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D I S S E R T A T I O N

A Novel Bioinformatics Approach for Disease and Mortality Risk Prediction

Ein neuer, bioinformatischer Ansatz der Prädiktion von Krankheits- und Mortalitätsrisiko

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3PM = Personalized medicine, targeted prevention, and predictive diagnostics
ACTH = Adrenocorticotropic hormone
ANOVA = Analysis of variance
ATP = Adenosine triphosphate
CM = Childhood maltreatment
CRH = Corticotropin releasing hormone
CRP = C-reactive protein
CTQ = Childhood Trauma Questionnaire
DALYs = Disability-adjusted life years
DHEA-S = Dehydroepiandrosterone sulfate
ELISA = Enzyme-Linked Immunosorbent Assay
HDL = High-density lipoproteins
HPA = Hypothalamic–pituitary–adrenal (axis)
IL-6 = Interleukin-6
IL-6 = Interleukin-12
LDL = Low- density lipoproteins
MD = Mean difference
MIDJA = Midlife in Japan
MIDUS = Midlife in the United States
mtDNA = Mitochondrial deoxyribonucleic acid
NCDs = Non-communicable diseases
NFKB = Nuclear Factor-KappaB
OR = Odds ratio
PAMP = Pathogen-associated molecular pattern
PRR = Pattern recognition receptors
SD = Standard deviation
SE = Standard error (of mean difference)
WHR = Waist-to-hip ratio

Abstract (German)

Hintergrund. Die Prävalenz nicht übertragbarer Krankheiten nimmt ständig zu und erfordert einfache und kostengünstige Methoden zur Identifizierung von Personen, die für die Entwicklung dieser Krankheiten gefährdet sind.

Ziele. Die Studie zielt darauf ab, einen auf Biomarkern basierenden Risikoindikator einzuführen, diesen mit anerkannten gesundheitlichen Risikofaktoren zu verknüpfen, ihn mit der aktuellen Krankheitslast in Beziehung zu setzen und prospektiv Personen zu identifizieren, die ein Risiko für vorzeitige Sterblichkeit und eingeschränkte Alltagsfunktionen haben.

Methoden. Anhand der Konzentrationen von C-reaktivem Protein (CRP), Interleukin-6 (IL-6), Fibrinogen, Cortisol und Kreatinin wurden in einer US-amerikanischen Stichprobe (*N*=1234) K-Mean Cluster identifiziert und in einer japanischen Stichprobe (*N*=378) validiert. Die Assoziationen der Cluster mit biologischem Geschlecht, Alter, Body-Mass-Index (BMI), körperlicher Aktivität, Alkohol- und Rauchgewohnheiten sowie frühkindlichem Stress wurde untersucht. Die Odds Ratios für Depressionen, Herzkrankheiten, Bluthochdruck, Magengeschwüre, Schlaganfall und Krebs wurden zwischen den Clustern verglichen. Die Cluster wurden zur Vorhersage der Sterblichkeit und der Arbeitsunfähigkeit (=Krankheitstage in den letzten 30 Tagen) 10 Jahre nach der Biomarker-Erfassung verwendet.

Ergebnisse. Es wurden drei biochemische Cluster identifiziert und validiert. Ein Cluster war durch durchschnittliche Konzentrationen aller Biomarker gekennzeichnet (=Referenzcluster), eins durch durchschnittliche Konzentrationen von CRP, IL-6 und Fibrinogen und überdurchschnittliche Werte von Cortisol und Kreatinin (=metabo-endokrines Cluster), und eins durch überdurchschnittliche Werte von CRP, IL-6 und Fibrinogen und durchschnittliche Werte von Cortisol und Kreatinin (=Hochrisikocluster). Im Vergleich zu den anderen Clustern bestand der Hochrisikocluster aus einem höheren Anteil von Männern und aus Personen mit einem höheren BMI, geringerer körperlicher Aktivität und einer höheren Exposition gegenüber frühkindlichem Stress. Diese Gruppe wies die höchsten Odds Ratios für Depressionen, Herzerkrankungen, Bluthochdruck, Magengeschwüren, Schlaganfall und Krebs auf. Personen im Hochrisikocluster hatten 10

Jahre nach der Biomarker-Erfassung ein höheres Sterberisiko und eine höhere Anzahl an Krankheitstagen, unabhängig von Geschlecht, Alter und Krankheitslast.

Schlussfolgerungen. Die immunendokrinen Profile unterscheiden sich in der Verteilung von Geschlecht, Alter, BMI, körperlicher Aktivität und frühkindlichem Stress und stehen in Zusammenhang mit Krankheitslast. Sie sagen über das Geschlecht, das Alter und die Krankheitslast hinaus auch die Sterblichkeit und die Funktionsfähigkeit im Alltag im folgenden Jahrzehnt vorher. Die Ergebnisse unterstreichen die Bedeutung der biomarkerbasierten Erstellung von Risikoprofilen, die neue Ziele für Interventionen im Rahmen der Präventivmedizin bieten.

Abstract (English)

Theoretical Background. The prevalence of non-communicable diseases are continuously increasing requiring simple and inexpensive ways to identify individuals at risk for developing these disorders to target with preventive approaches.

Study Aims. The current study aims to introduce a novel biomarker-based risk indicator, to link this novel tool to commonly recognized health risk factors, to relate it to current disease burden, and to prospectively identify individuals at risk for premature mortality and reduced everyday functioning.

Methods. K-mean clusters were identified based on C-reactive protein (CRP), interleukin-6 (IL-6), fibrinogen, cortisol, and creatinine concentrations in a U.S. American sample (*N*=1,234) and validated in a Japanese sample (*N*=378). The association of the resulting clusters with biological sex, age, body mass index (BMI), physical activity, alcohol, and smoking habits as well as early-life stress were examined. Odds ratios for depression, heart disease, hypertension, peptic ulcer disease, stroke, and cancer were compared between individuals in the identified biochemical clusters. The identified clusters were used to predict mortality and the inability to work (=number of sick days during the last 30 days) 10 years following the biomarker assessment.

Results. Three distinct biochemical clusters were identified and validated. One of these clusters was characterized by average concentrations of all considered biomarkers (=reference cluster), one by average concentrations of CRP, IL-6, and fibrinogen but above-average levels of cortisol and creatinine (=metabo-endocrine cluster), and a third one characterized by above-average levels of CRP, IL-6, and fibrinogen, and average levels of cortisol and creatinine (=high-risk cluster). Compared to the other identified clusters, the high-risk cluster consisted of a significantly higher proportion of men vs. women, and individuals with a higher BMI, lower physical activity, and higher reported exposures to early-life stress. This cluster had the highest odds ratios for current diagnoses of depression, heart disease, hypertension, peptic ulcer disease, stroke, and cancer. Individuals in this biochemical risk cluster had a higher risk for mortality 10 years following the biomarker assessment, independently from sex, age, and disease burden

at baseline. Furthermore, individuals in the high-risk cluster reported the highest number of sick days.

Conclusions. Immune-endocrine profiles differ by sex, age, BMI, physical activity, and early-life stress, and they are associated with disease burden. Importantly, they are predictive of mortality and everyday functioning within the following decade, over and above sex, age, and baseline disease burden. The findings highlight the importance of biomarker-based risk profiling providing new targets for interventions in the context of preventive medicine in the transition from health to disease and disease-related mortality.

1. Introduction

1.1. Global burden of disease: Current situation and recent developments

In 1990, infectious diseases still represented a major cause of both mortality and Disability-Adjusted Life Years (DALYs), signifying the loss of the equivalent of one year of full health (Vos et al., 2020). Specifically, the three diseases causing the highest percentage of DALYs in 1990 were neonatal disorders (ranked first; 10.6% of DALYs), lower respiratory infections (second; 8.7% of DALYs), and diarrheal diseases (ranked third; 7.3% of DALYs) across all ages (Vos et al., 2020). However, between 1990 and 2019, a fundamental shift happened with Non-Communicable Diseases (NCDs) replacing infectious diseases as the leading cause of mortality and DALYs (Global Burden of Disease Collaborative Network, 2018; Vos et al., 2020). According to a recent Global Burden of Disease analysis, in 2019, neonatal disorders were still ranked first as causing the highest percentage of DALYs worldwide (7.3% of DALYs), whereas ischemic heart diseases were now ranked second (causing 7.2% of DALYs), and strokes were ranked third (causing 5.7% of DALYs) (Vos et al., 2020). In addition, the DALYs caused by mood disorders steadily increased within the past three decades (Global Burden of Disease Collaborative Network, 2018; Vos et al., 2020). In particular, depressive and anxiety disorders have increasingly been recognized as important causes of DALYs (with depressive disorders rising from rank 19 in 1990 to rank 13 in 2019, and anxiety disorders rising from rank 34 to 24 between 1990 and 2019) (Vos et al., 2020). NCDs including mental disorders, hence, cause a major burden to affected individuals measured as DALYs but, at the same time, they also imply a massive socioeconomic strain, particularly to the health-cares systems (Global Burden of Disease Collaborative Network, 2018). Due to the prolonged and expensive treatments that many NCDs require, it is not surprising that, altogether, NCDs caused 90% of the annual health-care spending in the United States in 2019 – and this upward trend can be seen in multiple sites across the globe, and it will continue to rise (Global Burden of Disease Collaborative Network, 2018; Martin et al., 2021).

Collectively, prevalence and incidence of NCD as well as health-care spending for their treatment are continuously increasing, marking them as a global pandemic (Global

Burden of Disease Collaborative Network, 2018; Martin et al., 2021; Vos et al., 2020). Thus, identifying individuals at risk for NCDs and providing them with pre-emptive interventions should be the highest priority to effectively decrease the economic as well as the individual burden of NCDs. To identify these vulnerable individuals early-on, it is pivotal to better understand the determinants of health and disease, and to grasp how different somatic components interact.

Over the past few decades, therefore, there has been a trend arising away from established, unidimensional risk measures such as clinical CRP cutoffs and towards a more holistic and systemic consideration of preventive medicine and particularly risk prediction. An important milestone in this development represents the Allostatic Load Index based on the concept of allostatic load that generally refers to the aggregate burden of chronic stress, life events, and environmental factors (McEwen, 1998). The Allostatic Load Index is a cumulative multi-system risk score resulting from a comprehensive analysis from seven different angles; i.e., (1) anthropometric measures (body-mass index; BMI and waist-to-hip ratio; WHR), (2) cardiovascular markers (systolic and diastolic blood pressure), (3) indicators of sympathetic (epinephrine and norepinephrine) and parasympathetic system nervous system (heart rate variability parameters), (4) lipids (total cholesterol, low-and high-density lipoproteins; LDL and HDL, and triglycerides), (5) glucose homeostasis parameters (glucose and insulin), (6) neuroendocrine markers (cortisol and dehydroepiandrosterone sulfate; DHEA-S), and (7) immune-inflammatory markers (C-reactive protein; CRP, fibrinogen and albumin) (Chen et al., 2012; McEwen, 1998). For each system, risk indices are computed as the proportion of biomarkers within a system for which an individual has been assigned to a predefined high-risk quartile (Chen et al., 2012). These sub system risk scores vary from 0 to 1 (indicating 0 - 100% of system biomarkers in high-risk range for a given individual) and the final value of the Allostatic Load Index is calculated as the sum of the seven sub system scores (ranging from 0 to 7) (Chen et al., 2012; McEwen, 1998).The Allostatic Load Index has become a widely-used measure in research and it has been found to be predictive for various disease outcomes as well as all-cause mortality (Gallo et al., 2014; Juster et al., 2010), while, however, there are also critical limitations related to its conceptualization (Bertele et al., 2021).

Among these limitations is the fact that summing up the separate risk indices for each of the seven angles makes it impossible to account for any interactions among different sub systems and to estimate the potential predictive value of these interactions (Bertele et al., 2021). This gap is unfortunate because the allostatic load index includes parameters that were indeed found to interact, such as BMI and blood pressure (M. Tanaka, 2020). Furthermore, as described in 1.2.4, metabolic, endocrine, and immunological processes are generally linked and so are their effects on disease risk. Another concern refers to practicability issues related to the Allostatic Load Index making it challenging to ultimately implement it into the health care system; while the different parameters needed to calculate the Allostatic Load Index can be assessed relatively easy, it still bears the risk that, under routine clinical circumstances, parameters are only available partly for the majority of patients (Bertele et al., 2021). It remains unclear if and how that might decrease the predictive value of the Allostatic Load in these patients.

Collectively, the Allostatic Load Index is a comprehensive concept that triggered an important rethinking in science and medicine but, at the same time, it artificially splits and then sums up physiological processes that are naturally woven into a systemic allostatic reaction, as even suggested by the creator of the Allostatic Load Index (Bertele et al., 2021; McEwen, 1993). In addition, the Allostatic Load Index lacks feasibility in clinical routine, which is highlighted by the fact that, to date, the Allostatic Load Index has not been widely implemented in the health care system.

However, given the rising number of NCDs, there is a critical necessity to establish measures for risk evaluation such as the Allostatic Load Index in routine clinical practice allowing to target individuals at an enhanced risk for NCDs with preventive steps – before they get ill (Bertele et al., 2021). This is underlined by an alarming finding of the Global Burden of Disease Study (2017) suggesting that, between 1990 and 2017, the total number of DALYs caused by NCDs per year rose from 1.2 to 1.6 billion years. With that, NCDs accounted for more than 60 percent of DALYs worldwide (Global Burden of Disease Collaborative Network, 2018). In addition to this individual burden caused by NCDs, they also strain society, due to massive related monetary and non-monetary costs (Benjamin et al., 2018; Global Burden of Disease Collaborative Network, 2018).

While it is increasingly becoming clear that early-on prevention is the most promising approach to disburden the health care system, this field also represents one of the major public health challenges; namely, due to limited resources, only individuals at an enhanced risk for NCDs can be provided with pre-emptive interventions. Hence, the first step towards decreasing the multifaceted burden due to NCDs is to develop and implement practicable and inexpensive ways to identify individuals at risk.
To properly conceptualize such an approach, it is essential to comprehend the to-date knowledge about the multiple body systems involved in health and disease with each of them being represented by specific easily measurable biomarkers. To review these different systems together with commonly assessed biomarkers are the main purposes of the next sections.

1.2. Biological alterations underlying health and disease

1.2.1. The metabolic system

The term “metabolic system” refers to all biochemical reactions and processes in our body cells that produce energy out of the food we eat (Salway, 2017). In particular, the metabolic system deals with three macronutrients that we supply to the body through food: carbohydrates, proteins, and fats (Salway, 2017). More specifically, the metabolic system cleaves carbohydrates into simple sugars, the primarily used fuel in the human body, it breaks proteins down into amino acids, cell signaling molecules that are key for maintenance, development, and immunity, and it turns fats into fatty acids, the primary storage method of fuel in the human body (Salway, 2017). Hence, the metabolic system is not only intricate and highly dynamic in its function, but it is also essential to sustain life (Salway, 2017).
Carbohydrates, lipids, and proteins all ultimately break down into glucose, which represents the primary metabolic fuel of the human body. Consequently, glucose metabolism is a crucial part of the metabolic system. During the process of glycolysis, at the cellular level, the body uses glucose to generate two molecules of adenosine triphosphate (ATP), a universal form of energy that can be used by cells and tissues

(Nakrani et al., 2022). Insulin is an essential player in glucose metabolism since it allows glucose to enter cells, tissues, and organs, where it is used to generate ATP. In this way, insulin also regulates blood glucose levels keeping it within the healthy range (Schandry, 2016). The glucose metabolism is impaired in many diseases, not only in diabetes mellitus but also in different types of cancer (Gillies et al., 2008; Permert et al., 1993) and even in neuropsychiatric disorders such as schizophrenia (Bryll et al., 2020). Regarding cancer, the main reason for the link to an impaired glucose metabolism (yielding blood glucose levels beyond the health range) is thought to be that elevated glycolysis in response to increased blood glucose levels yields the production of large amounts of acid, which provides cancer cells with a beneficial environment compared to normal parenchyma (Gillies et al., 2008). Schizophrenia, on the other hand, might be related to impaired glucose metabolism as the latter might compromise various cognitive processes that require ATP. Resulting dysfunctions in synaptic transmission may then yield neuronal death and, consequently, changes in different brain areas (Bryll et al., 2020). However, these are only a few examples illustrating the crucial role of the integrity of the glucose metabolism in health outcomes and, in turn, how an impaired glucose metabolism can increase the risk for multiple disease states.

Another important actor in the metabolic system are the mitochondria, also referred to as “power houses of the cell” (Know, 2018). The mitochondria are bacterial endosymbionts meaning that, about 1.5 billion years ago, an α-protobacterium was internalized by an ancestry of the eukaryotic cell (Archibald, 2015). Therefore, mitochondria possess their own genome, known as mtDNA (Anderson et al., 1981). As critical cellular organelles, the mitochondria oxidate food with oxygen from the air and, in this way, generate ATP (Gyllenhammer et al., 2020). In this process called oxidative phosphorylation, mitochondria produce important signaling molecules, that is, reactive oxygen species (ROS) (Gyllenhammer et al., 2020).

Besides this role as major energy supplier, the mitochondria also serve as a cellular signaling system that is highly sensitive to the environment (Chandel, 2015). To respond to changes in the environment resulting in altered bioenergetic needs of the organism, the mitochondrial structure and function both are highly dynamic and adaptive (Picard & McEwen, 2018). The mitochondria can, for example, rapidly scale up their bioenergetic

work during physical activity, immune activation, to respond to acute psychosocial stressors, as well as in any other case of enhanced energy demands (Picard & McEwen, 2018). Chronic exposure to psychological stress as opposed to acute stress episodes is accompanied by increased energy demand and can result in molecular and functional recalibrations among mitochondria causing mitochondrial allostatic load (Picard et al., 2014; Picard & McEwen, 2018). Generally defined as the cumulative damage to the body caused by responses to chronic stress (McEwen & Stellar, 1993), when referring to the mitochondria, allostatic load characterizes “deleterious structural and functional changes that mitochondria undergo in response to elevated glucose levels and stress-related pathophysiology” (Picard et al., 2014). Mitochondrial allostatic load is closely linked to health and disease outcomes, particularly via a “three-stage temporal and causal sequence of biological damage” (Picard et al., 2014): “First, overactivation or underactivation of primary mediators, such as glucocorticoids, induces direct effects and outcomes on cellular processes. Next, secondary outcomes (metabolic, cardiovascular, neural and second-order immune biomarkers) become dysregulated as indicated by their abnormal patterns, including lack of adaptation, prolonged response or blunted response. Finally, this process culminates in tertiary outcomes or clinical end points” (Picard et al., 2014). These clinical end points include but are not limited to hypertension, cardiovascular diseases, diabetes mellitus, neurodegeneration, physical and cognitive decline (Picard et al., 2014).

Collectively, due to its role as the body’s energy supplier, its cellular signaling characteristics, and due to its sensitivity to environmental changes, the metabolic system is a major determinant of health and disease and, thus, a key somatic component to consider in risk evaluation and disease prevention.

There are multiple ways to assess or measure aspects of the metabolic system such as mitochondrial parameters or aspects of glucose metabolism. However, to assess the first requires high expertise and analytic effort making it unsuitable to a routine clinical setting and the latter requires to be measured fasting because it is very sensitive to food intake (Gyllenhammer et al., 2020; Nakrani et al., 2022). In addition, fasting glucose levels are highly influenced by diseases related to the glucose metabolism; especially diabetes (Nakrani et al., 2022). To assess the status of the metabolic system in a global and stable

way, these measures are thus not the first choice as reliable, easily measurable biomarkers in routine clinical care. Instead, I have chosen creatinine to represent the metabolic system. It is becoming increasingly clear from recent research, that creatinine is more than an indicator of renal functioning or a biochemical compound that is left over from the energy metabolism. Rather, it occurs to be a promising indicator of current bioenergetic challenge that the body is confronted with, e.g., while coping with allostatic load (Bonilla et al., 2021; Kashani et al., 2020; Kazak & Cohen, 2020; Kreider & Stout, 2021). Although this research is still very young, a solid body of empirical work suggests creatinine and creatine metabolism in general as essential markers in the context of allostasis and, thus, in disease prediction (Bonilla et al., 2021; Kashani et al., 2020; Kazak & Cohen, 2020; Kreider & Stout, 2021). Creatinine can further be assessed relatively easy, reliable, and cost-efficient (Kashani et al., 2020). In addition, creatinine is known for its link to disease phenotypes. Specifically, altered levels of creatinine often co-occur with renal dysfunction (Perrone et al., 1992), hypertension (Coresh et al., 2001), diabetes, with high BMI and they are also known to increase with age (Culleton et al., 1999).

1.2.2. The endocrine system

Together with the immune system, the endocrine system is the most important regulation and communication system of the body. It uses hormones released into the blood stream as messengers to initiate its desired effects and reactions in the organism (Schandry, 2016). More specifically, a particular hormone needs to reach one of its respective receptors, for example at the membrane of the target cell, to initiate a cellular reaction (Schandry, 2016). Hormones are built and released by eight endocrine glands that, taken together, built one functional system (Schandry, 2016). Some endocrine glands are in the brain such as the hypothalamus, the pituitary gland, and the pineal gland, others such as the thyroid and parathyroid glands can be found in the neck, the thymus is in the upper chest, the adrenals are located on top of the kidneys, and the pancreas is in the abdomen (Schandry, 2016).

The endocrine glands produce 50 different hormones with multiple functions while, for the purposes of this dissertation, I will focus on one specific aspect of the endocrine system

and its respective operating hormones since they are crucial to human stress response and allostatic load processes; the hypothalamic–pituitary–adrenal (HPA) axis (Schandry, 2016).

The HPA axis is the major endocrine stress axis in the human body and regulates several physiological processes, including the metabolic system, immune responses, and the autonomic nervous system mediating the effects of stressors (Dedovic et al., 2009; Sheng et al., 2021). The HPA axis is a negative feedback system and its activation starts with the secretion of corticotropin releasing hormone (CRH) by the paraventricular nucleus in the hypothalamus which then is released into the blood stream and travels to the pituitary gland (Brown, 1994). Here, CRH yields the secretion of adrenocorticotropic hormone (ACTH) into the bloodstream by the pituitary gland (Brown, 1994). ACTH then binds to receptors in the adrenal gland which then initiate the secretion of cortisol (Brown, 1994). Most body cells have receptors for cortisol allowing it to have a range of effects on the metabolic system, the cardiovascular, and immune system (Buckingham, 2006; McEwen, 1998). As part of the above-mentioned negative feedback loop that the HPA axis represents, once secreted into the bloodstream, cortisol regulates its further secretion by binding to receptors in the hippocampus, the amygdala, and in the prefrontal cortex (Feldman & Weidenfeld, 1995; Herman et al., 2005; Herman & Cullinan, 1997).

Cortisol is one of the glucocorticoids and its effects include the increase of blood glucose levels, it also enhances the use of glucose in the brain as well as it scales up the availability of substances to repair cells and tissues (Kemeny, 2003; Wolkowitz & Rothschild, 2003). In addition, cortisol restrains all somatic functions that would be detrimental in an acute situation of fight-or-flight including the suppression of inflammatory and digestive activity, as well as it limits processes of reproduction and growth (Kemeny, 2003; Wolkowitz & Rothschild, 2003). Thus, an adaptive and dynamic cortisol response in cases of acute stress is essential for survival and serves to facilitate adequate coping with acute threats. However, the exposure to chronic stress yield an excessive activation of the HPA axis and a prolonged cortisol release (i.e., hypercortisolism), going along with an increase in allostatic load that, as described above, enhances the risk for numerous disease states (McEwen, 2004). Specifically, hypercortisolism has previously been associated with an increased risk for cardiovascular and metabolic comorbidities such as

hypertension, impaired glucose metabolism and diabetes type 2, dyslipidemia, obesity, and metabolic syndrome because it often involves DNA, tissue, and organ damage (Min, 2016; Pivonello et al., 2008; Steffensen et al., 2016).

Collectively, the endocrine system is an intricate system whose sub systems are spread across the body. As a key player in human stress response and homeostasis, it is very reactive to changes in the environment as well as very interactive with other systems such as the immune and the metabolic system. Hence, it is considered another main determinant of health and disease across the lifespan.

The endocrine system will be represented by cortisol in the context of this dissertation since cortisol is the end product of the HPA axis, making it an important player in health and disease. Furthermore, although there is no gold standard measure of allostatic load, cortisol is among the most used endocrinological measures representing some of the physiological adjustments of the system due to allostasis (Lee et al., 2015). In addition, cortisol can be measured reliably, easily, and in-expensively.

1.2.3. The immune system

The immune system is the body's own protection system, and it defends us against pathogens and uncontrolled cell growth. The immune system consists of three defense lines; physical barriers, the innate and the acquired immune system (Wittchen & Hoyer, 2011). The first includes the intact skin as well as mucous membranes that, together, manage to repel most pathogens. The innate immune system also protects from external pathogens, but it can also destroy degenerated body cells (Wittchen & Hoyer, 2011). To be able to detect a variety of pathogens, the innate immune system focuses on molecular structure patterns of pathogens, so-called Pathogen-Associated Molecular Patterns (PAMPs) which it can identify using Pattern Recognition Receptors (PRRs) such as phagocytes circulating in the blood stream (Wittchen & Hoyer, 2011). After binding to the PRRs, the process of phagocytosis begins. Here, the pathogen is first absorbed by the cell and then digested in the cell plasma (Wittchen & Hoyer, 2011). While the PRRs of the innate immune system are detective of a broad range of pathogens and can initiate a more unspecific defense reaction, the acquired immune system develops across the

lifespan learning to encounter pathogens with a more specific defense reaction (Wittchen & Hoyer, 2011). Because the organism encounters different viruses and bacteria, lymphocytes form particular memory cells that will detect and initiate a specific and effective defense reaction in case of a second contact with a specific irritant (Wittchen & Hoyer, 2011). Notably, once acquired, this specific immunity then can persist throughout the lifespan (Wittchen & Hoyer, 2011).

The processes initiated whenever the immune system responds to an irritant are commonly summarized under the term inflammation. In the context of an inflammation process, the immune system utilizes different messenger substances, called cytokines, to communicate and to coordinate the immune reaction (Wittchen & Hoyer, 2011). For example, it uses Interleukin-6 (IL-6) to stimulate acute phase responses and immune reactions (M. Tanaka, 2020). Even faster than the secretion of IL-6 is the release of C-reactive protein (CRP), an acute-phase reactant protein produced in the liver, accelerating “the removal of cellular debris and damaged or apoptotic cells and foreign pathogens” (Nehring et al., 2022). Another important actor in an acute-phase immune response to particularly tissue injury is fibrinogen (Budzynski & Shainoff, 1986; Luyendyk et al., 2019). Fibrinogen is a multifunctional glycoprotein and its role in driving acute inflammatory responses is two-folded: “The first phase is dominated by thrombin cleavage of fibrinogen integrated with an acute inflammatory response that functions to contain tissue damage, stop the loss of blood, and prevent microbial infection. The second phase is dominated by plasmin dissolution of fibrin and other matrix proteins integrated with reparative inflammatory cells working to remodel and repair damaged tissue” (Luyendyk et al., 2019).

As mentioned above, inflammation can be triggered by harmful irritants that encounter the organism during an acute infection but there are further causes and factors that can contribute to an increase in inflammation such as stress, obesity, and age. For example, it has been shown that (psychosocial) stress can yield inflammatory responses in the brain and peripherally (Calcia et al., 2016; Rohleder, 2014). This mainly takes place via interactions between the endocrine and immune system, as will be described in more detail below. Obesity can also cause inflammation since adipose tissue is known to produce and release pro-inflammatory mediators, including IL-6 (Lafontan, 2005). The

specific processes that link obesity to inflammation will be discussed as interactions between the metabolic and the immune system, in section 1.2.4.

An important question given the increasing risk for various disease states with age is whether inflammaging, i.e., chronic, low-grade inflammation occurring with age and in the absence of an acutely triggering infection or harmful stimuli (Franceschi et al., 2018; Kennedy et al., 2014), directly causes these pathological conditions or whether it acts more as a biological mediator between its etiology factors, that are being increasingly understood, and disease outcomes (for a review, see Ferrucci & Fabbri, 2018). Inflammaging goes along with a continuing activation of the innate immune system that is mainly initiated by endogenous signals (Franceschi et al., 2018; Kennedy et al., 2014). It is not yet fully understood what causes inflammaging and why it even exists. While, on the one hand, previous NCDs might be one reason for accelerated aging of the immune system, on the other hand, an established theory attempting to explain inflammaging is the antagonistic pleiotropy theory of ageing (Franceschi et al., 2017). This theory postulates that, because inflammation has been evolutionarily selected due to its protective effects in early life and adulthood, it may persist and have detrimental effects, however, throughout later adulthood when the principle of natural selection is not active anymore (Franceschi et al., 2017). Yet, inflammaging might not occur to the same extent as a determinative function of age. Instead, a comprehensive body suggests certain factors enhancing inflammaging such as “genetic susceptibility, central obesity, increased gut permeability, changes to microbiota composition, cellular senescence, oxidative stress caused by dysfunctional mitochondria, and immune cell dysregulation” (Ferrucci & Fabbri, 2018).

Collectively, just like with the endocrine stress response described above, inflammation in response to a current threat is adaptive and essential to sustain life. However, by damaging DNA, tissue and organs, chronic inflammation has been shown to yield an increased risk of cardiovascular disease, cancer, chronic kidney disease, dementia, and depression as well as for premature death (for reviews, see Ferrucci & Fabbri, 2018; Furman et al., 2019). Hence, inflammation plays a crucial role maintaining health, but it is also one of the main biological processes underlying disease and dysfunction.

For the scope of this dissertation, inflammation will be represented by CRP, IL-6, and fibrinogen to get a maximum systemic perspective on inflammation; by involving mediators secreted directly in immune cells but also in peripheral organs such as the liver. CRP is an acute-phase reactant protein secreted by the liver in the scope of an inflammatory reaction (Nehring et al., 2022). Among the various functions of CRP is the identification and elimination of pathogens and injured cells (Nehring et al., 2022). Elevated CRP concentrations have previously been associated with acute infectious states (Nehring et al., 2022) but also with chronic conditions such as neurodegenerative disorders (Luan & Yao, 2018), renal disease (Panichi et al., 2001), and obstructive pulmonary disease (Lazovic, 2012). The main function of IL-6 is to support the organism in responding to infections by stimulating acute phase responses and immune reactions (Gabay, 2006; T. Tanaka et al., 2014). IL-6, thus, is a proinflammatory cytokine and echoes the acute, liver-induced inflammatory response by the highly sensitive CRP (Gabay, 2006). IL-6 has previously been linked to atherosclerotic cardiovascular disease, heart failure, and all-cause mortality (Cainzos-Achirica et al., 2018). Fibrinogen is a multifunctional glycoprotein released into the blood stream and completes the picture as a systemic moderator of the inflammatory cascade (Budzynski & Shainoff, 1986; Luyendyk et al., 2019). The main function of fibrinogen is the production of fibrin to bind together platelets and plasma proteins (Budzynski & Shainoff, 1986; Luyendyk et al., 2019). As such, elevated concentrations of fibrinogen have previously been associated with pulmonary disease (Duvoix et al., 2013), cardiovascular disease (The Emerging Risk Factors Collaboration, 2012), and atherothrombotic disease (Green, 2006). CRP, IL-6, and fibrinogen are furthermore the most used measures of inflammation in the literature, and they can be assessed easily and robustly in a laboratory setting (Budzynski & Shainoff, 1986; Luyendyk et al., 2019; Nehring et al., 2022; M. Tanaka, 2020).

1.2.4. How and why these systems interact

Although the term “system” has been referred to the metabolic, the endocrine, and the immune system, it is important to note that they are rather “sub systems” making and sustaining a much more comprehensive and intricate system, that is, the human

organism. Each sub system has its respective tasks and, thus, takes on an essential role towards the aim of maintaining the organism's global health. However, the interactions among the different sub systems are at least as important to this superior aim as each sub system considered separately. These intersystemic interactions are multi-facetted and complex; yet, in the following, I will address them in a very selective and limited fashion to fit the scope of this dissertation.

Interactions between the metabolic and the endocrine system. The endocrine system not only influences gene expression and, with that, protein synthesis, but it also is the chief regulator of circadian alignment (Keay, 2017; Schandry, 2016). Consequently, the endocrine system represents an essential modulator of the metabolic system; more specifically, it regulates the synthesis of gut-peptides, glucose-insulin interactions, substrate oxidation, as well as leptin and ghrelin concentrations in blood (Keay, 2017; Schandry, 2016). These are, however, not unidirectional effect chains but they rather involve various feedback system loops in which, for example, the hypothalamus as a control center of the endocrine system receives feedback about initiated changes in the metabolism allowing the endocrine system to increase or decrease its stimulating effects on the metabolic system (Keay, 2017; Schandry, 2016).

Metabo-endocrine interactions are also crucial in the context of chronic stress and allostatic load. In these cases, levels of oxidative stress tend to increase due to the excess production of ROS by the mitochondria (Sato et al., 2010). This oxidative stress is thought to cause an overactivation of the HPA axis yielding an increased secretion of cortisol and, more importantly, resulting in a further increase in oxidative stress (Sato et al., 2010). This modulation role of the endocrine system in oxidative stress is crucial to allow the metabolic and the endocrine system to orchestrate as the body responds to all types of stressors (Vitale et al., 2013). However, in some cases, these dynamics can also yield a vicious cycle of accelerating aging (Vitale et al., 2013).

Interactions between the metabolic and the immune system. The mitochondria function as a central modulator of the immune system since they provide the energy for and regulate cell defense (for a review, see Meyer et al., 2018). The mitochondria are responsible for the initiation of cell responses to the activation of the innate immune system but also to cell stress or damage, e.g., due to allostatic load (for a review, see

Meyer et al., 2018). Here, mitochondrial components released to the cytoplasm or the extracellular space function as danger signals when identified by receptors of the innate immune system, activating the same (for a review, see Meyer et al., 2018). In turn, mitochondrial function can also be altered by immune activation (Yu et al., 2020).

Another clinically relevant context of interaction between the metabolic and the immune system represents obesity (de Heredia et al., 2012; Larabee et al., 2020). The excessive amount of adipose tissue involved in cases of obesity are increasingly understood to trigger chronic systemic inflammation (de Heredia et al., 2012; Larabee et al., 2020). More specifically, this connection is thought to be mediated by alterations in fatty acid induced inflammation, adipokine secretion, oxidative stress, adipose tissue hypoxia, and endoplasmic reticulum stress (de Heredia et al., 2012; Larabee et al., 2020). In addition, adipocytes synthesize adipokines including proinflammatory leptin whose production is increased in cases of obesity (de Heredia et al., 2012; Larabee et al., 2020). Leptin initiates monocyte proliferation and differentiation into macrophages, influencing the stimulation of natural killer cells, and yielding the secretion of pro-inflammatory cytokines including IL-6, and interleukin-12 (IL-12) (de Heredia et al., 2012; Larabee et al., 2020).

Collectively, metabo-immune interactions are not only crucial to maintain health but their coordination also plays a crucial role in allostatic load processes and, thus, in disease vulnerability and progression (for a review, see Meyer et al., 2018).

Interactions between the endocrine and the immune system. The endocrine and the immune system both are systems distributed widely across the body and they communicate extensively and bi-directionally (for a review, see Klein, 2021). In particular, cortisol, the end product of the HPA axis, is known for its anti-inflammatory and immunosuppressive effects (Coutinho & Chapman, 2011). However, it has been found that a continuous activation or overstimulation of the HPA axis, e.g., in cases of chronic stress, can induce inflammation, since peripheral mononuclear cells that have been stimulated with CRH in the context of an HPA axis activation, also increase the secretion of IL-6 and, hence, yield an activation of the immune system (for a review, see Angioni et al., 1993). In response to an excess and continuous secretion of cortisol, the immune system can, in turn, loose its sensitivity to the immune-suppressing effect of glucocorticoids, yielding an accumulation of cortisol and an increased secretion of

proinflammatory cytokines (Miller et al., 2002; Vitlic et al., 2014). Specifically, it is thought that white blood cells respond to the excessive expression of glucocorticoids with a counter-reaction, down-regulating the expression and/or function of receptors responsible for binding glucocorticoid hormones. This downregulation of receptors then reduces the ability of the immune system to respond to the anti-inflammatory effects of cortisol (Miller et al., 2002).

Together, endocrine-immune crosstalk is essential to allow the organism to respond to environmental changes and stressors in a systemic manner but, again, might result in a detrimental cycle towards disease susceptibility in cases of allostatic load.

Interplay of metabolic, endocrine, and immune system. As laid out above, all three sub systems stand in close communication and continuously interact to maintain homeostasis in the body (Angioni et al., 1993; Klein, 2021; McEwen, 1998, 2004; Straub, 2014; Wensveen et al., 2019). By initiating and potentiating each other's effects, these intersystemic communications allow extremely fast and effective responses of the body to acute stressors in the short-term (Meyer et al., 2018; Sato et al., 2010; Vitale et al., 2013). They also allow the body to adapt to longer-term environmental changes, perfectly tailoring its functioning to specific conditions. However, various risk factors such as chronic stress, obesity, substance abuse, or genetic predispositions might yield detrimental effects of these intersystemic interactions on disease risk (McEwen, 1998, 2004; Straub, 2014; Wensveen et al., 2019).

A specific context of interaction between the endocrine, the metabolic, and the immune system is represented by the Nuclear Factor-KappaB (NFKB) signaling pathway, a significant regulator of genes involved in development and progression of inflammatory processes. An activation of NFKB can involve two main pathways: the canonical and the noncanonical pathway (for a review, see Liu et al., 2017). The first can be activated by various stimuli, such as ligands of several cytokine receptors, PRRs, tumor necrosis factor receptor superfamily members, as well as T-cell and B-cell receptors, the latter is activated more selectively, by limited group of stimuli, such as ligands of a subset of tumor necrosis factor receptor superfamily members (for a review, see Liu et al., 2017). While the canonical NFKB is involved in most parts of the immune response, the noncanonical NFKB pathway represents as a supplementary signaling axis cooperating with the

canonical NFKB pathway to modulate specific functions of the adaptive immune system (for a review, see Liu et al., 2017). In general, NFKB is considered a downstream effector of the endocrine response to stressful psychosocial events and connects changes in neuroendocrine axis activity to the cellular response (Bierhaus et al., 2003). Consequently, both NFKB pathways play important roles in translating an endocrine stress signal into a cellular response involving the induction of an inflammatory reaction (Bierhaus et al., 2003; Mehet, 2007). Furthermore, during sympathetic activation in the context of stressful events, NFKB is also activated, yielding an increased secretion of pro-inflammatory cytokines (Bierhaus et al., 2003; De Bosscher et al., 2003; Mercurio & Manning, 1999). To downregulate this increase of inflammatory signaling in the system, the HPA axis enhances the secretion of cortisol (Bierhaus et al., 2003; De Bosscher et al., 2003; Mehet, 2007; Mercurio & Manning, 1999). In some cases, e.g., in cases of glucocorticoid resistance or insensitivity, these enhanced cortisol concentrations might, however, become chronical (Bierhaus et al., 2003; De Bosscher et al., 2003; Mercurio & Manning, 1999). Interestingly, NFKB is also activated by obesity and metabolic stress causing increased levels of uncontrolled inflammation observable in obese individuals (Catrysse & van Loo, 2017). At the same time, NFKB activation influences the metabolic system by contributing to insulin resistance in these cases (Catrysse & van Loo, 2017). Consequently, NFKB is a crucial mediator between the metabolic, the endocrine, and the immune system emphasizing again the close and inseparable connection of these three systems (Bierhaus et al., 2003; Catrysse & van Loo, 2017).

1.3. A novel bioinformatics approach for risk evaluation

Following the promising trend towards a more comprehensive and systemic perspective on risk evaluation as proposed by the Allostatic Load Index, I developed an innovative biomarker-based approach aimed at identifying individuals with a high burden of NCDs and at risk for premature mortality. The main objective here was to develop a sufficient measure that can reasonably be incorporated in routine diagnostics by involving as little effort as possible (Bertele et al., 2021). Here, dimension reduction is achieved through a multivariate approach. According to its conceptualization, the approach attempts to use

no more than five commonly and easily assessed biomarkers, i.e., CRP, IL-6, fibrinogen, cortisol, and creatinine to divide individuals into distinct clusters (Bertele et al., 2021). As described above, these five biomarkers cover a broad and diverse somatic functionality and are well-established representants of different systems, so that their combination allows a systemic view on the current somatic condition. In brief, the combination of CRP, fibrinogen, and IL-6 allow a consideration of systemic inflammation as a multi-level network. CRP as produced in the liver highly sensitive marker indicating the current degree of inflammation in the body, an additionally increased IL-6 from immune cells quantifies the level of manifestation of an inflammatory signal, and fibrinogen marks the extent to which repair processes of tissue damage have been initiated as a result of inflammation (Baumeister et al., 2016; Budzynski & Shainoff, 1986; Luyendyk et al., 2019; Nehring et al., 2022; Rückerl et al., 2007; M. Tanaka, 2020; Thompson et al., 2010). I thus expect that, if considered in combination, CRP, IL-6, and fibrinogen will allow to explain a maximum possible level of variance compared to a single inflammatory marker. The involvement of cortisol as the end product of the HPA axis allows conclusions about the endocrine allostatic load and creatinine adds information about the current global ATP demand (Kashani et al., 2020; Zorn et al., 2017). Employing a k-mean clustering approach based on these five biomarkers as opposed to their consideration as main factors furthermore allows to take linear and non-linear interactions among these biomarkers into account and to relate the resulting clusters to respective outcomes (Bertele et al., 2021).

1.4. Aims

Given the urgent need for innovative – sensitive and economic – tools for risk prediction and embracing the novel, systemic thinking in predictive, preventive, and personalized medicine, this thesis seeks to investigate the following aims in the Midlife in the United States (MIDUS) and the Midlife in Japan (MIDJA) study cohort, two large, prospective general population samples.

1. To develop a novel, cluster-based tool for risk evaluation using k-mean clustering

Hypothesis: K-mean clustering approach based on concentrations of CRP, IL-6, fibrinogen, cortisol, and creatinine allows to identify and replicate a number of distinct biochemical clusters in a U.S. American and Japanese cohort.

2. To identify risk/etiology factors of the identified biochemical clusters

Hypothesis: Because the identified biochemical clusters likely have a considerable environmental component, a number of risk factors (e.g., sex, age, smoking habits, early-life stress) related to the cluster assignment will be identifiable.

3. To examine disease burden in the identified biochemical clusters

Hypothesis: Current disease burden can be predicted based on the identified biochemical clusters.

4. To investigate the predictive value of the identified biochemical clusters regarding mortality and inability to work 10 years following the biomarker assessment.

Hypothesis: Mortality and inability to work (during the past 30 days) ten years after the biomarker assessment can be predicted based on the identified biochemical clusters.

1.5. Contribution of this dissertation

1.5.1. Contribution to the field of risk stratification, preventive, and personalized medicine

In this work, a novel approach to risk stratification is presented that aims to take a more holistic and systemic view of risk. This perspective of the body as a comprehensive and interactive network is also reflected in the methodology of the thesis, since it represents a pioneering step toward establishing the use of multivariate statistics in the context of biomarker-based risk prediction. From a statistical point of view, it thus allows an enhanced degree of individual variation to be explained despite its parsimonious conceptualization as a result of the limitation to the five biomarkers considered most representative of the endocrine, the metabolic, and the immune system (Franklin, 2005). This parsimony feature could allow the actual implementation of the proposed tool in routine diagnostics and help to identify individuals at increased risk for health and mortality outcomes in need of targeted prevention.

By helping to identify individuals at risk, who should be targeted with preemptive interventions, the novel tool presented here helps to pave the way for effective and cost-efficient prevention. First, by employing a multivariate network approach, it makes the identification of individuals at risk more precise, narrowing the number of individuals to be targeted with prevention, and thus, related costs. Second, the multivariate approach used here, advances the understanding of interactions among different biomarkers which might have valuable implications for the new and further development of preventive interventions. Third, the supplementary consideration of various risk factors including drug consumption, BMI, and physical activity in the context of the identified biochemical clusters might add to these implications for preventive medicine. Moreover, the consideration of environmental factors and their interplay with biochemical interactions in the context of health and mortality outcomes also advances the field of personalized medicine.

1.5.2. Contribution to the field of medical psychology

By including the exposure to childhood maltreatment (CM) such as child abuse and neglect as one of the risk factors examined in relation to the identified biochemical clusters, the thesis explores biochemical profiles as a potential somatic manifestation of early-life stress. If associations between CM exposure and cluster assignment will be found, it could improve our understanding of how CM can enhance the risk for detrimental longer-term outcomes, as has been demonstrated by a wide range of empirical studies (for a review, see Grummitt et al., 2021). Furthermore, a link between cluster assignments and CM exposure would also indicate that psychotherapy represents a leverage point in both prevention and intervention of biochemical profiles associated with disease and mortality, highlighting the necessity of involving interdisciplinary perspectives in this context, that is, those of medical psychology.

1.5.3. Contribution of this author

I conducted a comprehensive literature review on biomarker-based risk predictions, the considered outcomes and risk factors, as well as on the previously used methodological approaches. I acquired the MIDUS and the MIDJA data set and prepared both data sets for the planned analyses. I conceptualized and computed a novel risk evaluation tool based on biochemical clusters using k-mean clustering, I defined the factors and outcomes to be tested in relation to the clusters, and I conducted all respective analyses. As the first author of both publications, I composed the initial drafts of the manuscripts and incorporated suggestions from coauthors and journal reviewers/editors throughout the revision process. Moreover, I presented the findings in poster presentations at conferences and colloquia.

1.5.4. Contribution of this dissertation

This work is based on two publications that I wrote as the first author (Bertele et al. 2021, 2022). All instances in the following Methods and Results sections that include some extracts, tables, and figures from the publications related to this dissertation (Bertele et al., 2021, 2022), have been cited as such. This dissertation expands significantly on the work presented in the published articles. On top of a more profound and extensive review of the literature background laying the groundwork for the hypotheses tested in the articles and in the dissertation, several additional analyses and results are presented that are not included in the publications. The discussion integrates and addresses findings from both papers and, thus, is much more comprehensive than the discussions in the publications. Moreover, the discussion presented here includes a more extensive reflection of the clinical implications of the findings as well as their implications on other related fields such as public health, medical psychology as well as “3P Medicine” that is, personalized medicine, targeted prevention, and predictive diagnostics. Finally, the dissertation expands on the outlook for future research directions, that have been presented in the publications.

2. Methods

2.1. Study design

The study has cross-sectional and prospective elements. As depicted in Figure 1, the first step was to compute the k-mean cluster analysis based on CRP, IL-6, fibrinogen, cortisol, and creatinine in the U.S. American sample and to then validate the resulting biochemical clusters in the Japanese cohort. Second, I compared the distributions of biological sex, age, BMI, physical activity, alcohol consumption, smoking (lifetime) – all factors affecting risk for NCDs – among different clusters in the U.S. American cohort and did the same in the Japanese cohort, despite here, only information on sex, age, and BMI was available. Since information on the exposure to CM was available in the U.S. cohort, I compared the severities among biochemical clusters in this sample only. Next, in each cohort, the clusters were related to diagnoses of depression, heart disease, hypertension, peptic ulcer disease, stroke, and cancer at the time of biomarker assessment (T0) because these diseases represent highest prevalence worldwide, the fastest expansion in numbers, and the utmost comorbidities (Bertele et al., 2021; Global Burden of Disease Collaborative Network, 2018). Then, I investigated mortality rates as well as reported inability to work approximately ten years following the biomarker assessment (T1) between biochemical clusters in the U.S. American cohort.

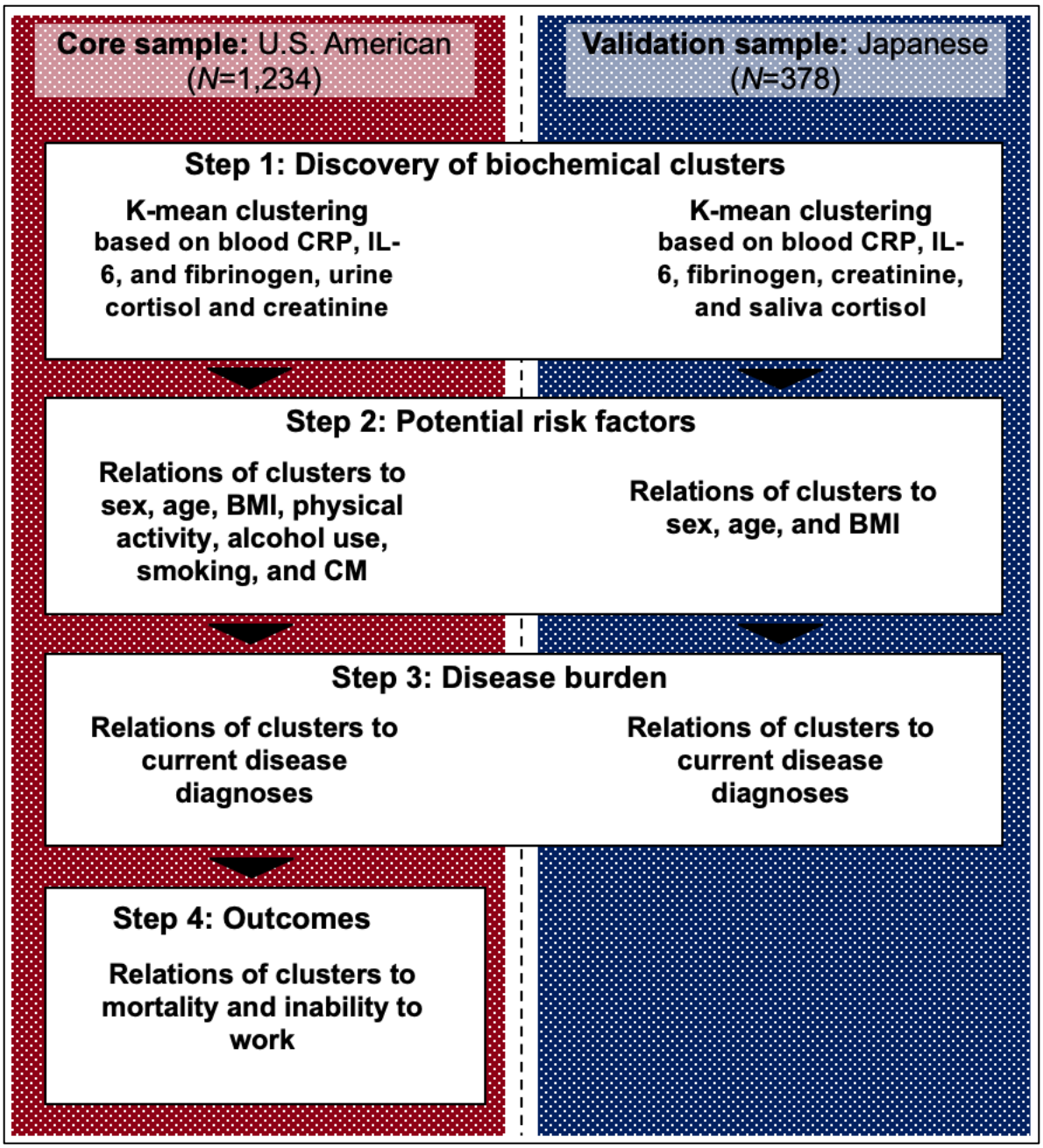

Figure 1: Study workflow chart, CRP=C-reactive protein, IL-6=Interleukin-6, BMI=Body Mass Index, CM=Childhood maltreatment. Source: Adapted from “How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine” N. Bertele et al., 2021, EPMA Journal, 12, p. 508 (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature.

Considered diseases were depression, heart disease, hypertension, peptic ulcer disease, stroke, and cancer.

2.2. Data collection and participants

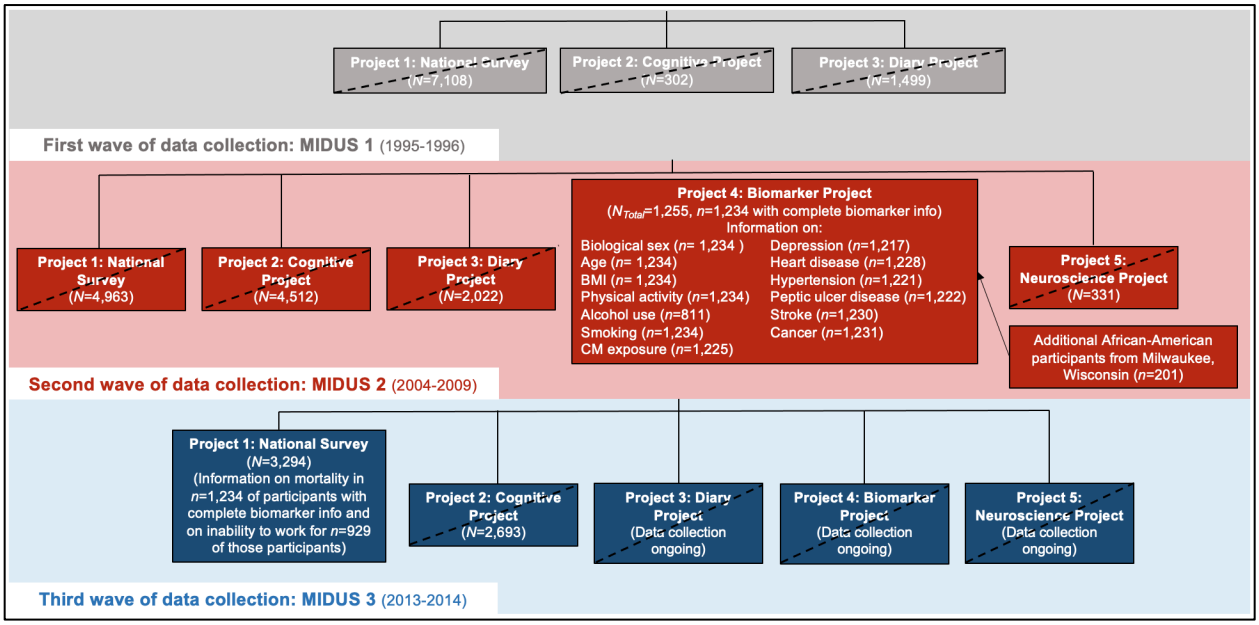

Figure 2: MIDUS: Data collection flowchart, MIDUS=Midlife in the United States, BMI=Body Mass Index, CM=Childhood maltreatment. Source: Own representation.

Irrelevant sub projects were crossed out.

As can be seen in Figure 2, MIDUS study is a population-based, longitudinal study with many assessment points and multiple sub projects. In this study, I focused on the second (MIDUS 2) and the third assessment timepoint (MIDUS 3). Specifically, I analyzed the biomarker sub sample of MIDUS 2 that has been assessed between 2004 and 2009 and consists of 1,255 participants (Dienberg Love et al., 2010). This sub sample contains biomarker data of 1,054 of the more than 7,000 participants from the large survey study starting in 1995, i.e., MIDUS 1, and of 201 additionally recruited African American participants from Milwaukee, Wisconsin (Radler & Lavender, 2020). The demographics of the whole MIDUS 2 sample as well as the biomarker sub sample used here are summarized in Table 1.

Table 1: Comparison of MIDUS 2 biomarker sub sample and the overall MIDUS 2 cohort.

Comparison of MIDUS 2 biomarker sub sample and the overall MIDUS 2 cohort.

	MIDUS 2 Biomarker Sub Sample	**Whole MIDUS 2 Cohort**
Biological Sex	56.8% female (*N*=1,234)	53.3 % female (*N*=4,963)
Age	*Mean:* 52.5 (*St. Dev.:* 11.7) (*N*=1,234)	*Mean:* 54.4 (*St. Dev.:* 12.5) (*N*=4,963)

Note: MIDUS=Midlife in the United States Study, St. Dev.= Standard deviation. Age was measured in years. Source: Own representation.

The MIDUS 2 biomarker data contained CRP, IL-6, fibrinogen, cortisol, and creatinine concentrations on 1,234 participants, information on sex, age, BMI, physical activity, and smoking (lifetime) all of those participants, and information on alcohol consumption was available in 811 of those participants. Information on the exposure to CM was obtained for 1,225 of the participants with complete biomarker data (i.e., info on CRP, IL-6, fibrinogen, cortisol, and creatinine concentrations). Information on depression diagnosis was available in 1,217 of the participants with complete biomarker info (i.e., info on CRP, IL-6, fibrinogen, cortisol, and creatinine concentrations), on hypertension for 1,221 participants, on heart disease for 1,228, on peptic ulcer disease for 1,222, on stroke for 1230, and on cancer for 1,231 participants. The third wave of data collection (MIDUS 3) took place from 2013 to 2014 and included information on mortality of 1,234 participants and on the inability to work of 929 participants of the MIDUS 2 biomarker sample (Figure 2).

MIDJA is the Japanese equivalent to MIDUS and, in this dissertation and related publications, I only focused on the biomarker subsample and one timepoint (2009-2010) (Markus et al., 2020). Table 2 shows the demographics for the whole MIDJA cohort (*N*=1,027) compared to the biomarker sub sample. The MIDJA biomarker project contained information on CRP, IL-6, fibrinogen, cortisol, and creatinine concentrations in 378 participants, information on sex, age, and BMI in all of those participants (Markus et al., 2020). Information on depression diagnosis was available in 378 of the participants with complete biomarker info (i.e., info on CRP, IL-6, fibrinogen, cortisol, and creatinine concentrations), on hypertension for 360 participants, on heart disease for 378, on peptic

ulcer disease for 355, on stroke for 360, and on cancer for 355 participants. Unfortunately, MIDJA includes no information regarding CM exposure nor any prospective data on mortality or the ability to work (Markus et al., 2020).

Table 2: Comparison of MIDJA 1 biomarker sub sample and the overall MIDJA 1 cohort.

Comparison of MIDJA 1 biomarker sub sample and the overall MIDJA 1 cohort.

	MIDJA 1 Biomarker Sub Sample	**Whole MIDJA 1 Cohort**
Biological Sex	56.1% female (*N*=378)	50.8 % female (*N*=1,027)
Age	*Mean:* 55.3 (*St. Dev.:* 14) (*N*=378)	*Mean:* 54.4 (*St. Dev.:* 14.2) (*N*=1,027)

Note: MIDJA=Midlife in the Japan Study, St. Dev.= Standard deviation. Age was measured in years.
Source: Own representation.

2.3. Biomarker assessment

The following biomarker assessment methods are described similarly in Bertele et al. (2021). In MIDUS, overnight fasting serum samples were obtained to assess CRP, IL-6, and fibrinogen concentrations, according to the manufacturer guidelines (Dade Behring Inc., Deerfield, IL for CRP and fibrinogen; R&D Systems, Minneapolis, Minnesota for IL-6) (Bertele et al, 2021; Crimmins et al., 2008). Citrated plasma levels of CRP and fibrinogen were assayed using immunonephelometric assay; IL-6 was assayed using Enzyme-Linked Immunosorbent Assay (ELISA) (Bertele et al, 2021; Gruenewald et al., 2012). 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 (Bertele et al, 2021; Gruenewald et al., 2012). Aiming to obtain a cumulative cortisol and creatinine measure, 12-hour overnight urine samples were collected between 7pm and 7am. Enzymatic Colorimetric Assays and Liquid Chromatography-Tandem Mass Spectrometry were performed at the Mayo Medical Laboratory in Rochester, Minnesota, U.S. (Bertele et al, 2021; Gruenewald et al., 2012). Participants were excluded from this procedure if they

had a renal failure or severe renal decline according to glomerular filtration rate (Bertele et al, 2021; Gruenewald et al., 2012).

In MIDJA, CRP, IL-6, and fibrinogen concentrations were assessed analogically to MIDUS, while cortisol was only available as assessed in saliva (three subsequent days, three times each day) (Bertele et al, 2021; Gruenewald et al., 2012). The resulting nine saliva measurements were averaged and used as a representative marker for an individual's cortisol concentration (Bertele et al., 2021; Kobayashi & Miyazaki, 2015). Creatinine was assessed in blood.

2.4. Assessment of biological sex, age, BMI, physical activity, alcohol consumption, lifetime smoking, and childhood maltreatment

Biological sex was assessed via self-report (C. D. Ryff et al., 2010). Age was assessed by subtracting the self-reported date of birth from the date of assessment (T0) (C. D. Ryff et al., 2010). Weight and height were assessed during the study visit (T0) and BMI was then calculated "by dividing weight in pounds by height in inches squared and multiplying by a conversion factor of 703" (C. D. Ryff et al., 2010). Physical activity was assessed via self-report, by asking participants: "Do you engage in regular exercise, or activity, of any type for 20 minutes or more at least 3 times a week?" (0=No, 1=Yes) (Bertele et al., 2021; C. D. Ryff et al., 2010). Alcohol consumption was also assessed via self-report ("In the past month, how often did you drink any alcoholic beverages, on the average?", 1=everyday, 2=5 or 6 drinks a week, 3=3 or 4 drinks a week, 4=1 or 2 drinks a week, 5=Less than one drink a week, 6=Never drinks) as well as smoking ("Have you ever smoked cigarettes regularly", 0=No, 1=Yes) (Bertele et al., 2021; C. D. Ryff et al., 2010). CM was assessed only in MIDUS and by using the short form of the Childhood Trauma Questionnaire (CTQ), a retrospective self-reported measure (Bernstein & Fink, 1998). The CTQ is a well-established measure, and it covers five subtypes of CM, that is, childhood emotional abuse (e.g., "I thought that my parents wished I had never been born."), physical abuse (e.g., "I was punished with a belt a board, a cord, or some other hard object."), sexual abuse (e.g., "Someone tried to touch me in a sexual way or tried to make me touch them."), emotional neglect (e.g., "There was someone in my family who

helped me feel that I was important or special”(R)), and physical neglect (e.g., “I didn't have enough to eat.”) (Bernstein & Fink, 1998). Each CM subtype is being assessed by five items and responses range from 1 (=Never true) to 5 (=Very often true) (Bernstein & Fink, 1998). The CTQ also contains a minimization/denial scale with three items (e.g., “I had the perfect childhood.”) (Bernstein & Fink, 1998). However, for the scope of this dissertation, only the subscales assessing the five different CM subtypes were considered. As suggested by Bernstein & Fink (1998), I built a sum score of all 25 items with higher values representing higher severities of CM. Consequently, the sum scores vary from 25 to 125 (Bernstein & Fink, 1998). According to Bernstein and Fink (1998) the CTQ was found to have high structural validity (via confirmatory factor analysis) and convergent validity (via correlations with therapists’ ratings). Furthermore, the CTQ has been shown to have high internal consistency, e.g., of 0.92 (Bernstein et al., 2003). Cronbach’s alpha in the current study was 0.95.

2.5. Assessment of disease burden

Since they represent highest prevalence worldwide, the fastest expansion in numbers, and utmost comorbidities, I focused on depression, heart disease, hypertension, stroke/Transient Ischemic Attack (TIA), peptic ulcer disease, and cancer (Global Burden of Disease Collaborative Network, 2018). Specifically, at T0, participants were asked if they were ever diagnosed with any of these diseases (0=No, 1=Yes) (Bertele et al., 2021).

2.6. Follow-up assessment of mortality

The MIDUS team used three different methods to obtain mortality data throughout October 2015. First, a National Death Index was conducted in 2009 that confirmed the death of 173 participants (Bertele et al., 2022; Elliot et al., 2018). Another 322 deaths were registered in the scope of tracing and mortality closeout interviews conducted by the University of Wisconsin Survey Center (UWSC) as part of MIDUS 3 (Bertele et al., 2022;

Elliot et al., 2018). Lastly, 57 deaths were confirmed via normal longitudinal sample maintenance (Bertele et al., 2022; Elliot et al., 2018).

2.7. Follow-up assessment of the inability to work

Participants' inability to work, as a measure of current everyday functioning, was assessed by a single item asking: "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?"(C. Ryff et al., 2015).

2.8. Statistical analysis

2.8.1. K-mean clustering

The following content has been described similarly in Bertele et al. (2021). K-mean clustering is a commonly used approach to classify multidimensional data into groups with characteristic patterns, and has previously been used in analyses of phenotypes based on biomarkers (Bertele et al., 2021; Franklin, 2005). K-mean cluster analysis panels the data points into a number of (k) clusters. An observation is assigned to the nearest cluster measured by Euclidean distance (Bertele et al., 2021; Franklin, 2005). In the current study, k-mean cluster analytics were used to identify distinct biochemical patterns (Bertele et al., 2021). The reason to prefer a k-mean generated cluster variable before the original biochemical indicator variables include that k-mean generated clusters help reduce the data and provide discrete memberships of biochemical patterns, which was a primary goal of this study (Bertele et al., 2021). Moreover, k-mean generated cluster variables allow to alleviate multicollinearity issues faced by directly using the original biomarkers in a regression model and, as a multivariate approach, they allow to account for linear and non-linear interactions among the original variables (Bertele et al., 2021; Franklin, 2005).

Because k-mean clustering is dependent on the participants' order in a data set (Bertele et al., 2021; Franklin, 2005), it is recommended to randomize the order before running

the k-mean clusters. After doing so, I performed a k-mean cluster analysis based on z-standardized CRP, IL-6, fibrinogen, cortisol, and creatinine concentrations in the MIDUS sample using IBM SPSS Statistics 27 (Bertele et al., 2021). To ensure the stability of clusters, the clustering process was repeated in two large subsamples (Bertele et al., 2021; Franklin, 2005): More specifically, I conducted a median-split based on age and performed the clustering for each group separately (Bertele et al., 2021). At the same time, this allowed me to test whether the clusters are age dependent. Further, validating k-mean cluster analysis were conducted for the whole MIDUS sample but after excluding participants with a BMI outside the health range (below 18 or above 35) (Bertele et al., 2021). The final validation step included the k-mean clustering based on CRP, IL-6, fibrinogen, cortisol, and creatinine concentrations in the MIDJA cohort (Bertele et al., 2021).

2.8.2. Examining the characteristics of identified biochemical clusters

In MIDUS, I first calculated means and standard deviations for age, BMI, alcohol consumption, and CM severity in each cluster. I also calculated the percentages of females/males, physical activity, and lifetime smoking in each cluster. Then, One-Way Analysis of Variance (ANOVA) models with multiple Bonferroni post-hoc tests were used to compare age, BMI, alcohol consumption, and CM exposure (represented by the total score of the CTQ). Pairwise χ^2-Tests adjusting for multiple comparisons were used to compare the distributions of biological sex, physical activity, and lifetime smoking between clusters.

At first, CM was treated just like the other investigated factors in this dissertation. However, as it has been shown during the analyses for my first publication (Bertele et al., 2021) that sex, age etc. differed significantly between clusters, in a second step, I created a General Linear Model (GLM) with pairwise comparisons including all other covariates (i.e., sex, age, BMI, physical activity, alcohol and smoking habits) to exactly reflect the analyses included in the publication (Bertele et al., 2021). To avoid issues resulting from heteroscedastic residual variances, here, I performed a bootstrapping procedure using 10,000 samples. This is considered the gold standard approach in this case since (1)

bootstrapping allows to identify robust parameter estimates without requiring homoscedasticity of residual variances, (2) clusters have been found to be stable (Bertele et al., 2021), and (3) none of the covariates included in the GLM are involved in the k-mean clustering process itself (Efron & Tibshirani, 1993).

In MIDJA, I first calculated means and standard deviations for age and BMI in each cluster. I also calculated the percentages of females/males in each cluster. Then, general linear models with bootstrapping (10,000 samples) and multiple comparisons controlling for multiple testing were used to compare age and BMI between clusters. Pairwise χ^2-Tests adjusting for multiple comparisons were used to compare the distributions of biological sex between clusters.

IBM SPSS Statistics 27 was used for all analyses described above.

2.8.3. Z-tests to compare the odds ratios for diseases between clusters

In both MIDUS and MIDJA, I first calculated the percentages of depression, heart disease, hypertension, peptic ulcer disease, stroke, and cancer diagnosis in each cluster. Then, *z*-tests using Bonferroni corrections for multiple testing were used to compare odds ratios (OR) for depression, heart disease, hypertension, peptic ulcer disease, stroke, and cancer between clusters.

To exploratively compare the k-mean cluster-based approach at hand to a well-established clinical biomarker that has previously been related to a broad range of NCDs, I evaluated and contrasted the number of diagnoses among individuals in the high-risk cluster to the number of diagnoses among individuals with CRP concentrations above the established clinical cut-off (>3mg/L) (Bertele et al., 2021; Pearson et al., 2003).

2.8.4. Logistic Regression Models to compare the mortality between clusters (with age-stratified analyses)

Mortality analyses were only conducted for the MIDUS cohort as this data was not available in MIDJA. First, percentages of deceased individuals (at T1) in each cluster were calculated (Bertele et al., 2022). As a second step, I conducted a logistic regression

analysis predicting mortality (at T1, 0=No, 1=Yes) by the biochemical clusters resulting from 28.1. (Bertele et al., 2022). In doing so, I applied the indicator method comparing each cluster to a reference cluster (defined based on its average levels on all considered biomarkers, as described in 2.1.) (Bertele et al., 2022). Also, I controlled for sex, age, and disease burden at T0 (Bertele et al., 2022). Regarding disease burden, I included diagnoses of depression, any cardio- or cerebrovascular disease (0=No diagnosis, 1=At least one diagnosis of heart disease, hypertension and/or stroke), peptic ulcer disease, and cancer as covariates (Bertele et al., 2022). Exploratively, the second step was repeated but this time separately in three different age groups (based on age at T0): 31-50 years, 51-70 years, and 71-90 years and then again, but separately for males vs. females.

IBM SPSS Statistics 27 was used for all analyses described above.

2.8.5. General Linear Models to compare the inability to work between clusters (with age-stratified analyses)

Analyses regarding the inability to work (at T1) were only conducted for the MIDUS cohort since this data was not available in MIDJA. First, the average days participants indicated that they were unable to work due to illness in the last 30 days (at T1) were calculated separately in each cluster (Bertele et al., 2022). Second, I performed a general linear model using Bonferroni pairwise comparisons and controlling for sex, age, and disease burden at T0 (as described in 2.8.4) to predict the days participants indicated that they were unable to work at T1 (Bertele et al., 2022). Exploratively, the second step was repeated but this time separately in two different age groups (based on age at T0): 31-50 years, and 51-70 years. Individuals above 70 years of age were excluded from the analyses since they were mostly retired. I also repeated the second step separately in males vs. females.

IBM SPSS Statistics 27 was used for all analyses described above.

3. Results

This section includes the main results reported in the publications (k-mean clustering based on biomarkers, relations of clusters to CM, current disease diagnoses, later mortality, and inability to work) as well as additional results of analyses that were not included in the publications (relations of clusters to sex, age, BMI, physical activity, alcohol use, and smoking habits, age-stratified analyses of mortality in the clusters, supplemental tables).

Some tables and graphs appeared in the publications upon which this dissertation is based, of which I am the sole first author (Bertele et al., 2021, 2022). Results reported in the publications are restated below in addition to results addressing additional patterns of relation investigated in this thesis.

3.1. Aim 1: Develop novel cluster-based tool for risk evaluation

3.1.1. U.S. American sample

Most of the considered biomarkers were positively correlated in the MIDUS cohort (Table 3).

Table 3: MIDUS: Descriptive statistics and correlations among biochemical markers.

MIDUS: Descriptive statistics and correlations among biochemical markers.

	CRP	IL-6	Fibrinogen	Cortisol	Creatinine
Mean	3.03	3.04	348.92	1.09	81.24
SD	4.78	3.05	87.85	1.13	53.7
CRP	-				
IL-6	0.39***	-			
Fibrinogen	0.49***	0.36**	-		
Cortisol	0.08**	-0.03	-0.02	-	
Creatinine	0.05	0.01	-0.06	0.38***	-

Note: MIDUS=Midlife in the United States cohort, CRP=C-reactive protein (ug/mL), IL-6=Interleukin-6 (pg/mL) and fibrinogen (mg/dL) were measured in blood, cortisol (ug/dL) and creatinine (mg/dL) were measured in urine.

** $p<0.01$, *** $p<0.001$, p-values are controlled for multiple testing according to Bonferroni.

All two-tailed.

Source: “How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine”, by N. Bertele et al., 2021, EPMA Journal, 12, p. 5 (supplemental material) (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature.

I evaluated the initial k-mean clustering results from k = 2 to 6 clusters. When k = 2, the patterns of clusters were not distinct enough; when k = 4 or above, some clusters were very small in size (i.e., smallest cluster portion < 5%) (Bertele et al., 2021). Through a combination of the parsimonious principle and engineering meaningful difference among clusters, k = 3 were selected for the subsequent analyses (Bertele et al., 2021). Figure 3 depicts the distributions of the three identified clusters with respect to the CRP, IL-6, fibrinogen, cortisol, and creatinine (all *z*-standardized).

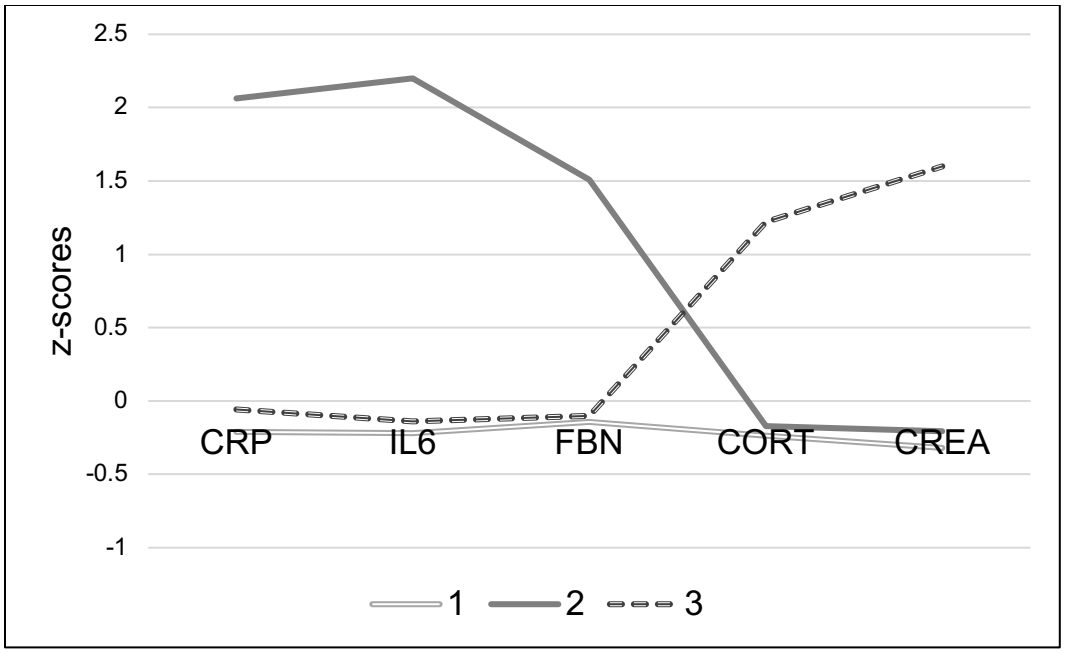

Figure 3: MIDUS: Biochemical markers (z-scores) and resulting clusters 1-3, CRP=C-reactive protein (ug/mL), IL-6=Interleukin-6 (pg/mL) and FBN=Fibrinogen (mg/dL) were measured in blood, CORT=cortisol (ug/dL) and CREA=creatinine (mg/dL) were measured in urine. Source: "How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine", by N. Bertele et al., 2021, EPMA Journal, 12, p. 510 (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature

$N_{cluster1} = 937$, $N_{cluster2} = 102$, $N_{cluster3} = 195$.

As depicted in Figure 3, cluster 1 is characterized by average levels in all biochemical measures and will thus be referred to as "reference cluster" in the following (Bertele et al., 2021). Cluster 2 was characterized by high and above-average levels of CRP, IL-6 and fibrinogen and will thus be referred to as "high-risk cluster" in the following (Bertele et al., 2021). Cluster 3 was characterized by high and above-average levels of cortisol and creatinine but average CRP, fibrinogen, and IL-6 concentrations and will be referred to as "metabo-endocrine cluster" in the following (Bertele et al., 2021).

3.1.2. Median split by age and k-mean clustering in BMI-restricted cohort

I replicated all three clusters in the younger MIDUS cohort (<54 years, Figure 4) as well as the reference and the endocrine-immune cluster in the older MIDUS cohort (>54 years, Figure 5) (Bertele et al., 2021). I further replicated all three clusters in the BMI-restricted MIDUS cohort (Figure 6) (Bertele et al., 2021).

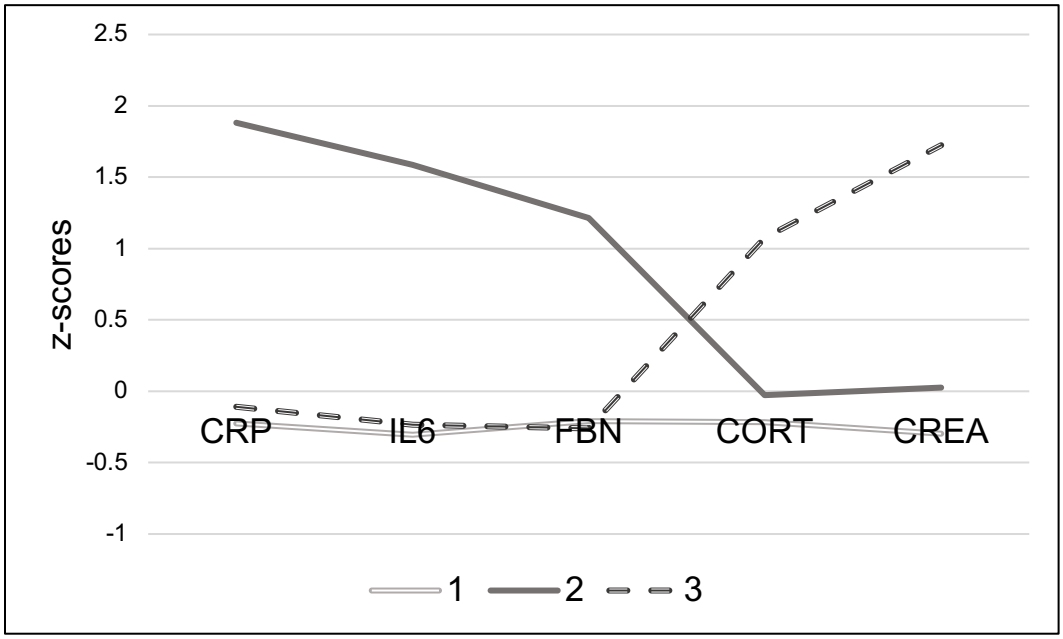

Figure 4: MIDUS young sample: biochemical markers (*z*-scores) by cluster, CRP=C-reactive protein (ug/mL), IL-6=interleukin-6 (pg/mL) and FBN=fibrinogen (mg/dL) were measured in blood, CORT=cortisol (ug/dL) and CREA=creatinine (mg/dL) were measured in urine. Source: Adapted from “How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine”, by N. Bertele et al., 2021, EPMA Journal, 12, p. 6 (supplemental material) (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature.

N_{total} = 624, $N_{cluster1}$ = 435, $N_{cluster2}$ = 59, $N_{cluster3}$ = 130. Only participants below the age median (i.e., 54 years) were included,

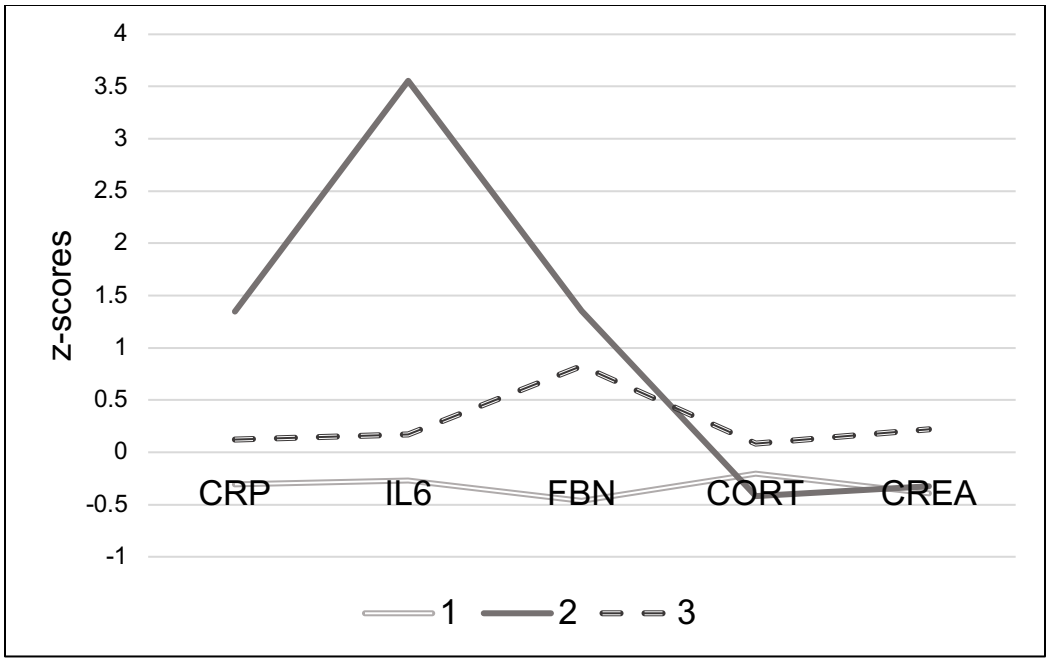

Figure 5: MIDUS old sample: Biochemical markers (*z*-scores) by cluster, CRP=C-reactive protein (ug/mL), IL-6=interleukin-6 (pg/mL) and FBN=fibrinogen (mg/dL) were measured in blood, CORT=cortisol (ug/dL) and CREA=creatinine (mg/dL) were measured in urine. Source: Adapted from "How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine", by N. Bertele et al., 2021, EPMA Journal, 12, p. 7 (supplemental material) (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature.

N_{total} = 601, $N_{cluster1}$ = 370, $N_{cluster2}$ = 28, $N_{cluster3}$ = 203. Only participants above the age median (i.e., 54 years) were included. Nine individuals were excluded due to CRP and/or cortisol levels more than 5 standard deviations above the mean.

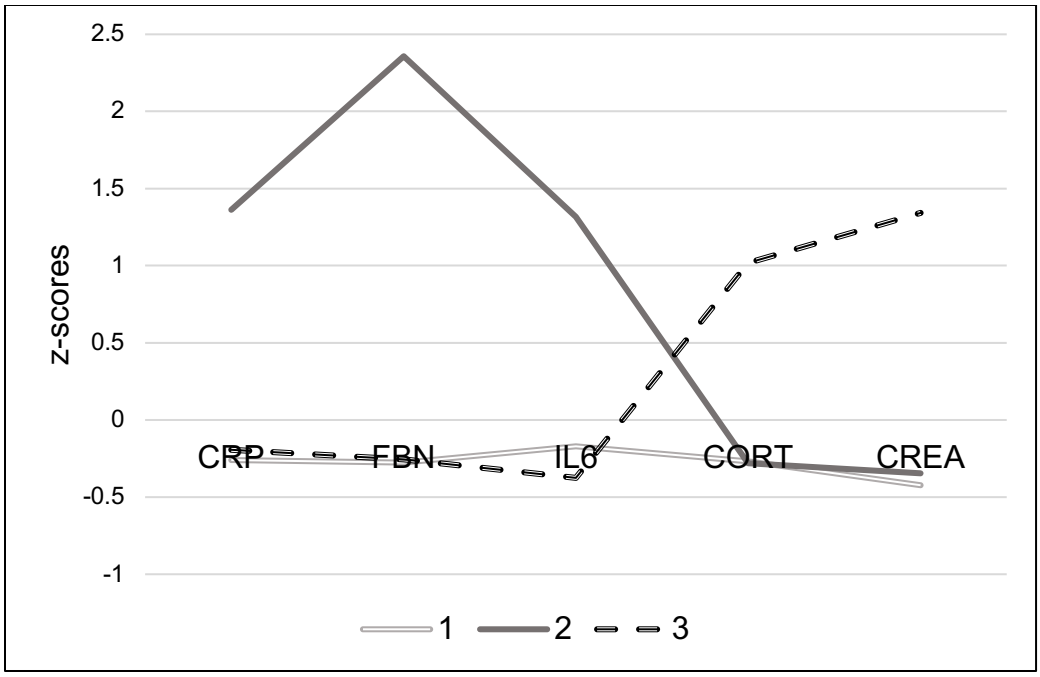

Figure 6: MIDUS BMI-restricted sample: Biochemical markers (*z*-scores) by cluster, CRP=C-reactive protein (ug/mL), IL-6=interleukin-6 (pg/mL) and FBN=fibrinogen (mg/dL) were measured in blood, CORT=cortisol (ug/dL) and CREA=creatinine (mg/dL) were measured in urine. Source: Adapted from "How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine", by N. Bertele et al., 2021, EPMA Journal, 12, p. 8 (supplemental material) (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature.

N_{total} = 1,004, $N_{cluster1}$ = 737, $N_{cluster2}$ = 63, $N_{cluster3}$ = 204. Participants with a Body Mass Index (BMI) below 18 and above 35 were excluded.

3.1.3. Replication in Japanese sample

In MIDJA, I also found significant intercorrelations between considered biomarkers (Table 4).

Table 4: MIDJA: Descriptive statistics and correlations among biochemical markers.

MIDJA: Descriptive statistics and correlations among biochemical markers.

	CRP	IL-6	Fibrinogen	Cortisol	Creatinine
Mean	0.67	1.55	319.06	7.43	0.74
SD	1.28	1.68	64.2	2.78	0.17
CRP	-				
IL-6	0.51***	-			
Fibrinogen	0.38***	0.26**	-		
Cortisol	0.12	0.08	0.05	-	
Creatinine	0.15*	0.2***	0.09	0.07	-

Note: MIDJA=Midlife in Japan cohort, CRP=C-reactive protein (ug/mL), IL-6=Interleukin-6 (pg/mL), fibrinogen (mg/dL) and creatinine (mg/dL) were measured in serum, cortisol was measured in saliva.
** $p<0.01$, *** $p<0.001$, p-values are controlled for multiple testing according to Bonferroni.
All two-tailed.
Source: “How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine”, by N. Bertele et al., 2021, EPMA Journal, 12, p. 5 (supplemental material) (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature.

The 3-cluster solution from MIDUS was replicated in the MIDJA cohort and the results are shown in Figure 7. Again, cluster 1 is characterized by average levels in all biochemical measures and will thus be referred to as “reference cluster” in the following (Bertele et al., 2021). Cluster 2 was characterized by high and above-average levels of CRP, IL-6 and fibrinogen and will thus be referred to as “high-risk cluster” in the following (Bertele et al., 2021). Cluster 3 was characterized by high and above-average levels of cortisol and creatinine but average CRP, fibrinogen, and IL-6 concentrations and will be referred to as “metabo-endocrine cluster” in the following (Bertele et al., 2021).

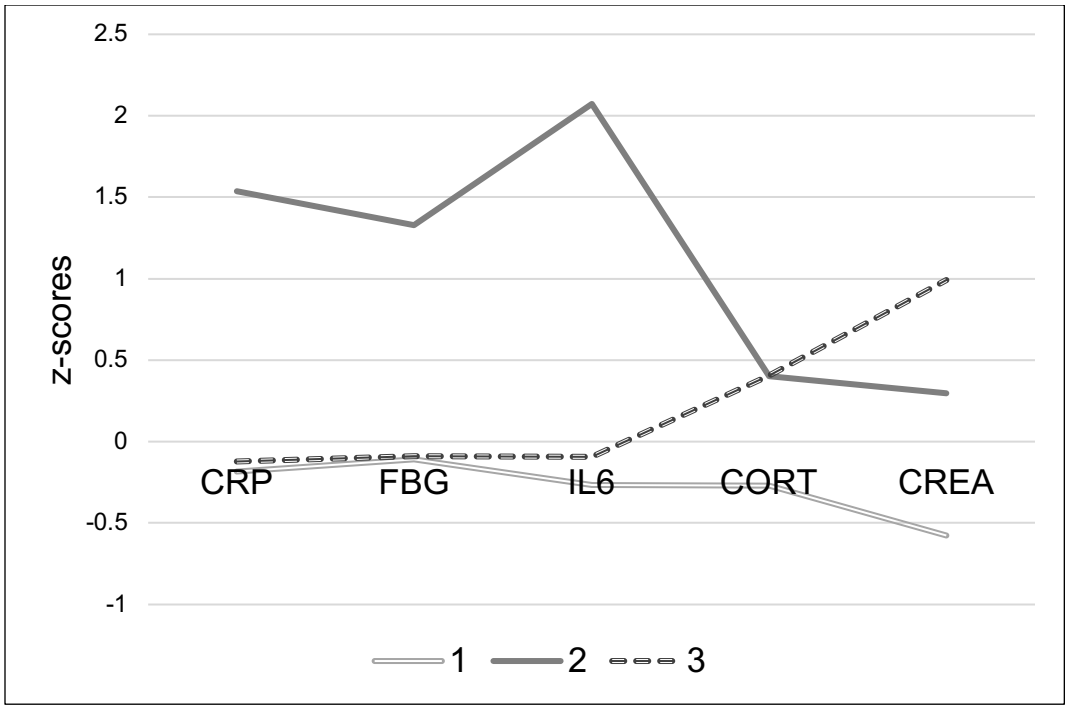

Figure 7: MIDJA: Biochemical markers (*z*-scores) and resulting clusters 1-3, CRP=C-reactive protein (ug/mL), IL-6=Interleukin-6 (pg/mL) and FBN=Fibrinogen (mg/dL), and CREA=creatinine (mg/dL) were measured in blood, CORT=cortisol (ug/dL) was measured in saliva. Source: “How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine”, by N. Bertele et al., 2021, EPMA Journal, 12, p. 510 (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature.

$N_{cluster1}$=233, $N_{cluster2}$=30, $N_{cluster3}$=115.

3.2. Aim 2: Identify risk/etiology factors of identified biochemical clusters

Distributions regarding biological sex, age, BMI, physical activity, alcohol consumption, smoking habits, and childhood maltreatment exposure by cluster are summarized in Table 5 for MIDUS. Below, I listed only the significant results/differences between clusters.

Regarding distributions of sex, after Bonferroni-correcting for multiple testing (i.e., multiplying *p*-values with three), I found that the high-risk cluster contained significantly less females compared to the reference cluster (χ^2=11.1, p<0.001), and so did the metabo-endocrine cluster (χ^2=52.1, p<0.001). Furthermore, the metabo-endocrine cluster involved significantly less females than the high-risk cluster (χ^2=54.9, p<0.001).

Regarding age, Bonferroni pairwise comparisons in the scope of a One-Way ANOVA have shown that individuals in the reference cluster were significantly older than in the metabo-endocrine cluster (Mean Difference (*MD)*=4.6, *SE*=0.91, p<0.001) and so were individuals in the high-risk cluster (*MD*=-4.8, *SE*=1.42, p<0.001).

Regarding BMI, Bonferroni pairwise comparisons in the scope of a One-Way ANOVA have shown that individuals in the high-risk cluster had a significantly higher BMI than individuals in the reference cluster (*MD*=-5.61, *SE*=0.66, p<0.001), as well as compared to individuals in the metabo-endocrine cluster (*MD*=3.5, *SE*=0.78, p<0.001). Furthermore, individuals in the metabo-endocrine cluster had significantly higher BMI compared to individuals in the reference cluster (*MD*=-2.11, *SE*=0.5, p<0.001).

Regarding physical activity, after Bonferroni-correcting for multiple testing (i.e., multiplying *p*-values with three), I found that a significantly higher percentage of individuals in the reference cluster engaged regular physical activity compared to the high-risk cluster (χ^2=24.7, p<0.001). Furthermore, a higher percentage of individuals assigned to the metabo-endocrine cluster engaged in regular physical activity compared to individuals assigned to the high-risk cluster (χ^2=6.9, p=0.027).

Regarding alcohol consumption, Bonferroni pairwise comparisons in the scope of a One-Way ANOVA have shown that individuals in the high-risk cluster drink significantly less alcohol than individuals in the reference cluster (*MD*=-0.44, *SE*=0.18, p=0.044).

Regarding the exposure to CM, Bonferroni pairwise comparisons in the scope of a One-Way ANOVA have shown that individuals in the high-risk cluster reported significantly higher CM severities compared to individuals in the reference cluster (*MD*=-4.55, *SE*=1.51, *p*=0.008) and compared to individuals in the metabo-endocrine cluster (*MD*=5.59, *SE*=1.76 *p*=0.005).

Table 5: Risk factors by cluster in MIDUS.

Risk factors by cluster in MIDUS.

Cluster	Reference	High-risk	Metabo-endocrine
Biological sex	76.2% female	59.6% female	31.3% female
Age[1] in years	55.3 (*SD*=11.6)	55.4 (*SD*=12.1)	50.6 (*SD*=11.2)
Body mass index	28.9 (*SD*=5.8)	34.5 (*SD*=9.3)	30.9 (*SD*=7)
Physical activity[2]	79.5%	57.8%	72.8%
Alcohol consumption[3]	3.7 (*SD*=1.4)	4.2 (*SD*=1.3)	3.8 (*SD*=1.3)
Smoking regularly (lifetime)	47%	55.9%	45.6%
Exposure to childhood maltreatment[4]	38 (*SD*=13.9)	42.5 (*SD*=18.4)	36.9 (*SD*=13.4)

Note: MIDUS=Midlife in the United States Study, *SD*=Standard deviation, [1]=measured at the time of biomarker assessment, [2]=Percentage of participants who responded with "yes" to: "Did you engage in regular exercise, or activity, of any type for 20 minutes or more at least 3 times/week?", [3]= Average response to the question "In the past month, how often did you drink any alcoholic beverages, on average?" (1=everyday, 2=5 or 6 days/week, 2=3 or 4 days/week, 4=1 or 2 days/week, 5=less than one

day/week, 6=never drinks), [4]=Exposure to childhood maltreatment is represented by the sum score of the Childhood Trauma Questionnaire.

* p<0.05, ** p<0.01, *** p<0.001.

Pairwise comparisons of age, BMI, and alcohol consumption are based on One-Way Analyses of Variance with Bonferroni pairwise comparisons, pairwise comparisons of sex, physical activity, and smoking are based on Bonferroni-corrected χ^2 tests.

Source: Adapted from "How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine", by N. Bertele et al., 2021, EPMA Journal, 12, p. 3 (supplemental material) (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature.

For the MIDJA cohort, only information in biological sex, age, and BMI was available and results by cluster are summarized in Table 6. Below, I list the significant results/differences.

Regarding distributions of sex, after Bonferroni-correcting for multiple testing (i.e., multiplying p-values with three), I found that the high-risk cluster contained significantly less females compared to the reference cluster (χ^2=9.1, p=0.009). Furthermore, the metabo-endocrine cluster included significantly fewer females than the reference cluster (χ^2=90.8, p<0.001), and than the high-risk cluster (χ^2=7.1, p=0.024).

Regarding age, Bonferroni pairwise comparisons in the scope of a One-Way ANOVA have shown that individuals in the high-risk cluster were significantly older than in the reference cluster (*MD*=-11.7, *SE*=2.8, p<0.001) and in the metabo-endocrine cluster (*MD*=-8.14, *SE*=2.91, p=0.016).

Regarding BMI, Bonferroni pairwise comparisons in the scope of a One-Way ANOVA have shown that individuals in the high-risk cluster had a significantly higher BMI than individuals in the reference cluster (*MD*=-1.7, *SE*=0.59, p=0.012), as well as compared to individuals in the metabo-endocrine cluster (*MD*=-1.25, *SE*=0.32 p<0.001).

Table 6: Risk factors by cluster in MIDJA.

Risk factors by cluster in MIDJA.

Cluster	Reference	High-risk	Metabo-endocrine
Biological sex	75.6% female	48.1% female	23% female
Age[1] in years	53.3 (*SD*=13.5)	65 (*SD*=11.9)	56.9 (*SD*=14.4)
Body mass index	22.1 (*SD*=3)	23.8 (*SD*=2.6)	23.3 (*SD*=2.8)

Note: MIDJA=Midlife in Japan Study, *SD*=Standard deviation, [1]=measured at the time of biomarker assessment. No information on physical activity, alcohol consumption or smoking habits was available in MIDJA.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Pairwise comparisons of age and Body Mass Index are based on One-Way Analyses of Variance with Bonferroni pairwise comparisons, pairwise comparisons of sex are based on Bonferroni-corrected χ^2 tests.

Source: Adapted from “How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine”, by N. Bertele et al., 2021, EPMA Journal, 12, p. 3 (supplemental material) (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature.

3.2.1. Full-factorial model including CM and all covariates

Full-factorial GLMs using the continuous CM score and controlling for sex, age, BMI, physical activity, alcohol use, and smoking habits indicated (Table 7 and 3.1) that CM exposure was the highest in the high-risk cluster, followed by the reference and the metabo-endocrine clusters (all *p*s<0.001).

Table 7: MIDUS: General linear model predicting childhood maltreatment by cluster.

MIDUS: General linear model predicting childhood maltreatment by cluster.

Dependent variable: CTQ sum score

Source	Type III Sum of Squares	df	Mean Square	F	*p*
Corrected Model	1563901920.14[a]	709	2205785.5	123936.67	<0.001
Intercept	241538242.56	1	241538242.56	13571331.2	<0.001
Cluster	3019986.43	2	1509993.22	84842.13	<0.001
Sex	116191.31	1	116191.31	6528.45	<0.001
Age	96700306.77	49	1973475.65	110883.86	<0.001
BMI	1327678509.49	650	2042582.32	114766.76	<0.001
Physical activity	484217.85	1	484217.85	27206.79	<0.001
Alcohol	3254689.1	5	650937.82	36574.3	<0.001
Smoking	28830.93	1	28830.93	1619.93	<0.001
Error	143451028.84	8060096	17.8		
Total	13564939758.34	8060806			
Corrected Total	1707352948.99	8060805			

a. R^2= 0.92 ($R^2_{adjusted}$= 0.92)

Note: Bootstrapping was performed using 10,000 samples.

MIDUS=Midlife in the United States cohort, CTQ=Childhood Trauma Questionnaire.

Source: Adapted from “How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine”, by N. Bertele et al., 2021, EPMA Journal, 12, p. 11 (supplemental material) (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature.

Table 7.1: Pairwise comparisons of childhood maltreatment among clusters.

Pairwise comparisons of childhood maltreatment among clusters.

Dependent Variable: CTQ sum score

(I) Cluster	(J) Cluster	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Reference	High-risk	-6.92	0.01	<0.001	-6.93	-6.9
	Metabo-endocrine	0.68	0.01	<0.001	0.67	0.69
High-risk	Reference	6.92	0.01	<0.001	6.9	6.93
	Metabo-endocrine	7.6	0.01	<0.001	7.59	7.61
Metabo-endocrine	Reference	-0.68	0.01	<0.001	-0.69	-0.67
	High-risk	-7.6	0.01	<0.001	-7.61	-7.58

Note: Based on observed means. The error term is Mean Square (Error) = 17.7.

p-values are corrected for multiple testing (Bonferroni,

i.e., multiplied by three due to three pairwise comparisons).

Bootstrapping was performed using 10,000 samples.

CTQ = Childhood Trauma Questionnaire.

Source: Adapted from “How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine”, by N. Bertele et al., 2021, EPMA Journal, 12, p. 11 (supplemental material) (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature.

3.3. Aim 3: Examine disease burden in identified biochemical clusters

In MIDUS, the high-risk cluster had the highest ORs for all considered diseases compared to the reference and the metabo-endocrine clusters (Figure 8).

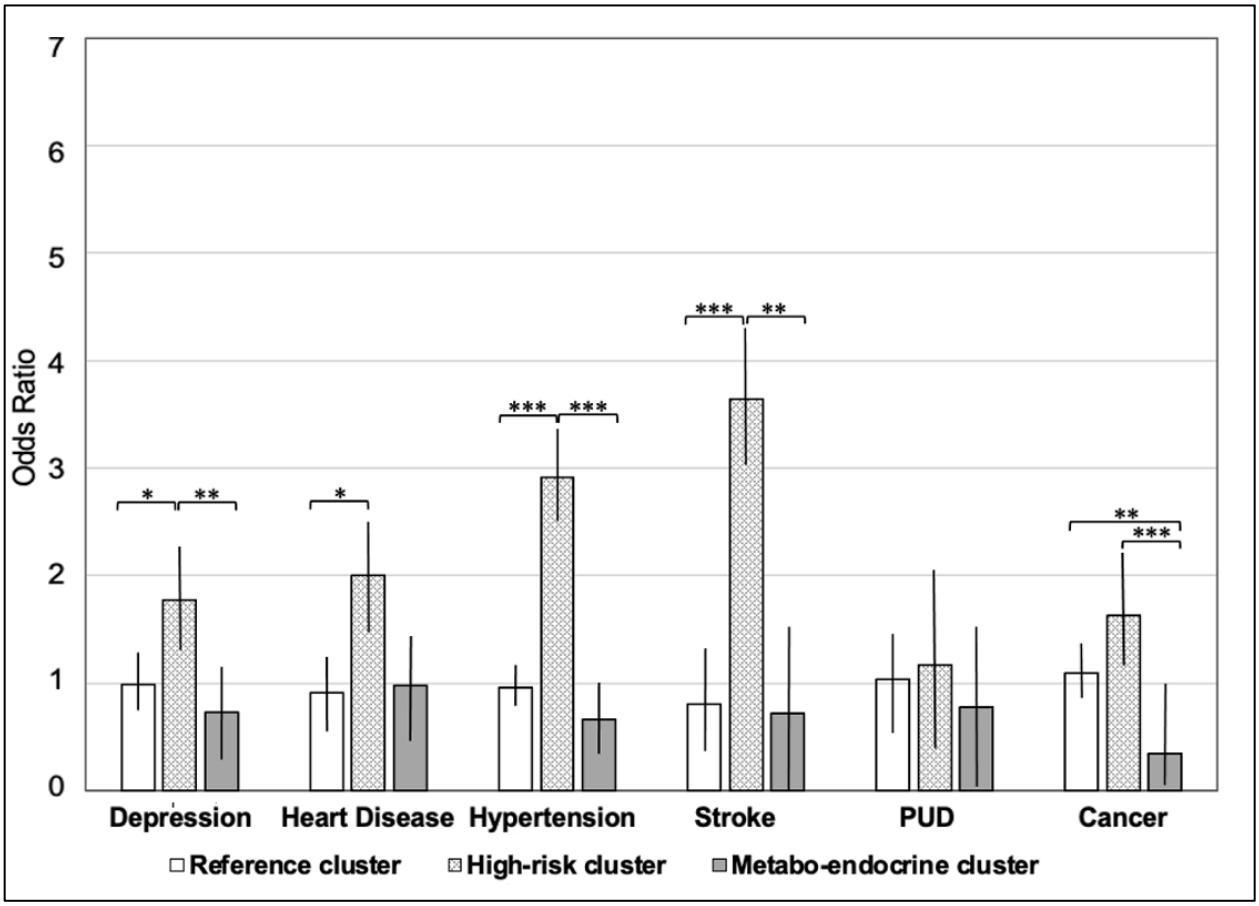

Figure 8: MIDUS: Odds ratios for diseases by cluster, MIDUS=Midlife in the United States sample, PUD=Peptic Ulcer Disease. Source: “How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine”, by N. Bertele et al., 2021, EPMA Journal, 12, p. 511 (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature.

Error bars display 95% confidence intervals. Comparisons of odds ratios were conducted with log odds ratios using *z*-tests. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, *p*-values are controlled for multiple testing according to Bonferroni. All two-tailed.

In MIDJA, the metabo-endocrine cluster had the highest ORs for heart disease, hypertension, and PUD, the high-risk cluster had the highest ORs for stroke and cancer, and reference cluster had the highest ORs for depression (Figure 9).

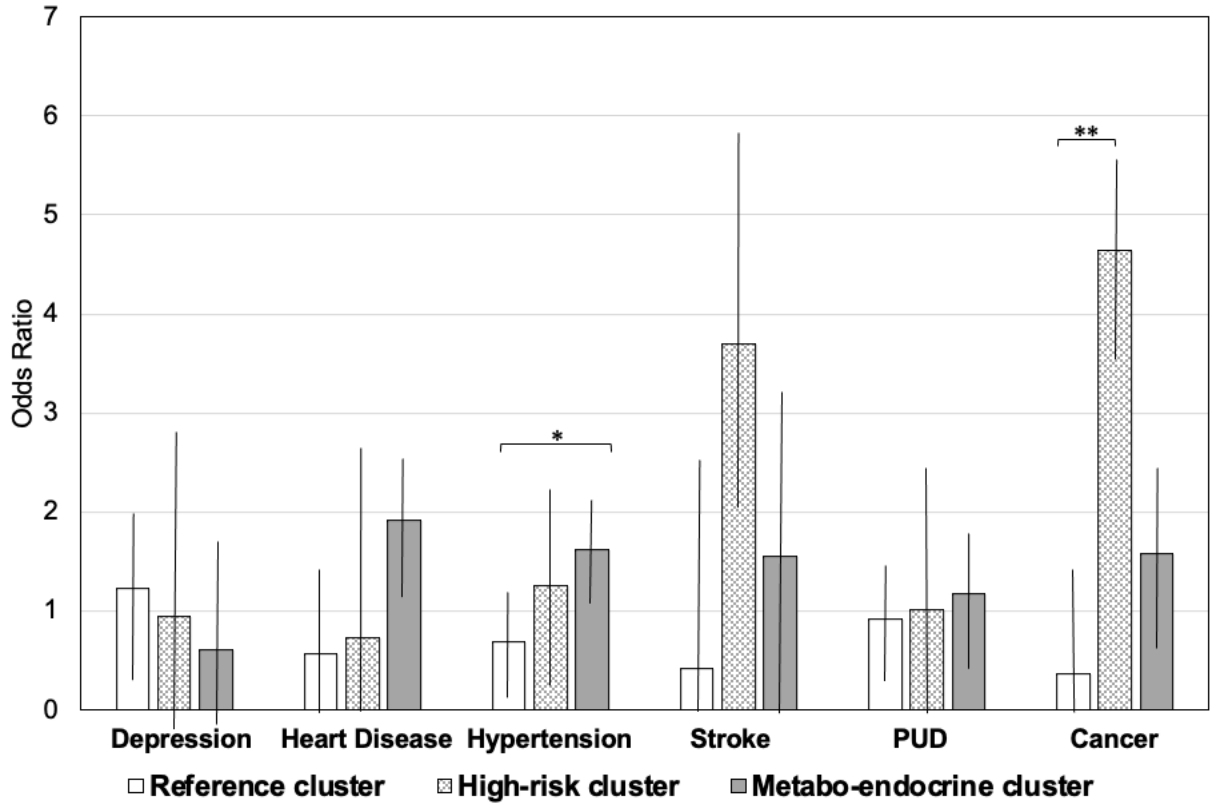

Figure 9: MIDJA: Odds ratios for diseases by cluster, MIDJA=Midlife in Japan sample, PUD=Peptic Ulcer Disease. Source: “How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine”, by N. Bertele et al., 2021, EPMA Journal, 12, p. 511 (https://doi.org/10.1007/s13167-021-00255-0). Copyright 2021 by Springer Nature.

Error bars display 95% confidence intervals. Comparisons of odds ratios were conducted with log odds ratios using *z*-tests. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, *p*-values are controlled for multiple testing according to Bonferroni. All two-tailed.

To compare the k-mean cluster-based approach to a well-established clinical biomarker that is associated with a broad range of NCDs, the number of diagnoses among individuals in the high-risk cluster was compared to the number of diagnoses among individuals with CRP concentrations above the clinical cut-off (>3mg/L) (Bertele et al.,

2021; Pearson et al., 2003). The disease burden in the high-risk cluster was higher with 1.6 diagnoses (*SD*=1.16; 0.9 diagnoses for individuals not assigned to the high-risk cluster) compared to individuals above the CRP-Cutoff with 1.2 diagnoses (*SD*=1.07; 0.9 diagnoses for individuals below the cutoff) (Bertele et al., 2021).

3.4. Aim 4: Investigate predictive value of biochemical clusters

3.4.1. 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 (Bertele et al., 2022).

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=0.043, see Table 8) (Bertele et al., 2022). The indicator comparison between the reference cluster and the high-risk cluster was significant (B=0.82, standard error (SE)=0.33, p=0.012); the comparison between the metabo-endocrine and the reference cluster was not significant (B=0.18, SE=0.32, p=0.59) (Bertele et al., 2022). 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=0.048) (Bertele et al., 2022).

Table 8: Logistic regression analyses predicting mortality.

Logistic regression analyses predicting mortality.

	B	Standard error	Wald	df	*p*	Exp(*B*)
Cluster (general)			6.3	2	0.043	
Reference vs. high-risk cluster	0.82	0.33	6.24	1	0.012	2.27
Reference vs. metabo-endocrine	0.18	0.32	0.3	1	0.59	1.19
Sex	-0.61	0.22	7.6	1	0.006	0.54
Age	0.1	0.01	84.78	1	<0.001	1.1
Depression	0.72	0.25	8.44	1	0.004	2.05
Cerebro- and cardiovascluar disease	0.74	0.23	10.54	1	0.001	2.1
Peptic ulcer disease	0.01	0.45	0	1	0.98	1.01
Cancer	0.27	0.26	1.1	1	0.29	1.3
Constant	-7.4	0.84	78.34	1	<0.001	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.

Source: "Biochemical clusters predict mortality and reported inability to work 10 years later", by N. Bertele et al., 2022, Brain, Behavior, Immunity - Health, 21(100432), p. 3 (https://doi.org/10.1016/j.bbih.2022.100432). Copyright 2022 by Elsevier Inc.

3.4.2. Mortality in age-stratified sample and separately for each biological sex

As described above, I divided each cluster into three age groups (31-50, 51-70, and 71-90 years old) and repeated the primary mortality analysis to compare the outcomes between different clusters among each age group (see Table 9).

Table 9: Sample sizes after splitting by cluster and by age group.

Sample sizes after splitting by cluster and by age group.

Cluster	Age group	*N*
Reference	31-50y	352
	51-70y	479
	71-90y	106
High-risk	31-50y	36
	51-70y	55
	71-90y	11
Metabo-endocrine	31-50y	108
	51-70y	78
	71-90y	9

Note: y=years. Source: Own representation.

As shown in Figure 10, descriptively, the odds ratios were the highest in the high-risk cluster across all age groups. According to *z*-tests comparing the odds ratios between different clusters within each age group, the difference between the reference and the high-risk cluster among 51–70-year-old was significant (z=-2.45, p=0.021). The difference regarding mortality risk between the reference and the high-risk cluster among 71–90-year-old was marginally significant (z=-1.97, p=0.073).

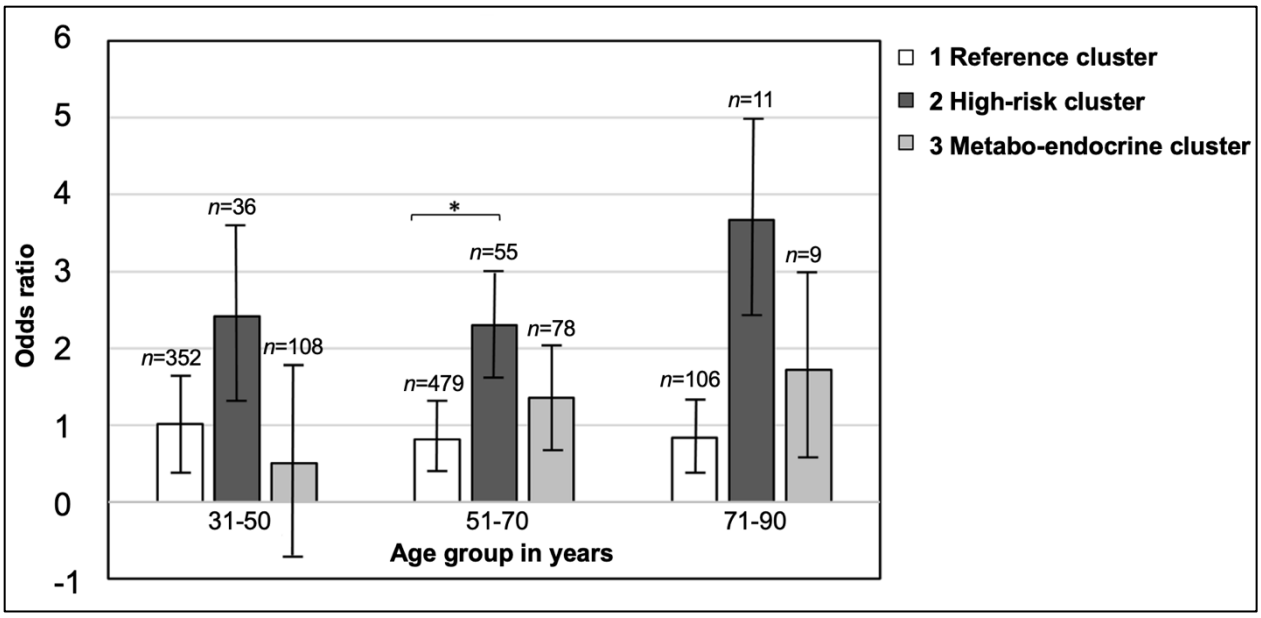

Figure 10: Odds ratios for mortality by cluster in different age groups. Source: Own representation.

Age has been assessed at the time of biomarker assessment. Mortality has been assessed over 10 years following the biomarker assessment. * $p<0.001$.

Odds ratios for mortality by cluster separately for males and females are depicted in Figure 11. 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) (Bertele et al., 2022).

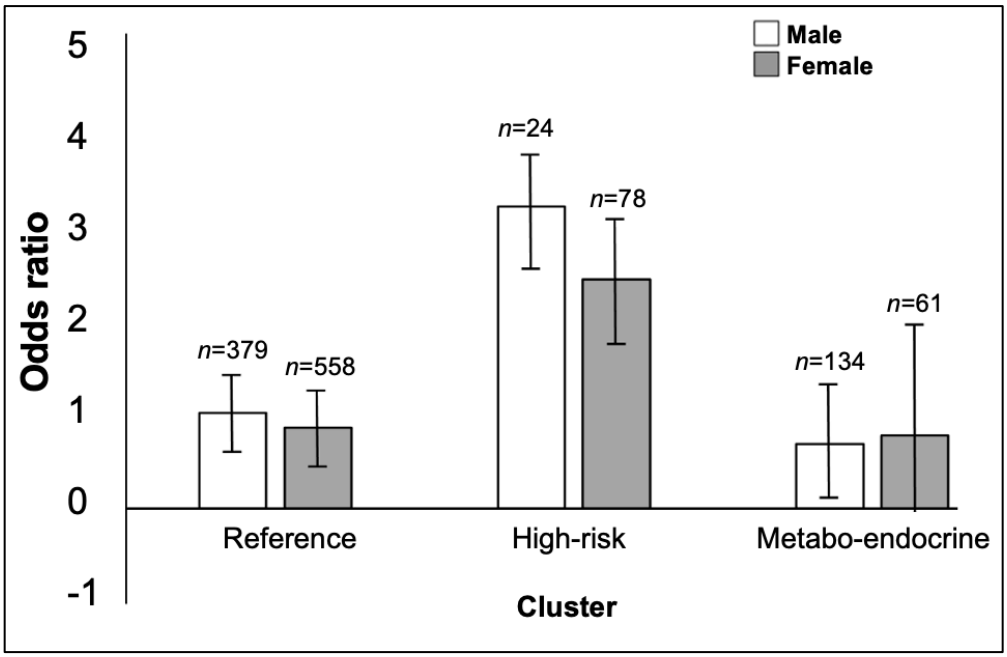

Figure 11: Bar chart Depicting odds ratios for mortality by sex and by cluster. Source: “Biochemical clusters predict mortality and reported inability to work 10 years later”, by N. Bertele et al., 2022, Brain, Behavior, Immunity - Health, 21(100432), p. 1 (supplemental material) (https://doi.org/10.1016/j.bbih.2022.100432). Copyright 2022 by Elsevier Inc.

Odds ratios were calculated separately for each sex; e.g., odds ratio in females in the reference cluster equals odds for mortality in females in the reference cluster divided by the odds for mortality in all females (across clusters). Error bars display 95% confidence intervals. No significance tests were performed due to the small sample sizes.

3.4.3. 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 (Bertele et al., 2022). 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 (Bertele et al., 2022).

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 ($F(2,790)=3.3$, $p=0.037$, Table 10) (Bertele et al., 2022). Pairwise comparisons according to Bonferroni, indicated that the differences between the reference and the high-risk cluster ($z=2.28$, $p=0.008$) and between the high-risk and the metabo-endocrine cluster were significant ($z=2.97$, $p=0.001$) (Table 10.1) (Bertele et al., 2022). The effect sizes (Cohen's d) for the group differences were 0.35 (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 (Bertele et al., 2022).

Table 10: General linear models predicting inability to work.

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	<0.001
Intercept	1226.84	1	1226.84	58.29	<0.001
Cluster	139.05	2	69.53	3.3	0.037
Sex	0.49	1	0.49	0.02	0.88
Age	1033.58	36	28.71	1.36	0.08
Depression	114.33	1	114.33	5.43	0.02
Cerebro- and cardiovascular disease	207.69	1	207.69	9.87	0.002
Peptic ulcer disease	556.65	1	556.65	26.44	<0.001
Cancer	7.48	1	7.48	0.36	0.55
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.

Source: "Biochemical clusters predict mortality and reported inability to work 10

years later", by N. Bertele et al., 2022, Brain, Behavior, Immunity - Health, 21(100432), p. 4 (https://doi.org/10.1016/j.bbih.2022.100432). Copyright 2022 by Elsevier Inc.

Table 10.1: General linear models predicting inability to work: Bonferroni pairwise comparisons between clusters.

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	95% Confidence Interval Upper Bound
Reference	High-risk	-2.28	0.76	0.008	-4.09	-0.47
	Metabo-endocrine	0.69	0.44	0.33	-0.35	1.74
High-risk	Reference	2.28	0.76	0.008	0.47	4.09
	Metabo-endocrine	2.97	0.84	0.001	0.97	4.98
Metabo-endocrine	Reference	-0.69	0.44	0.33	-1.74	0.35
	High-risk	-2.97	0.84	0.001	-4.98	-0.97

Note: Source: "Biochemical clusters predict mortality and reported inability to work 10 years later", by N. Bertele et al., 2022, Brain, Behavior, Immunity - Health, 21(100432), p. 5 (https://doi.org/10.1016/j.bbih.2022.100432). Copyright 2022 by Elsevier Inc.

3.4.4. Inability to work in age-stratified sample and separately for each biological sex

As depicted in Figure 12, the number of days participants indicated they were unable to work due to illness were the highest in the high-risk cluster compared to the other two clusters across both age groups. While the cluster variable exhibited a significant effect predicting the reported days participants between 31 and 50 years of age were unable to work ($F(2,335)=6.34$, $p=0.002$, Table 11) (Bertele et al., 2022). Bonferroni pairwise comparisons have shown that the difference between the reference and the high-risk cluster ($z=4.42$, $p<0.001$) and between the high-risk cluster and the metabo-endocrine cluster ($z=4.29$, $p=0.001$) were significant (Table 11.1) (Bertele et al., 2022). Among

individuals between 51 and 70 years of age, the cluster variable also exhibited a marginally significant effect predicting reported days participants were unable to work ($F(2,448)=2.67$, $p=0.07$, Table 11). Bonferroni pairwise comparisons have shown that the difference between the high-risk cluster and the metabo-endocrine cluster ($z=2.73$, $p=0.054$) was marginally significant (Table 11.1).

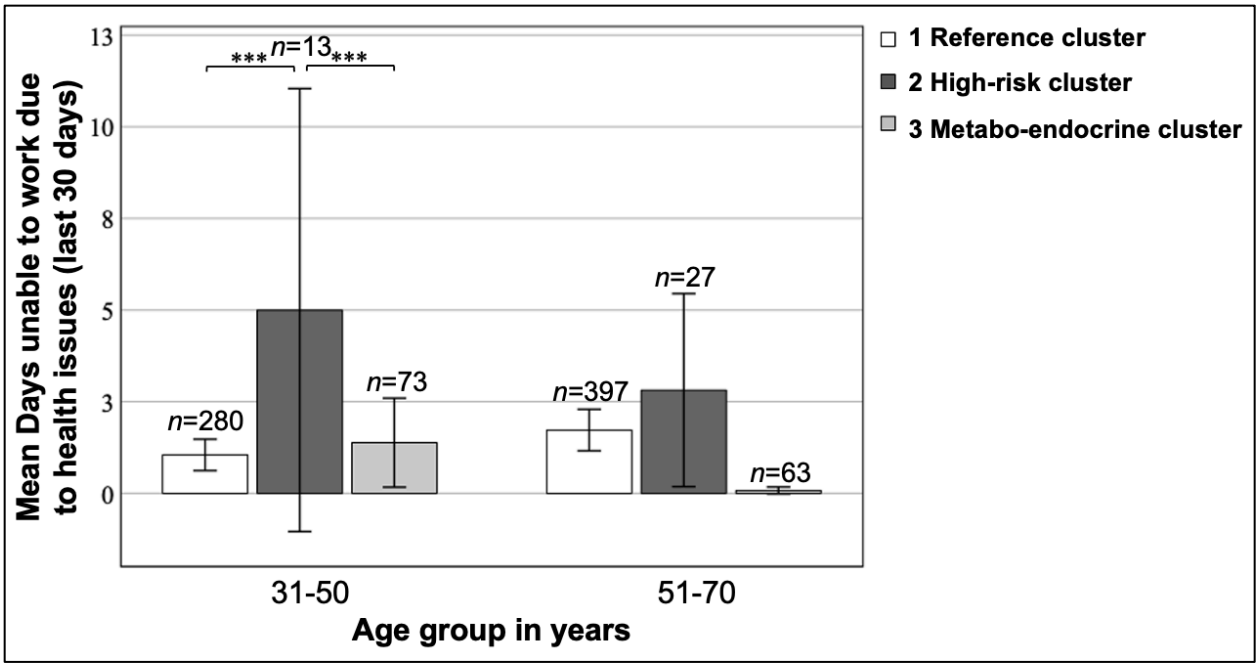

Figure 12: Mean sick days by cluster in different age groups. Source: Own representation.

Age has been assessed at the time of biomarker assessment. The inability to work has been assessed via self-report approximately 10 years following the biomarker assessment; that is, by asking participants: “How many days have you been unable to work due to illness in the last 30 days?”. Participants over the age of 70 were excluded from the analysis

Table 11: General linear models predicting inability to work by age group.

General linear models predicting inability to work by age group.

Age group	Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
31-50y	Corrected Model	1364.09[a]	23	59.31	3.99	<0.001	0.22
	Intercept	941.24	1	941.24	63.36	<0.001	0.16
	Cluster	188.28	2	94.14	6.34	0.002	0.04
	Sex	18.88	1	18.88	1.27	0.26	0
	Age	176.44	16	11.03	0.74	0.75	0.03
	Depression	181.11	1	181.11	12.19	<0.001	0.04
	Cerebro- and cardiovascular disease	65.86	1	65.86	4.43	0.036	0.01
	Peptic ulcer disease	591.82	1	591.82	39.84	<0.001	0.11
	Cancer	0.11	1	0.11	0.01	0.93	0
	Error	4976.54	335	14.86			
	Total	6832	359				
	Corrected Total	6340.64	358				
51-70y	Corrected Model	1449.23[b]	26	55.74	2.23	<0.001	0.12
	Intercept	379.85	1	379.85	15.19	<0.001	0.03
	Cluster	133.65	2	66.83	2.67	0.07	0.011
	Sex	13.12	1	13.12	0.53	0.47	0
	Age	921.86	19	48.52	1.94	0.01	0.08
	Depression	4.07	1	4.07	0.16	0.69	0
	Cerebro- and cardiovascular disease	104.75	1	104.75	4.19	0.041	0.01
	Peptic ulcer disease	146.2	1	146.2	5.85	0.016	0.01
	Cancer	1.66	1	1.66	0.07	0.8	0
	Error	11200.45	448	25			
	Total	13652	475				
	Corrected Total	12649.69	474				

a. R Squared = 0.22 (Adjusted R Squared = 0.16)

b. R Squared = 0.12 (Adjusted R Squared = 0.07)

Note: y=years. Age has been assessed at the time of biomarker assessment. The inability to work has been assessed via self-report approximately 10 years following the biomarker assessment; that is, by asking participants: “How many days have you been unable to work due to illness in the last 30 days?”. Participants over the age of 70 were excluded from the analysis. Source: Own representation.

Table 11.1: General linear models predicting inability to work: Bonferroni pairwise comparisons between clusters by age group.

General linear models predicting inability to work: Bonferroni pairwise comparisons between clusters by age group.

Age group	Cluster	vs. Cluster	*Z* of mean difference	Standard error of mean difference	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
31-50y	Reference	High-risk	-4.42	1.14	<0.001	-7.16	-1.69
		Metabo-endocrine	-0.13	0.51	1	-1.36	1.10
	High-risk	Reference	4.42	1.14	<0.001	1.69	7.16
		Metabo-endocrine	4.29	1.2	0.001	1.40	7.18
	Metabo-endocrine	Reference	0.13	0.51	1	-1.10	1.36
		High-risk	-4.29	1.2	0.001	-7.18	-1.40
51-70y	Reference	High-risk	-1.24	0.99	0.64	-3.63	1.15
		Metabo-endocrine	1.50	0.68	0.087	-0.15	3.14
	High-risk	Reference	1.24	0.99	0.64	-1.15	3.63
		Metabo-endocrine	2.73	1.15	0.054	-0.04	5.50
	Metabo-endocrine	Reference	-1.50	0.69	0.087	-3.14	0.15
		High-risk	-2.73	1.15	0.054	-5.50	0.04

Note: y=years. Age has been assessed at the time of biomarker assessment. The inability to work has been assessed via self-report approximately 10 years following the biomarker assessment; that is, by asking participants: “How many days have you been unable to work due to illness in the last 30 days?”. Participants over the age of 70 were excluded from the analysis. Source: Own representation.

The average days of sickness by cluster and sex are illustrated in Figure 13. 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. Own representation.

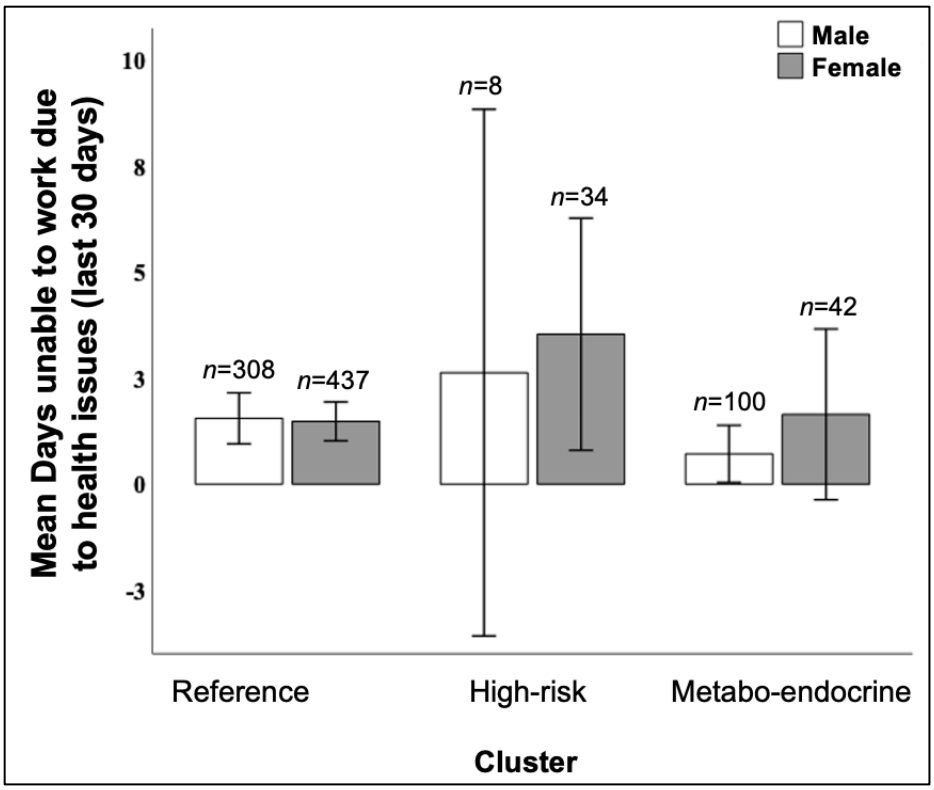

Figure 13: Bar chart depicting the number of sick days by sex and by cluster. Source: “Biochemical clusters predict mortality and reported inability to work 10 years later”, by N. Bertele et al., 2022, Brain, Behavior, Immunity - Health, 21(100432), p. 2 (supplemental material) (https://doi.org/10.1016/j.bbih.2022.100432). Copyright 2022 by Elsevier Inc.

The inability to work has been assessed via self-report approximately 10 years following the biomarker assessment; that is, by asking participants: “How many days have you been unable to work due to illness in the last 30 days?”. Participants over the age of 70 were excluded from the analysis. Error bars display 95% confidence intervals. No significance tests were performed due to the small sample sizes.

4. Discussion

The study on which this dissertation is based is, to the author's knowledge, the first to use a multivariate cluster-based approach to study the prevalence, correlates, and predictive value of biomarker profiles in the general population and, therefore, represents an important, novel contribution to the field of risk assessment and prediction. Previous research has been preoccupied investigating the value of single biomarkers in health and disease states (e.g., Li et al., 2017; Nowakowski, 2014). This study hence offers a novel avenue embedding the emerging systemic perspective in medicine into risk prediction as well. These and other strengths of the study, as well as its limitations, are outlined below, along with further evaluation of the results.

Beyond this, the findings have significant implications for public health disciplines, predictive, preventive, and personalized (3P) medicine, medical psychology, and clinical practice. The findings also point to important directions for future research, including the need to longitudinally study potential risk factors for the emergence of a high-risk biochemical profile such as biological sex, age, BMI, physical activity, and the exposure to early life stress such as CM. These practical implications and future directions are also discussed in the following.

4.1. Study contribution and strengths

4.1.1. Discovery of an advanced, cost-efficient risk indicator

Findings revealed three distinct and interculturally stable biochemical clusters observable in the general population of the United States and Japan (Bertele et al., 2021). The reference cluster is characterized by average levels of all biomarkers, the high-risk cluster by high inflammation-related mediators coupled with average concentrations of cortisol and creatinine, and the metabo-endocrine cluster by above-average levels of cortisol and creatinine (Bertele et al., 2021). The stability of clusters is supported by their replication in the MIDJA sample as well as in the BMI-restricted, in the younger (below age median) and in the older MIDUS cohort (above age median; here only the reference and the high-risk cluster were replicated) (Bertele et al., 2021). Notably, this novel, cluster-based tool

allowed to identify individuals with a high disease burden more precisely than established measures such as the CRP-cutoff (Bertele et al., 2021). Beyond this additional precision in discovering individuals with a current disease burden, the clusters efficiently predicted mortality and inability to work approximately 10 years after biomarker assessment, over and above prior disease burden (Bertele et al., 2022). Specifically, a biochemical profile of enhanced systemic inflammation coupled with low cortisol and creatinine like in the high-risk cluster, seems to be of high predictive value indicating doubled mortality rates in the decade following the biomarker assessment and substantially increased sick days reported in the working population previously assigned to this cluster (Bertele et al., 2022). I discuss potential mechanisms linking the presence of a high-risk biochemical profile and these detrimental outcomes in 4.1.2.

In addition to the predictive value of the high-risk cluster as suggested by the findings of this study, analyses regarding potential correlates and risk factors revealed valuable knowledge gain and implications for further research. First, in both the U.S. and the Japanese cohort, the odds for a biochemical high-risk profile seem to be significantly higher in men compared to women suggesting the male sex as a possible risk factor for potentially detrimental biochemical profiles. A finding worth to be investigated further, since it could reveal one potential pathway linking male sex to earlier mortality, as suggested by several lines of evidence (for a review, see Zhang et al., 2021). The finding that individuals assigned to the high-risk cluster were significantly older than in the other two clusters could further reveal a contributing role of age to the emergence of systemic inflammation together with average concentrations of cortisol and creatinine, i.e., high-risk biochemical profile, which would be in line with the large body of literature suggesting an increase in systemic inflammation with age (Chung et al., 2019). However, the age-stratified analyses suggest caution by interpreting these findings as I discuss in sections 4.1.2 and 4.1.3. Another important finding is that BMI was significantly higher in the high-risk cluster compared to the reference cluster in both the U.S. and the Japanese cohort, adding to the empirical evidence suggesting a BMI above the health range and obesity as central contributors to chronic systemic inflammation and further detrimental conditions (e.g., Ellulu et al., 2016). Important mechanisms in this pattern of relation and, thus, potential targets for intervention might be the above-described NFKB signaling pathway

(Bierhaus et al., 2003; Catrysse & van Loo, 2017). Further research, ideally in prospective longitudinal studies, is necessary to better understand the role of BMI in the emergence of a biomarker profile as in the metabo-endocrine cluster. In the U.S. cohort, I could further identify the absence of regular physical activity as a potential risk factor for the emergence of a high-risk biochemical profile. Although this cross-sectional finding is supported by various studies suggesting physical activity as a protective factor for inflammation (e.g., Mathur & Pedersen, 2008), further, longitudinal studies are needed to support the causal contribution of (lacking) physical activity to the emergence of "risky" biochemical profiles. Interestingly, individuals assigned to the high-risk cluster indicated to drink significantly less alcohol than individuals assigned to the reference cluster. One explanation for this finding could be the increased and salient disease burden in individuals in the high-risk cluster (already at T0), causing them to limit their alcohol consumption. To make ultimate conclusions about the directions of these effects, however, it would be necessary to assess alcohol consumption prior to the emergence of the biochemical risk profile. Finally, a valuable finding of this study is that the exposure to CM was significantly higher in the high-risk biochemical cluster as compared to both other clusters. This underlines the previously suggested relation of CM to systemic inflammation as well as various disease and mortality outcomes (Danese et al., 2007; Grummitt et al., 2021). Additionally, the present findings contribute the idea of the identified high-risk profile as a potential mediator in the pattern of relation between CM and disease outcomes.

Relating clusters to disease diagnoses at the time of biomarker assessment, in MIDUS, the high-risk cluster showed the highest ORs for depression, heart disease, hypertension, stroke, and cancer (Bertele et al., 2021). These findings are in line with previous literature postulating that CRP, IL-6, and fibrinogen are associated with depression (Gimeno et al., 2009; Toker et al., 2005), coronary heart disease (Danesh, 2000; Danesh et al., 1998, 2004; Luc et al., 2003), blood pressure (Piché et al., 2005), stroke (Di Napoli et al., 2001; Welsh et al., 2008; Zhou et al., 2016), and cancer (Allin & Nordestgaard, 2011; Qian et al., 2019). Yet, as opposed to these previous studies, the clustering approach used in this study accounted for well-known collinearities between biomarkers and thus lends weight to a more holistic perspective (Bertele et al., 2021). While findings lend support for the results of previous studies suggesting a link between inflammation and disease states

(Chung et al., 2009), they also demonstrate that it might not be one specific biomarker but a specific biochemical pattern (i.e., high CRP, IL-6, fibrinogen coupled with low cortisol and creatinine) that is associated with diseases (Bertele et al., 2021). The observation that individuals in the high-risk cluster, descriptively, indicated a higher disease burden than individuals above the clinically well-established CRP cutoff lends further support to this multivariate, cluster-based viewpoint (Bertele et al., 2021). Notably, no differences in the ORs for peptic ulcer disease are observed between clusters despite the role of inflammation in its pathology (Bertele et al., 2021; Lanas & Chan, 2017). Future research may aim to further investigate the role of inflammation in the pathology of peptic ulcer disease (Bertele et al., 2021).

While the high-risk cluster can be considered as being associated with higher disease burden, being assigned to the metabo-endocrine cluster may potentially be protective against developing disease in MIDUS (Bertele et al., 2021). I found that the ORs for most diseases were lower in the metabo-endocrine cluster compared to the high-risk cluster but also compared to the reference cluster (Bertele et al., 2021). Concerning cancer, this difference became significant. In 4.1.4, I will discuss potential biological mechanisms underlying this pattern of relation. Nonetheless, longitudinal studies may examine the consequences of this specific biochemical pattern that is the metabo-endocrine cluster. Additionally, these longitudinal studies will help to better understand to what extent biochemical profiles are dynamic vs. stable in different individuals across the lifespan and especially during disease states. Here, the ultimate aim should be to identify factors that influence “risky” biochemical profiles that could then be targeted to prevent the manifestation in form of a pathological state.

In MIDJA, however, the high-risk cluster only seems to be a vulnerable cluster regarding stroke and cancer, whereas for the other diseases considered, the reference cluster or the metabo-endocrine cluster indicated the highest burden (Bertele et al., 2021). One relevant and possibly limiting aspect here might be the fact that the MIDJA cohort (*N*=378) and especially the high-risk cluster were very small in size (*N*=30) (Bertele et al., 2021). Hence, these findings bear the risk of compromised reliability (Bertele et al., 2021). However, if replicated in further studies, these findings could be attributed to cross-cultural differences, that is, that specific biochemical profiles may be linked to different

comorbidities and outcomes in Japan than in the U.S. since certain moderating mechanisms (e.g., BMI, nutrition, medication etc.) vary between cultures (Bertele et al., 2021; Kalat, 2009). The fact that disease burden in MIDJA was much lower compared to MIDUS despite approximately 8% of participants were assigned to the high-risk cluster in each cohort, lends additional weight to this perspective (Bertele et al., 2021). Moreover, the different results in the two cohorts underline the significance of individual characteristics in disease susceptibility noted above and the role of interactions between these cultural, lifestyle, and biochemical factors; while a U.S. American individual with the biochemical risk profile might present with a high disease burden, this might not be the case for a Japanese individual despite similar biochemical profile (Bertele et al., 2021).

4.1.2. Replication of the finding that chronic systemic inflammation predicts premature mortality under consideration of additional contributors

The findings of this study replicate the well-established link between chronic systemic inflammation and the risk for premature mortality in a large, community-based sample (Kantor et al., 2019; Weber et al., 2021). Mechanisms linking systemic inflammation to premature mortality might not only be the disease susceptibility directly associated with systemic inflammation (for a review, see Furman et al., 2019) and accelerated aging processes caused by inflammation (for a review, see Ferrucci & Fabbri, 2018) but also the fact that inflammation is often linked to other temporally preceding factors augmenting disease susceptibility and aging processes across the lifespan such as early life stress (e.g., Danese et al., 2007), diet (e.g., Navarro et al., 2016), and obesity (e.g., Ellulu et al., 2016), as also underlined by the findings of the current study. However, due to the cross-sectional character of the current study, it remains unclear whether lifestyle factors such as diet and obesity have influenced cluster assignments. Future studies should assess these factors and control for them in the clustering process to clarify the direction of the identified effects.

Previous literature particularly studied the role of separate inflammatory markers in predicting mortality and functionality without taking other biomarkers under consideration (Bertele et al., 2021, 2022; Li et al., 2017; Nowakowski, 2014). Moreover, previous studies

particularly considered clinical samples presenting with a given pathological burden to examine the link between inflammation and mortality (e.g., individuals suffering from chronic obstructive pulmonary disease (Mendy et al., 2018), HIV-infected patients (Tien et al., 2010), or kidney disease patients (Alves et al., 2018)) making it challenging to differentiate influences by inflammation from effects caused by disease states themselves (Bertele et al., 2022). The current study elaborates on these previous findings by using biochemical clusters based on multiple biomarkers, that cover a broad range of somatic functionality, as predictors for mortality; all while controlling for disease burden at the time of biomarker assessment (Bertele et al., 2021). Relating these biochemical clusters to mortality, I found that the most detrimental biomarker profile is characterized by systemic inflammation paired with average cortisol and creatinine, that is, when inflammation persists in an uncontrolled and unresolved manner (Bertele et al., 2021; Chung et al., 2019).

Interestingly, an assignment to the metabo-endocrine cluster (characterized by high concentrations of cortisol and creatinine and average levels of inflammation), did not imply higher subsequent mortality when compared to the reference cluster (Bertele et al., 2022). This is consistent with the findings regarding baseline disease burden, where the metabo-endocrine cluster did not present with a higher disease burden than the reference cluster (Bertele et al., 2021).

Analyses in each age group separately suggest that an assignment to the high-risk cluster might be especially unfavorable between 51-70 years of age (at the time of biomarker assessment) with respect to mortality risk, potentially suggesting an association of the high-risk cluster to premature death. This is underlined by the finding that the odds ratio for mortality among 51–70-year-old in the high-risk cluster was even higher than in the other two clusters at the age of 71-90 years. To further investigate this observation, future studies involving a larger sample size are needed.

When comparing mortality outcomes of the biochemical clusters in males vs. females, I observed a tendency towards higher mortality in males potentially suggesting that an assignment to the high-risk cluster is more unfavorable in males than in females (Bertele et al., 2022). If replicated in larger studies, future studies should examine the moderating role of sex steroids in these observed patterns of relation (Bertele et al., 2022).

4.1.3. Chronic systemic inflammation as an eminent risk factor for everyday functioning

Relating the biochemical clusters to the reported inability to work the last 30 days in the decade following the biomarker assessment, I found that individuals with high inflammation coupled with cortisol and creatinine concentrations below average, that is, individuals assigned to the high-risk cluster, reported the highest number of sick days (Bertele et al., 2022). Importantly, this finding regarding the high-risk biochemical profile, that was associated with higher disease burden at baseline, was independent of sex, age and different disease states at baseline (Bertele et al., 2022). Still, the biochemical risk profile may have yielded an accelerated disease progression in individuals assigned to this cluster, causing the observed high number of sick days (Bertele et al., 2022). Unfortunately, examining whether biochemical profiles might play a moderating role in the link between baseline disease states and sick days 10 years later was not possible in the current study due to the limited sample size (Bertele et al., 2022). I thus recommend for future studies to focus on these potential moderating effects using larger, population-based samples (Bertele et al., 2022).

Among 31–50-year-old, an assignment to the high-risk cluster was associated with a significantly higher number of days individuals were unable to work due to illness 10 years following the biomarker assessment; independently from the disease burden at the time of biomarker assessment. Thus, systemic inflammation starting at a relatively young age might be especially detrimental with respect to the future ability to work. Furthermore, the increased number of sick days at a relatively young age (31-50 years) in individuals in the high-risk cluster might be an early indicator for the susceptibility of severe diseases as well as later (premature) mortality outcomes.

Comparing the number of sick days between the biochemical clusters in males and females, a descriptive tendency towards a higher number of sick days in females compared to males was observed, as opposed to the higher OR for mortality in males than in females in this cluster. This could point to differential effects of the high-risk biochemical profile in males vs. females and underlines the importance to investigate gender-specific factors of the biochemical risk profile as well as its longer-term effects.

4.1.4. Biological mechanisms underlying the link between biochemical risk profiles and disease burden, mortality, and inability to work

The biological mechanisms linking the identified biochemical risk profile to various adverse outcomes are intricate and of multifaceted, dynamic, and especially of continuous character (Bertele et al., 2022; Wang, 2021). First, uncontrolled systemic inflammation might result in multiple detrimental but pre-pathological conditions (Wang, 2021). These include the manifestation of a chronic state of allostatic load involving excessive levels of oxidative stress, and, therefore, impaired stem cell reproductivity, immunosenesence (i.e., aging of the immune system), as well as functional and structural damage of cellular DNA (Bertele et al., 2021, 2022; Wang, 2021). Certain lifestyle and environmental factors (i.e., poor diet, obesity, psychological stress) that are often present in individuals with elevated systemic inflammation might further contribute to same (Ellulu et al., 2016; Navarro et al., 2016). Second, these pre-disease states might then manifest with time, spreading across the organism, augmenting, and yielding other unfavorable processes such as an increased biomolecular entropy and accelerated cellular aging (Wang, 2021). The result might be a vicious cycle resulting in manifest disease states that then impact the subjective quality of life, the ability to work, and ultimately, increase the risk for (premature) mortality, as suggested by the current findings (Bertele et al., 2021, 2022).

Regarding the metabo-endocrine cluster and its potential protective character, it remains unclear what the underlying biological mechanisms might be, if the current findings are replicated in larger, longitudinal studies. Generally, solid lines of previous literature suggest a link between hypercortisolism as in the metabo-endocrine cluster and both disease burden and mortality (Min, 2016; Steffensen et al., 2016). Yet, this discrepancy with some of the present findings might further highlight the significance of considering cortisol in interaction with other biomarkers and somatic processes as a matter of principle when examining its longer-term outcomes (Bertele et al., 2021, 2022). Specifically, a biochemical profile characterized by low inflammation and high cortisol and creatinine, like the metabo-endocrine cluster, might represent an indicator of integrity of the glucocorticoid negative feedback system, protecting from negative outcomes (Bertele et al., 2021, 2022; Kalat, 2009).

4.2. Study limitations

This work has a number of strengths including the validation of the clusters in an independent, Japanese sample and the representative character of cohorts. However, the findings have some limitations. First, the part of the study investigating correlates and comorbidities of the identified biochemical clusters is cross-sectional which prohibits causal inferences. Second, the Japanese cohort was relatively small in size. It is, therefore, possible that ORs with respect to disease diagnoses lack reliability. Third, some given methodological inconsistencies (urine cortisol and creatinine levels in MIDUS, average saliva levels of cortisol and blood levels of creatinine in MIDJA) between cohorts may have compromised the k-mean clustering process. Forth, diseases were assessed via self-report, which bears the risk of a report bias. Moreover, CM has been assessed retrospectively bearing the potential of report and memory biases. This said, the significance of self-reported assessment of CM when investigating its correlates and longer-term effects has recently been demonstrated (Danese & Widom, 2020). Because information on CM severities was not available in the Japanese sample, the association between CM and the biochemical clusters observed in the U.S. sample must be interpreted with caution until replicated in other, independent cohorts. Fifth, the sample size for the mortality analysis was relatively small, which may have affected the power to determine the impact of each cluster on mortality, particularly in different age groups. The small sample sizes resulting from the splitting into three age groups also disallowed to test for potential interactions between the biochemical clusters and different diseases over the lifespan. Sixth, inability to work in the past 30 days has been assessed by a single item and via self-report, bearing the risk of reporting and memory bias. While previous literature lends support to this assessment method (Cunny & Perri, 1991; Fisher et al., 2016), using only one item disallows to monitor the psychometric properties of the measurement. Seventh, mortality analyses and the analysis of the inability to work considered disease burden in a limited fashion concentrating on diagnoses of depression, heart disease, stroke, hypertension, peptic ulcer disease and cancer. Despite these six diseases cover pathology in multiple somatic systems, other covariates and diseases that may also be relevant might not have been included, which could limit the power of the results.

4.3. Public health implications

4.3.1. Implications for Personalized Medicine, Targeted Prevention, and Predictive Diagnostics

It is increasingly becoming clear from recent literature, that many well-established risk factors such as a BMI outside the normal range (Golubnitschaja et al., 2021), genetic risk factors (Huang & Hu, 2015; Lopizzo et al., 2015), etc., that are expected to help identify individuals at an enhanced risk for certain diseases, are not independent from the individual environment and do not behave the same way in different individuals (Bertele et al., 2021). More specifically, the presence of a particular risk factor may have little predictive value for adverse outcomes unless it is considered systemically, that is, in the context of other physiological, environmental, psychological, and biochemical parameters and processes (Bertele et al., 2021; Golubnitschaja et al., 2021; Huang & Hu, 2015; Lopizzo et al., 2015).

The approach presented here combines this systemic perspective with the necessary scientific parsimony. The presented results advance our understanding of the interplay of diverse risk factors and hence, provide valuable implications on Personalized Medicine, Targeted Prevention, and Predictive Diagnostics (i.e., 3PM). In particular, the assessment of CRP, IL-6, fibrinogen, cortisol, and creatinine should be assessed as a matter of principle in all 3PM disciplines to provide a systemic insight into an individual's current health condition (Bertele et al., 2021). High inflammatory signaling (CRP, IL-6, and fibrinogen approximately 1.5 SDs above the general population average) coupled with low compensation (average cortisol and creatinine), is a detrimental biochemical profile associated with unfavorable longer-term outcomes, especially when prevalent in younger individuals (Bertele et al., 2022). Hence, if observed in a patient, this profile should be taken as a reason for further investigation (especially with respect to artery health/stroke and cancer) and for offering personalized treatment options (Bertele et al., 2021, 2022). Depending on the patient's condition, these treatments may involve anti-inflammatory drugs, nutrient substitutions, and treatment supplements, for example, nutrition and exercise plans (Bertele et al., 2021, 2022).

4.3.2. Implications for Medical Psychology

A crucial question relevant to clinical and preventative targets refers to the etiology of the clusters that have been identified. The finding that individuals in the high-risk cluster reported higher CM exposure than the other two clusters, could provide initial evidence for early-life stress as a potential factor in the etiology of this cluster (Bertele et al., 2021). Recent findings suggesting CM as a leading cause of a number of diseases and mortality lend weight to this perspective (for a review, see Grummitt et al., 2021), while the link between CM and morbidity/mortality may, in part, be mediated by systemic inflammation observable in adults that have been exposed to CM (Bertele et al., 2021, 2022). Since CM exposure has also been shown to be linked to telomere shortening (Shalev, 2012; Shalev & Belsky, 2016), to cognitive decline (Barnes et al., 2009; Pesonen et al., 2013), and to accelerate age-induced effects on neurogenesis (Ruiz et al., 2018), individuals assigned to the high-risk cluster might be particularly susceptible to accelerated aging processes (Bertele et al., 2021, 2022). This may then yield high levels of oxidative stress and hence, cell damage and tissue injury, but also other age-accelerating processes yet to be studied (Bertele et al., 2021, 2022; Calder et al., 2013; Chatterjee, 2016; Straub, 2017).

4.4. Clinical implications

Individuals assigned to the biochemical risk profile should be examined with a special focus on stress including early-life stress and especially CM (Bertele et al., 2021). In cases where CM is prevalent, health care providers should study its role in the patient's individual condition pattern thoroughly and offer psychotherapy (Bertele et al., 2021). On the one hand, doing so could help to advance the understanding of the etiology factors of the identified biochemical clusters, and, on the other hand, it could provide valuable implications with regard to tailored, personalized (preemptive) interventions (Bertele et al., 2021, 2022). Specifically, trauma and stress focused psychotherapy might play a crucial role in these interventions (Bertele et al., 2021, 2022). Furthermore, it might be essential to monitor the nutrient balance (micronutrients, minerals and vitamins, in

particular) of at-risk individuals since it has been shown that an anti-inflammatory diet can buffer age-accelerating processes (Bertele et al., 2021, 2022; Cheng et al., 2010; Stromsnes et al., 2021). If needed, nutritional supplements should be administered (Bertele et al., 2021, 2022). Ultimately, such studies would be of highest clinical value as they would help to better understand whether certain interventions can influence biochemical profiles and, consequently, disease burden and other negative outcomes.

4.5. Future research directions

While the selection of CRP, IL-6, fibrinogen, cortisol and creatinine was mainly led by a conceptual perspective, aiming to keep the number of required parameters to a minimum and after studying the endocrine, the metabolic, the immune system, and their interactions in the literature. In the end, however, the five biomarkers chosen here are only one option to approach the clustering process and future studies might as well involve additional biomarkers tested in routine care settings. Doing so, it would be interesting to employ a more empirical approach for the selection of the most relevant biomarkers; more specifically, to start the clustering process with a high number of different biomarkers and narrow them down by investigating how many biomarkers it takes to still distinguish the clusters precisely with respect to disease burden and outcomes. This approach could not only help to further precise this novel tool, but it might also help to gain additional knowledge about the role of different somatic systems, their interactions, and their role in health and disease. Future research should employ longitudinal designs including multiple assessment time-points to further examine the predictive value of the identified biochemical clusters with respect to long-term well-being, mental and physical health, and mortality as well as to identify the most effective point of intervention. Additionally, longitudinal designs would help to better understand the way biochemical profiles behave along the lifespan, in the context of critical events, disease states, and ultimately, they might reveal ways to influence these trajectories. Ideally, future research should study different cultures enabling to better understand the generalizability of the predictive power of the identified biochemical clusters. Moreover, future studies may identify additional factors to be considered in combination with the

biochemical clusters presented here, helping to improve and precise disease prediction and, hence, to improve both targeted prevention and personalized interventions (Bertele et al., 2021, 2022). For these future, more comprehensive studies, I would suggest to examine the role of perceived stress, early-life stress, sex steroids, genetic and epigenetic factors, and mitochondrial function in the context of biochemical risk profiles and their longer-term effects.

5. Conclusion

This work identified three biochemical clusters in two independent population-based cohorts and then tested and validated their predictive character regarding mortality and inability to work the last 30 days in the decade following the biomarker assessment. Results suggest that a specific inflammatory-endocrine biochemical pattern represents a valuable risk indicator for mortality and a proficient predictor for functionality a decade later; over and above the disease burden reported at baseline and particularly in younger adults (Bertele et al., 2021, 2022). Since the herein presented biochemical clustering method is relatively in-expensive, it might as well be incorporated into routine care and diagnostics to help identify individuals at risk for premature mortality and other detrimental subsequent outcomes (Bertele et al., 2021, 2022). Individuals identified in this way could then be provided with tailored, early-on treatments targeting biological alterations manifesting after the exposure to early-life stress and to other vulnerability factors, that are being increasingly understood, avoiding the development of a biochemical risk profile in the first place (Bertele et al., 2021, 2022).

Accordingly, future research should further validate the predictive power of the identified biochemical profiles regarding other subsequent outcomes. Simultaneously, preventive medical research should concentrate on developing early-on interventions that can be flexibly tailored according to a patient's biochemical profile and other individual characteristics (Bertele et al., 2021, 2022). Furthermore, research should focus on identifying etiology factors of the biochemical profiles, advancing the understanding of the role of early-life stress and other life history factors, as well as (epi-)genetic factors in this context (Bertele et al., 2021, 2022). On the other hand, future research should also dedicate considerable attention to the identification of potentially protecting, that is, resilience, factors occurring in the context of biochemical (risk) profiles and their outcomes (Bertele et al., 2021, 2022).

References

Allin, K. H., & Nordestgaard, B. G. (2011). Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. *Critical Reviews in Clinical Laboratory Sciences*, *48*(4), 155–170. https://doi.org/10.3109/10408363.2011.599831

Alves, F. C., Sun, J., Qureshi, A. R., Dai, L., Snaedal, S., Bárány, P., Heimbürger, O., Lindholm, B., & Stenvinkel, P. (2018). The higher mortality associated with low serum albumin is dependent on systemic inflammation in end-stage kidney disease. *PLOS ONE*, *13*(1), e0190410. https://doi.org/10.1371/journal.pone.0190410

Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H., Staden, R., & Young, I. G. (1981). Sequence and organization of the human mitochondrial genome. *Nature*, *290*(5806), 457–465. https://doi.org/10.1038/290457a0

Angioni, S., Petraglia, F., Gallinelli, A., Cossarizza, A., Franceschi, C., Muscettola, M., Genazzani, A. D., Surico, N., & Genazzani, A. R. (1993). Corticotropin-releasing hormone modulates cytokines release in cultured human peripheral blood mononuclear cells. *Life Sciences*, *53*(23), 1735–1742. https://doi.org/10.1016/0024-3205(93)90160-5

Archibald, J. M. (2015). Endosymbiosis and Eukaryotic Cell Evolution. *Current Biology*, *25*(19), R911–R921. https://doi.org/10.1016/j.cub.2015.07.055

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. https://doi.org/10.1016/j.chiabu.2008.09.013

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. *Molecular Psychiatry*, *21*(5), 642–649. https://doi.org/10.1038/mp.2015.67

Benjamin, E. J., Virani, S. S., Callaway, C. W., Chamberlain, A. M., Chang, A. R., Cheng, S., Chiuve, S. E., Cushman, M., Delling, F. N., Deo, R., de Ferranti, S.

D., Ferguson, J. F., Fornage, M., Gillespie, C., Isasi, C. R., Jiménez, M. C., Jordan, L. C., Judd, S. E., Lackland, D., … Muntner, P. (2018). Heart Disease and Stroke Statistics—2018 Update: A Report From the American Heart Association. *Circulation*, *137*(12). https://doi.org/10.1161/CIR.0000000000000558

Bernstein, D. P., & Fink, L. (1998). *Childhood Trauma Questionnaire: A Retrospective Self-report*. Harcourt Brace & Company.

Bernstein, D. P., Stein, J. A., Newcomb, M. D., Walker, E., Pogge, D., Ahluvalia, T., Stokes, J., Handelsman, L., Medrano, M., Desmond, D., & Zule, W. (2003). Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse & Neglect*, *27*(2), 169–190. https://doi.org/10.1016/S0145-2134(02)00541-0

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. *EPMA Journal*. https://doi.org/10.1007/s13167-021-00255-0

Bertele, N., Karabatsiakis, A., Talmon, A., & Buss, C. (2022). Biochemical clusters predict mortality and reported inability to work 10 years later. *Brain, Behavior, & Immunity - Health*, *21*(100432). https://doi.org/10.1016/j.bbih.2022.100432

Bierhaus, A., Wolf, J., Andrassy, M., Rohleder, N., Humpert, P. M., Petrov, D., Ferstl, R., von Eynatten, M., Wendt, T., Rudofsky, G., Joswig, M., Morcos, M., Schwaninger, M., McEwen, B., Kirschbaum, C., & Nawroth, P. P. (2003). A mechanism converting psychosocial stress into mononuclear cell activation. *Proceedings of the National Academy of Sciences*, *100*(4), 1920–1925. https://doi.org/10.1073/pnas.0438019100

Bonilla, D. A., Kreider, R. B., Stout, J. R., Forero, D. A., Kerksick, C. M., Roberts, M. D., & Rawson, E. S. (2021). Metabolic Basis of Creatine in Health and Disease: A Bioinformatics-Assisted Review. *Nutrients*, *13*(4), 1238. https://doi.org/10.3390/nu13041238

Brown, R. E. (1994). *An introduction to neuroendocrinology*. Cambridge University Press.

Bryll, A., Skrzypek, J., Krzyściak, W., Szelągowska, M., Śmierciak, N., Kozicz, T., & Popiela, T. (2020). Oxidative-antioxidant imbalance and impaired glucose metabolism in schizophrenia. *Biomolecules*, *10*(3), 384. https://doi.org/10.3390/biom10030384

Buckingham, J. C. (2006). Glucocorticoids: Exemplars of multi-tasking. *British Journal of Pharmacology*, *147*(S1), S258–S268. https://doi.org/10.1038/sj.bjp.0706456

Budzynski, A. Z., & Shainoff, J. R. (1986). Fibrinogen and fibrin: Biochemistry and pathophysiology. *Critical Reviews in Oncology/Hematology*, *6*(2), 97–146. https://doi.org/10.1016/S1040-8428(86)80019-1

Cainzos-Achirica, M., Enjuanes, C., Greenland, P., McEvoy, J. W., Cushman, M., Dardari, Z., Nasir, K., Budoff, M. J., Al-Mallah, M. H., Yeboah, J., Miedema, M. D., Blumenthal, R. S., Comin-Colet, J., & Blaha, M. J. (2018). The prognostic value of interleukin 6 in multiple chronic diseases and all-cause death: The Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis*, *278*, 217–225. https://doi.org/10.1016/j.atherosclerosis.2018.09.034

Calcia, M. A., Bonsall, D. R., Bloomfield, P. S., Selvaraj, S., Barichello, T., & Howes, O. D. (2016). Stress and neuroinflammation: A systematic review of the effects of stress on microglia and the implications for mental illness. *Psychopharmacology*, *233*(9), 1637–1650. https://doi.org/10.1007/s00213-016-4218-9

Calder, P. C., Ahluwalia, N., Albers, R., Bosco, N., Bourdet-Sicard, R., Haller, D., Holgate, S. T., Jönsson, L. S., Latulippe, M. E., Marcos, A., Moreines, J., M'Rini, C., Müller, M., Pawelec, G., van Neerven, R. J. J., Watzl, B., & Zhao, J. (2013). A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. *British Journal of Nutrition*, *109*(S1), S1–S34. https://doi.org/10.1017/S0007114512005119

Catrysse, L., & van Loo, G. (2017). Inflammation and the Metabolic Syndrome: The tissue-specific functions of NF-κB. *Trends in Cell Biology*, *27*(6), 417–429. https://doi.org/10.1016/j.tcb.2017.01.006

Chandel, N. S. (2015). Evolution of mitochondria as signaling organelles. *Cell Metabolism*, *22*(2), 204–206. https://doi.org/10.1016/j.cmet.2015.05.013

Chatterjee, S. (2016). Oxidative stress, inflammation, and disease. In *Oxidative Stress and Biomaterials* (pp. 35–58). Elsevier. https://doi.org/10.1016/B978-0-12-803269-5.00002-4

Chen, E., Miller, G. E., Lachman, M. E., Gruenewald, T. L., & Seeman, T. E. (2012). Protective factors for adults from low-childhood socioeconomic circumstances: The Benefits of Shift-and-Persist for Allostatic Load. *Psychosomatic Medicine*, *74*(2), 178–186. https://doi.org/10.1097/PSY.0b013e31824206fd

Cheng, W.-H., Bohr, V. A., & de Cabo, R. (2010). Nutrition and aging. *Mechanisms of Ageing and Development*, *131*(4), 223–224. https://doi.org/10.1016/j.mad.2010.03.011

Chung, H. Y., Cesari, M., Anton, S., Marzetti, E., Giovannini, S., Seo, A. Y., Carter, C., Yu, B. P., & Leeuwenburgh, C. (2009). Molecular inflammation: Underpinnings of aging and age-related diseases. *Ageing Research Reviews*, *8*(1), 18–30. https://doi.org/10.1016/j.arr.2008.07.002

Chung, H. Y., Kim, D. H., Lee, E. K., Chung, K. W., Chung, S., Lee, B., Seo, A. Y., Chung, J. H., Jung, Y. S., Im, E., Lee, J., Kim, N. D., Choi, Y. J., Im, D. S., & Yu, B. P. (2019). Redefining chronic inflammation in aging and age-related diseases: Proposal of the Senoinflammation Concept. *Aging and Disease*, *10*(2), 367. https://doi.org/10.14336/AD.2018.0324

Coresh, J., Wei, G. L., McQuillan, G., Brancati, F. L., Levey, A. S., Jones, C., & Klag, M. J. (2001). Prevalence of high blood pressure and elevated serum creatinine level in the United States: Findings from the Third National Health and Nutrition Examination Survey (1988-1994). *Archives of Internal Medicine*, *161*(9), 1207. https://doi.org/10.1001/archinte.161.9.1207

Coutinho, A. E., & Chapman, K. E. (2011). The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Molecular and Cellular Endocrinology*, *335*(1), 2–13. https://doi.org/10.1016/j.mce.2010.04.005

Crimmins, E. M., Vasunilashorn, S., Kim, J. K., Hagedorn, A., & Saito, Y. (2008). A comparison of biological risk factors in two populations: The United States and

Japan. *Population and Development Review*, *34*(3), 457–482. https://doi.org/10.1111/j.1728-4457.2008.00232.x

Culleton, B. F., Larson, M. G., Evans, J. C., Wilson, P. W. F., Barrett, B. J., Parfrey, P. S., & Levy, D. (1999). Prevalence and correlates of elevated serum creatinine levels: The Framingham Heart Study. *Archives of Internal Medicine*, *159*(15), 1785. https://doi.org/10.1001/archinte.159.15.1785

Cunny, K. A., & Perri, M. (1991). Single-item vs multiple-item measures of health-related quality of life. *Psychological Reports*, *69*(1), 127–130. https://doi.org/10.2466/pr0.1991.69.1.127

Danese, A., Pariante, C. M., Caspi, A., Taylor, A., & Poulton, R. (2007). Childhood maltreatment predicts adult inflammation in a life-course study. *Proceedings of the National Academy of Sciences*, *104*(4), 1319–1324. https://doi.org/10.1073/pnas.0610362104

Danese, A., & Widom, C. S. (2020). Objective and subjective experiences of child maltreatment and their relationships with psychopathology. *Nature Human Behaviour*, *4*(8), 811–818. https://doi.org/10.1038/s41562-020-0880-3

Danesh, J. (2000). Low grade inflammation and coronary heart disease: Prospective study and updated meta-analyses. *BMJ*, *321*(7255), 199–204. https://doi.org/10.1136/bmj.321.7255.199

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), 1477-1482. https://doi.org/10.1001/jama.279.18.1477

Danesh, J., Wheeler, J. G., Hirschfield, G. M., Eda, S., Eiriksdottir, G., Rumley, A., Lowe, G. D. O., Pepys, M. B., & Gudnason, V. (2004). C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *New England Journal of Medicine*, *350*(14), 1387–1397. https://doi.org/10.1056/NEJMoa032804

De Bosscher, K., Vanden Berghe, W., & Haegeman, G. (2003). The interplay between the glucocorticoid receptor and nuclear factor-κB or activator protein-1: Molecular

mechanisms for gene repression. *Endocrine Reviews*, *24*(4), 488–522. https://doi.org/10.1210/er.2002-0006

de Heredia, F. P., Gómez-Martínez, S., & Marcos, A. (2012). Obesity, inflammation and the immune system. *Proceedings of the Nutrition Society*, *71*(2), 332–338. https://doi.org/10.1017/S0029665112000092

Dedovic, K., Duchesne, A., Andrews, J., Engert, V., & Pruessner, J. C. (2009). The brain and the stress axis: The neural correlates of cortisol regulation in response to stress. *NeuroImage*, *47*(3), 864–871. https://doi.org/10.1016/j.neuroimage.2009.05.074

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. https://doi.org/10.1161/01.STR.32.1.133

Dienberg Love, G., Seeman, T. E., Weinstein, M., & Ryff, C. D. (2010). Bioindicators in the MIDUS National Study: Protocol, measures, sample, and comparative context. *Journal of Aging and Health*, *22*(8), 1059–1080. https://doi.org/10.1177/0898264310374355

Duvoix, A., Dickens, J., Haq, I., Mannino, D., Miller, B., Tal-Singer, R., & Lomas, D. A. (2013). Blood fibrinogen as a biomarker of chronic obstructive pulmonary disease. *Thorax*, *68*(7), 670–676. https://doi.org/10.1136/thoraxjnl-2012-201871

Efron, B., & Tibshirani, R. (1993). *An introduction to the bootstrap*. Chapman & Hall.

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 Psychology*, *37*(3), 262–270. https://doi.org/10.1037/hea0000585

Ellulu, M. S., Khaza'ai, H., Rahmat, A., Patimah, I., & Abed, Y. (2016). Obesity can predict and promote systemic inflammation in healthy adults. *International Journal of Cardiology*, *215*, 318–324. https://doi.org/10.1016/j.ijcard.2016.04.089

Feldman, S., & Weidenfeld, J. (1995). Neural mechanisms involved in the corticosteroid feedback effects on the hypothalamo-pituitary-adrenocortical axis. *Progress in Neurobiology*, *45*(2), 129–141. https://doi.org/10.1016/0301-0082(94)00039-K

Ferrucci, L., & Fabbri, E. (2018). Inflammageing: Chronic inflammation in ageing, cardiovascular disease, and frailty. *Nature Reviews Cardiology*, *15*(9), 505–522. https://doi.org/10.1038/s41569-018-0064-2

Fisher, G. G., Matthews, R. A., & Gibbons, A. M. (2016). Developing and investigating the use of single-item measures in organizational research. *Journal of Occupational Health Psychology*, *21*(1), 3–23. https://doi.org/10.1037/a0039139

Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., & Santoro, A. (2018). Inflammaging: A new immune–metabolic viewpoint for age-related diseases. *Nature Reviews Endocrinology*, *14*(10), 576–590. https://doi.org/10.1038/s41574-018-0059-4

Franceschi, C., Garagnani, P., Vitale, G., Capri, M., & Salvioli, S. (2017). Inflammaging and 'Garb-aging.' *Trends in Endocrinology & Metabolism*, *28*(3), 199–212. https://doi.org/10.1016/j.tem.2016.09.005

Franklin, J. (2005). The elements of statistical learning: Data mining, inference and prediction. *The Mathematical Intelligencer*, *27*(2), 83–85. https://doi.org/10.1007/BF02985802

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D. W., Fasano, A., Miller, G. W., Miller, A. H., Mantovani, A., Weyand, C. M., Barzilai, N., Goronzy, J. J., Rando, T. A., Effros, R. B., Lucia, A., Kleinstreuer, N., & Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. *Nature Medicine*, *25*(12), 1822–1832. https://doi.org/10.1038/s41591-019-0675-0

Gabay, C. (2006). Interleukin-6 and chronic inflammation. *Arthritis Research & Therapy*, *8*(Suppl 2), S3. https://doi.org/10.1186/ar1917

Gallo, L. C., Fortmann, A. L., & Mattei, J. (2014). Allostatic load and the assessment of cumulative biological risk in biobehavioral medicine: Challenges and opportunities. *Psychosomatic Medicine*, *76*(7), 478–480. https://doi.org/10.1097/PSY.0000000000000095

Gillies, R. J., Robey, I., & Gatenby, R. A. (2008). Causes and consequences of increased glucose metabolism of cancers. *Journal of Nuclear Medicine*, *49*(Suppl 2), 24S-42S. https://doi.org/10.2967/jnumed.107.047258

Gimeno, D., Kivimäki, M., Brunner, E. J., Elovainio, M., De Vogli, R., Steptoe, A., Kumari, M., Lowe, G. D. O., Rumley, A., Marmot, M. G., & Ferrie, J. E. (2009). Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. *Psychological Medicine*, *39*(3), 413–423. https://doi.org/10.1017/S0033291708003723

Global Burden of Disease Collaborative Network. (2018). *Global Burden of Disease Study 2017 (GBD 2017) Results*. Institute for Health Metrics and Evaluation (IHME).

Golubnitschaja, O., Liskova, A., Koklesova, L., Samec, M., Biringer, K., Büsselberg, D., Podbielska, H., Kunin, A. A., Evsevyeva, M. E., Shapira, N., Paul, F., Erb, C., Dietrich, D. E., Felbel, D., Karabatsiakis, A., Bubnov, R., Polivka, J., Polivka, J., Birkenbihl, C., … Kubatka, P. (2021). Caution, “normal” BMI: Health risks associated with potentially masked individual underweight—EPMA Position Paper 2021. *EPMA Journal*. https://doi.org/10.1007/s13167-021-00251-4

Green, F. R. (2006). Fibrinogen polymorphisms and atherothrombotic disease. *Annals of the New York Academy of Sciences*, *936*(1), 549–559. https://doi.org/10.1111/j.1749-6632.2001.tb03543.x

Gruenewald, T. L., Karlamangla, A. S., Hu, P., Stein-Merkin, S., Crandall, C., Koretz, B., & Seeman, T. E. (2012). History of socioeconomic disadvantage and allostatic load in later life. *Social Science & Medicine*, *74*(1), 75–83. https://doi.org/10.1016/j.socscimed.2011.09.037

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. *JAMA Pediatrics*, *175*(12),1269–1278. https://doi.org/10.1001/jamapediatrics.2021.2320

Gyllenhammer, L. E., Entringer, S., Buss, C., & Wadhwa, P. D. (2020). Developmental programming of mitochondrial biology: A conceptual framework and review. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1926), 20192713. https://doi.org/10.1098/rspb.2019.2713

Herman, J. P., & Cullinan, W. E. (1997). Neurocircuitry of stress: Central control of the hypothalamo–pituitary–adrenocortical axis. *Trends in Neurosciences*, *20*(2), 78–84. https://doi.org/10.1016/S0166-2236(96)10069-2

Herman, J. P., Ostrander, M. M., Mueller, N. K., & Figueiredo, H. (2005). Limbic system mechanisms of stress regulation: Hypothalamo-pituitary-adrenocortical axis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *29*(8), 1201–1213. https://doi.org/10.1016/j.pnpbp.2005.08.006

Huang, T., & Hu, F. B. (2015). Gene-environment interactions and obesity: Recent developments and future directions. *BMC Medical Genomics*, *8*(Suppl.1), S2. https://doi.org/10.1186/1755-8794-8-S1-S2

Juster, R.-P., McEwen, B. S., & Lupien, S. J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neuroscience & Biobehavioral Reviews*, *35*(1), 2–16. https://doi.org/10.1016/j.neubiorev.2009.10.002

Kalat, J. W. (2009). *Biological psychology* (10th ed). Wadsworth, Cengage Learning.

Kantor, E. D., Udumyan, R., Giovannucci, E. L., Valdimarsdottir, U. A., Signorello, L. B., Montgomery, S., & Fall, K. (2019). Association of blood marker of inflammation in late adolescence with premature mortality. *JAMA Pediatrics*, *173*(11), 1095-1097. https://doi.org/10.1001/jamapediatrics.2019.2835

Kashani, K., Rosner, M. H., & Ostermann, M. (2020). Creatinine: From physiology to clinical application. *European Journal of Internal Medicine*, *72*, 9–14. https://doi.org/10.1016/j.ejim.2019.10.025

Kazak, L., & Cohen, P. (2020). Creatine metabolism: Energy homeostasis, immunity and cancer biology. *Nature Reviews Endocrinology*, *16*(8), 421–436. https://doi.org/10.1038/s41574-020-0365-5

Keay, N. (2017, September 29). Temporal considerations in endocrine/metabolic interactions Part 1. *Blog of the British Journal of Sports Medicine*. https://blogs.bmj.com/bjsm/2017/09/29/temporal-considerations-endocrinemetabolic-interactions-part-1/

Kemeny, M. E. (2003). The psychobiology of stress. *Current Directions in Psychological Science*, *12*(4), 124–129. https://doi.org/10.1111/1467-8721.01246

Kennedy, B. K., Berger, S. L., Brunet, A., Campisi, J., Cuervo, A. M., Epel, E. S., Franceschi, C., Lithgow, G. J., Morimoto, R. I., Pessin, J. E., Rando, T. A., Richardson, A., Schadt, E. E., Wyss-Coray, T., & Sierra, F. (2014). Geroscience: Linking aging to chronic disease. *Cell*, *159*(4), 709–713. https://doi.org/10.1016/j.cell.2014.10.039

Klein, J. R. (2021). Dynamic interactions between the immune system and the neuroendocrine system in health and disease. *Frontiers in Endocrinology*, *12*. https://doi.org/10.3389/fendo.2021.655982

Know, L. (2018). *Mitochondria and the future of medicine: The key to understanding disease, chronic illness, aging, and life itself*. Chelsea Green Publishing.

Kobayashi, H., & Miyazaki, Y. (2015). Distribution characteristics of salivary cortisol measurements in a healthy young male population. *Journal of Physiological Anthropology*, *34*(1), 30. https://doi.org/10.1186/s40101-015-0068-0

Kreider, R. B., & Stout, J. R. (2021). Creatine in health and disease. *Nutrients*, *13*(2), 447. https://doi.org/10.3390/nu13020447

Lafontan, M. (2005). FAT CELLS: Afferent and efferent messages define new approaches to treat obesity. *Annual Review of Pharmacology and Toxicology*, *45*(1), 119–146. https://doi.org/10.1146/annurev.pharmtox.45.120403.095843

Lanas, A., & Chan, F. K. L. (2017). Peptic ulcer disease. *The Lancet*, *390*(10094), 613–624. https://doi.org/10.1016/S0140-6736(16)32404-7

Larabee, C. M., Neely, O. C., & Domingos, A. I. (2020). Obesity: A neuroimmunometabolic perspective. *Nature Reviews Endocrinology*, *16*(1), 30–43. https://doi.org/10.1038/s41574-019-0283-6

Lazovic, B. (2012). Correlation of CRP and serum level of fibrinogen with severity of disease in chronic obstructive pulmonary disease patients. *Medical Archives*, *66*(3), 159-160. https://doi.org/10.5455/medarh.2012.66.159-160

Lee, D. Y., Kim, E., & Choi, M. H. (2015). Technical and clinical aspects of cortisol as a biochemical marker of chronic stress. *BMB Reports*, *48*(4), 209–216. https://doi.org/10.5483/BMBRep.2015.48.4.275

Li, Y., Zhong, X., Cheng, G., Zhao, C., Zhang, L., Hong, Y., Wan, Q., He, R., & Wang, Z. (2017). Hs-CRP and all-cause, cardiovascular, and cancer mortality risk: A

meta-analysis. *Atherosclerosis*, *259*, 75–82. https://doi.org/10.1016/j.atherosclerosis.2017.02.003

Liu, T., Zhang, L., Joo, D., & Sun, S.-C. (2017). NF-κB signaling in inflammation. *Signal Transduction and Targeted Therapy*, *2*(1), 17023. https://doi.org/10.1038/sigtrans.2017.23

Lopizzo, N., Bocchio Chiavetto, L., Cattane, N., Plazzotta, G., Tarazi, F. I., Pariante, C. M., Riva, M. A., & Cattaneo, A. (2015). Gene-environment interaction in major depression: Focus on experience-dependent biological systems. *Frontiers in Psychiatry*, *6*. https://doi.org/10.3389/fpsyt.2015.00068

Luan, Y., & Yao, Y. (2018). The clinical significance and potential role of C-reactive protein in chronic inflammatory and neurodegenerative diseases. *Frontiers in Immunology*, *9*, 1302. https://doi.org/10.3389/fimmu.2018.01302

Luc, G., Bard, J.-M., Juhan-Vague, I., Ferrieres, J., Evans, A., Amouyel, P., Arveiler, D., Fruchart, J.-C., & Ducimetiere, P. (2003). C-reactive protein, interleukin-6, and fibrinogen as predictors of coronary heart disease: The PRIME Study. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *23*(7), 1255–1261. https://doi.org/10.1161/01.ATV.0000079512.66448.1D

Luyendyk, J. P., Schoenecker, J. G., & Flick, M. J. (2019). The multifaceted role of fibrinogen in tissue injury and inflammation. *Blood*, *133*(6), 511–520. https://doi.org/10.1182/blood-2018-07-818211

Markus, H. R., Coe, C. L., Ryff, C. D., Karasawa, M., Kawakami, N., & Kitayama, S. (2020). *Survey of Midlife in Japan (MIDJA): Biomarker Project, 2009-2010*. Inter-university Consortium for Political and Social Research [distributor]. https://doi.org/10.3886/ICPSR34969.v4

Martin, A. B., Hartman, M., Lassman, D., Catlin, A., & The National Health Expenditure Accounts Team. (2021). National health care spending in 2019: Steady growth for the fourth consecutive year: Study examines national health care spending for 2019. *Health Affairs*, *40*(1), 14–24. https://doi.org/10.1377/hlthaff.2020.02022

Mathur, N., & Pedersen, B. K. (2008). Exercise as a mean to control low-grade systemic inflammation. *Mediators of Inflammation*, *2008*, 1–6. https://doi.org/10.1155/2008/109502

McEwen, B. S. (1993). Stress and the individual: Mechanisms leading to disease. *Archives of Internal Medicine*, *153*(18), 2093-2101. https://doi.org/10.1001/archinte.1993.00410180039004

McEwen, B. S. (1998). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York Academy of Sciences*, *840*(1), 33–44. https://doi.org/10.1111/j.1749-6632.1998.tb09546.x

McEwen, B. S. (2004). Protection and damage from acute and chronic stress: Allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Annals of the New York Academy of Sciences*, *1032*(1), 1–7. https://doi.org/10.1196/annals.1314.001

McEwen, B. S., & Stellar, E. (1993). Stress and the individual. Mechanisms leading to disease. *Archives of Internal Medicine*, *153*(18), 2093–2101.

Mehet, D. K. (2007). *Regulation of the transcription factor nuclear factor kappa B in the neuroendocrine response to stress*. Imperial College London.

Mendy, A., Forno, E., Niyonsenga, T., & Gasana, J. (2018). Blood biomarkers as predictors of long-term mortality in COPD. *The Clinical Respiratory Journal*, *12*(5), 1891–1899. https://doi.org/10.1111/crj.12752

Mercurio, F., & Manning, A. M. (1999). NF-κB as a primary regulator of the stress response. *Oncogene*, *18*(45), 6163–6171. https://doi.org/10.1038/sj.onc.1203174

Meyer, A., Laverny, G., Bernardi, L., Charles, A. L., Alsaleh, G., Pottecher, J., Sibilia, J., & Geny, B. (2018). Mitochondria: An organelle of bacterial origin controlling inflammation. *Frontiers in Immunology*, *9*(536), 1–8. https://doi.org/10.3389/fimmu.2018.00536

Miller, G. E., Cohen, S., & Ritchey, A. K. (2002). Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoid-resistance model. *Health Psychology*, *21*(6), 531–541. https://doi.org/10.1037/0278-6133.21.6.531

Min, Le. (2016). Functional hypercortisolism, visceral obesity, and metabolic syndrome. *Endocrine Practice*, *22*(4), 506–508. https://doi.org/10.4158/EP161197.CO

Nakrani, M. N., Wineland, R. H., & Anjum, F. (2022). Physiology, glucose metabolism. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK560599/

Navarro, S. L., Kantor, E. D., Song, X., Milne, G. L., Lampe, J. W., Kratz, M., & White, E. (2016). Factors associated with multiple biomarkers of systemic inflammation. *Cancer Epidemiology Biomarkers & Prevention*, *25*(3), 521–531. https://doi.org/10.1158/1055-9965.EPI-15-0956

Nehring, S. M., Goyal, A., Bansal, P., & Patel, B. C. (2022). C-reactive protein. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK441843/

Nowakowski, A. C. (2014). Chronic inflammation and quality of life in older adults: A cross-sectional study using biomarkers to predict emotional and relational outcomes. *Health and Quality of Life Outcomes*, *12*(1), 141. https://doi.org/10.1186/s12955-014-0141-0

Panichi, V., Migliori, M., De Pietro, S., Taccola, D., Bianchi, A. M., Norpoth, M., Metelli, M. R., Giovannini, L., Tetta, C., & Palla, R. (2001). C-reactive protein in patients with chronic renal diseases. *Renal Failure*, *23*(3–4), 551–562. https://doi.org/10.1081/JDI-100104737

Pearson, T. A., Mensah, G. A., Alexander, R. W., Anderson, J. L., Cannon, R. O., Criqui, M., Fadl, Y. Y., Fortmann, S. P., Hong, Y., Myers, G. L., Rifai, N., Smith, S. C., Taubert, K., Tracy, R. P., & Vinicor, F. (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. https://doi.org/10.1161/01.CIR.0000052939.59093.45

Permert, J., Ihse, I., Jorfeldt, L., von Schenck, H., Arnqvist, H. J., & Larsson, J. (1993). Pancreatic cancer is associated with impaired glucose metabolism. *The European Journal of Surgery = Acta Chirurgica*, *159*(2), 101–107.

Perrone, R. D., Madias, N. E., & Levey, A. S. (1992). Serum creatinine as an index of renal function: New insights into old concepts. *Clinical Chemistry*, *38*(10), 1933–1953.

Pesonen, A.-K., Eriksson, J. G., Heinonen, K., Kajantie, E., Tuovinen, S., Alastalo, H., Henriksson, M., Leskinen, J., Osmond, C., Barker, D. J. P., & Räikkönen, K.

(2013). Cognitive ability and decline after early life stress exposure. *Neurobiology of Aging*, *34*(6), 1674–1679. https://doi.org/10.1016/j.neurobiolaging.2012.12.012

Picard, M., Juster, R.-P., & McEwen, B. S. (2014). Mitochondrial allostatic load puts the “gluc” back in glucocorticoids. *Nature Reviews Endocrinology*, *10*(5), 303–310. https://doi.org/10.1038/nrendo.2014.22

Picard, M., & McEwen, B. S. (2018). Psychological stress and mitochondria: A systematic review. *Psychosomatic Medicine*, *80*(2), 141–153. https://doi.org/10.1097/PSY.0000000000000545

Piché, M.-È., Lemieux, S., Weisnagel, S. J., Corneau, L., Nadeau, A., & Bergeron, J. (2005). Relation of high-sensitivity C-reactive protein, interleukin-6, tumor necrosis factor-alpha, and fibrinogen to abdominal adipose tissue, blood pressure, and cholesterol and triglyceride levels in healthy postmenopausal women. *The American Journal of Cardiology*, *96*(1), 92–97. https://doi.org/10.1016/j.amjcard.2005.02.051

Pivonello, R., De Martino, M. C., De Leo, M., Lombardi, G., & Colao, A. (2008). Cushing’s Syndrome. *Endocrinology and Metabolism Clinics of North America*, *37*(1), 135–149. https://doi.org/10.1016/j.ecl.2007.10.010

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 Journal*, *10*(4), 365–381. https://doi.org/10.1007/s13167-019-00194-x

Radler, B., & Lavender, K. (2020). *The Midlife in the United States (MIDUS) Study-Accessing and Using the MIDUS Data*. https://hdl.handle.net/2027.42/154574

Rohleder, N. (2014). Stimulation of systemic low-grade inflammation by psychosocial stress. *Psychosomatic Medicine*, *76*(3), 181–189. https://doi.org/10.1097/PSY.0000000000000049

Rückerl, R., Greven, S., Ljungman, P., Aalto, P., Antoniades, C., Bellander, T., Berglind, N., Chrysohoou, C., Forastiere, F., Jacquemin, B., von Klot, S., Koenig, W., Küchenhoff, H., Lanki, T., Pekkanen, J., Perucci, C. A., Schneider, A., Sunyer, J., & Peters, A. (2007). Air pollution and inflammation (Interleukin-6, C-reactive

protein, fibrinogen) in myocardial infarction survivors. *Environmental Health Perspectives*, *115*(7), 1072–1080. https://doi.org/10.1289/ehp.10021

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. https://doi.org/10.1016/j.psyneuen.2018.07.012

Ryff, C., Almeida, D., Ayanian, J., Binkley, N., Carr, D. S., Coe, C., Davidson, R., Grzywacz, J., Karlamangla, A., Krueger, R., Lachman, M., Love, G., Mailick, M., Mroczek, D., Radler, B., Seeman, T., Sloan, R., Thomas, D., Weinstein, M., & Williams, D. (2015). *Midlife in the United States (MIDUS 3), 2013-2014: Version 7* (Version v7) [Data set]. Inter-University Consortium for Political and Social Research. https://doi.org/10.3886/ICPSR36346.V7

Ryff, C. D., Seeman, T., & Weinstein, M. (2010). *Midlife in the United States (MIDUS 2): Biomarker Project, 2004-2009: Version 9* (Version v9) [Data set]. Inter-University Consortium for Political and Social Research. https://doi.org/10.3886/ICPSR29282.V9

Salway, J. G. (2017). *Metabolism at a glance* (Fourth edition). John Wiley & Sons Inc.

Sato, H., Takahashi, T., Sumitani, K., Takatsu, H., & Urano, S. (2010). Glucocorticoid generates ROS to induce oxidative injury in the hippocampus, leading to impairment of cognitive function of rats. *Journal of Clinical Biochemistry and Nutrition*, *47*(3), 224–232. https://doi.org/10.3164/jcbn.10-58

Schandry, R. (2016). *Biologische Psychologie: Mit Online-Material* (4., überarbeitete Auflage). Beltz.

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. https://doi.org/10.1002/bies.201200084

Shalev, I., & Belsky, J. (2016). Early-life stress and reproductive cost: A two-hit developmental model of accelerated aging? *Medical Hypotheses*, *90*, 41–47. https://doi.org/10.1016/j.mehy.2016.03.002

Sheng, J. A., Bales, N. J., Myers, S. A., Bautista, A. I., Roueinfar, M., Hale, T. M., & Handa, R. J. (2021). The hypothalamic-pituitary-adrenal axis: Development, programming actions of hormones, and maternal-fetal interactions. *Frontiers in Behavioral Neuroscience*, *14*. https://doi.org/10.3389/fnbeh.2020.601939

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. *European Journal of Endocrinology*, *175*(6), R247–R253. https://doi.org/10.1530/EJE-16-0434

Straub, R. H. (2014). Interaction of the endocrine system with inflammation: A function of energy and volume regulation. *Arthritis Research & Therapy*, *16*(1), 203. https://doi.org/10.1186/ar4484

Straub, R. H. (2017). The brain and immune system prompt energy shortage in chronic inflammation and ageing. *Nature Reviews Rheumatology*, *13*(12), 743–751. https://doi.org/10.1038/nrrheum.2017.172

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. https://doi.org/10.3390/biomedicines9080922

Tanaka, M. (2020). Improving obesity and blood pressure. *Hypertension Research*, *43*(2), 79–89. https://doi.org/10.1038/s41440-019-0348-x

Tanaka, T., Narazaki, M., & Kishimoto, T. (2014). IL-6 in inflammation, immunity, and disease. *Cold Spring Harbor Perspectives in Biology*, *6*(10), a016295–a016295. https://doi.org/10.1101/cshperspect.a016295

The Emerging Risk Factors Collaboration. (2012). C-reactive protein, fibrinogen, and cardiovascular disease prediction. *New England Journal of Medicine*, *367*(14), 1310–1320. https://doi.org/10.1056/NEJMoa1107477

Thompson, A. M. S., Zanobetti, A., Silverman, F., Schwartz, J., Coull, B., Urch, B., Speck, M., Brook, J. R., Manno, M., & Gold, D. R. (2010). Baseline repeated measures from controlled human exposure studies: Associations between ambient air pollution exposure and the systemic inflammatory biomarkers IL-6 and fibrinogen. *Environmental Health Perspectives*, *118*(1), 120–124. https://doi.org/10.1289/ehp.0900550

Tien, P. C., Choi, A. I., Zolopa, A. R., Benson, C., Tracy, R., Scherzer, R., Bacchetti, P., Shlipak, M., & Grunfeld, C. (2010). Inflammation and mortality in HIV-infected adults: Analysis of the FRAM study cohort. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *55*(3), 316–322. https://doi.org/10.1097/QAI.0b013e3181e66216

Toker, S., Shirom, A., Shapira, I., Berliner, S., & Melamed, S. (2005). The association between burnout, depression, anxiety, and inflammation biomarkers: C-reactive protein and fibrinogen in men and women. *Journal of Occupational Health Psychology*, *10*(4), 344–362. https://doi.org/10.1037/1076-8998.10.4.344

Vitale, G., Salvioli, S., & Franceschi, C. (2013). Oxidative stress and the ageing endocrine system. *Nature Reviews Endocrinology*, *9*(4), 228–240. https://doi.org/10.1038/nrendo.2013.29

Vitlic, A., Lord, J. M., & Phillips, A. C. (2014). Stress, ageing and their influence on functional, cellular and molecular aspects of the immune system. *AGE*, *36*(3), 9631. https://doi.org/10.1007/s11357-014-9631-6

Vos, T., Lim, S. S., Abbafati, C., Abbas, K. M., Abbasi, M., Abbasifard, M., Abbasi-Kangevari, M., Abbastabar, H., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abolhassani, H., Aboyans, V., Abrams, E. M., Abreu, L. G., Abrigo, M. R. M., Abu-Raddad, L. J., Abushouk, A. I., … Murray, C. J. L. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: A systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, *396*(10258), 1204–1222. https://doi.org/10.1016/S0140-6736(20)30925-9

Wang, Z. (2021). The entropy perspective on human illness and aging. *Engineering*, S2095809921003866. https://doi.org/10.1016/j.eng.2021.08.014

Weber, B., Biery, D. W., Singh, A., Divakaran, S., Berman, A. N., Wu, W. Y., Brown, J. M., Hainer, J., Nasir, K., Liao, K., Bhatt, D. L., Di Carli, M. F., & Blankstein, R. (2021). Association of inflammatory disease and long-term outcomes among young adults with myocardial infarction: The Mass General Brigham YOUNG-MI Registry. *European Journal of Preventive Cardiology, 29*(2), 352–359. https://doi.org/10.1093/eurjpc/zwaa154

Welsh, P., Lowe, G. D. O., Chalmers, J., Campbell, D. J., Rumley, A., Neal, B. C., MacMahon, S. W., & Woodward, M. (2008). Associations of proinflammatory cytokines with the risk of recurrent stroke. *Stroke*, *39*(8), 2226–2230. https://doi.org/10.1161/STROKEAHA.107.504498

Wensveen, F. M., Šestan, M., Turk Wensveen, T., & Polić, B. (2019). 'Beauty and the beast' in infection: How immune–endocrine interactions regulate systemic metabolism in the context of infection. *European Journal of Immunology*, *49*(7), 982–995. https://doi.org/10.1002/eji.201847895

Wittchen, H.-U., & Hoyer, J. (Eds.). (2011). *Klinische Psychologie & Psychotherapie* (2., überarbeitete und erweiterte Auflage). Springer.

Wolkowitz, O. M., & Rothschild, A. J. (Eds.). (2003). *Psychoneuroendocrinology: The scientific basis of clinical practice* (1st ed). American Psychiatric Pub.

Yu, H., Hu, W., Song, X., & Zhao, Y. (2020). Immune modulation of platelet-derived mitochondria on memory CD4+ T cells in humans. *International Journal of Molecular Sciences*, *21*(17), 6295. https://doi.org/10.3390/ijms21176295

Zhang, J., Jin, Y., Jia, P., Li, N., & Zheng, Z.-J. (2021). Global gender disparities in premature death from cardiovascular disease, and their associations with country capacity for noncommunicable disease prevention and control. *International Journal of Environmental Research and Public Health*, *18*(19), 10389. https://doi.org/10.3390/ijerph181910389

Zhou, Y., Han, W., Gong, D., Man, C., & Fan, Y. (2016). Hs-CRP in stroke: A meta-analysis. *Clinica Chimica Acta*, *453*, 21–27. https://doi.org/10.1016/j.cca.2015.11.027

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. https://doi.org/10.1016/j.psyneuen.2016.11.036

Statutory declaration

“I, Nina Spägele (born Bertele), by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic “A Novel Bioinformatics Approach for Disease and Mortality Risk Prediction” / “Ein neuer, bioinformatischer Ansatz der Prädiktion von Krankheits- und Mortalitätsrisiko” independently and without the support of third parties, and that I used no other sources and aids than those stated. All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility. Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons. My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice. I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty. The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

Date Signature

Declaration of your own contribution to the publications

Nina Spägele (born Bertele) contributed the following to the below listed publications:

I was the first author of both papers and contributed significantly to all stages of the process. Based on consultations with my supervisor about research in the field, I developed the concept and related hypotheses on how biomarker patterns could be used to predict mortality and other outcomes. I conducted all statistical analysis myself using data from two large existing cohorts of the Midlife in the United States Study and the Midlife in Japan Study. I wrote the first draft of the paper and, under supervision from my first supervisor, incorporated comments from coauthors and reviewers across several rounds of revision. I created all tables and figures included in the papers and dissertation.

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Signature and date of first supervising university professor / lecturer

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Signature and date of doctoral student

Extract from journal summary list

EPMA journal

Journal Data Filtered By: **Selected JCR Year: 2020** Selected Editions: SCIE,SSCI
Selected Categories: **"MEDICINE, RESEARCH and EXPERIMENTAL"**
Selected Category Scheme: WoS
Gesamtanzahl: 140 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	NATURE MEDICINE	114,401	53.440	0.184050
2	Science Translational Medicine	45,509	17.956	0.103780
3	JOURNAL OF CLINICAL INVESTIGATION	132,296	14.808	0.114560
4	JOURNAL OF EXPERIMENTAL MEDICINE	74,803	14.307	0.062280
5	MOLECULAR ASPECTS OF MEDICINE	8,136	14.235	0.006640
6	Annual Review of Medicine	7,553	13.739	0.009800
7	EMBO Molecular Medicine	11,474	12.137	0.020440
8	TRENDS IN MOLECULAR MEDICINE	13,213	11.951	0.014720
9	Theranostics	23,558	11.556	0.034890
10	Clinical and Translational Medicine	2,201	11.492	0.003110
11	MOLECULAR THERAPY	24,333	11.454	0.030250
12	Wiley Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology	3,763	9.182	0.003760
13	Molecular Therapy-Nucleic Acids	8,812	8.886	0.014970
14	EXPERIMENTAL AND MOLECULAR MEDICINE	8,780	8.718	0.013260
15	JOURNAL OF BIOMEDICAL SCIENCE	6,621	8.410	0.007330
16	JCI Insight	15,237	8.315	0.054040
17	EBioMedicine	15,647	8.143	0.040730
18	Inflammation and Regeneration	743	7.354	0.001450
19	npj Vaccines	1,342	7.344	0.003850
20	Molecular Therapy-Oncolytics	1,582	7.200	0.002970
21	AMYLOID-JOURNAL OF PROTEIN FOLDING DISORDERS	2,202	7.141	0.003280
22	Translational Research	5,766	7.012	0.007980
23	Cold Spring Harbor Perspectives in Medicine	10,709	6.915	0.016100
24	Stem Cell Research & Therapy	13,356	6.832	0.018900
25	Molecular Therapy-Methods & Clinical Development	3,268	6.698	0.008200
26	EPMA Journal	1,507	6.543	0.001330

BBI Health

BBI Health is a very novel, official journal of the Psychoneuroimmunology Research Society (PNIRS) that doesn't have journal metrics yet.

Publications

EPMA Journal
https://doi.org/10.1007/s13167-021-00255-0

RESEARCH

How biomarker patterns can be utilized to identify individuals with a high disease burden: a bioinformatics approach towards predictive, preventive, and personalized (3P) medicine

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Abstract
Prevalences of non-communicable diseases such as depression and a range of somatic diseases are continuously increasing requiring simple and inexpensive ways to identify high-risk individuals to target with predictive and preventive approaches. Using *k*-mean cluster analytics, in study 1, we identified biochemical clusters (based on C-reactive protein, interleukin-6, fibrinogen, cortisol, and creatinine) and examined their link to diseases. Analyses were conducted in a US American sample (from the Midlife in the US study, $N = 1234$) and validated in a Japanese sample (from the Midlife in Japan study, $N = 378$). In study 2, we investigated the link of the biochemical clusters from study 1 to childhood maltreatment (CM). The three identified biochemical clusters included one cluster (with high inflammatory signaling and low cortisol and creatinine concentrations) indicating the highest disease burden. This high-risk cluster also reported the highest CM exposure. The current study demonstrates how biomarkers can be utilized to identify individuals with a high disease burden and thus, may help to target these high-risk individuals with tailored prevention/intervention, towards personalized medicine. Furthermore, our findings raise the question whether the found biochemical clusters have predictive character, as a tool to identify high-risk individuals enabling targeted prevention. The finding that CM was mostly prevalent in the high-risk cluster provides first hints that the clusters could indeed have predictive character and highlight CM as a central disease susceptibility factor and possibly as a leverage point for disease prevention/intervention.

Keywords Biomarker patterns · Personalized medicine (PPPM/3PM) · Patient stratification · Risk assessment · Childhood maltreatment · Psychiatric disorders

Introduction

The global burden of disease—current situation

Prevalence and incidence of non-communicable diseases (NCD) are continuously increasing in numbers, causing a strong socio-economic as well as a medical burden to the healthcare systems. Economically speaking, the US healthcare costs have steadily increased for 4 consecutive years, to reach 3.8 trillion US dollars in 2019 [1, 2]. NCD caused 90% of these costs as they result in massive long-term treatment costs and are often present with comorbidities [1, 2]. Thus, the prevention of NCD, and in this context the identification of at-risk individuals and sensitive biomarkers of disease risk, is more important than ever as it represents a leverage point to reduce the economic as well as the individual burden of diseases.

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The contribution of the current study

The two-consecutive study presented here demonstrates how routinely assessed biomarkers can be bioinformatically clustered and utilized to identify individuals with a high disease burden. Specifically, in study 1, we employed a clustering approach based on C-reactive protein (CRP), interleukin-6 (IL-6), fibrinogen, cortisol, and creatinine concentrations in a US cohort and validated the identified clusters in a Japanese cohort (for a study overview, see Fig. 1). We then linked these biochemical clusters to documented diseases including depression, heart disease, hypertension, stroke, peptic ulcer disease (PUD), and cancer. In study 2, we tested the association of childhood maltreatment (CM), a well-established early-life risk factor for developing mental and somatic disorders, with diseases as well as with the identified biochemical clusters from study 1.

General methods

Description of the study populations

US American sample

Data were drawn from the biomarker subsample of the *Midlife in the United States* (MIDUS) study between 1995 and 1996 [3]. For more information about the project, please see http://www.midus.wisc.edu/data/index.php.

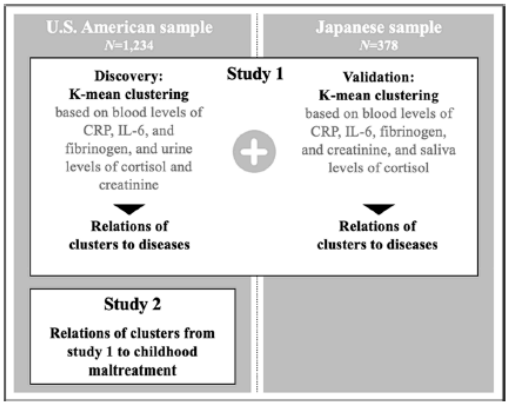

Fig. 1 Study workflow chart. *Note*: CRP = C-reactive protein, IL-6 = Interleukin-6. All one-time measures except saliva cortisol in the Japanese sample which was averaged across three time points (morning, noon, evening) for a total of 3 days

A total of 1255 individuals participated in the biomarker study, and of those complete biomarker data was available from 1234 individuals.

Japanese Sample

Data were drawn from the *Midlife in Japan* (MIDJA) study (N = 1027). In 2009–2010 biomarker data were generated for a subset of these participants ($N = 378$). Data were obtained analogically to MIDUS.

Study 1

Introduction

The importance of risk evaluation in personalized medicine, targeted prevention, and predictive diagnostics

According to the Global Burden of Disease study (2017), between 1990 and 2017, disability-adjusted life years (DALYs) due to NCD increased from 1.2 to 1.6 billion. With that, NCD caused more than 60% of DALYs worldwide [4]. But NCD cause not only individual suffering but also burden society as a whole, due to massive monetary and non-monetary costs [4, 5]. Relying on interventions—no matter how effective they are—after individuals are already ill is therefore a pivotal fallacy. Instead, current developments require simple and inexpensive ways to identify high-risk individuals to target with both preventive and interventive approaches. Furthermore, it is increasingly becoming clear that many well-established risk factors (such as body mass index (BMI) outside the normal range [6], genetic risk factors [7, 8]) supposedly helping to identify individuals at high risk for certain diseases are not independently from the individual environment and do not behave the same way across different individuals, highlighting the importance of personalized, tailored approaches in the context of preventive medicine. The presence of one particular risk factor might not have much predictive character for negative outcomes without being considered systemically/holistically, that is, in the context of other physiological, environmental, psychological, and biochemical parameters and processes [e.g., 6–8]. Despite these intricacies, at the same time, disease-predictive measures should be cost-efficient making it possible to implement them in the healthcare system.

The allostatic load index: chances and limitations

One particular concept that has become well-established in the literature is the concept of allostatic load (referring to the cumulative burden of chronic stress and adverse life events) with its suggested allostatic load index (ALI) [9]. ALI is a

cumulative multi-system risk score based on physiological and biochemical measures [10]. For each system, risk indices are calculated as the proportion of biomarkers for which an individual falls into predefined high-risk quartiles.

As a systemic risk score, ALI is predictive for various outcomes, including all-cause mortality [11, 12], while there are some critical limitations concerning its conceptualization. First, calculating a risk score as the sum of different system risk scores does not allow to account for intersystemic interactions and the possible predictive effect of these interactions. This gap is unfortunate as ALI includes parameters that indeed are not independent of each other, such as BMI and blood pressure [13]. Another concern refers to practicability and implementation of ALI into the healthcare system. While ALI considers parameters that can be assessed relatively simple, it is still likely that, for most individuals, parameters are only partially available, possibly limiting the predictive power of ALI. Together, ALI is a profound concept but artificially splits physiological processes that are woven into a holistic allostatic reaction, as acknowledged by the developers of ALI [14]. Furthermore, ALI lacks practicability, which is underlined by the fact that, to date, ALI has not been implemented in routine diagnostics.

A novel biochemical clustering approach

Given the rising number of NCD, there is an urgent necessity to develop an approach that is practicable, cost-efficient, and at best, based on biomarkers that are assessed in clinical routine allowing to identify high-risk individuals to target with specific preventive steps. The current study aimed to develop and validate an easily accessible measure that can realistically be implemented in routine diagnostics. Towards this aim and building on ALI, five biomarkers were chosen as they cover broad physiological functionality; CRP, fibrinogen, and IL-6 are pro-inflammatory markers (i.e., positively associated with inflammation), cortisol as the end product of the hypothalamus–pituitary–adrenal axis is an immune-modulatory mediator playing a crucial role in stress response, and creatinine is important for cellular energy metabolism [15–19]. Contrary to ALI, employing a clustering approach based on these biomarkers allows to account for linear and non-linear interactions among them and to link the resulting clusters to a range of mental and somatic diseases. To examine the association between biochemical clusters and diseases, we focused on depression, heart disease, hypertension, stroke, PUD, and cancer as these represent globally the highest prevalence, the fastest increase in numbers, and the utmost comorbidities [4]. We first clustered biochemical markers and related them to odds ratios (ORs) for diseases in a US population sample and then repeated this process in a Japanese cohort. To ensure representativity, both samples were recruited via random-digit-dialing qualifying them for studies with results generalizable to the population. Towards our aim to ensure that the selected biomarkers and their clustering demonstrate robust applicability across different cultures and ethnicities [20], we chose one US American and one Japanese sample to generate and validate the biochemical clusters.

Methods

Collection of biosamples and the assessment of biochemical markers

MIDUS Blood samples were collected after overnight fasting for the assessment of CRP, IL-6, and fibrinogen, according to the manufacturer guidelines (Dade Behring Inc., Deerfield, IL for CRP and fibrinogen; R&D Systems, Minneapolis, Minnesota for IL-6) [20]. Plasma levels of CRP and fibrinogen were assayed using immunonephelometric assay; IL-6 was quantitatively assessed using enzyme-linked immunosorbent assay (ELISA). The laboratory inter-assay coefficient of variance was 5.7% for CRP, 13% for IL-6, and 2.6% for fibrinogen, all below the 20% acceptable range [21].

To obtain a cumulative cortisol and creatinine measure, 12-h overnight urine samples were collected between 7 PM and 7 AM. Enzymatic colorimetric assays and liquid chromatography-tandem mass spectrometry were performed at the Mayo Medical Laboratory in Rochester, Minnesota. Data were excluded if participants had a renal failure or severe renal decline according to glomerular filtration rate [21].

MIDJA CRP, IL-6, and fibrinogen were assessed analogically to MIDUS, while cortisol was assessed in saliva (3 subsequent days, three times each day) and creatinine was assessed in blood. The 9 saliva measurements were averaged and used as a representative marker for cortisol concentrations [22]. We used blood levels of creatinine.

Diseases

Depression, heart disease, hypertension, stroke/transient ischemic attack (TIA), PUD, and cancer were assessed via self-report. Participants were asked if they were ever diagnosed with any of these diseases before/at the time of study participation.

Statistical analyses

First, the potential collinearity of the biomarker levels was assessed by calculating Pearson correlations among CRP, fibrinogen, IL-6, creatinine, and cortisol. After randomizing the order of participants [23], we performed a *k*-mean

cluster analysis with these markers in the MIDUS sample using IBM SPSS Statistics 27. To ensure the stability of clusters, we repeated the clustering process in subsamples [23]: Specifically, we conducted a median split based on age and performed the clustering for each group separately to assess whether the clusters are age-dependent. For the same purpose, we repeated the clustering procedure after excluding participants with a BMI outside the health range (below 18 or above 35). The next step was to repeat biochemical clustering that was performed for the whole MIDUS sample, in the MIDJA cohort. Finally, *z*-tests were used to compare ORs for diseases among clusters.

Results

Preliminary analyses

In both MIDUS and MIDJA samples, biomarkers were positively correlated (see SI Tables 4 and 5).

In MIDUS, 24.1% of the participants (currently or previously) had depression, 11.5% heart disease, 37.1% hypertension, 4.3% stroke/TIA, 5.3% PUD, and 13.6% cancer. In MIDJA, 4.5% of the participants had depression, 5.6% heart disease, 19.3% hypertension, 1.1% stroke/TIA, 8.3% PUD, and 5.1% cancer.

K-mean clustering

We used *z*-standardized biomarkers for *k*-mean clustering and evaluated the clustering results from $k=2$ to 6 clusters for MIDUS. When $k=2$, the patterns of clusters were not distinct enough; when $k=4$ or above, some clusters were very small in size. Through a combination of the parsimonious principle and engineering meaningful difference among clusters, $k=3$ were selected for the subsequent analyses. Figure 2 illustrates the distributions of the three identified clusters with respect to the biochemical markers. We replicated all three clusters in the younger MIDUS cohort as well as clusters 1 and 2 in the older MIDUS cohort (SI Figs. 7 and 8). We further replicated all three clusters in the BMI-restricted MIDUS cohort (SI Fig. 9).

Then, the 3-cluster solution from MIDUS was validated in the MIDJA sample; the results are shown in Fig. 3.

As depicted in Figs. 2 and 3, cluster 1 is characterized by average levels in all biochemical measures. Cluster 2 is characterized by high and above-average levels for CRP, IL-6, and fibrinogen. Cluster 3 is characterized by high and above-average levels for cortisol and creatinine but average concentrations of CRP, fibrinogen, and IL-6.

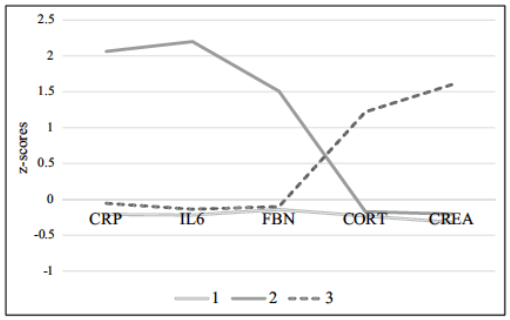

Fig. 2 MIDUS: biochemical markers (z-scores) and resulting clusters 1–3. *Note*: CRP = C-reactive protein (µg/mL), IL-6 = interleukin-6 (pg/mL), and FBN = fibrinogen (mg/dL) were measured in blood, and cortisol (µg/dL) and creatinine (mg/dL) were measured in urine. $N_{cluster1}=937$, $N_{cluster2}=102$, $N_{cluster3}=195$

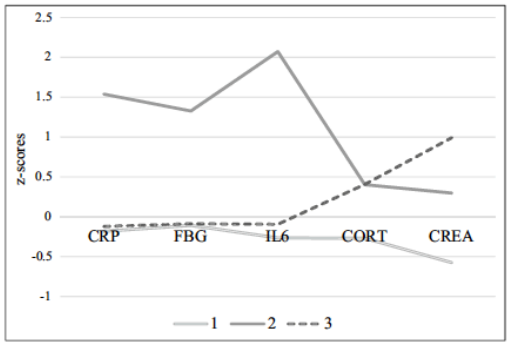

Fig. 3 MIDJA: biochemical markers (z-scores) and resulting clusters 1–3. *Note*: CRP = C-reactive protein (µg/mL), IL-6 = interleukin-6 (pg/mL) and FBN = fibrinogen (mg/dL), and creatinine (mg/dL) were measured in blood, and cortisol (µg/dL) was measured in saliva. $N_{cluster1}=233$, $N_{cluster2}=30$, $N_{cluster3}=115$

Associations between biochemical clusters and disease states

MIDUS Cluster 2 had the highest ORs for all considered diseases compared to the clusters 1 and 3 (Fig. 4, SI 10).

MIDJA Cluster 3 had the highest ORs for heart disease, hypertension, and PUD, cluster 2 had the highest ORs for stroke and cancer, and cluster 1 had the highest ORs for depression (Fig. 5, SI 10.1).

To compare this cluster-based approach to a well-established clinical biomarker that is associated with a broad range of NCD, the number of diagnoses among individuals in cluster 2 was compared to the number of diagnoses

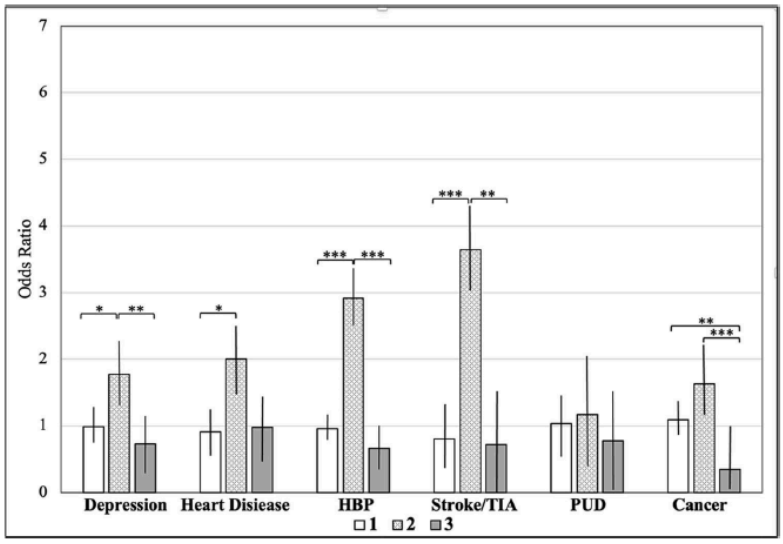

Fig. 4 MIDUS: odds ratios for diseases by cluster. *Note*: MIDUS = Midlife in the US sample, HPB = high blood pressure, TIA = transient ischemic attack, PUD = peptic ulcer disease. Error bars display 95% confidence intervals. Comparisons of odds ratios were conducted with log odds ratios using *z*-tests. * $p < .05$, ** $p < .01$, *** $p < .001$, *p*-values are controlled for multiple testing according to Bonferroni. All two-tailed

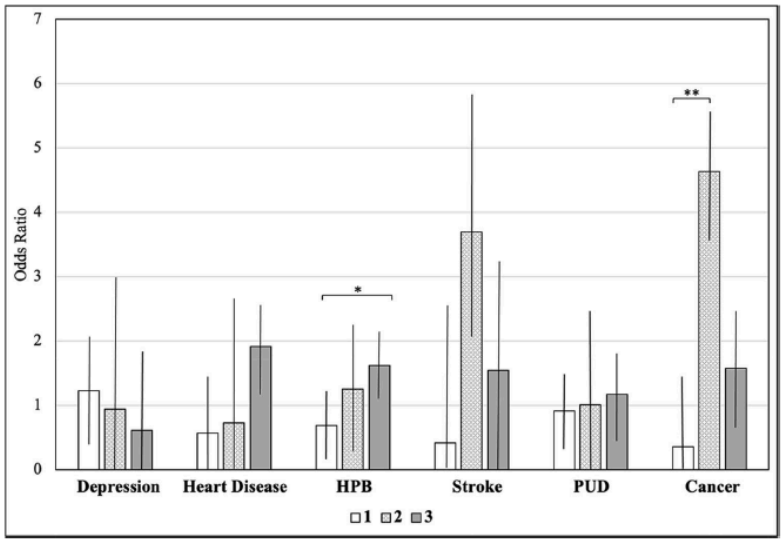

Fig. 5 MIDJA: odds ratios for diseases by cluster. *Note*: MIDJA = Midlife in Japan sample, HPB = high blood pressure, TIA = transient ischemic attack, PUD = peptic ulcer disease. Error bars display 95% confidence intervals. Comparisons of odds ratios were conducted with log odds ratios using *z*-tests. * $p < .05$, ** $p < .01$, *** $p < .001$, *p*-values are controlled for multiple testing according to Bonferroni. All two-tailed

among individuals with CRP concentrations above the clinical cutoff (> 3 mg/L) [24]. The disease burden in cluster 2 was higher with 1.6 diagnoses ($SD = 1.16$; 0.9 diagnoses for individuals not assigned to cluster 2) compared to individuals above the CRP cutoff with 1.2 diagnoses ($SD = 1.07$; 0.9 diagnoses for individuals below the cutoff).

Discussion

Three biochemical clusters in the general population

Findings reveal three distinct and interculturally stable biochemical clusters observable in the general population.

Cluster 1 is characterized by average levels of all biomarkers, cluster 2 by high inflammation-related mediators coupled with low cortisol and creatinine, and cluster 3 by high levels of cortisol and creatinine. The stability of clusters is supported by their replication in the MIDJA sample as well as in the BMI-restricted, in the younger (below age median) and in the older MIDUS cohort (above age median; here only clusters 1 and 2 were replicated). However, we did not replicate cluster 3 in the older MIDUS cohort. One explanation could be that, due to an age-related increase in systemic inflammation [25], older individuals were not assigned to cluster 3, which is characterized by low inflammation.

The link of biochemical clusters to disease states

Relating clusters to diseases, in MIDUS, cluster 2 showed the highest ORs for depression, heart disease, hypertension, stroke, and cancer (Fig. 4). These findings are supported by previous evidence suggesting that CRP, IL-6, and fibrinogen are associated with depression [26, 27], coronary heart disease [28–31], blood pressure [32], stroke [33–35], and cancer [36, 37]. However, contrary to these previous studies, the clustering approach used in this study allowed to account for well-known collinearities between biomarkers and thus promotes a more holistic perspective. Specifically, findings build on previous studies suggesting a link between inflammation and diseases [25] by demonstrating that it might not be one specific biomarker but a specific biochemical pattern (i.e., high CRP, IL-6, fibrinogen coupled with low cortisol and creatinine) that is associated with diseases. This idea is supported by the observation that individuals in cluster 2, descriptively, indicate a higher disease burden than individuals above the clinically well-established CRP cutoff.

Interestingly, we found no differences in the ORs for PUD between clusters despite the role of inflammation in its pathology [38]. Future research may aim to further examine the role of inflammatory signaling in the pathology of PUD.

While the cluster with high levels of CRP, IL-6, and fibrinogen can be considered a high-risk cluster, cluster 3 with high levels of cortisol and creatinine but low inflammation may be considered a protective cluster in MIDUS. We found that ORs for most diseases were lower in cluster 3 not only as compared to the high-risk cluster but also as compared to cluster 1 with average levels of all biomarkers. Concerning cancer, this difference became significant, potentially suggesting a protective character of this cluster. This would be in contrast to studies suggesting a link between hypercortisolism and disease outcomes [39, 40]. However, the combination of low inflammation and high cortisol and creatinine as in cluster 3 might indicate the integrity of the glucocorticoid negative feedback system, protecting from negative health outcomes [41]. Longitudinal studies may examine the consequences of this specific biochemical pattern. Towards this aim, we will examine MIDUS follow-up data (10 years after biomarker assessments) with respect to mortality outcomes.

In MIDJA, cluster 2 only seems to be a high-risk cluster for stroke and cancer while for other considered diseases, cluster 1 or cluster 3 indicates the highest burden. One aspect to consider here is that the MIDJA sample ($N = 378$) and especially cluster 2 were very small in size ($N = 30$). It is, therefore, possible that the present findings lack reliability. However, different biochemical patterns may be associated with different outcomes in the Japanese compared to the US American population because moderating mechanisms such as BMI, nutrition, and medication differ between populations [41]. This idea is supported by the finding that although in both MIDUS and MIDJA, approximately 8% of participants were assigned to cluster 2, the disease burden in MIDJA was much lower compared to MIDUS. This highlights the importance of individual aspects in disease susceptibility mentioned above and the role of interactions among different cultural, lifestyle, and biochemical factors; while an assignment of a US American individual to cluster 2 might be associated with a high disease burden, this might not be the case for a Japanese individual with the similar biochemical profile. Future studies should aim to examine the found biochemical clusters in other cultural contexts promoting a better understanding of their associative and predictive character in multiple populations. From a preventive perspective, this may also help to further precise targeted prevention, that is, to better understand which biochemical profile is associated with what disease susceptibility under what conditions.

Limitations

Our work has several strengths such as the validation of the clusters in an independent, Japanese sample and the representative character of cohorts. Yet, the findings face limitations. First, the present study is cross-sectional not allowing causal inferences. Second, the MIDJA sample size was relatively small. It is, therefore, possible that the ORs lack reliability. Third, methodological inconsistencies (urine cortisol and creatinine levels in MIDUS, average saliva levels of cortisol and blood levels of creatinine in MIDJA) between the cohorts may have impacted the clustering process. Fourth, diseases were assessed via self-report, which bears the risk of a report bias.

Conclusion

While the interactions among biomarkers make the distinction of their outcomes challenging, the design of the current study helps to gain a better understanding regarding the biochemical patterns that are present in the general population and how these patterns contribute to different physiological states on

a systemic scale. We identified and replicated three distinct biochemical signatures in two mid-life populations including one cluster with collinearly occurring elevated levels of CRP, fibrinogen, and IL-6 as well as low concentrations of cortisol and creatinine that indicated the highest prevalence of stroke and cancer.

Future longitudinal studies should aim to test the predictive character of the clusters found in this study, because, if clusters are indeed predictive in terms of risk evaluation, then they would represent a valuable clinical tool for both diagnostics and prevention of diseases. Specifically, if high-risk individuals can be identified by the clustering approach presented here, then these individuals could be provided with personalized treatment options including psychotherapy, anti-inflammatory drugs, and treatment supplements, e.g., nutrition and exercise plans.

Study 2

Introduction

The role of childhood maltreatment in disease susceptibility

Childhood maltreatment (CM) is an umbrella term that includes any act of emotional, physical, and sexual abuse as well as emotional and physical neglect experienced until the age of 18 [42]. CM can have a myriad of negative effects on survivors' mental and somatic health. The association between CM and inflammation is well established and may underlie the increased prevalence of somatic and mental disorders in CM-exposed individuals [16, 43–45]. Thus, CM, which is still an underestimated phenomenon in somatic/clinical settings, might be a disruptive factor in the context of both personalized medicine and targeted prevention, as it may amplify and interact with other disease susceptibility factors, resulting in a massive increase and expansion of an individual's disease risk and development. Therefore, in study 2, the association of CM with disease prevalence as well as with the assignment to the biochemical clusters was investigated.

We used the MIDUS sample for these analyses, as CM was not assessed in MIDJA. Based on previous literature, we expected to find higher exposure of CM in clusters with high inflammation as compared to clusters with low inflammation [16, 43–45].

Methods

Assessment of childhood maltreatment

CM was assessed using the Childhood Trauma Questionnaire (CTQ; Bernstein and Fink [46]). As a retrospective self-report measure with 28 items, the CTQ assesses five types of CM: emotional, physical, and sexual abuse, emotional, and physical neglect as well as the tendency to minimize CM [46].

Statistical analyses

Cutoff values for moderate CM exposure were used to create dichotomous variables for each CTQ subscale (emotional abuse ≥ 13; physical abuse ≥ 10; sexual abuse ≥ 8; emotional neglect ≥ 15; and physical neglect ≥ 10) [46]. A composite variable was then computed indicating exposure to at least one category of moderate to severe abuse or neglect (CM+) vs. no or low exposure (CM−) [46]. Using the moderate cutoff variable, prevalences of CM were calculated for the whole sample. Next, we compared general disease burden as well as the prevalence of specific diseases in individuals without and with CM experiences using χ^{2-}tests and *t*-tests. Then, a continuous total score of the CTQ was calculated by summing up the scores across all items. This continuous score was used to create a general linear model (GLM) with pairwise comparisons correcting for sex, age, BMI, physical activity, alcohol, and smoking habits as well as for multiple testing (Bonferroni) comparing CM among clusters. To avoid issues resulting from heteroscedastic residual variances, we performed a bootstrapping (10,000 samples). Bootstrapping, which allows finding robust parameter estimates (i.e., independently from the homoscedasticity assumption of residual variances), is considered the gold standard approach since our clusters are stable and since none of the covariates included in the GLM is involved in the clustering process [47].

Results

One-third (36.1%) of participants reported at least moderate CM on at least one CTQ subscale. Individuals exposed to CM had a higher overall disease burden with 1.12 ($SD = 1.03$) diagnoses on average compared to 0.85 ($SD = 0.93$) diagnoses in individuals without CM history ($t(1192) = -4.549$, $p < 0.001$). This difference was mainly driven by the higher prevalence of depression in CM-exposed individuals (36.2%) compared to individuals without CM (16.9%, $\chi^2(1) = 61.72$, $p < 0.001$).

CM exposure differed between biochemical clusters, with 45.1% of individuals in cluster 2 reporting at least moderate CM on at least one of the CTQ subscales (28.4% without CM), compared to 35.9% in cluster 1 (37.1% without CM) and 30.8% in cluster 3 (43.6% individuals without CM). GLMs using the continuous CM score indicated (SI

Table 13) the highest CM exposure in cluster 2, followed by clusters 1 and 3 (all $ps < 0.001$).

Discussion

Childhood maltreatment and disease burden: a mediating role for biochemical profiles?

The CM prevalences found here are in line with meta-analytic findings [48] as well as the result that CM-exposed individuals have a higher disease burden compared to non-exposed individuals is supported by previous evidence [49–51]. Given the association of CM to inflammatory processes [16, 43–45], one mechanism possibly linking CM to diseases might be the biochemical clusters from study 1. As we found that cluster 2 had the highest CM exposure and also the highest disease prevalences, specific biochemical profiles may underlie the association between CM and disease burden. If that is the case, clusters may represent a future leverage point for targeted prevention, enabling CM-exposed individuals to overcome the abusive experience and their stress burden-related health consequences through e.g. psychotherapy and support groups before it comes to the onset and manifestation in the form of severe disease. However, this idea faces the limitation that we could not statistically test this mediation of the biochemical clusters in the link between CM and disease prevalences as both the possible mediator (clusters) and the dependent variables (disease yes/no) were categorial. To get a deeper insight into this issue, our aim with the MIDUS follow-up data (10 years after biomarker assessments) is to examine whether CM-exposed individuals in cluster 2 indeed show more detrimental outcomes than CM-exposed individuals in the other two clusters.

Limitations

The present findings should be considered in light of the limitation that we used retrospective self-reported measures of CM. Therefore, report and memory biases are possible. Although the value of self-reported measures of CM when investigating its correlates and outcomes has been emphasized [52], future studies should also aim to relate CM assessed via official reports to the found biochemical clusters and to diseases. CM was not available in the Japanese cohort; therefore, the associative nature of CM with the identified clusters in the US sample needs future replication in independent cohorts. As this study was cross-sectional, causal inferences cannot be drawn without subsequent research.

Conclusions

Findings complement existing literature indicating detrimental longer-term implications of CM on survivors' health. Results highlight the importance of identifying CM as early as possible before it manifests itself biologically and possibly increases disease vulnerability. We thus encourage professionals in preventive and medical care contexts to be attentive to reports of CM and to consider these in individual treatments; validated screening instruments are available in multiple languages (e.g., CTQ) [46].

Conclusions and expert recommendations in the framework of 3P medicine

The contribution of the current findings

Our findings suggest three distinct biochemical signatures that are replicable and interculturally stable. One of them is a high-risk cluster indicated by its high disease burden. Due to the cross-sectional character of this study, it might also be that the biochemical clusters are consequences of diseases; however, study 2 demonstrating a strong link between the high-risk cluster and CM provides first hints that the clusters could be indeed pre-disease markers affecting the vulnerability to diseases. Future studies should aim to test the predictive character of clusters to evaluate their applicability as pre-disease markers. Furthermore, integrating CM screenings in standard medical practice may be a promising way for identifying individuals at risk and for developing tailored prevention and intervention techniques.

Implications and recommendations for personalized medicine, targeted prevention, and predictive diagnostics

The assessment of CRP, IL-6, fibrinogen, cortisol, and creatinine should be mandatory in all 3PM (i.e., personalized medicine, targeted prevention, and predictive diagnostics) disciplines to get a global insight into an individual's current health condition. High inflammatory signaling coupled with low compensation, that is, with low cortisol and creatinine, is a detrimental biochemical profile associated with a high disease burden and should be taken as a reason for further examination (especially with respect to artery condition/stroke and cancer) and for personalized treatments involving anti-inflammatory drugs, nutrient substitutions, and treatment supplements, e.g., nutrition and exercise plans. Furthermore, individuals with this biochemical profile should

be examined with a special focus on early-life stress and especially CM. In cases where CM is prevalent, its role in the patient's individual condition pattern should be examined thoroughly and psychotherapy or other stress reducing interventions should be offered/employed.

Future research directions to foster the understanding of biochemical profiles in personalized medicine, targeted prevention, and predictive diagnostics

Future research should examine the predictive character of the found biochemical clusters with respect to long-term well-being, mental and physical health, and mortality. Ideally, these studies should examine different cultures promoting a better understanding of the generalizability and limitedness of the predictive power of the identified biochemical clusters. Furthermore, this future research may suggest additional factors to be taken into account together with the biochemical clusters, helping to advance and precise disease prediction and, hence, to improve both targeted prevention and personalized interventions.

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Data availability All analyzed data are available publicly: http://www.midus.wisc.edu/data/index.php.

Code availability Not applicable.

Declarations

Ethics approval The Midlife in the United States Study data collection was reviewed and approved by the Education and Social/Behavioral Sciences and the Health Sciences IRBs at the University of Wisconsin-Madison. The Midlife in Japan Study was approved by the IRB of the University of Tokyo.

Consent to participate Informed consent was obtained by all participants in both the Midlife in the United States Study and in the Midlife in Japan Study.

Consent to publish After reviewing the manuscript, all authors agreed with its publication in the current form.

Competing interests The authors declare no competing interests.

References

1. Buttorff C, Ruder T, Bauman M. Multiple chronic conditions in the United States [Internet]. RAND Corporation; 2017 [cited 2021 May 4]. Available from: http://www.rand.org/pubs/tools/TL221.html
2. Martin AB, Hartman M, Lassman D, Catlin A. The National Health Expenditure Accounts Team. National Health Care spending in 2019: steady growth for the fourth consecutive year: study examines national health care spending for 2019. Health Aff. 2021;40:14–24.
3. Ryff CD, Seeman T, Weinstein M. Midlife in the United States (MIDUS 2): Biomarker Project, 2004–2009: Version 9 [Internet]. Inter-University Consortium for Political and Social Research; 2010 [cited 2021 Mar 27]. Available from: https://www.icpsr.umich.edu/icpsrweb/NACDA/studies/29282/versions/V9.
4. Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2017 (GBD 2017) Results. Seattle, U.S.: Institute for Health Metrics and Evaluation (IHME); 2018.
5. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, et al. Heart disease and stroke statistics—2018 update: a report from the American Heart Association. Circulation [Internet]. 2018 [cited 2021 May 4];137. Available from: https://www.ahajournals.org/doi/https://doi.org/10.1161/CIR.0000000000000558.
6. Golubnitschaja O, Liskova A, Koklesova L, Samec M, Biringer K, Büsselberg D, et al. Caution, "normal" BMI: health risks associated with potentially masked individual underweight—EPMA Position Paper 2021. EPMA Journal [Internet]. 2021 [cited 2021 Aug 18]; Available from: https://link.springer.com/https://doi.org/10.1007/s13167-021-00251-4.
7. Lopizzo N, Bocchio Chiavetto L, Cattane N, Plazzotta G, Tarazi FI, Pariante CM, et al. Gen-environment interaction in major depression: focus on experience-dependent biological systems. Front Psychiatry [Internet]. 2015 [cited 2021 Aug 18];6. Available from: http://www.frontiersin.org/Schizophrenia/https://doi.org/10.3389/fpsyt.2015.00068/abstract.
8. Huang T, Hu FB. Gene-environment interactions and obesity: recent developments and future directions. BMC Med Genomics. 2015;8:S2.
9. McEwen BS. Stress, adaptation, and disease: allostasis and allostatic load. Ann N Y Acad Sci. 1998;840:33–44.
10. Chen E, Miller GE, Lachman ME, Gruenewald TL, Seeman TE. Protective factors for adults from low-childhood socioeconomic circumstances: the benefits of shift-and-persist for allostatic load. Psychosom Med. 2012;74:178–86.
11. Gallo LC, Fortmann AL, Mattei J. Allostatic load and the assessment of cumulative biological risk in biobehavioral medicine: challenges and opportunities. Psychosom Med. 2014;76:478–80.
12. Juster R-P, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci Biobehav Rev. 2010;35:2–16.
13. Tanaka M. Improving obesity and blood pressure. Hypertens Res. 2020;43:79–89.
14. McEwen BS. Stress and the individual: mechanisms leading to disease. Arch Intern Med. 1993;153:2093.

15. Thompson AMS, Zanobetti A, Silverman F, Schwartz J, Coull B, Urch B, et al. 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. 2010;118:120–4.
16. Baumeister D, Akhtar R, Ciufolini S, Pariante CM, Mondelli V. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. Mol Psychiatry. 2016;21:642–9.
17. Rückerl R, Greven S, Ljungman P, Aalto P, Antoniades C, Bellander T, et al. Air pollution and inflammation (interleukin-6, C-reactive protein, fibrinogen) in myocardial infarction survivors. Environ Health Perspect. 2007;115:1072–80.
18. Kashani K, Rosner MH, Ostermann M. Creatinine: from physiology to clinical application. Eur J Intern Med. 2020;72:9–14.
19. Zorn JV, Schür RR, Boks MP, Kahn RS, Joëls M, Vinkers CH. Cortisol stress reactivity across psychiatric disorders: a systematic review and meta-analysis. Psychoneuroendocrinology. 2017;77:25–36.
20. Crimmins EM, Vasunilashorn S, Kim JK, Hagedorn A, Saito Y. A comparison of biological risk factors in two populations: the United States and Japan. Popul Dev Rev. 2008;34:457–82.
21. Gruenewald TL, Karlamangla AS, Hu P, Stein-Merkin S, Crandall C, Koretz B, et al. History of socioeconomic disadvantage and allostatic load in later life. Soc Sci Med. 2012;74:75–83.
22. Kobayashi H, Miyazaki Y. Distribution characteristics of salivary cortisol measurements in a healthy young male population. J Physiol Anthropol. 2015;34:30.
23. Franklin J. The elements of statistical learning: data mining, inference and prediction. Math Intell. 2005;27:83–5.
24. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, et al. 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. 2003;107:499–511.
25. Chung HY, Cesari M, Anton S, Marzetti E, Giovannini S, Seo AY, et al. Molecular inflammation: underpinnings of aging and age-related diseases. Ageing Res Rev. 2009;8:18–30.
26. Gimeno D, Kivimäki M, Brunner EJ, Elovainio M, De Vogli R, Steptoe A, et al. Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. Psychol Med. 2009;39:413–23.
27. Toker S, Shirom A, Shapira I, Berliner S, Melamed S. The association between burnout, depression, anxiety, and inflammation biomarkers: C-reactive protein and fibrinogen in men and women. J Occup Health Psychol. 2005;10:344–62.
28. Danesh J. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. BMJ. 2000;321:199–204.
29. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med. 2004;350:1387–97.
30. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte sount with coronary heart disease: meta-analyses of prospective studies. JAMA. 1998;279:1477.
31. Luc G, Bard J-M, Juhan-Vague I, Ferrieres J, Evans A, Amouyel P, et al. C-reactive protein, interleukin-6, and fibrinogen as predictors of coronary heart disease: the PRIME study. Arterioscler Thromb Vasc Biol. 2003;23:1255–61.
32. Piché M-È, Lemieux S, Weisnagel SJ, Corneau L, Nadeau A, Bergeron J. Relation of high-sensitivity C-reactive protein, interleukin-6, tumor necrosis factor-alpha, and fibrinogen to abdominal adipose tissue, blood pressure, and cholesterol and triglyceride levels in healthy postmenopausal women. Am J Cardiol. 2005;96:92–7.
33. Zhou Y, Han W, Gong D, Man C, Fan Y. Hs-CRP in stroke: a meta-analysis. Clin Chim Acta. 2016;453:21–7.
34. Welsh P, Lowe GDO, Chalmers J, Campbell DJ, Rumley A, Neal BC, et al. Associations of proinflammatory cytokines with the risk of recurrent stroke. Stroke. 2008;39:2226–30.
35. Di Napoli M, Papa F, Bocola V. Prognostic influence of increased C-reactive protein and fibrinogen levels in ischemic stroke. Stroke. 2001;32:133–8.
36. Allin KH, Nordestgaard BG. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. Crit Rev Clin Lab Sci. 2011;48:155–70.
37. Qian S, Golubnitschaja O, Zhan X. Chronic inflammation: key player and biomarker-set to predict and prevent cancer development and progression based on individualized patient profiles. EPMA Journal. 2019;10:365–81.
38. Lanas A, Chan FKL. Peptic ulcer disease. Lancet. 2017;390:613–24.
39. Le M. Functional hypercortisolism, visceral obesity, and metabolic syndrome. Endocr Pract. 2016;22:506–8.
40. Steffensen C, Pereira AM, Dekkers OM, Jørgensen JOL. Diagnosis of endocrine disease: prevalence of hypercortisolism in type 2 diabetes patients: a systematic review and meta-analysis. Eur J Endocrinol. 2016;175:R247–53.
41. Kalat JW. Biological psychology. 10th ed. Belmont, California, U.S.: Wadsworth, Cengage Learning. 2009.
42. Gilbert R, Widom CS, Browne K, Fergusson D, Webb E, Janson S. Burden and consequences of child maltreatment in high-income countries. Lancet. 2009;373:68–81.
43. Coelho R, Viola TW, Walss-Bass C, Brietzke E, Grassi-Oliveira R. Childhood maltreatment and inflammatory markers: a systematic review. Acta Psychiatr Scand. 2014;129:180–92.
44. Danese A, Moffitt TE, Pariante CM, Ambler A, Poulton R, Caspi A. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. Arch Gen Psychiatry. 2008;65:409.
45. Osborn M, Widom CS. Do documented records and retrospective reports of childhood maltreatment similarly predict chronic inflammation? Psychol Med. 2020;50:2406–15.
46. Bernstein D, Fink L. Manual for the childhood trauma questionnaire. San Antonio, U.S.: The Psychological Corporation. 1998.
47. Efron B, Tibshirani R. An introduction to the bootstrap. New York: Chapman & Hall; 1993.
48. Stoltenborgh M, Bakermans-Kranenburg MJ, Alink LRA, van IJzendoorn MH, . The prevalence of child maltreatment across the globe: review of a series of meta-analyses: Prevalence of child Maltreatment across the globe. Child Abuse Rev. 2015;24:37–50.
49. Basu A, McLaughlin KA, Misra S, Koenen KC. Childhood maltreatment and health impact: the examples of cardiovascular disease and type 2 diabetes mellitus in adults. Clin Psychol Sci Pract. 2017;24:125–39.
50. Batten SV, Aslan M, Maciejewski PK, Mazure CM. Childhood maltreatment as a risk factor for adult cardiovascular disease and depression. J Clin Psychiatry. 2004;65:249–54.
51. Hovdestad WE, Shields M, Shaw A, Tonmyr L. Childhood maltreatment as a risk factor for cancer: findings from a population-based survey of Canadian adults. BMC Cancer. 2020;20:70.
52. Danese A, Widom CS. Objective and subjective experiences of child maltreatment and their relationships with psychopathology. Nat Hum Behav. 2020;4:811–8.

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

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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

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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

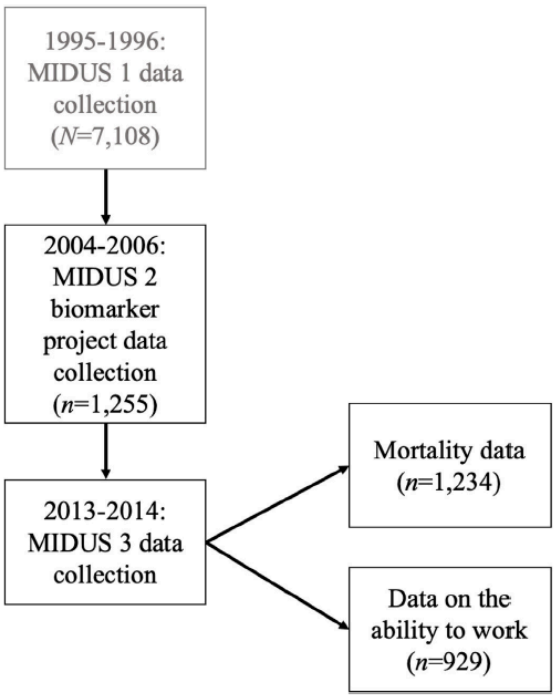

Fig. 1. Overview of the data collection Process.
Note: MIDUS = Midlife in the United States study.

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.

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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.

Curriculum vitae

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List of publications

Bertele, N., Talmon, A., Gross, J. J. (2020). Childhood Maltreatment and Narcissism: The Mediating Role of Dissociation. *Journal of Interpersonal Violence*. Impact-Factor: 6.14

Bertele, N., & Talmon, A. (2021). Sibling Sexual Abuse: A Review of Empirical Studies in the Field, *Trauma, Violence & Abuse*. Impact-Factor: 10.57

Bertele, N., Karabatsiakis, A., Buss, C., & Talmon, A. (2021). How Biomarker Patterns can be Utilized to Identify Individuals With a High Disease Burden. *EPMA Journal*. Impact-Factor: 6.37

Gyllenhammer, L. E., Rasmussen, J. M., **Bertele, N.**, Halbing, A., Entringer, S., Wadhwa, P. D., & Buss, C. (2021). Maternal inflammation during pregnancy and offspring brain development: the role of mitochondria. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. Impact-Factor: 6.2

Bertele, N., Karabatsiakis, A., Talmon, A., & Buss, C. (2022). Biochemical Clusters Predict Mortality and the Reported Inability to Work 10 Years Later. *BBI Health, 21*. Impact-Factor: N/A

Bertele, N., Talmon, A., Gross, J. J., Schmahl, C., Schmitz, M., & Niedfeld, I. (2022). Childhood Maltreatment and Borderline Personality Disorder: The Mediating Role of Difficulties with Emotion Regulation. *Journal of Personality Disorders, 36*(3), 264-276. Impact-Factor: 3.13

Bertele, N., Wendling, C., Reinken, V., Gross, J., Talmon, A. (2022). Somatic Symptom Profiles Are Associated with Pre-Treatment Depression and Anxiety Symptom Severity but Not Inpatient Therapy Outcomes. *Journal of Psychotherapy Research.* Impact-Factor: 3.77

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