-6), fibrinogen, cortisol and creatinine) in participants, age 35–81 years, of the open access Midlife in the United States Study (MIDUS) dataset. These clusters have been validated in an independent cohort of Japanese mid-life adults. Among these clusters, the one characterized by high inflammation coupled with low cortisol and creatinine concentrations was associated with the highest disease burden, referred to as high-risk cluster in the following. The current study aims to further examine the nature of this cluster and specifically whether it predicts mortality and the reported inability to work the last 30 days 10 years after the biomarker assessment.

*Methods and findings:* Longitudinally assessed health data from N = 1234 individuals were analyzed in the current study. Logistic regression analyses were performed to predict mortality within one decade after first assessment (T0 = first assessment; T1 = second assessment). General linear models were used to predict the number of days study participants were unable to work due to health issues in the last 30 days (assessed at T1, analyses restricted to individuals <70 years of age). Biological sex, disease burden, and age at T0 were used as covariates in all analyses. Individuals in the previously identified high-risk cluster had a higher risk for mortality (22% of individuals deceased between T0 and T1 versus 10% respectively 9% in the two other clusters). Logistic regression models predicting mortality resulted in a significant difference between individuals from the high-risk cluster compared to those from an identified reference cluster (indicator method, $p = .012$), independently of age and disease burden. Furthermore, individuals in the high-risk cluster reported a higher number of disability days during the past 30 days (3.4 days in the high-risk cluster versus 1.5 respectively 1.0 days in the reference clusters) assessed at T1. All pairwise comparisons involving the high-risk cluster were significant (all $ps < .001$).

*Conclusions:* Immune-endocrine profiles are predictive of mortality within the following decade over and above age and disease burden. The findings thus highlight the importance of biomarker-based risk profiling that may provide new targets for interventions in the context of preventive medicine in the transition from health to disease and disease-related mortality.

## 1. Introduction

Chronic systemic inflammation is closely linked to a broad range of diseases and thus, predicts lower overall functioning and all-cause mortality. In a recent study using a bioinformatics approach, we identified three biochemical clusters in the general population that were associated with differences in disease burden in assigned individuals (Bertele et al., 2021). This novel, cluster-based approach represents a promising step in the direction of more advanced and comprehensive methods of health-risk evaluation, while picking up the trend towards a more personalized perspective on medicine. In the current paper, we explored the predictive value of the identified clusters for mortality and aspects of

* Corresponding author. Institute of Medical Psychology, Charité – Universitätsmedizin Berlin, Luisenstraße 57, 10117, Berlin, Germany.
*E-mail address:* claudia.buss@charite.de (C. Buss).

https://doi.org/10.1016/j.bbih.2022.100432
Received 17 February 2022; Accepted 18 February 2022

everyday functioning, over and above the associated variation in disease burden.

Inflammation results from the process by which the immune system reacts to harmful stimuli, such as pathogens, damaged cells, or toxic compounds (Parham, 2021). By releasing C-reactive protein (CRP), interleukin-6 (IL-6), and fibrinogen, the body tries to eliminate the initial cause of insult (Parham, 2021). Thus, intermittent acute inflammation is critical for survival. However, the presence of certain social, psychological, environmental, and biological factors has been linked to the prevention of resolution of acute inflammation, causing a prolonged reactivity of the immune system (i.e., chronic systemic inflammation). Chronic systemic inflammation, which induces increased oxidative stress resulting in tissue injury, cellular damage and increased cellular allostatic load (Straub, 2017; Calder et al., 2013; Chatterjee, 2016), significantly contributes to the risk for a broad range of age-associated diseases, like cardio- and cerebrovascular diseases (Danesh, 2000; Danesh et al., 1998, 2004), diabetes type 2 (Wang et al., 2013), stroke (Welsh et al., 2008; Zhou et al., 2016; Di Napoli et al., 2001), and cancer (Allin and Nordestgaard, 2011; Qian et al., 2019).

However, chronic inflammation is *systemic*, that is, it does not occur as an isolated process but is closely interwoven with other somatic alterations, particularly with metabolic and endocrine actions (Cruz et al., 2018; Knight et al., 2021; Milrad et al., 2018). It is thus likely that the longer-term implications of systemic inflammation vary based on an individual's overall somatic health condition. As a consequence, systemic inflammation may be more detrimental in one individual compared to another, depending on interindividual variation in other aspects of physiology. Interestingly, this perspective is barely reflected in previous research in the field of inflammation. Rather, studies frequently used either one single or a set of a few inflammatory markers (Pearson et al., 2003; Sabatine et al., 2007) but concurrent consideration of other concurrent pathophysiological processes is not reported as common practice in the literature.

To consider inflammatory responses as isolated processes in research, despite their well-known interactions with other functions, might limit their predictive power with respect to longer-term outcomes. More pivotally, this constrained perspective might obscure essential knowledge for preventive approaches and treatment. At the same time, there is an urgent need for interdisciplinary tools to effectively predict individuals at risk for diseases and premature mortality to identify targets for prevention and intervention due to the rising numbers of non-communicable diseases and the massive related financial burden for the healthcare systems (Global Burden of Disease Collaborative Network, 2018).

We have recently proposed a novel, cluster-identification tool (based on routinely assessed biomarkers; CRP, IL-6, fibrinogen, cortisol, and creatinine) enabling to assign adults from a representative cohort study to one of three biochemical clusters (1). Among these three clusters, we found one cluster of high inflammation coupled with low creatinine and cortisol concentrations, i.e., high-risk cluster (1). This cluster indicated the highest disease burden compared to the other two clusters.

The current study aimed to test the predictive value of the previously identified biochemical high-risk cluster for mortality and the reported inability to work the last 30 days due to illness 10 years following the biomarker assessment over and above age and disease burden at baseline assessment.

## 2. Methods

### 2.1. Participants and setting

In the scope of the study Midlife in the United States Study (MIDUS), a total of $N = 7108$ individuals between 25 and 74 years of age were recruited from January 1995 to September 1996 from a national random-digit-dial sample of adults living in the 48 contiguous states (Ryff et al., 2010). Participants from MIDUS 1 were reinvited for a follow-up study with an emphasis on biomarkers (2004–2006), referred to as MIDUS 2, yielding a response rate of 70 percent ($n = 4963$). Additionally, a supplement sample of African Americans ($n = 592$) was recruited from Milwaukee, Wisconsin. Overall, a representative subset of 1255 individuals participated in the biomarker sub study, and of those complete biomarker data (regarding CRP, IL-6, fibrinogen, cortisol, and creatinine) was available from 1234 individuals. Informed consent was obtained by all participants. More than half of the 1234 participants were female (56.8%) and the average age at the time of biomarker assessment (T0) was 52.52 years ($SD = 11.71$). From 2013 to 2014, the third data collection took place, referred to as MIDUS 3, including mortality data from all 1234 participants (T1) and survey data that included self-reports on the reported inability to work the last 30 days from 929 of the participants of the MIDUS 2 biomarker subsample (T1). For an overview of the data collection process, please see Fig. 1.

For more information about the MIDUS project, please see [http://www.midus.wisc.edu/data/index.php].

### 2.2. Measures

#### 2.2.1. Biomarkers and biochemical clusters

Detailed information on biomarker assessment and generating biochemical clusters is provided in Bertele et al. (1). In brief, plasma levels of CRP and fibrinogen were assayed using immunonephelometric assays; IL-6 was quantitatively assessed using Enzyme-Linked Immunosorbent Assays (ELISA). The laboratory inter-assay coefficient of variance was 5.7% for CRP, 13% for IL-6, 2.6% for fibrinogen, all below the 20% acceptable range (Gruenewald et al., 2012). To obtain a cumulative cortisol and creatinine measure 12-h overnight urine samples were collected between 7 p.m. and 7 a.m. (22).

Biochemical clusters were created using k-mean clustering based on the levels of CRP, IL-6, fibrinogen, cortisol and creatinine, as these

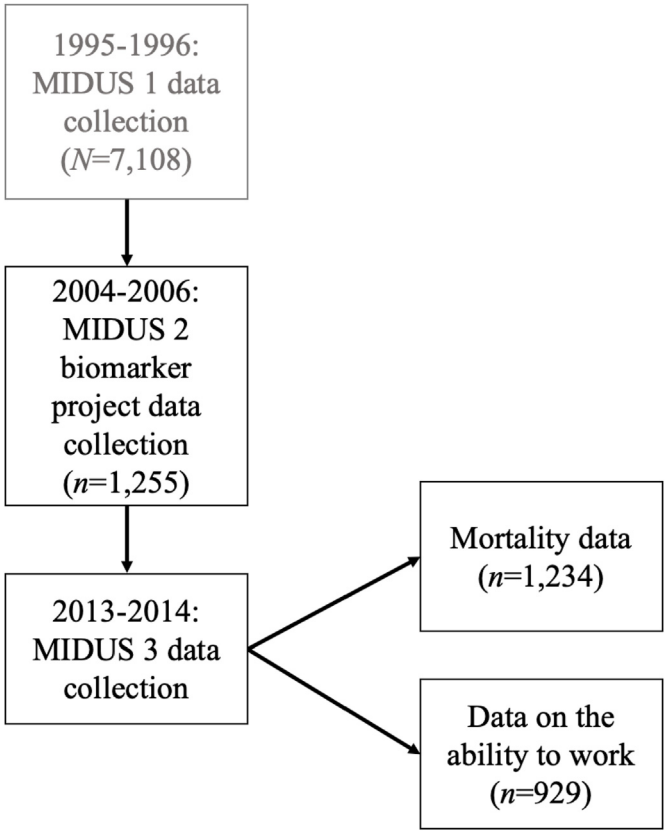


**Fig. 1.** Overview of the data collection Process.
*Note:* MIDUS = Midlife in the United States study.

biomarkers cover broad physiological functioning; CRP, fibrinogen, and IL-6 are pro-inflammatory markers (i.e., positively associated with inflammation), cortisol is the end product of the hypothalamus-pituitary–adrenal axis is an immune-modulatory mediator playing a crucial role in the stress response (Bertele et al., 2021; Thompson et al., 2010; Baumeister et al., 2016; Rückerl et al., 2007; Kashani et al., 2020; Zorn et al., 2017). The final k-mean clustering solution distinguished three different biomarker patterns; one with average levels on all five biomarkers, one with high concentrations of CRP, IL-6, and fibrinogen coupled with low cortisol and creatinine (i.e., high-risk cluster), and one with average CRP, IL-6, and fibrinogen but high concentrations of both cortisol and creatinine (i.e., metabo-endocrine cluster). The first cluster was used as a reference group (i.e., reference cluster) in all analyses, as this was the largest group ($N = 937$, see Table 1) and individuals assigned to this cluster indicated average levels on all biomarkers and low disease burden, suggesting a low pathological character of this cluster (1).

#### 2.2.2. Mortality

Through October 2015, mortality data on all MIDUS participants was obtained using three different methods; (1) A National Death Index (NDI) search in 2009 confirmed the death of 173 participants; (2) 322 deaths were recorded during tracing and mortality closeout interviews conducted by the University of Wisconsin Survey Center (UWSC) as part of MIDUS 3 (2013–2015), and (3) 57 deaths were recorded during normal longitudinal sample maintenance (Elliot et al., 2018).

#### 2.2.3. Ability to work

In the scope of MIDUS 3 (2013–2015), participants' functioning/ability to work was assessed by a single item by which information was obtained on the number of days the respondents had been unable to work during the last 30 days (exact wording in the survey: "In the past 30 days, how many days were you completely unable to go to work or carry out your normal household work activities because of your physical health or mental health?").

#### 2.2.4. Covariates

Biological sex was assessed dichotomously (0 = male, 1 = female). Depression, peptic ulcer disease, and cancer were assessed dichotomously (diagnosis yes = 1 vs. no = 0) at T0 (MIDUS 2). If an individual reported at least one cerebro- or cardiovascular disease (heart disease, hypertension, or stroke), they were assigned 1, when an individual reported none of these diseases, they were assigned 0.

### 2.3. Data analyses

All analyses were conducted using IBM SPSS Statistics 27. As a first step, the percentages of deceased individuals in each cluster were calculated. Second, we conducted a logistic regression analysis controlling for sex, age, and disease burden at T0 to predict mortality (yes vs. no) by the biochemical clusters. In doing so, we applied the indicator method comparing the high-risk and the metabo-endocrine cluster to the reference group. As a third step, we calculated the average days participants indicated that they were unable to work due to illness in the last 30 days separately in each cluster. Fourth, a General Linear Model (GLM) using Bonferroni pairwise comparisons and controlling for sex, age, and disease burden at T0 was performed to predict the days participants indicated that they were unable to work.

**Table 1**
Demographics by cluster.

| | Total sample ($N = 1234$) | Reference cluster ($n = 937$) | High-risk cluster ($n = 102$) | Metabo-endocrine cluster ($n = 195$) |
|---|---|---|---|---|
| Sex | 56.8% female | 76.2% female | 59.6% female | 31.3% female |
| Age | $M = 52.52$ $SD = 11.71$ | $M = 55.25$ $SD = 11.61$ | $M = 55.40$ $SD = 12.09$ | $M = 50.63$ $SD = 11.24$ |
| BMI | $M = 29.77$ $SD = 6.626$ | $M = 28.88$ $SD = 5.81$ | $M = 34.49$ $SD = 9.26$ | $M = 30.9$ $SD = 6.99$ |

*Note:* BMI=Body Mass Index.

## 3. Results

### 3.1. Primary analysis

#### 3.1.1. Biochemical clusters and mortality

Between T0 and T1, 9.8% of the individuals assigned to the reference cluster deceased ($N_{deceased} = 92$, $N_{total} = 937$), 21.6% in the high-risk cluster ($N_{deceased} = 22$, $N_{total} = 102$), and 8.7% in the metabo-endocrine cluster ($N_{deceased} = 17$, $N_{total} = 195$), respectively.

Logistic regression analyses using the indicator method and controlling for sex, age, and disease burden at T0 revealed a significant association between assignment to the clusters and mortality ($p = .043$, see Table 2). The indicator comparison between the reference cluster and the high-risk cluster was significant ($B = 0.82$, standard error ($SE$) $= 0.33$, $p = .012$); the comparison between the metabo-endocrine and the reference cluster was not significant ($B = 0.18$, $SE = 0.32$, $p = .59$). Likelihood ratio tests revealed that removing the cluster variable as a predictor, the model would explain significantly less variance in mortality (Model Log Likelihood: −316.16, Change in −2 Log Likelihood: $\chi^2(2) = 5.95$, $p = .048$).

Odds ratios for mortality by cluster separately for males and females can be found in the supplemental material (see Fig. S1). Comparing the odds ratios between males and females ((Number of deceased males/number of non-deceased males)/(deceased females/non-deceased females)), there was a tendency towards higher mortality in males vs. females across clusters (odds ratio (OR) = 1.96), but especially in the high-risk cluster (OR = 2.29).

#### 3.1.2. Reported inability to work

The number of days participants reported that they were unable to work due to illness (in the last 30 days) varied across clusters. While, on average, individuals in the reference cluster were 1.51 ($SD = 5.08$, $N_{respondents} = 745$) days unable to work, individuals in the high-risk cluster were 3.36 ($SD = 7.68$, $N_{respondents} = 42$) days unable to work, and individuals in the metabo-endocrine cluster were 0.99 ($SD = 4.52$, $N_{respondents} = 142$) days unable to work.

The GLM with pairwise comparisons controlling for sex, age, and disease burden at T0 revealed a significant association between cluster assignment and the reported inability to work the last 30 days ($F(2,790)$

**Table 2**
Logistic regression analyses predicting mortality.

| | $B$ | Standard error | Wald | df | $p$ | Exp($B$) |
|---|---|---|---|---|---|---|
| Cluster (general) | | | 6.3 | 2 | .043 | |
| Reference vs. high-risk cluster | .82 | .33 | 6.24 | 1 | .012 | 2.27 |
| Reference vs. metabo-endocrine | .18 | .32 | .3 | 1 | .59 | 1.19 |
| Sex | -.61 | .22 | 7.6 | 1 | .006 | .54 |
| Age | .1 | .01 | 84.78 | 1 | <.001 | 1.1 |
| Depression | .72 | .25 | 8.44 | 1 | .004 | 2.05 |
| Cerebro- and cardiovascular disease | .74 | .23 | 10.54 | 1 | .001 | 2.1 |
| Peptic ulcer disease | .01 | .45 | 0 | 1 | .98 | 1.01 |
| Cancer | .27 | .26 | 1.1 | 1 | .29 | 1.3 |
| Constant | −7.4 | .84 | 78.34 | 1 | <.001 | 0 |

*Note:* Nagelkerke's $R^2 = 0.29$. Results of the group comparisons are based on the indicator method. Sex is coded as follows: 0 = male, 1 = female, chronological age was assessed at the time of biomarker assessment. Depression, cerebro- and cardiovascular disease, peptic ulcer disease, and cancer have been assessed via self-report (yes vs. no). Cerebro- and cardiovascular diseases include heart disease, hypertension, and stroke.

= 3.3, $p = .037$, Table 3). Pairwise comparisons according to Bonferroni, indicated that the differences between the reference and the high-risk cluster ($z = 2.28$, $p = .008$) and between the high-risk and the metabo-endocrine cluster were significant ($z = 2.97$, $p = .001$) (see Table 4). The effect sizes (Cohen's d) for the group differences were .35 (95% confidence interval: 0.04-0.66) for the high-risk cluster vs. the reference cluster and 0.1 for (95% confidence interval: −0.08–0.28) the metabo-endocrine cluster vs. the reference cluster.

The average days of sickness by cluster and sex can be found in the supplements (see Fig. S2). There was a descriptive tendency towards a higher number of sick days in males in the high-risk cluster compared to females assigned to the high-risk cluster.

## 4. Discussion

The findings of the current study reveal an association between a biochemical profile that is characterized by high inflammation and low cortisol and creatinine concentrations (i.e., high-risk cluster) and mortality 10 years later independent of age, sex, and disease burden at biomarker assessment. Furthermore, in those alive 10 years post biomarker assessment, the same cluster negatively predicted functionality (i.e., reported inability to work the last 30 days) (1). Previous studies are in concordance with these results by suggesting that chronic systemic inflammation predicts mortality and lower every day functioning (e.g., measured as cognitive impairment) (Gorelick, 2010; Paine et al., 2015; C-reactive protein concen, 2010). Mechanisms that might link systemic inflammation to these detrimental outcomes might be the increased disease susceptibility associated with systemic inflammation (Furman et al., 2019) as well as the fact that inflammation often occurs in individuals who present with a variety of risk factors that augment disease susceptibility across the lifespan such as poor diet (Navarro et al., 2016) and obesity (Ellulu et al., 2016). According to the Free Radical Theory of Aging, systemic inflammation can induce a chronic state of allostatic load, accompanied by high levels of oxidative stress. In the long-term, this may yield impaired stem cell reproductivity, immunosenescence (i.e., aging of the immune system), and cellular aging (35) resulting from increased biomolecular entropy as well as functional and structural damage of cellular DNA (Wang, 2021). Consequently, due to these (accelerated) aging processes, individuals might be more susceptible to poor health and consequently, for premature mortality (Harman, 1992).

However, previous studies on systemic inflammation and longer-term outcomes commonly considered single inflammatory markers as predictors for mortality and functionality without taking other related biomarkers into account (29–31). Furthermore, previous research mostly involved clinical samples with a high pathological burden to investigate the link between inflammation and mortality (e.g., individuals suffering from chronic obstructive pulmonary disease (Mendy et al., 2018), patients infected with HIV (Tien et al., 2010), kidney disease patients (Alves et al., 2018)), making it challenging to distinguish inflammatory risk for earlier mortality from the established disease phenotypes. The current study expands on previous findings by using our previously proposed biomarker clusters based on multiple biomarkers, that cover a broad range of somatic functioning, as predictors for mortality and the reported inability to work the last 30 days in a large non-clinical population sample 10 years after biomarker assessment; all while controlling for disease burden at baseline (1). With that, the novel approach of risk evaluation might be more precise with respect to its predictive value (1) and, as described below, might reveal valuable and innovative implications for treatment and intervention.

Relating the biochemical clusters to mortality and the reported inability to work the last 30 days, we found that individuals with high inflammation and low cortisol and creatinine concentrations had the highest risk for mortality and impaired functionality (1). Importantly, the high-risk biochemical profile, that was associated with higher disease burden at baseline, was associated with mortality and functionality 10 year later independent of age and different disease states at baseline. It is possible though that the biochemical risk profile led to an accelerated disease progression among individuals assigned to this cluster, resulting in higher rates of mortality and lower everyday functioning. Investigating the potential moderating role of the biochemical risk profiles of the association between disease states at baseline and mortality and functionality 10 years later was not possible in the current study due to the limited sample size. Future studies should test these potential interactions in larger, population-based studies.

Belonging to the metabo-endocrine cluster, which was characterized by high concentrations of cortisol and creatinine accompanied by average inflammatory markers, was not associated with higher mortality rates compared to the reference cluster. This is in line with the observations from our previous study (Bertele et al., 2021), where the metabo-endocrine cluster did not show a higher disease burden than the reference cluster. These observations seem in contrast with previous research linking hypercortisolism to disease states and mortality (Min, 2016; Steffensen et al., 2016). However, this discrepancy might further emphasize the importance of considering biomarkers in the context of other biomarkers and somatic processes as a matter of principle when examining longer-term outcomes.

An important question relevant for preventative targets is related to the etiology of the identified clusters. In a previous study, we reported that individuals assigned to the high-risk cluster were significantly more likely to report experiences of childhood maltreatment (CM; that is, experiences of child abuse and neglect), pointing to early-life stress as one possible etiological factor of this cluster (1). In line with this are findings of a recent review suggesting CM as a leading contributor to a number of diseases and mortality (Grummitt et al., 2021), which may in part be mediated by the well-established low-grade, systemic inflammatory states in individuals exposed to CM. Our results suggest that assessment of additional biomarkers, in addition to inflammatory mediators, may increase precision of risk profiling. Moreover, CM exposure is known to be associated with telomere shortening (Shalev and Belsky, 2016; Shalev, 2012), to accelerate age-induced effects on neurogenesis (Ruiz et al., 2018) and cognitive decline (Barnes et al., 2009; Pesonen et al., 2013). Individuals assigned to the high-risk cluster, hence, might be especially affected by accelerated aging processes due to increased systemic inflammation causing high levels of oxidative stress and hence, cell damage and tissue injury, but also due to other age-accelerating processes yet to be studied (3–5). Further advancing the understanding of the etiology factors of the identified biochemical clusters would reveal

**Table 3**
General linear models predicting inability to work.

| Source | Type III Sum of Squares | df | Mean Square | *F* | *p* |
|---|---|---|---|---|---|
| Corrected Model | 2377.81 | 43 | 55.3 | 2.62 | <.001 |
| Intercept | 1226.84 | 1 | 1226.84 | 58.29 | <.001 |
| Cluster | 139.05 | 2 | 69.53 | 3.3 | .037 |
| Sex | .49 | 1 | .49 | .02 | .88 |
| Age | 1033.58 | 36 | 28.71 | 1.36 | .08 |
| Depression | 114.33 | 1 | 114.33 | 5.43 | .02 |
| Cerebro- and cardiovascular disease | 207.69 | 1 | 207.69 | 9.87 | .002 |
| Peptic ulcer disease | 556.65 | 1 | 556.65 | 26.44 | <.001 |
| Cancer | 7.48 | 1 | 7.48 | .36 | .55 |
| Error | 16628.85 | 790 | 21.05 | | |
| Total | 20484 | 834 | | | |
| Corrected Total | 19006.66 | 833 | | | |

*Note:* $R^2 = 0.13$ (Adjusted $R^2 = 0.08$). Sex is coded as follows: 0 = male, 1 = female, age was assessed at the time of biomarker assessment. Depression, cerebro- and cardiovascular disease, peptic ulcer disease, and cancer have been assessed via self-report (yes vs. no). Cerebro- and cardiovascular diseases include heart disease, hypertension, and stroke. At T1, participants' functionality/ability to work was assessed by a single item by which information was obtained on the number of days the respondents had been unable to work during the last 30 days.

**Table 4**
General linear models predicting inability to work: Bonferroni pairwise comparisons between clusters.

| Cluster | vs. Cluster | Z of mean difference | Standard error of mean difference | P | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Reference | High-risk | −2.28 | .76 | .008 | −4.09 | -.47 |
| | Metaboendocrine | .69 | .44 | .33 | -.35 | 1.74 |
| High-risk | Reference | 2.28 | .76 | .008 | .47 | 4.09 |
| | Metabo- endocrine | 2.97 | .84 | .001 | .97 | 4.98 |
| Metabo-endocrine | Reference | -.69 | .44 | .33 | −1.74 | .35 |
| | High-risk | −2.97 | .84 | .001 | −4.98 | -.97 |

valuable implications with respect to more tailored, personalized (pre-emptive) interventions. In the context of such interventions, for example, trauma-focused psychotherapy might play a crucial role and it might be of importance to monitor the nutrient balance (micronutrients, minerals and vitamins, in particular) of at-risk individuals as well as to administer supplements when needed, as it has been shown that an anti-inflammatory diet for example can buffer some of the age-accelerating processes (for reviews, see (Cheng et al., 2010; Stromsnes et al., 2021)).

Comparing the longer-term outcomes of the biochemical clusters in males and females, we observed a descriptive tendency towards higher mortality and the reported inability to work the last 30 days in males, potentially suggesting that an assignment to the high-risk cluster is more detrimental in males than in females (see S5 and S6). If replicated in larger studies the moderating role of sex steroids of the associations observed here should be examined.

The assessment of CRP, IL-6, fibrinogen, cortisol, and creatinine concentrations, represent a valuable approach for identifying individuals at-risk for premature mortality and for impaired functionality in the following years. As it is relatively in-expensive, the assessment of these five biomarkers might be integrated into routine diagnostics and treatments and could help identify individuals at risk that could be offered tailored interventions including early-on therapy approaches focusing on biological mechanisms initiated after CM exposure, that are being increasingly understood, preventing the manifestation of biochemical risk profiles in the first place.

Our work has several strengths such as the representative general population sample and the use of gold standard biomarker assessment methods. Yet, the findings have some limitations. First, the sample size for the mortality analysis was relatively small, possibly decreasing the power to detect effects of each cluster on mortality. The small sample size also prohibited the examination of potential interactions between the biochemical clusters and each disease state over the lifespan. Future longitudinal studies involving larger sample sizes should investigate whether an assignment to the high-risk cluster might accelerate disease progression in these individuals. Second, the reported inability to work the last 30 days was assessed via self-report, which bears the risk of a reporting bias and via a single item. Although this procedure is supported by previous literature (Fisher et al., 2016; Cunny and Perri, 1991), the use of only one item prohibits to test the psychometric properties of the assessment. In addition, disease burden has been addressed in a limited fashion focusing on depression, heart disease, stroke, hypertension, peptic ulcer disease and cancer. Although the chosen disease states cover pathology in various somatic systems, it is still possible that other relevant covariates/diseases were not considered potentially limiting the generalizability of our results. Furthermore, the selection of available biomarkers in MIDUS was limited. In addition to the five biomarkers chosen here, future studies should also consider additional biomarkers and parameters to cover an even broader range of functionality. For example, estrogen and testosterone concentrations (representing the reproductive systems and known for their influence on aging processes and disease risk (Ohnaka, 2017; Gurvich et al., 2018)), mitochondrial integrity (as an additional indicator of the metabolic system (Bratic and Larsson, 2013; Lee et al., 2012)), as well as nutritional balance parameters (known for their crucial role in healthy and unhealthy aging processes over the lifespan, for a review see Cheng et al. (2010)) might be candidates worth including.

This study validated the predictive character of three previously identified biochemical clusters with respect to mortality and the reported inability to work the last 30 days 10 years following the biomarker assessment (1). Our findings suggest that an inflammatory-endocrine profile characterized by high inflammation coupled with low cortisol and creatinine concentrations represents a valuable risk marker for mortality and functionality a decade later; over and above the disease burden reported at baseline. Future studies should further validate the predictive power of the identified high-risk cluster with respect to other longer-term outcomes and they should also focus on the development of effective early-on targeted interventions personalized based on biochemical risk profiles. Moreover, additional etiology factors of biochemical risk profiles should be identified, enhancing our understanding of the role of early-life stress and other life history factors as well as genetic risk and epigenetic factors in this context. In addition, future research should aim at identifying potential protecting, that is resilience, factors in individuals with biochemical risk profiles.

## Funding

The work was, in part, supported by the European Research Council (ERC-STG 639766).

## Declaration of competing interest

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version, This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue, The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2022.100432.

## References

Allin, K.H., Nordestgaard, B.G., 2011. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. Crit. Rev. Clin. Lab Sci. 48 (4), 155–170. Aug.

Alves, F.C., Sun, J., Qureshi, A.R., Dai, L., Snaedal, S., Bárány, P., et al., 2018. The higher mortality associated with low serum albumin is dependent on systemic inflammation in end-stage kidney disease, 1. In: Shearer, G. (Ed.), PLoS ONE, vol. 13, e0190410. Jan 3.

Barnes, J.E., Noll, J.G., Putnam, F.W., Trickett, P.K., 2009. Sexual and physical revictimization among victims of severe childhood sexual abuse. Child Abuse Neglect 33 (7), 412–420. Jul.

Baumeister, D., Akhtar, R., Ciufolini, S., Pariante, C.M., Mondelli, V., 2016. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. Mol. Psychiatr. 21 (5), 642–649. May.

Bertele, N., Karabatsiakis, A., Buss, C., Talmon, A., 2021. How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach

towards predictive, preventive, and personalized (3P) medicine [Internet] EPMA J. Sep 29 [cited 2021 Oct 6]; Available from https://link.springer.com/10.1007/s13167-021-00255-0.
Bratic, A., Larsson, N.-G., 2013. The role of mitochondria in aging. J. Clin. Invest. 123 (3), 951–957. Mar 1.
C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 375 (9709), 2010, 132–140. Jan.
Calder, P.C., Ahluwalia, N., Albers, R., Bosco, N., Bourdet-Sicard, R., Haller, D., et al., 2013. A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. Br. J. Nutr. 109 (S1), S1–S34. Jan 23.
Chatterjee, S., 2016. Oxidative Stress, Inflammation, and Disease. in: Oxidative Stress and Biomaterials [Internet]. Elsevier [cited 2021 Nov 3]. pp. 35–58. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128032695000024.
Cheng, W.-H., Bohr, V.A., de Cabo, R., 2010. Nutrition and aging. Mech. Ageing Dev. 131 (4), 223–224. Apr.
Cruz, L.A.B., Barral-Netto, M., Andrade, B.B., 2018. Distinct inflammatory profile underlies pathological increases in creatinine levels associated with Plasmodium vivax malaria clinical severity. Escalante AA. PLoS Neglected Trop. Dis. 12 (3), e0006306. Mar 29.
Cunny, K.A., Perri, M., 1991. Single-item vs multiple-item measures of health-related quality of life. Psychol. Rep. 69 (1), 127–130. Aug.
Danesh, J., 2000. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. BMJ 321 (7255), 199–204. Jul 22.
Danesh, J., Collins, R., Appleby, P., Peto, R., 1998. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA 279 (18), 1477. May 13.
Danesh, J., Wheeler, J.G., Hirschfield, G.M., Eda, S., Eiriksdottir, G., Rumley, A., et al., 2004. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N. Engl. J. Med. 350 (14), 1387–1397. Apr.
Di Napoli, M., Papa, F., Bocola, V., 2001. Prognostic influence of increased C-reactive protein and fibrinogen levels in ischemic stroke. Stroke 32 (1), 133–138. Jan.
Elliot, A.J., Turiano, N.A., Infurna, F.J., Lachman, M.E., Chapman, B.P., 2018. Lifetime trauma, perceived control, and all-cause mortality: results from the Midlife in the United States Study. Health Psychol. 37 (3), 262–270. Mar.
Ellulu, M.S., Khaza'ai, H., Rahmat, A., Patimah, I., Abed, Y., 2016. Obesity can predict and promote systemic inflammation in healthy adults. Int. J. Cardiol. 215, 318–324. Jul.
Fisher, G.G., Matthews, R.A., Gibbons, A.M., 2016. Developing and investigating the use of single-item measures in organizational research. J. Occup. Health Psychol. 21 (1), 3–23. Jan.
Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., et al., 2019. Chronic inflammation in the etiology of disease across the life span. Nat. Med. 25 (12), 1822–1832. Dec.
Global Burden of Disease Collaborative Network, 2018. Global Burden of Disease Study 2017 (GBD 2017) Results. Institute for Health Metrics and Evaluation (IHME), Seattle, U.S.
Gorelick, P.B., 2010. Role of inflammation in cognitive impairment: results of observational epidemiological studies and clinical trials: Gorelick. Ann. N. Y. Acad. Sci. 1207 (1), 155–162. Oct.
Gruenewald, T.L., Karlamangla, A.S., Hu, P., Stein-Merkin, S., Crandall, C., Koretz, B., et al., 2012. History of socioeconomic disadvantage and allostatic load in later life. Soc. Sci. Med. 74 (1), 75–83. Jan.
Grummitt, L.R., Kreski, N.T., Kim, S.G., Platt, J., Keyes, K.M., McLaughlin, K.A., 2021. Association of Childhood Adversity with Morbidity and Mortality in US Adults: A Systematic Review [Internet]. JAMA Pediatr. Oct 4 [cited 2021 Oct 6]; Available from. https://jamanetwork.com/journals/jamapediatrics/fullarticle/2784732.
Gurvich, C., Hoy, K., Thomas, N., Kulkarni, J., 2018. Sex differences and the influence of sex hormones on cognition through adulthood and the aging process. Brain Sci. 8 (9), 163. Aug 28.
Harman, D., 1992. Free radical theory of aging. Mutation Research/DNAging. 275 (3–6), 257–266. Sep.
Kashani, K., Rosner, M.H., Ostermann, M., 2020. Creatinine: from physiology to clinical application. Eur. J. Intern. Med. 72, 9–14. Feb.
Knight, E.L., Jiang, Y., Rodriguez-Stanley, J., Almeida, D.M., Engeland, C.G., Zilioli, S., 2021. Perceived stress is linked to heightened biomarkers of inflammation via diurnal cortisol in a national sample of adults. Brain Behav. Immun. 93, 206–213. Mar.
Lee, H.-C., Wei, Y.-H., 2012. Mitochondria and aging [cited 2021 Nov 5]. In: Scatena, R., Bottoni, P., Giardina, B. (Eds.), Advances in Mitochondrial Medicine [Internet], vol. 942. Springer Netherlands, Dordrecht, pp. 311–327. Advances in Experimental Medicine and Biology. http://link.springer.com/10.1007/978-94-007-2869-1_14. Available from.
Mendy, A., Forno, E., Niyonsenga, T., Gasana, J., 2018. Blood biomarkers as predictors of long-term mortality in COPD. Clin. Res. J 12 (5), 1891–1899. May.
Milrad, S.F., Hall, D.L., Jutagir, D.R., Lattie, E.G., Czaja, S.J., Perdomo, D.M., et al., 2018. Depression, evening salivary cortisol and inflammation in chronic fatigue syndrome: a psychoneuroendocrinological structural regression model. Int. J. Psychophysiol. 131, 124–130. Sep.
Min, Le, 2016. Functional hypercortisolism, visceral obesity, and metabolic syndrome. Endocr. Pract. 22 (4), 506–508. Apr.
Navarro, S.L., Kantor, E.D., Song, X., Milne, G.L., Lampe, J.W., Kratz, M., et al., 2016. Factors associated with multiple biomarkers of systemic inflammation. Cancer Epidemiol. Biomarkers Prev. 25 (3), 521–531. Mar.
Ohnaka, K., 2017. [Aging and homeostasis. Sex hormones and aging.]. Clin. Calcium 27 (7), 947–954.
Paine, N.J., Bosch, J.A., Ring, C., Drayson, M.T., Veldhuijzen van Zanten JJCS, 2015. Induced mild systemic inflammation is associated with impaired ability to improve cognitive task performance by practice: inflammation and reduced practice effect in cognitive task. Psychophysiology 52 (3), 333–341. Mar.
Parham, P., 2021. The Immune System.
Pearson, T.A., Mensah, G.A., Alexander, R.W., Anderson, J.L., Cannon, R.O., Criqui, M., et al., 2003. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the centers for disease control and prevention and the American heart association. Circulation 107 (3), 499–511. Jan 28.
Pesonen, A.-K., Eriksson, J.G., Heinonen, K., Kajantie, E., Tuovinen, S., Alastalo, H., et al., 2013. Cognitive ability and decline after early life stress exposure. Neurobiol. Aging 34 (6), 1674–1679. Jun.
Qian, S., Golubnitschaja, O., Zhan, X., 2019. Chronic inflammation: key player and biomarker-set to predict and prevent cancer development and progression based on individualized patient profiles. EPMA J. 10 (4), 365–381. Dec.
Rückerl, R., Greven, S., Ljungman, P., Aalto, P., Antoniades, C., Bellander, T., et al., 2007. Air pollution and inflammation (Interleukin-6, C-reactive protein, fibrinogen) in myocardial infarction survivors. Environ. Health Perspect. 115 (7), 1072–1080. Jul.
Ruiz, R., Roque, A., Pineda, E., Licona-Limón, P., José Valdéz-Alarcón, J., Lajud, N., 2018. Early life stress accelerates age-induced effects on neurogenesis, depression, and metabolic risk. Psychoneuroendocrinology 96, 203–211. Oct.
Ryff, C.D., Seeman, T., Weinstein, M., 2010. Midlife in the United States (MIDUS 2): Biomarker Project, 2004-2009: Version 9 [Internet]. Inter-University Consortium for Political and Social Research [cited 2021 Mar 27]. Available from. https://www.icpsr.umich.edu/icpsrweb/NACDA/studies/29282/versions/V9.
Sabatine, M.S., Morrow, D.A., Jablonski, K.A., Rice, M.M., Warnica, J.W., Domanski, M.J., et al., 2007. Prognostic significance of the centers for disease control/American heart association high-sensitivity C-reactive protein cut points for cardiovascular and other outcomes in patients with stable coronary artery disease. Circulation 115 (12), 1528–1536. Mar 27.
Shalev, I., 2012. Early life stress and telomere length: investigating the connection and possible mechanisms: a critical survey of the evidence base, research methodology and basic biology. Bioessays 34 (11), 943–952. Nov.
Shalev, I., Belsky, J., 2016. Early-life stress and reproductive cost: a two-hit developmental model of accelerated aging? Med. Hypotheses 90, 41–47. May.
Steffensen, C., Pereira, A.M., Dekkers, O.M., Jørgensen, J.O.L., 2016. Diagnosis OF endocrine disease: prevalence of hypercortisolism in type 2 diabetes patients: a systematic review and meta-analysis. Eur. J. Endocrinol. 175 (6), R247–R253. Dec.
Straub, R.H., 2017. The brain and immune system prompt energy shortage in chronic inflammation and ageing. Nat. Rev. Rheumatol. 13 (12), 743–751. Dec.
Stromsnes, K., Correas, A.G., Lehmann, J., Gambini, J., Olaso-Gonzalez, G., 2021. Anti-inflammatory properties of diet: role in healthy aging. Biomedicines 9 (8), 922. Jul 30.
Thompson, A.M.S., Zanobetti, A., Silverman, F., Schwartz, J., Coull, B., Urch, B., et al., 2010. Baseline repeated measures from controlled human exposure studies: associations between ambient air pollution exposure and the systemic inflammatory biomarkers IL-6 and fibrinogen. Environ. Health Perspect. 118 (1), 120–124. Jan.
Tien, P.C., Choi, A.I., Zolopa, A.R., Benson, C., Tracy, R., Scherzer, R., et al., 2010. Inflammation and mortality in HIV-infected adults: analysis of the FRAM study cohort. JAIDS J. Acquir. Immun. Def. Syndr. 55 (3), 316–322. Nov 1.
Wang, Z., 2021. The Entropy Perspective on Human Illness and Aging. Engineering. Oct; S2095809921003866.
Wang, X., Bao, W., Liu, J., OuYang, Y.-Y., Wang, D., Rong, S., et al., 2013. Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. Diabetes Care 36 (1), 166–175. Jan 1.
Welsh, P., Lowe, G.D.O., Chalmers, J., Campbell, D.J., Rumley, A., Neal, B.C., et al., 2008. Associations of proinflammatory cytokines with the risk of recurrent stroke. Stroke 39 (8), 2226–2230. Aug.
Zhou, Y., Han, W., Gong, D., Man, C., Fan, Y., 2016. Hs-CRP in stroke: a meta-analysis. Clin. Chim. Acta 453, 21–27. Jan.
Zorn, J.V., Schür, R.R., Boks, M.P., Kahn, R.S., Joëls, M., Vinkers, C.H., 2017. Cortisol stress reactivity across psychiatric disorders: a systematic review and meta-analysis. Psychoneuroendocrinology 77, 25–36. Mar.