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Optimising Nutrition to Alleviate Age-Associated Functional Decline

Edited by
Tomasz Kostka

Printed Edition of the Special Issue Published in *Nutrients*

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Editor

Tomasz Kostka

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Editor
Tomasz Kostka
Department of Geriatrics
Medical University of Lodz
Lodz
Poland

Editorial Office
MDPI
St. Alban-Anlage 66
4052 Basel, Switzerland

This is a reprint of articles from the Special Issue published online in the open access journal *Nutrients* (ISSN 2072-6643) (available at: www.mdpi.com/journal/nutrients/special_issues/Optimising_Nutrition_to_Alleviate_Age-Associated_Functional_Decline).

For citation purposes, cite each article independently as indicated on the article page online and as indicated below:

LastName, A.A.; LastName, B.B.; LastName, C.C. Article Title. *Journal Name* **Year**, *Volume Number*, Page Range.

ISBN 978-3-0365-2429-0 (Hbk)
ISBN 978-3-0365-2428-3 (PDF)

Contents

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Editorial

Special Issue: "Optimising Nutrition to Alleviate Age-Associated Functional Decline"

Tomasz Kostka

Department of Geriatrics, Healthy Ageing Research Centre, Medical University of Lodz, Plac Hallera 1, 90-647 Lodz, Poland; tomasz.kostka@umed.lodz.pl

Appropriate nutrition is a cornerstone of preventive gerontology. This Special Issue of *Nutrients* provides new insights on nutritional assessment and potential modifications of nutritional behaviours and supplements to prevent age-associated disorders and increase life expectancy in different populations of older subjects.

This Special Issue includes five original articles and four systematic reviews. The first review discusses the synergistic action of selected antioxidant micronutrients (vitamin C, vitamin E, selenium, and zinc) for inhibiting oxidative stress and DNA damage. Micronutrients are involved in every cellular/biochemical process. Seniors are prone to micronutrient deficiencies due to age-associated physiological changes and often poor diet. Moreover, the lack of micronutrients has an indirect impact on the genome. Their low levels reduce the activity of antioxidant enzymes and therefore inhibit the efficiency of defense against free radicals, which can lead to the formation of DNA lesions. The more DNA damage in the genetic material, the faster aging at the cellular level and the higher the risk of pathological processes (e.g., carcinogenesis). Targeted supplementation of crucial antioxidative micronutrients such as selenium, zinc, vitamin C, and vitamin E seems to have the potential to positively influence the condition of an ageing organism, including minimizing inflammation, enhancing antioxidative defense, and limiting the formation of DNA lesions. Consequently, it may lead to lowering the risk and incidence of age-related diseases such as cardiovascular diseases, neurodegenerative diseases, and malnutrition [1].

The continuous increase in life expectancy results in a growing risk of cancer, which consequently increases the population of older adults with cancer. In the second review, problems associated with diet and nutrition in the elderly undergoing active cancer therapy have been presented. As epigenetics, an emerging element of the regulation of gene expression, is involved in both aging and cancer and the epigenetic profile can be modulated by the diet, it seems to be a candidate to assist with planning a nutritional intervention in elderly populations with cancer. Nutritional interventions modulating the epigenetic profile, including caloric restriction and basal diet with modifications (elimination diet, supplementary diet), are discussed as the ways to improve the efficacy of cancer therapy and maintain the quality of life of older adults with cancer [2].

Nutritional interventions have been shown to be especially effective for cardiometabolic risk. The Mediterranean diet, with olive oil as a vital component, has both health benefits and acceptable adherence. The third review provides an updated overview of current knowledge on the benefits of olive oil most relevant to menopause-associated metabolic syndrome, including an analysis of the components with the greatest health impact, their effect on basic mechanisms of disease, and the state of the art regarding their action on the main features of metabolic syndrome [3].

Neurological diseases have steadily increasing significance for the health of aging populations. Citicoline is a chemical compound involved in the synthesis of cell membranes with a promising role in neurology. Citicoline is often used to enhance cognitive functions. In the fourth review, accessible databases were searched for articles regarding citicoline use in neurological diseases. The review found that citicoline has been proven to enhance

Citation: Kostka, T. Special Issue: "Optimising Nutrition to Alleviate Age-Associated Functional Decline". *Nutrients* **2021**, *13*, 2824. https://doi.org/10.3390/nu13082824

Received: 18 July 2021
Accepted: 4 August 2021
Published: 17 August 2021

cognitive functions among healthy individuals and improve prognosis after stroke. In an animal model of nerve damage and neuropathy, citicoline stimulated regeneration and lessened pain. Citicoline has a wide range of effects and could be an essential substance in the treatment of many neurological diseases [4].

Depression is one of the diseases with increasing prevalence in the older population. The first original article analyzes the relationship between nutritional status and depression symptoms severity in 1975 older outpatients. Women with higher-severity depression symptoms had significantly lower nutritional status, shorter education time, smaller calf circumference, and higher waist to height ratio. Men with depression symptoms had lower nutritional status, shorter education, and smaller calf circumference. In the model of stepwise multiple regression, nutritional status and education years were the only independent variables predicting the severity of depression symptoms in both women and men. Results obtained in the study indicate a strong relationship between proper nutritional status and education level with severity of depression symptoms in older women and men [5].

The second original article analyses the role of serotonin and other tryptophan (TRP) metabolites generated in the kynurenine pathway in the pathogenesis of depression. Ninety subjects in three groups, 30 subjects each, were enrolled in this study: controls (healthy young adults, group I) and older individuals without (group II) or with (group III) symptoms of mild and moderate depression. The average daily intake of TRP was significantly lower in group III than the remaining two groups, but group III was also characterised by higher urinary levels of L-kynurenine, kynurenic acid, xanthurenic acid, and quinolinic acid compared with younger adult individuals and older patients without mood disorders. Therefore, mild and moderate depression in the elderly may be associated with a lower intake of TRP and changes in its kynurenine metabolic pathway, which suggests a potential dietary TRP-based intervention in this group of patients [6].

The last three original articles assess nutritional status in hospitalised older adults. The first one presents an optimal set of variables that are independently associated with the mortality risk of 433 older comorbid adults that have been discharged from the geriatric ward. Stepwise backward variable selection and the iterative Bayesian model averaging approaches to the Cox proportional hazards models were used. The results of the multivariable analysis identified seven explanatory variables that were independently associated with the length of survival. The mortality rate was higher in males than in females; it increased with the comorbidity level and C-reactive proteins plasma level but was negatively affected by a person's mobility, geriatric nutritional risk index (GNRI), and lymphocyte count, as well as the vitamin D plasma level [7].

The second study compares two widely recommended short nutrition assessment tools—Nutrition Risk Screening 2002 (NRS-2002) and Subjective Global Assessment Form (SGA)—with other Comprehensive Geriatric Assessment (CGA) measurements in 622 consecutively hospitalised older subjects. Both NRS-2002 and SGA were inversely related to anthropometric measurements, functional assessment tests, and Mini-Mental State Examination (MMSE) and positively associated with the Vulnerable Elders Survey-13 (VES-13) score. Comparison of well-nourished subjects and patients with suggested problems with nutrition according to NRS-2002 (0–2 vs. 3–7) and SGA (A vs. B + C) gave comparable results. Both nutritional scales at given cut-off points similarly discriminated anthropometric data and other CGA tools in the populations of well-nourished vs. malnourished hospitalised older subjects. In conclusion, the authors recommend using both NRS-2002 and SGA to detect malnutrition or risk of malnutrition in routine clinical practice of the geriatric department ward [8].

The last original article emphasises the role of albumin as a useful marker of in-hospital malnutrition and frailty. The prevalence of preexisting hypoalbuminemia at the time of discharge from the hospital was investigated using a sample of 9428 patients. Analysis of albumin levels at admission and at discharge was conducted by classes of albuminemia and then stratified by age. At the time of admission, hypoalbuminemia was found to be

present in more than half of the sample, with no sex differences. The serum albumin level tended to decrease with age. The condition of marked and mild hypoalbuminemia was more prevalent in patients over 65 years of age. The authors conclude that albumin levels should be integrated into the routine assessment of patients, especially when dealing with nutritionally fragile populations [9].

In conclusion, the present Special Issue presents several aspects of assessment of nutritional status and prevention and treatment of nutritional deficiencies in different populations of older adults. Undoubtedly, future research will deepen our knowledge on this crucial public health issue.

Conflicts of Interest: The author declares no conflict of interest.

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Review

Nutrition Can Help DNA Repair in the Case of Aging

Julia Kaźmierczak-Barańska, Karolina Boguszewska and Boleslaw T. Karwowski *

DNA Damage Laboratory of Food Science Department, Faculty of Pharmacy, Medical University of Lodz, ul. Muszynskiego 1, 90-151 Lodz, Poland; julia.kazmierczak-baranska@umed.lodz.pl (J.K.-B.); karolina.boguszewska@umed.lodz.pl (K.B.)

* Correspondence: boleslaw.karwowski@umed.lodz.pl; Tel.: +48-42-677-91-40

Received: 24 September 2020; Accepted: 29 October 2020; Published: 1 November 2020

Abstract: Micronutrients such as vitamins and trace elements are crucial for maintaining the health of all organisms. Micronutrients are involved in every cellular/biochemical process. They play roles in proper heart and brain functioning, influence immunological responses, and antioxidant defense systems. Therefore, prolonged deficiency in one or more micronutrients leads to cardiovascular or neurodegenerative disorders. Keeping micronutrients at adequate levels is especially important for seniors. They are prone to deficiencies due to age-associated functional decline and often to a diet poor in nutrients. Moreover, lack of micronutrients has an indirect impact on the genome. Their low levels reduce the activity of antioxidant enzymes, and therefore inhibit the efficiency of defense against free radicals which can lead to the formation of DNA lesions. The more DNA damage in the genetic material, the faster aging at the cellular level and a higher risk of pathological processes (e.g., carcinogenesis). Supplementation of crucial antioxidative micronutrients such as selenium, zinc, vitamin C, and vitamin E seems to have the potential to positively influence the condition of an aging organism, including minimizing inflammation, enhancing antioxidative defense, and limiting the formation of DNA lesions. In consequence, it may lead to lowering the risk and incidence of age-related diseases such as cardiovascular diseases, neurodegenerative diseases, and malnutrition. In this article, we attempt to present the synergistic action of selected antioxidant micronutrients (vitamin C, vitamin E, selenium, and zinc) for inhibiting oxidative stress and DNA damage, which may impede the process of healthy aging.

Keywords: micronutrients; aging; DNA damage; genome stability; neurodegenerative disorders

1. Introduction

Malnutrition, according to the definition, is an imbalance at the cellular level between the demand for nutrients and their intake. The fulfillment of nutritional needs supports proper growth and maintenance of the body's vital functions [1]. Malnutrition should not be associated only with skinny people with an anorectic appearance. It is a common clinical problem, with numerous causes, such as poverty, caring negligence, aging, chronic somatic diseases, or deliberate action to reduce weight. Therefore, it is difficult to estimate its actual scale. According to the WHO, approximately 45% of children's deaths (under age five) are caused by malnutrition, mainly in destitute and middle-income countries. Interestingly, these countries also have an increasing percentage of overweight and obese children. In that case, eating high-energy but low-nutrient meals results in qualitative malnutrition. The same applies to adults for whom stressful lifestyle and increasingly inappropriate eating habits lead to dietary deficiencies. In obese people, malnutrition can result from a shortage of nutrients, vitamins, and microelements which are necessary for the proper functioning of the body. In addition, low-calorie or elimination diets (such as a vegan diet), if a balanced eating plan is not adhered to, can cause malnutrition by increasing the risk of protein and vitamin deficiencies [2].

One of the more interesting forms of malnutrition is the so-called anorexia of aging, i.e., the loss of appetite associated with aging. It concerns approximately 25% of Europeans over 65 years of age [3]. The risk of anorexia in seniors is higher due to physiological changes associated with aging, coexisting diseases, and medical treatments. Moreover, elderly people often struggle with psychosocial problems such as poverty or social isolation, which strongly predisposes them to loss of appetite. Anorexia is an independent risk factor for death in an older population [4]. As it is associated with qualitative and/or quantitative nutritional deficiencies, immune functions, metabolism, and antioxidative defense systems are weakened. A shortage of polyunsaturated fatty acids (PUFAs), vitamins, micro- and macroelements is partly responsible for geriatric syndromes such as frailty (i.e., drastic functional decline leading to multiorgan impairment). A special model of nutrition for longevity has not yet been identified, but a well-balanced diet with a sufficient quantity of nutrients promotes healthy aging in contrast to malnutrition, which increases susceptibility to disease.

Although aging is a natural process and not a disease, older people are more prone to illness. The feeble and malnourished organism can suffer from, among others, impairment of the immune system. *Inflammaging* (low-grade chronic inflammation) develops with age and may speed up the deterioration processes and worsen other age-related disorders [5,6]. Age-specific conditions also coincide with chronic subliminal inflammation. Fagiolo et al. observed mononuclear peripheral blood cells in the elderly population as compared with healthy young people [7]. The results showed a higher concentration of tumor necrosis factor (TNF-α) and proinflammatory cytokines, interleukin 6 (IL-6) and interleukin 1 (IL-1), during 72 h incubation with the mitogen. Additionally, elevated levels of IL-6 or TNF-α affected nutrition control centers, suppressed appetite, changed sensory sensations, and inhibited muscle protein synthesis [8], all of which could promote the development of anorexia. Subsequently, it could be a cause of inadequate nutrient intake or malfunctioning absorption.

Malnourished older people can have deficits of most micronutrients, including zinc, selenium, vitamin C, vitamin E, riboflavin, electrolytes, and others. Most importantly, micronutrients directly affect (e.g., vitamin C and vitamin E) or indirectly (e.g., selenium and zinc) the activity of antioxidant defense systems (e.g., antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT)) [9]. The proper operation of these antioxidant systems is highly important for the whole organism. They protect the cell against endo- and exogenous pro-oxidative factors, including reactive oxygen species (ROS). The choice of the four presented micronutrients was dictated primarily by their antioxidant properties implemented through antioxidant enzymes. Selenium, in the form of selenocysteine (Sec), is present in the active center of selenoproteins, including GPX, in which the main function is to neutralize H_2O_2 and organic peroxides. Vitamin E also neutralizes peroxides and its action is synergistic to vitamin C, selenium, and zinc. Zinc is a component of enzymes from the group of SOD, which catalyzes the dissolution reaction of the $O_2^{\bullet -}$ to H_2O_2 and O_2. Vitamin C reduces ROS level (i.e., $O_2^{\bullet -}$, $^{\bullet}OH$, and $^1O_2$) but at the same time, it regenerates the oxidized form of vitamin E to its reduced form. Micronutrients selected for this review benefit from the presence of each other and sustain the overall effectiveness of antioxidant defense of the organism. The synergistic effect of the presented microelements on the antioxidant network is illustrated in Figure 1.

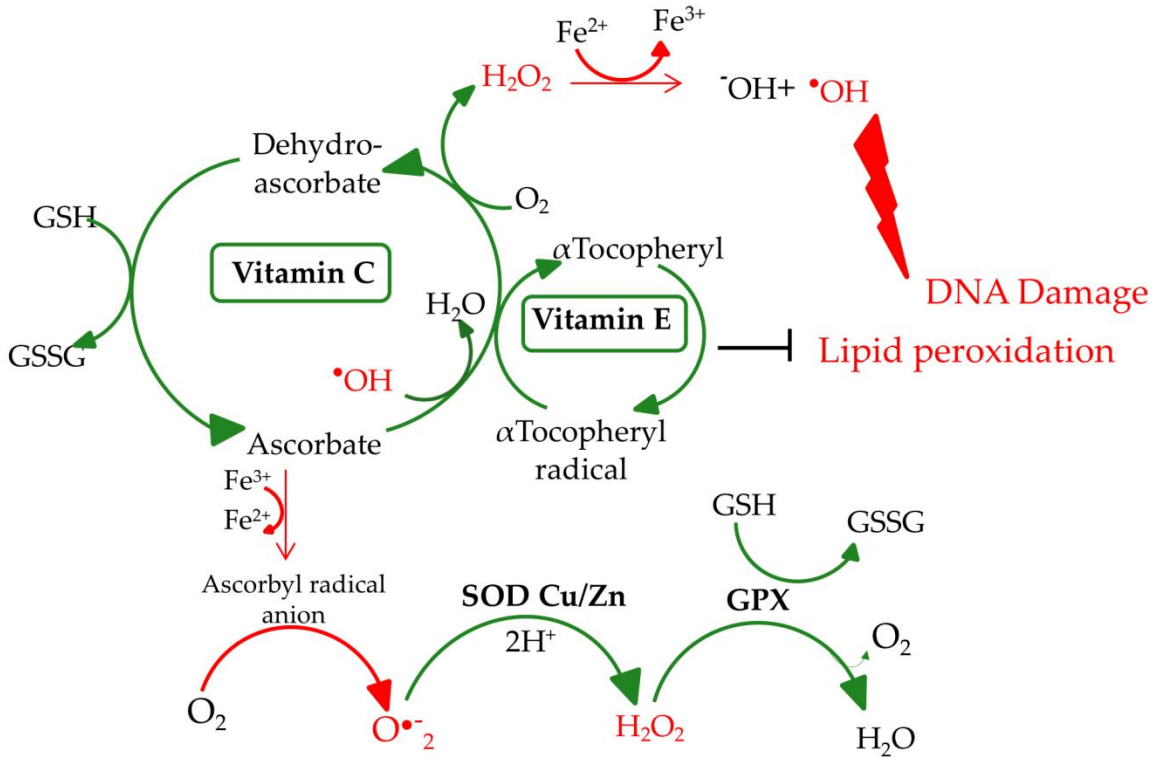

Figure 1. Antioxidant network. SOD Cu/Zn, Cu/Zn superoxide dismutase; GPX, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide [9].

The proper operation of antioxidant systems is highly important for the whole organism. Antioxidants protect the cell against endo- and exogenous pro-oxidative factors, including ROS. Their levels increase, especially, in the case of physiological stress, for example, inflammatory processes, malnutrition, and anorexia [10]. In addition, physiological decline connected with age can cause an increase in ROS levels. High ROS levels and lack of nutrients in an older body are even more dangerous as they accelerate aging and the incidence of age-related disorders. ROS formed during physiological reactions are necessary for proper gene expression and cell differentiation but in excess, ROS lead to the formation of DNA lesions and can impact the integrity of the nuclear and mitochondrial DNA (mtDNA). H_2O_2 does not pose a direct threat due to its moderate activity, but, in certain conditions, it can be transformed into highly reactive $^{\bullet}OH$ (Figure 1) [11]. Due to its low redox potential, guanine is the most susceptible to oxidation. The major type of guanine lesions is 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and its enol form 8-hydroxy-2′-deoxyguanosine (8-OHdG) formed 10^5 times per day per cell (Figure 2) [12].

Oxidative lesions are detected and corrected mainly by base excision repair (BER) which is the only mechanism able to correct single nucleotide lesions. DNA repair mechanisms are also affected by nutritional deficiencies which can lead to decreased repair efficiency and subsequent mutations resulting in pathological conditions (e.g., carcinogenesis or neurodegeneration) [13].

This review focuses on selected micronutrients involved in the DNA repair processes and antioxidant protection of the body in the case of malnutrition and anorexia-related deficiencies in the elderly population.

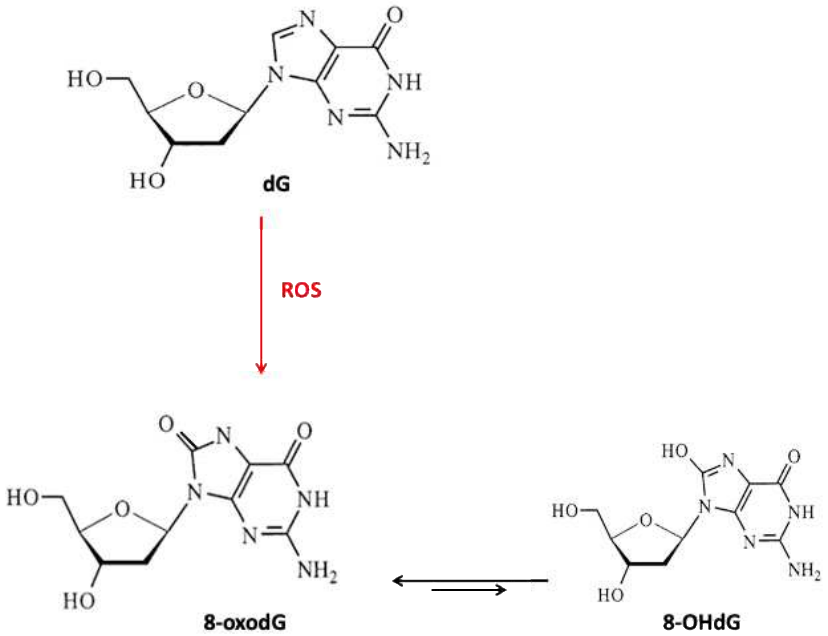

Figure 2. Guanosine and its oxidative modifications. dG, 2′-deoxyguanosine; 8-oxodG, 8-oxo-7, 8-dihydro-2′-deoxyguanosine; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; ROS, reactive oxygen species.

2. Biochemical Aspects of Selected Micronutrients

The most common biochemical problems that affect patients with anorexia nervosa are dehydration or electrolyte disturbances due to insufficient supply of micro- and macroelements in the diet. Physiologically, the concentration of K^+ ions is much higher inside the cell, while the concentrations of Na^+, Ca^{2+}, and Cl^- ions are higher in the extracellular space. In anorectic patients, hypokalaemia is a frequent problem. It is dangerous due to the possible consequences such as cardiac arrhythmias, abnormal nerve conduction, or paralysis in striated and smooth muscles, etc. [14]. Moreover, hypophosphatemia has also been observed in patients with an anorexia-related condition, which affects phosphate functions (including ATP synthesis) and impairs glucose metabolism, enzyme phosphorylation, and acid-base management. The biochemical imbalance can lead to the development of, for example, osteomalation, rhabdomyolysis, central nervous system disorders, or hemolysis [15]. Other most important microelements, which regulate the overall well-being of the human organism, are trace elements and vitamins such as selenium, zinc, vitamin E, and vitamin C, which are discussed in this article.

2.1. Selenium

Selenium (Se) is present in the active centers of many proteins and enzymes in the form of Sec residues. Selenoproteins play crucial roles in the proper functioning of the whole organism; selenoprotein K (SELENOK, SELK) participates in the construction of protein-protein complexes, selenoprotein M (SELENOM, SELM, SEPM) is involved in the protection of neurons against oxidative stress, and selenoprotein N (SELENON, SELN, SEPN1) is involved in the regeneration of skeletal muscle tissue [16]. Iodothyronine deiodinases (DIO1-3) which are involved in the formation of thyroid hormones are also an example of selenoproteins. Selenium is associated with an immuno-inflammatory and proinflammatory response. Its deficiency in endothelial cells results in reduced production of prostaglandins (PGI2, PGE2, and PGF2α). Selenoproteins also regulate macrophages' migration and phagocytosis [17].

Selenoproteins have antioxidant properties and are involved in the regulation of the antioxidant defense system. A low level of Se causes reduced cells' resistance to free radicals. In this context, selenoprotein P (SELENOP, SELP, SEPP1), thioredoxin reductase (TXNRD1, TRXR1), and glutathione

peroxidases (GPX) are the most important. SELP acts as an antioxidant, while TRXR1 provides proper cell growth, DNA synthesis, replication, and apoptosis' inhibition [18,19]. GPX are involved in the protection against oxidative damage by conversion of H_2O_2 to water (Figure 1). Interestingly, the level of glutathione (GSH), which is a cofactor for the antioxidant enzymes (GPX and glutathione transferase), is reduced in people suffering from anorexia. Therefore, their ability to detoxify electrophilic metabolites and neutralize ROS is impaired [20]. It can lead to peroxidation of membrane lipids, oxidation of unsaturated fatty acids, reduced membranes' fluidity/permeability, and, as a consequence, to pathological conditions such as atherosclerosis, diabetes mellitus, or rheumatoid arthritis [21].

Severe selenium deficiency often manifests as cardiomyopathies and heart failures which are often seen in anorexia patients. Kashin–Beck disease is a musculoskeletal disorder with abnormal bone development, growth inhibition, joint pain, and edema with reduced mobility, which is caused by the Se shortage in an unbalanced diet. Therefore, monitoring the selenium level in a patient's body and fluids can be helpful and is recommended from the therapeutic point of view. Lack of Se can also result in neurological symptoms (e.g., depression), which are often observed in people with anorexia or malnutrition [22,23].

Selenium is considered to be a factor of prolonged life expectancy. Recently, Hammad et al. demonstrated the relationship between Se and replication senescence in human embryonic fibroblasts (WI-38). The authors highlighted that the lack of Se was related to increased ROS levels in aging cells and decreased antioxidant defense (including the activity of selenoproteins) [24]. The addition of selenium increases the number of cell divisions and reduces aging markers (β-galactosidase (SABG) and heterochromatin foci (SAHF)), while its deficiency accelerates senescence and reduces the cell's proliferative capacity [25]. Mice's diet enriched with Se enhances the activity/level of SOD, GPX, and total antioxidant capacity (T-AOC) [26]. Selenium may also reduce oxidative stress in peripheral blood lymphocytes, and therefore improves healthy aging [27].

An elderly population usually has reduced selenium levels [28,29]. Adding Se to seniors' diets may be an important factor in preventing age-related diseases and improving their quality of life (QoL). A study on 347 elders (age > 80) showed that low plasma selenium levels correlated with high levels of IL-6 and C-reactive protein (CRP) [30]. Moreover, all-cause mortality was higher in people with low selenium (≤105.3 μg/L). The authors suggested that higher selenium levels had a positive effect on age-related inflammation. Interestingly, the synergistic effect of Se and vitamin E for quenching free radicals was observed. Patients with selenium-related diseases often have vitamin E deficit (discussed in further sections) [31]. Having in mind that excess of Se can induce adverse effects (diarrhea, fatigue, hair loss, and joint pain) [32], a carefully planned and advised diet enriched in selenium may potentially improve seniors' QoL.

2.2. Zinc

Zinc (Zn) is essential for proper functioning of the cells. It plays an important role in transcription regulation. Zn deficiency often occurs in a malnourished organism and can lead to growth retardation, delayed pubescence, impaired wound healing, dermatitis, decreased appetite, and mental lethargy [33]. Zn is involved in metabolic processes, for example, immune response, as well as neurobehavioral and physical development. Its deficiency impacts antibody production, cytokine production (interleukin 2 (IL-2) and interferon γ (IFNg)), cell signaling, proliferation, and the function of B, T helper, and natural killer (NK) cells [34,35].

Moreover, zinc is present in zinc-finger domains of many proteins such as transcription factors and regulatory proteins. The presence of Zn^{2+} ions is also crucial for the stability of DNA binding proteins because the zinc-finger domain is directly involved in the binding process of the nucleic acid molecule [36]. Moreover, zinc neutralizes the $O_2^{\bullet -}$ as a component of the Zn/Cu-SOD and is a crucial element (as part of a catalytic domain) of the metalloproteinases [18]. Metallothioenins (MTs) are a family of highly conserved cysteine-rich metalloproteins [37]. MTs have strong antioxidant properties, i.e., they can scavenge ROS and detoxify heavy metals ions [38]. The availability of microelements,

such as selenium or zinc, regulates MTs production and cellular accumulation. MTs' expression increases during stress conditions (e.g., inflammation). It is of interest to describe the so-called redox cycle with MTs. The sulfone group confers redox activity to the Zn-MT complex and can be oxidized and reduced with simultaneous release and binding of Zn in an oxidoreductive environment. Zinc released from MT is available to other molecules. This process is modulated by GSH and glutathione disulfide (GSSG). A more oxidized state results in the release of zinc and a more reduced state promotes MT stabilization [39]. A reduction in oxidized MTs restores its ability to bind Zn. The MT genes have been characterized as one of the few longevity genes. Transgenic mice with *MT* overexpression live longer. Yang et al. observed that their cardiomyocytes inhibited age-related cytochrome C release and generated lower levels of superoxides as compared with control mice. The authors highlighted MTs' direct impact on cardiac aging and lifespan [40].

Zinc lowers the level of proinflammatory cytokines and markers of oxidative stress. Studies conducted on healthy adults (age 55–87) have shown that monocytic cells of zinc supplemented people generated significantly less TNF. A six-month supplementation led to a significant reduction in TNF levels (1897 ± 1004 pg/mL to 1411 ± 786 pg/mL) as compared with the placebo group (1728 ± 498 pg/mL to 2698 ± 785 pg/mL). Moreover, there was a significant decrease in plasma oxidative stress markers (malondialdehyde (MDA), 4-hydroxyalkenals (HAE), and 8-OHdG) in the supplemented group, with no change in the placebo group (8-OHdG, 0.63 ± 0.16 ng/mL to 0.50 ± 0.14 ng/mL ($p = 0.030$) in the supplemented group vs. 0.66 ± 0.13 ng/mL to 0.68 ± 0.13 ng/mL in the placebo group; MDA + HAE, 1.66 ± 0.343 μmol/L to 1.35 ± 0.18 μmol/L ($p = 0.0002$) in the supplemented group vs. 1.70 ± 0.30 μmol/L to 1.71 ± 0.35 μmol/L in the placebo group) [41]. The authors concluded that zinc, as a non-mutagenic, relatively non-toxic, effective anti-inflammatory and antioxidative agent, could be beneficial for preventing chronic disorders associated with oxidative stress in an elderly population.

2.3. Vitamin E

Vitamins E is a group of fat-soluble compounds with strong antioxidant properties as they inhibit lipid peroxidation. Vitamin E (α-tocopherol (α-T) and vitamin E) has a synergistic effect with vitamin C, selenium, and zinc. As a constituent of the cellular membranes, vitamin E is the main antioxidant of PUFA. It inhibits oxidation of cellular macromolecules as donating electron interrupts the chain reaction of phospholipids' oxidation in membranes at the propagation stage (Figure 1). It can be postulated that by protecting cell membranes, vitamin E delays cellular aging [42] and has a beneficial effect on vascular and cardiac function [43]. Moreover, its moiety is built into the ceramides that are part of the intercellular spaces of the stratum corneum and due to strong antioxidant properties, it protects the epidermis, and therefore increases its resistance to UV radiation [44].

A shortage of vitamin E harms the external cell bilayer and can lead to cancer, cardiovascular diseases, as well as infectious and inflammatory processes. Vitamin E deficiency is well characterized by an isolated lack of vitamin E in ataxia with vitamin E deficiency (AVED). AVED is an autosomal recessive neurodegenerative disorder caused by a mutation in the α-tocopherol protein transfer gene (*α-TTP*) with clinical manifestations being progressive spinocerebellar ataxia, loss of deep sensation (proprioceptivity), and areflexia. However, high doses of vitamin E (800 mg/day) inhibit the symptoms' progression and may even reverse some neurological symptoms [45]. It the case of anorectic and malnourished people, level of vitamin E is often reduced, for example, due to insufficient intake [46,47]. Vitamin E deficiency may result in neuropathies, as well as progressive necrosis of the nervous system and muscles. Patients with anorexia may also experience cardiovascular complications, arrhythmias, peripheral edema, and even sudden cardiac arrest [48].

On the one hand, there is no consistent evidence that a diet enriched with vitamin E protects against chronic diseases or cancer [49,50]. On the other hand, studies conducted on a population with very low levels of micronutrients due to poor living conditions indicated that vitamin-mineral supplementation (including vitamin E) potentially reduced the risk of cancer [51]. It seems that

supplementation is effective in a population with low intake and concentration of antioxidant nutrients such as older people; the potential benefits possibly outweigh the side effects [52].

2.4. Vitamin C

It is important to supply this vitamin through food, as the human body cannot synthesize vitamin C (vitamin C, ascorbic acid) due to the lack of L-gulonolactone oxidase. Vitamin C is primarily involved in the synthesis of collagen, catecholamines, and L-carnitine [53]. It is a cofactor for numerous enzymes (e.g., hydrolases, oxygenases, and dioxygenases) and is involved in many metabolic processes (e.g., synthesis of adrenaline from tyrosine). Ascorbic acid, as a water-soluble antioxidant, acts as the body's primary defense against ROS occurring in the water phase. Vitamin C leads to the formation of well-soluble ferrous salts, by reducing Fe^{3+} to Fe^{2+}, which can be more easily absorbed from the gastrointestinal tract [54]. At the intracellular level, ascorbic acid is possibly considered to be an ideal antioxidant, it is present in the cell in the right quantity (it varies in different types of cells, fluids, and tissues [55]), neutralizes a large number of free radicals, and is regenerated to some extent [56]. Vitamin C inactivates free radicals, and thus breaks the oxidative chain. Moreover, it strengthens the action and regeneration of α-tocopherol by reducing its radical formed after the reaction of tocopherol with free radical [42].

The level of vitamin C in the plasma decreases with age [57,58]. On the one hand, clinical studies have indicated a relationship between serum vitamin C levels and the risk of cardiovascular disease (e.g., peripheral arterial disease or stroke). Patients with a low vitamin C level (27.8 μmol/L as compared with a control group 51.7 μmol/L, $p < 0.0001$) have a significantly increased CRP (2.51 mg/L vs. control 4.80 mg/L, $p < 0.0001$) and are at higher risk of developing fatal cardiovascular disease [59]. In addition, serum vitamin C levels are inversely associated with stroke incidence [60]. Similar observations apply to patients suffering from diabetes or hypertension, as their serum vitamin C levels are low [61]. On the other hand, studies have shown that daily vitamin C intake did not reduce serious cardiovascular events, cancer outcomes, or cardiovascular mortality [62,63]. Nevertheless, it seems likely that population groups with low vitamin C status may benefit from additional vitamin C intake [64]. It is especially visible in the case of seniors suffering from anorexia, and therefore with an inadequate intake of vitamin C; the high levels of oxidative damage should be observed in this group.

Moreover, vitamin C is involved in the regulation of gene expression [65]. The authors showed that ascorbate deficiency reduced the expression of the TET1-dependent (methylcytosine oxidase ten-eleven translocation proteins) genes crucial for germline development. Reproductive cells lacking vitamin C have a different gene expression profile than controls because vitamin C is a cofactor of TET hydroxylases (involved in the demethylation of DNA). Reduced expression of *TET* genes accompanies many types of cancers [66], which indicates the role of *TET* genes as tumor suppressors [67]. Vitamin C seems to be required for DNA demethylation, and thus proper epigenetic regulation. Studies have indicated that ascorbic acid is a DNA protector, i.e., a group of healthy subjects showed a significant decrease in the level of 8-oxodG (a marker of oxidative stress) in the plasma and urine after supplementation with 500 mg/day of vitamin C [68]. Additionally, a gene expression analysis has indicated that DNA repair processes were enhanced in cells treated with vitamin C, which we discuss below [69].

3. Micronutrients, DNA Damage, and Repair

The human body changes with age. Aging is a set of complex biochemical and physiological phenomena determined by the molecular processes that occur at the cellular level. The aging process is influenced by numerous factors ranging from genetic predispositions to lifestyle choices (e.g., diet), performed kind of work, and living conditions. It is a lifelong process constantly shaped by environmental factors which constitute more than 50% of overall human well-being [70]. Modern awareness about the diet as the major factor influencing our health goes as far back as the 19th century, when the phrase "you are what you eat" originated. However, changes occur at different times and intensities in various organs, and they reach the greatest intensity in old age.

According to one of the theories, aging is associated with the accumulation of oxidative changes in macromolecules and cellular structures [71]. Free radicals in physiological quantity play an important role in cell signaling [72,73] but in excess, they could damage the cell. Oxidation may impair the function of cell membranes and proteins (e.g., enzymes and receptors), or may lead to the formation of DNA lesions. The brain is significantly affected by oxidative damage. Intensive metabolism, high content of fatty acids, and relatively low activity of antioxidant enzymes contribute to age-related neurodegenerative disease [74]. ROS are also generated in mitochondria as a part of the electron transport chain (ETC) when so-called electron leakage occurs. When radicals' production is too high, the function and structure of mitochondria are disrupted, including the integrity of the cristae and the inner membrane. Hindered mitochondrial function can induce a further increase in oxidative stress and subsequent DNA damage [75]. Moreover, the proximity of the respiratory chain may impair the integrity of mtDNA which leads to a decrease in mitochondrial activity with age [76]. Maintaining stable concentrations of ROS in the cells is a major determinant of longevity and healthy aging. Cellular aging is also associated with extensive and irreversible DNA damage within telomeric or non-telomeric genome sequences [77–79].

Fundamental factors of aging include oxidative stress, cellular damage, low effectiveness of damage prevention, and inhibited DNA repair. The oxidative DNA lesions manifest as base modifications, strand breaks, or DNA adducts. The most common markers of oxidative DNA damage are 8-oxodG and 8-OHdG. Wolf et al. showed that older rats had higher concentrations of 8-OHdG as compared with young rats [80]. The level of 8-OHdG increased with rats' age in their heart, skeletal muscles, liver, peripheral blood, or brain. The most significant increase in 8-OHdG level was observed in the heart and peripheral blood lymphocytes (from 0.157 OD at four months to 0.370 OD at 12 months of age and 0.220 OD at four months to 0.550 OD at 24 months of age, respectively), which authors suggested was related to the DNA repair efficiency. Another study analyzed the level of 8-oxodG in human leukocytes in different age groups in correlation with the concentration of ascorbate in the plasma [81]; the 8-oxodG level increased in leukocytes' DNA with age. The authors concluded this could have been related to the decrease in antioxidant defense with age (lower ascorbate level).

For chronic malnutrition, the lack of antioxidants is pro-oxidative, i.e., it increases oxidative stress and impairs ROS neutralization via GSH [82]. Studies have shown that the consumption of antioxidative micronutrients reduced the level of DNA damage or improved DNA repair efficiency. Moreover, micronutrients are important for maintaining genome stability. Their deficiencies can lead to DNA damage formation similar to those resulting from radiation (DNA strands' or chromosomes' breaks) [83,84]. Figure 3 presents the main effects of selected micronutrients on genome stability concerning their deficiency or normal level in the body.

3.1. Selenium

Selenium is involved in the protection against the negative effects of ROS action. Selenium is a ROS scavenging agent and an element of selenoproteins which catalyzes reactions of ROS removal from an organism. However, it is also known to have an impact on genome stability (Figure 3). It has been shown to inhibit DNA adducts formation with, for example, carboplatin, polychlorinated biphenyl (PCB), or 7,12-dimethylbenz[α]anthracene (DMBA), and lower the number of chromosome breaks, gain, or loss resulting from carcinogens [85]. Moreover, selenium seemed to support the repair of oxidative DNA damage. The potential of repair by an incision was significantly higher in protein extracts from cells pretreated with Se as compared with a control (30% vs. 20% excision, respectively). Mice with Se deficit had upregulated genes induced by DNA damage, which suggested that Se deficiency could be a stress factor for the cell [86].

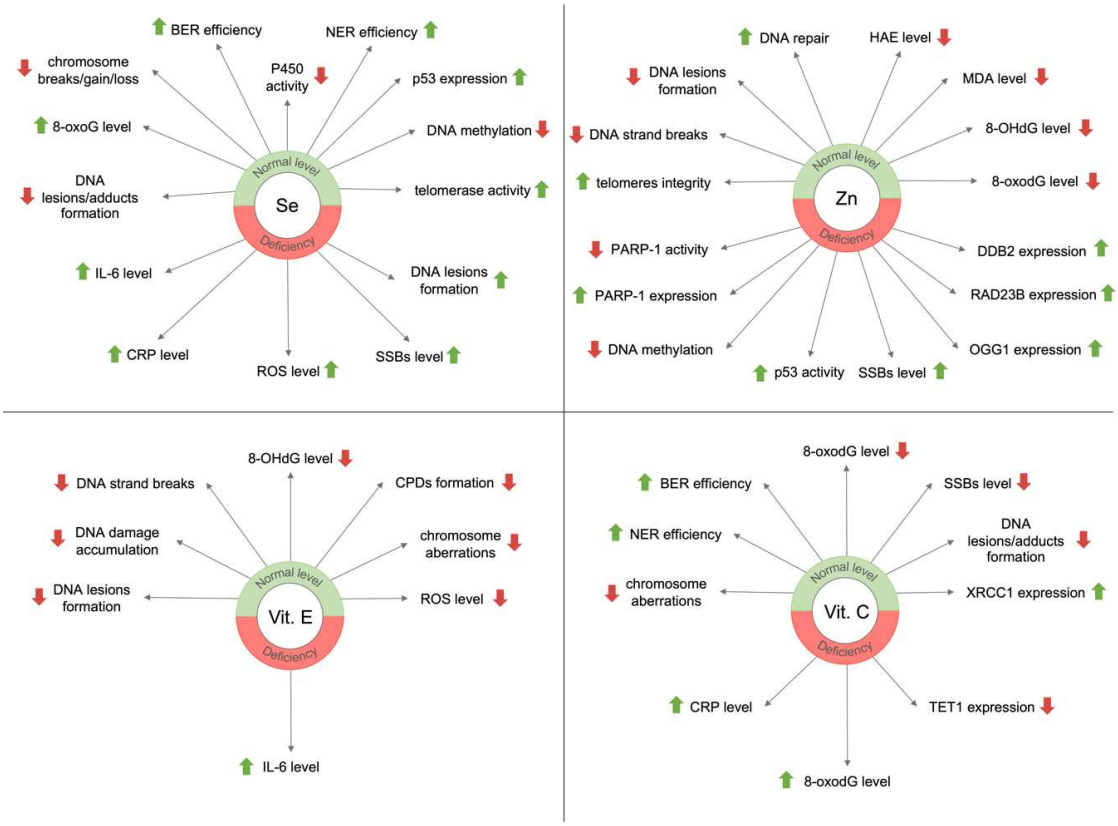

Figure 3. Effects of selected micronutrients on genome stability. BER, base excision repair; NER, nucleotide excision repair; 8-oxoG, 8-oxo-7,8-dihydroguanine; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; SSBs, single-strand breaks; P450, cytochrome P450; IL-6, interleukin 6; CRP, C-reactive protein; ROS, reactive oxygen species; CPDs, cyclobutane pyrimidine dimers; HAE, 4-hydroxyalkenals; MDA, malondialdehyde; PARP-1, poly[ADP-ribose] polymerase 1; OGG1, 8-oxoguanine glycosylase 1; DDB2, damage-specific DNA binding protein 2; RAD23B, RAD23 homolog B; XRCC1, X-ray repair cross-complementation group 1; TET1, methylcytosine oxidase ten-eleven translocation proteins.

Another study examined the influence of selenium on the level of DNA damage in a group of 43 people (age 50–75) [87]. Subjects with an initial Se level below the average of 100 ng/mL had higher levels of DNA damage in peripheral blood lymphocytes. The authors suggested that serum Se levels should be kept above 100 ng/mL as DNA damage prevention. Se supplementation is also beneficial in patients with a *BRCA1* mutation [88]. BRCA1 is involved in the repair of DNA double-stranded breaks (DSBs), acts as a tumor suppressor, and maintains genome stability. Urine collected from supplemented (300 µg/day of sodium selenite) *BRCA1* mutation carriers contained a higher level of 8-oxo-7,8-dihydroguanine (8-oxoG, a product of BER system) as compared with a non-supplemented group. The median of 8-oxoG, in the urine samples of *BRCA1* mutation carriers with supplementation, reached 7.75 nmol/mmol creatinine as compared with 5.77 nmol/mmol creatinine in *BRCA1* mutation carriers with no supplementation. Additionally, about a 26% reduction in the 8-oxoG background level in cellular DNA was observed in supplemented patients. These results indicate that Se supplementation enhances the repair of oxidative damage. Selenium can influence gene expression and activate silenced genes (e.g., antioxidant enzymes or tumor suppressors) through epigenetic modulation of histones and DNA in prostate cancer cells (LNCaP). Xiang et al. showed that selenite treatment caused partial demethylation of promoter DNA and re-expression of glutathione S-transferase, decreased overall DNA methylation, and restored expression of the tumor suppressor adenomatous polyposis coli

(APC) and cellular stress response 1 (CSR1). The authors concluded that Se could play a role in the chemoprevention of prostate and other cancers through epigenetic regulation of anticancer genes [89].

Seleno-L-methionine (Se-Met) is a naturally occurring selenium-containing amino acid and it appears to selectively regulate the nucleotide excision repair (NER) pathway. Studies on human fibroblasts have shown that pretreatment with Se-Met (10 μL) protected the mouse embryonic fibroblast (MEF) cells from UV-induced DNA damage and induced p53-dependent DNA repair [90]. Similar results apply to human prostate adenocarcinoma cells [91]. Pretreating cell culture with 10 μM of Se-Met protects DNA against damage induced by UVA (50 J/cm) or H_2O_2 (200 μM). Studies conducted in hemodialysis patients with chronic kidney disease have shown that the subjects had a lower concentration of selenium than healthy people (40.6 ± 10.4 ng/mL vs. 52.7 ± 9.7 ng/mL, $p < 0.0001$). Moreover, the number of DNA single-strand breaks (SSBs) in white blood cells was significantly higher (DNA damage expressed as the tail moment (0.73 ± 0.84) as compared with the control group (0.25 ± 0.24; $p < 0.01$). After a three-month supplementation (200 μg/day of selenium as Se-rich yeast), 2.6 times lower levels of oxidative damage were observed in hemodialysis patients as compared with a control group [92].

Evidence that an older population needs higher selenium intake also comes from a study on primary human keratinocytes collected from normal skin biopsies [93]. Keratinocytes obtained from older subjects (age 60–70) were more susceptible to UVA-induced damage. Additionally, to inhibit the cytotoxic effect of radiation, the cells required eight times higher doses of Se than those from younger people (age 20–30). Se supplementation may be beneficial for the elderly as it can activate telomerase and p53 expression [85]. Furthermore, selenium inhibits the cytochrome P450 system's activity (phase I proteins). It converts chemical carcinogens into their reactive forms leading to the formation of various DNA lesions [94–96].

Interestingly, studies have shown that selenium deficiency can promote cancer development in humans [97,98]. While Se supplementation in a well-nourished population is rather modest, it may be healthful in older people with low levels of Se [99]. Se deficiency arises from many factors such as improper intake, lack of accompanying nutrients (e.g., methionine), bowel diseases impacting Se absorption, or variations of Se-related genes [85]. Despite plenty of studies about the influence of selenium on DNA damage, DNA repair, and aging, there are no clear guidelines for selenium daily supplementation, because the adequate level of Se is highly individual [100]. Current intake recommendations vary in the range of 25–150 μg/day (depending on sex and the recommending country) [85]. However, it must be taken into consideration that too much selenium can be as damaging as too little and it should be supplemented according to individual needs, especially in an elderly population.

3.2. Zinc

Zinc is a crucial element for the overall well-being of the human organism and its genome stability (Figure 3). It is involved in apoptosis, cell proliferation, protection against free radicals, and DNA repair pathways. Zn plays a role as a cofactor of the antioxidant enzymes (e.g., Zn/Cu-SOD) and also of DNA repair-related enzymes such as 8-oxoguanine glycosylase 1 (OGG1), apurinic/apyrimidinic endonuclease (APE), and poly(ADP-ribose) polymerase 1 (PARP-1). Zinc is also a part of a tumor suppressor p53 protein which is responsible for the cell cycle arrest, thus, allowing DNA to repair before replication starts. A lack of Zn upregulates p53 activity (however, 50% Zn depletion results in non-functional p53) and affects DNA repair response [83]. A different study indicated the important role of zinc in the activity of the PARP-1 protein [101]. PARP-1 functions in various repair mechanisms including BER, NER, and DSBs repair [102,103]. It contains the zinc finger motif and is crucial in DNA repair; it detects DNA damage and facilitates the selection of the repair path. Zn deficiency, which is characteristic of an older population, reduces PARP-1 activity, and thus reduces the effectiveness of BER [101]. PARP-1 also has a vital role in inflammatory processes that accompany aging organisms [104].

A shortage of Zn can lead to the accumulation of oxidative DNA lesions. OGG1 and PARP-1 expression levels are higher ($p < 0.05$) in zinc-deficient cells, in addition to a significant increase in DNA strand breaks ($p < 0.05$) [105]. Studies on primary human fibroblasts showed that zinc deficiency induced oxidative stress and DNA damage (SSBs). It also modulated the expression of DNA repair enzymes [106]. Microarray analysis have showed that a lack of zinc affected genes involved in DNA damage, DNA repair, and oxidative stress; two-fold upregulation of damage-specific DNA binding protein 2 (DDB2) and 0.5-fold downregulation of RAD23 homolog B have been observed.

Dietary restriction and repletion of Zn affect DNA integrity. A study on healthy men (age 19–50) showed an increased number of DNA strand breaks during six weeks of Zn restriction. The study proved the importance of dietary Zn for genome stability, because the level of DNA breaks dropped after Zn repletion [107]. After xix weeks of low zinc consumption, DNA damage was significantly increased in the peripheral blood cells (mean tail moment increased by 57%, $p < 0.05$). Interestingly, zinc supplementation reduced DNA damage (mean tail moment decreased by 39.9%, $p < 0.01$). Moreover, a clinical study on 200 patients (age 65–80) showed that Zn supplementation (20 mg/day) improved genome stability and telomeres integrity [108]. After 12 weeks of supplementation, the activity of Cu/Zn SOD in erythrocytes was significantly higher in the Zn group vs. the control group (activity increased by 33.07% in the Zn group, while the placebo group showed only a 2.45% increase (relative to the initial value)). This study also showed a decrease in the micronuclei (MNi) and DNA damage formation as compared with the non-supplemented group (MNi per 1000 binucleates, 6.930 vs. 11.125, $p = 0.001$). Additionally, patients in the supplemented group had a lower level of 8-oxodG in the telomeric regions (8-oxodG/kbp telomere, 6.820 vs. 9.937, $p = 0.291$, respectively). An insufficient dietary intake of Zn results in increased level of oxidative stress and subsequent lesions and it can affect cellular response to those lesions. A deficiency of zinc may increase the incidence of cancer which is especially dangerous for older people as they are already more prone to carcinogenesis. Cancer development and aging are also related to hypermethylation of CpG islands in DNA. Interestingly, studies have shown that Zn depletion led to hypomethylation [109].

Similar to selenium [110], zinc doses should be selected individually, because excess Zn, similar to a deficiency, is harmful and pro-oxidative [111]. Cases of poisoning by drinks containing 2500 mg/L of zinc have been observed. An in vitro study showed that the optimal Zn concentration for DNA damage prevention ranged between 4 and 16 μM [105], while a study on healthy men showed that 11 mg/day of Zn helped to reduce DNA damage [107]. Levels of Zn that are too high can induce DSBs, bases oxidation, and chromosomes' instability [105]. However, recommendations are yet unclear, as human studies on Zn's influence on the genome are still lacking. Many variables must be considered while planning supplementation for malnourished patients, especially older patients.

3.3. Vitamin E

Vitamin E is the most important agent scavenging lipid peroxyl radicals. It can inhibit lipid peroxidation, H_2O_2 action on DNA, and lower oxidative stress resulting from environmental mutagenic factors (e.g., smoke and food additives). The diet of rats enriched with vitamin E (300 mg/kg for six months) significantly decreased the number of chromosomal aberrations in the bone marrow [112]. Other interesting studies have been carried out on animals where α-tocopherol was administered prior to irradiation. In mice pretreated with 100 mg/kg/day of vitamin E, irradiated with 2 Gy, a statistically significant decrease in the incidence of MNi in polychromatic erythrocytes (PCE) was observed [113]. In the supplemented group (200 mg/kg/day of vitamin E) statistically significant protection of the bone marrow against radiation was also detected (expressed as an increase in the PCE/(PCE + NCE) ratio as compared with the positive control, i.e., 7.3% vs. 3.4%, $p < 0.05$). These results suggested that vitamin E could have radioprotective effects. Vitamin E is a well-known ROS scavenging agent that protects against UV-induced DNA damage [114]. It may prevent the formation of cyclobutane pyrimidine dimers (CPDs) developed as a result of UVA in human skin cells. Pre- or posttreatment with vitamin E (0.1 mM) results in lower oxidation and DNA damage. Moreover, studies have shown that α-tocopherol

protected the DNA of liver cancer cells from oxidative lesions resulting from ionizing radiation [115]. The level of 8-OHdG increased after irradiation (5 cGy) but the effects were reversed by vitamin E enrichment which indicated its genoprotective properties. The protective effect of α-tocopherol against neurodegeneration in prematurely aging mice has also been described [116]. Xpg-/-mice mimic symptoms of Cockayne syndrome patients, i.e., they are highly sensitive to nutritional deficiencies. The authors suggested that vitamin E supplementation inhibited the accumulation of DNA damage and oxidative stress in liver and brain tissues which both significantly deteriorate with age. Studies have shown that vitamin E reduced the formation of DNA damage such as DNA strand breaks or modifications of 8-OHdG. A study on a group of 21 healthy non-smoking men (age 28.9 ± 1.3) showed that an increase in vitamin E intake by an additional 80 mg/day in a high PUFA diet (15%) decreased DNA damage. A high-fat diet causes lymphocytes to be more prone to DNA strand breakage and an increase in vitamin E intake can probably remove this effect [117].

Vitamin E supplementation for older people must be planned carefully as the proper intake depends on many factors such as background level of vitamin E, its supplemented form, duration of treatment, and possible genetic variations (which may alter vitamin absorption or metabolism) [116]. Different forms of vitamin E can have different impacts on oxidative status in older adults, as the study on 71 patients (age 50–55) showed. [118]. Tocotrienol rich fraction and α-tocopherol were administered for six months and DNA damage level dropped for tocotrienol rich fraction in female subjects after six months. The study showed that the form of vitamin E mattered and could have different effects, which were also dependent on sex. Interestingly, vitamin E (100 μM) has been proven to be potentially beneficial for oncological patients due to its antioxidant action and lack of interference with camptothecin (chemotherapeutic) [119]. Nonetheless, other data have shown no beneficial effect of vitamin E supplementation on cellular DNA damage [120,121]. A positive correlation has been described between serum vitamin E levels and the level of 8-OHdG in peripheral blood lymphocytes in premenopausal non-smoking women (age 45–50) [122]. In another study, healthy men (age 50–70) received 500 mg of vitamin E, but there was no effect observed on micronucleus formation after eight weeks of supplementation [120].

While in vitro studies have proven the likely positive effect of vitamin E on DNA damage (Figure 3), human studies have not fully confirmed it. However, lower levels of circulating α-tocopherol are associated with reduced immune function (increased levels of inflammatory markers) and QoL in the elderly [123]. Among 69 elderly subjects (mean age 78.9) the elevated level of IL-6 was observed and accompanied by a decrease in the concentration of vitamin E (R-(0.277), $p < 0.01$). The results correlated with poor physical and mental health. The authors suggested that insufficient intake of antioxidants (including vit. E) led to reduced QoL and increased the risk of age-related diseases. The influence of α-tocopherol on DNA damage is yet to be confirmed to recommend adequate doses for supplementation in an older population.

3.4. Vitamin C

Vitamin C is a crucial antioxidative agent with the ability to enhance genome stability (Figure 3). Studies have shown that its adequate intake could lower the number of chromosome aberrations, DNA adducts, and strand breakage [124]. Insufficient consumption of vitamin C led to an increase in the level of oxidative DNA lesions [125]. Fraga et al. showed that a low/poor dietary supply of vitamin C resulted in a two-fold increase in the level of 8-oxodG in sperm DNA. A different study on 112 patients with coronary artery disease showed that low levels of ascorbate and GSH in peripheral blood lymphocytes were accompanied by more frequent chromosomal aberrations [126].

An interesting study conducted on a group of 139 subjects examined the correlation of air pollution, markers of oxidative DNA damage (including 8-oxodG), and DNA repair gene expression [127]. The authors selected genes coding enzymes involved in the repair of 8-oxodG in BER and non-homologous end-joining (NHEJ), i.e., human 8-oxoguanine glycosylase 1 (*hOGG1*), apurinic/apyrimidinic endodeoxyribonuclease 1 (*APEX1*), X-ray repair cross-complementation group

1 (*XRCC1*), *XRCC4*, *XRCC5*, *XRCC6*, and DNA ligase 4 (*LIG4*). The group of subjects living in a more polluted environment had a lower concentration of 8-oxodG in urine (4.16 vs. 4.99 nmol/mmol creatinine) with simultaneously higher plasma ascorbate levels (11.8 vs. 8.3 mg/L) as compared with subjects living in relatively cleaner regions. The authors speculated that higher plasma levels of ascorbate resulted in higher XRCC1 expression in some people and a later increase in BER efficiency (resulting in lower levels of 8-oxodG in urine), thereby protecting the body from oxidative DNA damage. A different study tested 340 healthy Norwegians for dietary and genetic factors influencing DNA damage and repair capacity [128]. Subjects did not undergo a special diet or supplementation. The Food Frequency Questionnaire and tests of fasting blood samples were used. The levels of DNA strand breaks, oxidized lesions, and the activity of BER and NER were measured. The results showed a significant correlation between diet and level of DNA damage. The quantity of strand breaks and oxidized lesions of purines and pyrimidines was higher when subjects consumed fewer vegetables and fruit. The study also showed that female subjects who consumed less fruit had approximately a 20% higher level of DNA damage. The authors observed, among other things, that NER efficiency was 0.141-fold higher for subjects with a higher level of ascorbate and total DNA damage was 0.037-fold lower.

It seems that antioxidant supplementation is especially important for poorly nourished people [129]. Guarnieri et al. showed that the basic level of DNA repair by an incision in mononuclear blood cells was significantly lower in poorly nourished patients as compared with well-nourished patients. At the same time, poorly nourished people had higher levels of oxidized guanine. Vitamin C supplementation was potentially beneficial, because an increase in DNA repair incision capacity was observed, which was not seen in well-nourished subjects. It is possible that the influence of vitamin C on DNA depends on the preexisting level of this vitamin (the protective effect is observed for >50 μmol/L vitamin C in plasma) and individual level of oxidative stress (resulting from environmental factors such as smoking or exposure to mutagenic chemicals). Moreover, vitamin C should be supplemented for longer periods and together with other antioxidants (e.g., vitamin E) to observe possible positive effects [124].

Oxidative stress is widely recognized as the epigenetic factor of aging. Antioxidative micronutrients play a key role in reducing the inflammatory response associated with poor health outcomes in the elderly population. In addition, enzymatic capacity of cellular antioxidants declines with age, therefore, older people with lower peripheral antioxidant parameters and reduced antioxidant capacity are more susceptible to age-related diseases, disability, weakness, and higher mortality throughout a five-year follow-up [130,131]. These factors explain the increasing trend towards researching the effects of antioxidants on aging and preventing age-related disease.

4. Conclusions

Micronutrients are an important part of the antioxidant defense mechanisms. Oxidative metabolism inevitably leads to the production of ROS which can cause further oxidation, particularly of cell membranes and nucleic acids. The cell can counteract oxidative damage with endo- and exogenous antioxidants and repair systems. The damaging potential of free radicals is directly inhibited by the action of ascorbate, tocopherols, or enzyme systems, for example, Zn/Cu-SOD and GPX (dependent on selenium). Therefore, vitamins and trace elements (e.g., selenium and zinc) supplied with the diet are crucial for proper functioning of antioxidant enzymes [9]. In older people, the level of oxidative damage increases, thereby disrupting healthy aging at the molecular level. Shortage of microelements, such as vitamin C, E, zinc, and selenium, makes DNA more susceptible to oxidation. One of the most interesting examples are telomeres, which shorten with age. DNA lesions in their sequence (e.g., guanine oxidation) can result in SSBs or DSBs [132], which increase the risk of age-related diseases such as cancer, cardiovascular, neurodegenerative diseases (e.g., dementia), and diabetes [77]. Anorexia of aging is a state of severe deficiencies of microelements involved in antioxidant protection and maintaining genome stability. It accelerates and aggravates the course of the aging process and seriously disrupts the integrity of genetic information, causing SSBs, DSBs, and oxidative DNA damage.

These micronutrient deficiencies can be as harmful as DNA lesions resulting from UV rays and chemical agents' activity.

Understanding the influence of nutrition on cellular and molecular pathways should enable the development of nutritional strategies for maintaining health and possibly for treating and preventing diseases triggered by dietary deficiencies. The elderly population is particularly vulnerable to various deficits due to reduced intake of food rich in vitamins, micro- and macroelements. Micronutrients are essential for the maintenance of physical and cognitive functions in an aging body. Their insufficient consumption can possibly lead to deterioration of health and general QoL. Anorexia of aging additionally worsens the health condition of older patients, as it exacerbates the natural decrease in micronutrient levels that occur with age [133]. Nevertheless, oral supplementation should be recommended with caution in the elderly population, i.e., only for patients with diagnosed deficiencies, under medical supervision, and for a finite time. A well-balanced diet rich in vegetables and fruit should be the most important part of prophylaxis of age-related diseases such as cardiovascular disease, neurodegenerative diseases, or age-related anorexia, as well as a way to promote healthy aging with a high QoL.

Author Contributions: J.K.-B., concept and design, acquisition, analysis, and interpretation of literature data, drafting the article, critical review in relation to important intellectual content, revision of the article, final approval of the version to be published; K.B., acquisition, analysis, and interpretation of literature data, drafting the article, critical review in relation to important intellectual content, revision of the article, final approval of the version to be published; B.T.K., supervision, critical review in relation to important intellectual content, final approval of the version to be published. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Medical University of Lodz (503/3-045-02/503-31-001-19-00) and by the National Science Center, Poland (grant no. 2016/23/B/NZ7/03367).

Conflicts of Interest: The authors declare no conflict of interest.

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Review

Nutrition in Cancer Therapy in the Elderly—An Epigenetic Connection?

Janusz Blasiak [1,*], Jan Chojnacki [2], Elzbieta Pawlowska [3], Joanna Szczepanska [4] and Cezary Chojnacki [2]

1 Department of Molecular Genetics, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland
2 Department of Clinical Nutrition and Gastroenterological Diagnostics, Medical University of Lodz, 90-647 Lodz, Poland; jan.chojnacki@umed.lodz.pl (J.C.); cezary.chojnacki@umed.lodz.pl (C.C.)
3 Department of Orthodontics, Medical University of Lodz, 92-216 Lodz, Poland; elzbieta.pawlowska@umed.lodz.pl
4 Department of Pediatric Dentistry, Medical University of Lodz, 92-216 Lodz, Poland; joanna.szczepanska@umed.lodz.pl
* Correspondence: janusz.blasiak@biol.uni.lodz.pl

Received: 24 September 2020; Accepted: 28 October 2020; Published: 1 November 2020

Abstract: The continuous increase in life expectancy results in a steady increase of cancer risk, which consequently increases the population of older adults with cancer. Older adults have their age-related nutritional needs and often suffer from comorbidities that may affect cancer therapy. They frequently are malnourished and present advanced-stage cancer. Therefore, this group of patients requires a special multidisciplinary approach to optimize their therapy and increase quality of life impaired by aging, cancer, and the side effects of therapy. Evaluation strategies, taking advantage of comprehensive geriatric assessment tools, including the comprehensive geriatric assessment (CGA), can help individualize treatment. As epigenetics, an emerging element of the regulation of gene expression, is involved in both aging and cancer and the epigenetic profile can be modulated by the diet, it seems to be a candidate to assist with planning a nutritional intervention in elderly populations with cancer. In this review, we present problems associated with the diet and nutrition in the elderly undergoing active cancer therapy and provide some information on epigenetic aspects of aging and cancer transformation. Nutritional interventions modulating the epigenetic profile, including caloric restriction and basal diet with modifications (elimination diet, supplementary diet) are discussed as the ways to improve the efficacy of cancer therapy and maintain the quality of life of older adults with cancer.

Keywords: cancer; older adults; nutrition; malnutrition; epigenetic regulation of gene expression; DNA methylation; epigenetic diet; caloric restriction

1. Introduction

Aging of societies implies an increasing number of cancer diagnoses in the elderly [1]. As older adults have substantially different nutritional needs than their younger counterparts, the question is whether such differences will result in a different response to cancer therapy in the categories of both the efficacy in the target tissue and unwanted side effects. Any kind of cancer therapy is a serious challenge and burden for the patient, so it should be adjusted to the nutritional status of the patient and vice versa. Nutritional studies among older adults with cancer are considered a major area of interest in geriatric oncology, as most studies on diet and nutrition in cancer have been conducted in younger adults [2].

In general, the care of older adults with cancer is complex due to competing comorbidities, multiple drugs usage, deficit in cognitive functions, and other features complicating the care. On the other hand, cancer chemotherapy may be associated with adverse events, including vomiting and mouth sores, that may influence the nutritional status of cancer patients. Furthermore, cancer is frequently associated with weight loss and a dietary intervention may be recommended in such cases. A European study showed that over 70% of elderly cancer patients presented undernutrition, defined as weight loss of 10% or greater [3].

Epigenetic regulation of gene expression is an emerging field in human molecular genetics, physiology, and pathology. The epigenetic profile of the genome (the epigenome) is established by DNA methylation, chemical modifications of chromatin, and the action of non-coding RNAs. In contrary to its genetic counterpart, the epigenetic profile is erased in the germ cells and can be modulated at any stage of development by environmental and lifestyle influences. This fact is exploited in epigenetic therapies with the use of drugs modulating the epigenetic profile (epidrugs) [4]. Many studies show that diet and nutrition influence epigenetic mechanisms playing a role in the pathogenesis of many diseases, including cancer (reviewed in [5]). On the other hand, the epigenetic profile is modulated by aging. Therefore, epigenetics seems to be a natural candidate to link nutrition with cancer therapy in older adults. In this review, we discuss the main problems associated with nutrition in older adult cancer patients undergoing active therapy, as well as the role of the epigenetic profile in aging and cancer transformation, and present a perspective of epigenetic nutritional intervention in elderly cancer patients.

2. Management of Older Adult Cancer Patients

Although chronologic age is one of the main determinants of therapeutic strategy in cancer, older adults have other conditions that may influence morbidity and mortality independently of metrical age (Figure 1) [6].

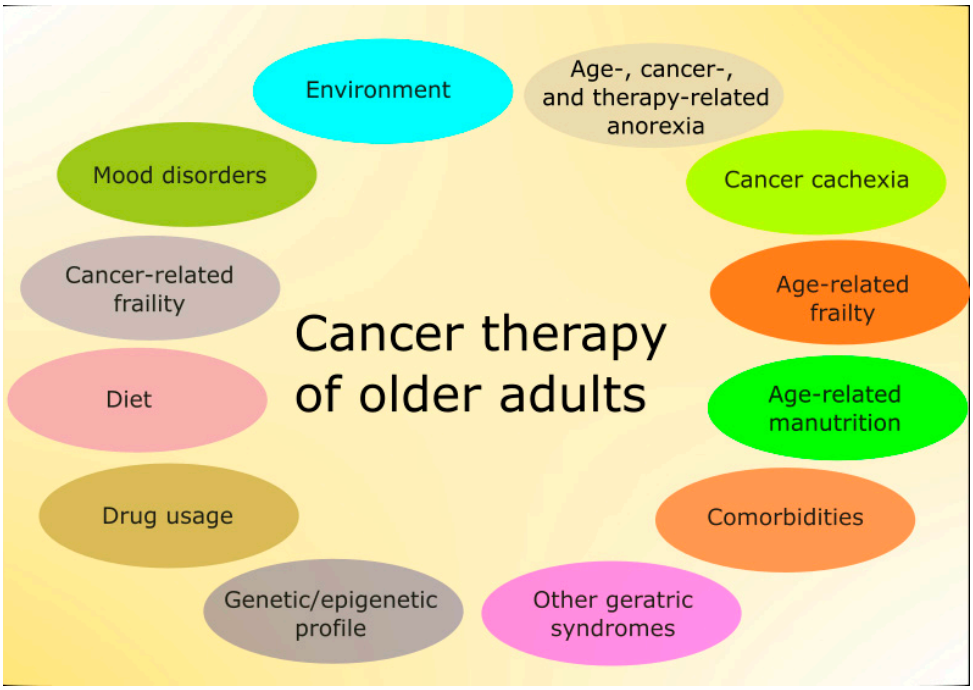

Figure 1. Main factors affecting therapy in older adults with cancer. Some of these factors are mutually dependent and some partly overlap. Environment is understood here in a broad sense and also includes family and social relationships. Some factors, such as the diet, are of general significance, but have several features specific for this group of patients.

These conditions include cognitive impairment, delirium, incontinence, malnutrition, falls, gait disorders, pressure ulcers, sleep disorders, sensory deficits, fatigue, dizziness, and others. They are widespread in older adults and may have a major influence on quality of life and disability. Therefore, doctors should have a tool to quickly assess various aspects of elderly patients to develop an optimal therapeutic strategy as well as monitor and evaluate its consequences. They are listed in the comprehensive geriatric assessment (CGA), a process used to evaluate and manage fit, frail, or vulnerable older people (Figure 2) [7].

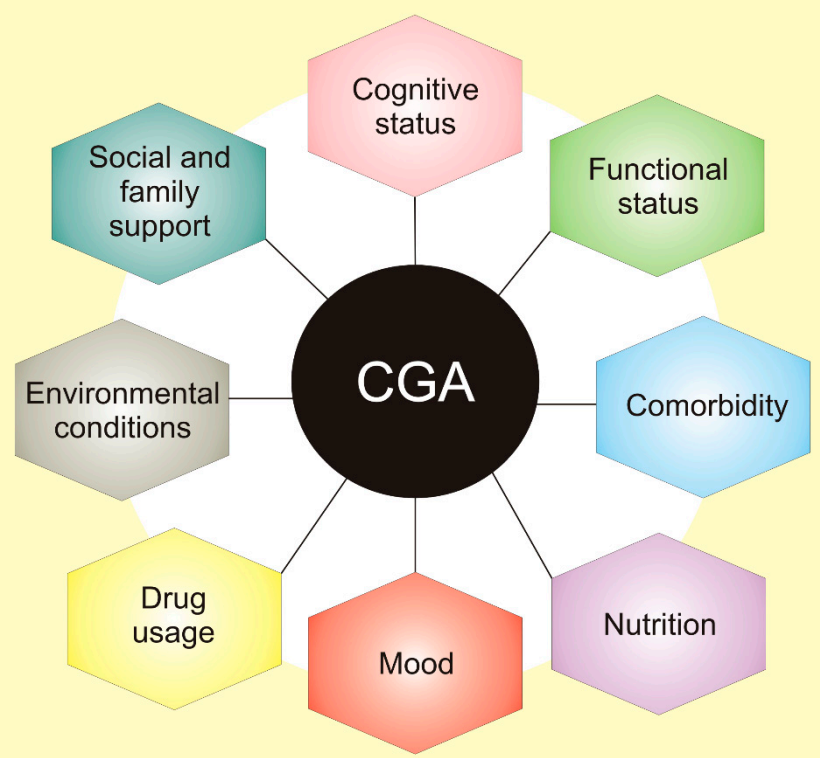

Figure 2. Comprehensive geriatric assessment (CGA) is an organized evaluation method to provide a multidisciplinary assessment of and care for the elderly. It assesses physical medical conditions, including comorbidity, the disease severity, immunization status, and others. Assessment of functional status refers to an elderly person's ability to perform daily tasks and determines several core functions, including balance and mobility. Other areas of CGA include issues contained in broad categories of assessment of social health and environment.

CGA involves not only medical diagnoses but also functional deficiency and the environmental and social matters that disturb patient wellbeing. It creates problem lists and shows aim-driven interventions to face them. Eventually, it delivers and organizes a complex plan for therapy, rehabilitation, support, and long-term care [7].

CGA factors that may be useful in oncology care of older adults are physical function, comorbid medical conditions, cognitive function, psychological state, social support, polypharmacy, and geriatric syndromes [6]. Financial consideration is also included in these factors, often with social support. These factors should be considered in a decision-making process in the treatment of older adults with cancer. Comorbid medical conditions seem to be critical for life expectancy and treatment tolerance, which is essential to maintain quality of life. Moreover, these comorbid conditions usually, if not always, affect the treatment. Therefore, the basic question a doctor should answer is whether a patient is more likely to die of cancer or other comorbid conditions, which is a complex and challenging task in the case of older adults [6]. From the point of view of this review, comorbidity resulting from nutritional status is of a prime interest. However, it is not easy to determine the involvement of dietary factors in

the pathogenesis of many serious diseases influencing cancer treatment in the elderly, including other cancers. That is why we will focus on the existing nutritional status of older cancer patients.

In general, weight loss in late life was associated with an increased mortality [8]. Malnutrition in older adults with cancer may diminish tolerance to therapy and result in a worse response to treatment [9]. The risk associated with nutritional status in the senior population can be quickly evaluated with the Mini-Nutritional Assessment (MNA), a part of CGA, including anthropometric measurements; questions related to lifestyle, mobility, and medications; a brief dietary questionnaire; and self-perception of health and nutrition [10]. It can be an alternative or supplement for self-reported practical markers of frailty, including weight loss and low Body Mass Index (BMI), which was established as less than 18.5 kg/m^2 by the World Health Organization [11].

In a multicenter study, Soubeyran et al. enrolled over 300 patients older than 70 years with various types of advanced cancer [12]. They evaluated their state with various aspects of baseline abbreviated CGA and concluded that a low MNA score and poor mobility predicted an early death—within 6 months from the start of chemotherapy. These studies confirm that a poor nutritional status in older adults with cancer is correlated with a bad prognosis. The authors underlined that the MNA test in these patients likely reflected the consequences of advanced disease and that the MNA questionnaire contained 18 questions not related directly with nutrition. Yet another method for comprehensive nutritional assessment of adult oncology patients to determine the strategy of nutritional intervention is SGA (Subjective Global Assessment) and its variant, PG-SGA (Patient Generated-Subjective Global Assessment) [13].

Aaldriks et al. enrolled 143 patients aged 70 years or older with advanced colorectal cancer receiving adjuvant or palliative chemotherapy [14]. Before chemotherapy, they were assessed by MNA, Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE), Groningen Frailty Indicator (GFI), and Mini Mental State Examination (MMSE). The authors observed that malnutrition and frailty were strongly linked with an increased mortality risk in patients undergoing palliative chemotherapy and a poor score on MNA was correlated with a worse tolerance of chemotherapy. Therefore, nutrition was again shown to be an important factor in the cancer care of older adults.

Comorbidity is one of the most important issues addressed in geriatric assessment. As older age is associated with frailty, diabetes, and cancer, Liuu et al. investigated older adults with cancer from the prospective single-center cohort ANCRAGE (Analyses of CanceR in AGEd) in order to determine the influence of type 2 diabetes mellitus (T2DM) and its vascular complications on frailty and adverse outcomes during 8-year follow-up [15]. They recruited nearly 1100 patients $\geq$ 75 years with cancer, and about 30% of them presented a metastatic disease, and frailty was common in this group (84%). After adjustment for age, gender, and metastatic status, frail T2DM patients with vascular complications displayed the highest risk of all-cause death. In the context of this review, the most important result of this study was that death was more often due to non-cancer causes, which supports the complexity of considerations surrounding the care of older adults with cancer. On the other hand, it is not easy to assess the real role of cancer in deaths whose immediate reason was T2DM, as cancer and T2DM have much in common and affect each other [16].

3. Nutrition, Aging, and Cancer in the Elderly

Older adults show diminished energetic demands, but they still need some essential nutrients, which are especially important as their total intake of food is lower than average. Therefore, their diet should be carefully chosen with limited amounts of products with sugar and fat and a dominating proportion of products with high nutrient density. However, cancer as a systemic disease may enforce alterations to such carefully established diets, and cancer therapy may require further changes. Despite common use of dietary supplements after cancer diagnosis, no consensus has been achieved for their recommendation by medical authorities, including the World Cancer Research Fund and the American Cancer Society [2].

Many dietary supplements administrated to cancer patients contain antioxidants that may neutralize reactive oxygen species (ROS) that play a role in the process of carcinogenesis, as they may induce mutations fueling cancer transformation [17,18]. However, many regimes of chemotherapy and radiotherapy produce ROS that can damage biological molecules, including proteins and DNA, in cancer cells. Supplementary antioxidants add to the cellular antioxidant defense system, containing antioxidant enzymes, DNA repair, and low-weight antioxidants. Many studies suggest that this system declines with aging [19,20]. At present, clinical recommendations say that cancer patients, independently of age, should rather not take antioxidants during therapy [21–23]. In a recent study, Ambrosone et al. concluded that the use of antioxidant supplements during chemotherapy, as well as iron and vitamin B12, might increase the risk of breast cancer recurrence and mortality [24]. However, this study did not stratify patients according to age, and the main age of patients enrolled in the study was about 50 years.

Malnutrition arises from an inflammatory state, which advances anorexia and resulting weight loss. Malnutrition is common in cancer patients, as up to 40% of all cancer patients display weight loss at the time of diagnosis [25,26]. However, older adults may show weight loss as a result of various comorbidities and other geriatric syndromes, so it is not easy to precisely determine cachexia among them. On the other hand, obesity, the other face of malnutrition, is increasingly becoming an issue affecting cancer survival [27,28]. This problem may be especially important in older cancer patients, as obesity occurs with aging, despite a reduction in food consumption (reviewed in [29]). Weight gain and obesity among older adults may occur with concomitant reduction in muscle mass and sarcopenia [30]. However, steroids and hormonal therapy in a long-term cancer treatment may stimulate the development of diabetes and cardiovascular disease at which older adults, especially with obesity, are at risk [31,32]. Therefore, nutritional research is needed among obese older cancer patients to establish prognosis of the disease course [33].

4. Nutrition and Cancer Therapy in the Elderly

Nutrition care during active cancer therapy should be directed to increase the efficacy of the therapy, reduce unwanted side effects, prevent nutritional deficiencies, and maintain weight and quality of life [34]. Nutritional status is an independent predictor of survival, and poor nutritional status is associated with worse outcomes for older patients undergoing cancer therapy [12,35,36]. On the other hand, malnutrition may be a risk factor for unwanted side effects of chemotherapy [37,38].

Chemotherapy influences patients' nutritional status, as more than half of patients undergoing chemotherapy experience vomiting, mucositis, nausea, and parageusia [39]. Similar effects can be expected in a substantial proportion of cancer patients undergoing radiotherapy [40]. Consequently, malnutrition is an important element that should be considered in the planning of and during cancer therapy. Optimally, malnutrition should be recognized prior to surgery, chemotherapy, and radiotherapy, or any other therapy, and treated with a nutritional intervention [41]. Therefore, nutritional interventions should be fundamental and adjuvant for any kind of cancer therapy as a kind of multidisciplinary follow-up [9]. When patients are of an advanced age, this issue becomes more complex and requires some additional and specific approaches.

Muscle mass loss and fatty muscle infiltration are frequently used to assess malnutrition, sarcopenia, and cachexia and to monitor the side effects of cancer therapy [42]. Cancer-independent, significant muscle mass loss in older adults is an important factor that should be considered in such assessments.

Apart from problems associated with cancer therapy and directly related to the diet and nutrition status, some other factors should be considered in the cancer care of older patients. Hoppe et al. presented data from 12 centers in France with older (age ≥70 years) cancer patients receiving first-line chemotherapy [43]. They observed that a substantial portion of patients, 50 of 364, experienced an early functional decline between the beginning of chemotherapy and its second cycle. This decline was determined as a decrease of ≥0.5 points on the Activities of Daily Living scale [44]. Factors associated with early functional decline were evaluated with the use of various geriatric assessments,

including abbreviated CGA, MNA. They observed that early functional decline resulting from first-line chemotherapy was associated with baseline depression and instrumental dependencies. Both these features may cause nutritional problems and impede nutritional interventions.

The diet seems to be the only element during cancer therapy that can be perceived by a patient as a fully controllable means to maintain energy and activity and successfully overcome the therapy [9]. This seems especially important in the case of head and neck cancer as well as cancer of the gastrointestinal tract, as patients with these cancers are particularly prone to problems with nutrition due to the location of tumor and area of treatment [45,46].

From the clinical point of view, future research should concentrate on energy balance among older adults and their body composition during cancer treatment, biomarkers for cachexia, and personalized multi-disciplinary interventions [47]. From a scientific standpoint, it is important to determine the process of cellular aging in cancer cells and relate it to organismal aging.

5. Epigenetic Mechanisms in Cancer Transformation and Aging

Cancer, a disease of genes, results from the accumulation of genetic and epigenetic alterations and their clonal expansion in proliferating cells (Figure 3).

Cancer is predominantly a disease of later life, as it needs time to disrupt controls in multiple cells. Age is frequently considered to be the most serious cancer risk factor, but it is difficult to fully accept this view, especially in cancers underlined by germ mutations or some juvenile leukemias [1]. Genomic and epigenomic instability seem to be crucial for cancer development. Epigenetic dysregulation plays a role in all stages of cancer transformation. It can be induced by genetic changes, first mutations in genes encoding epigenetic regulators, or by tissue inflammation affecting cell signaling, resulting in altered chromatin organization [48].

Dysregulated DNA methylation is likely the best-known epigenetic effect in cancer transformation [49]. Loss of DNA methylation at some specific repetitive elements and regulatory sites has been associated with increased genomic instability and chromosomal aberrations, resulting in fusion genes often encoding oncoproteins [50,51].

Modulation of chromatin structure through covalent modification of histone N-terminal tails is an essential way to change DNA accessibility during its transcription, replication, damage repair, and a series of other cellular processes [52,53]. The biological outcome of histone modifications is expressed either by a direct modulation of nucleosomal structure or by recruiting downstream proteins that play a role of 'reader' or 'effector'. The histone code is read to recruit proteins that can alter the chromatin structure. Many enzymes involved in establishing the code can contribute to cancer transformation when their activity is aberrant [54].

The role of non-coding RNAs in cancer is an emerging area of research [55].

Genetic, epigenetic, and environmental events driving the process of aging are mutually coupled, as environmental factors, such as smoking, are associated with the production of molecules that may damage DNA and induce mutations (Figure 4). On the other hand, mutations induced by products of normal cellular metabolism may affect the expression of genes responsible for the detoxification of environmental DNA-damaging agents. Furthermore, there is a mutual dependence between aging and genetic, epigenetic, and environmental factors that promote aging. This dependence is a kind of vicious cycle, as the declines in some functions linked with aging may result in an enhanced susceptibility to environmental factors that in turn may result in further declines in these age-related functions.

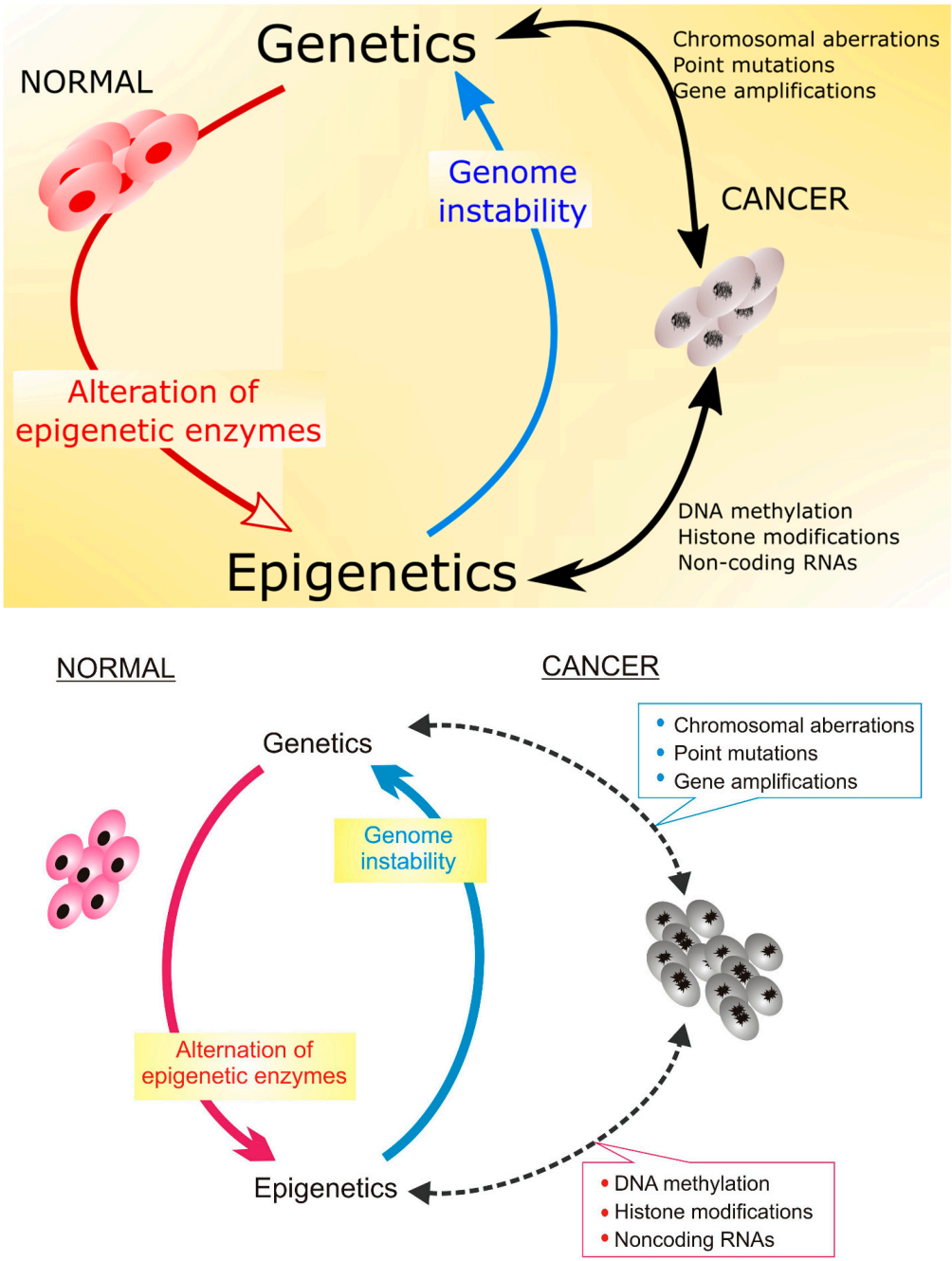

Figure 3. The interplay between genetic and epigenetic factors in cancer transformation. Genomic instability, typical for most cancers, results in an increased number of mutations in genes encoding modifiers of the epigenetic profile. On the other hand, epigenetic changes, including DNA methylation, histone modification, and changes in non-coding RNAs, affect the expression of genes responsible for the maintenance of DNA, resulting in an increased number of chromosomal aberrations, DNA point mutations, amplifications, and other changes increasing genome instability.

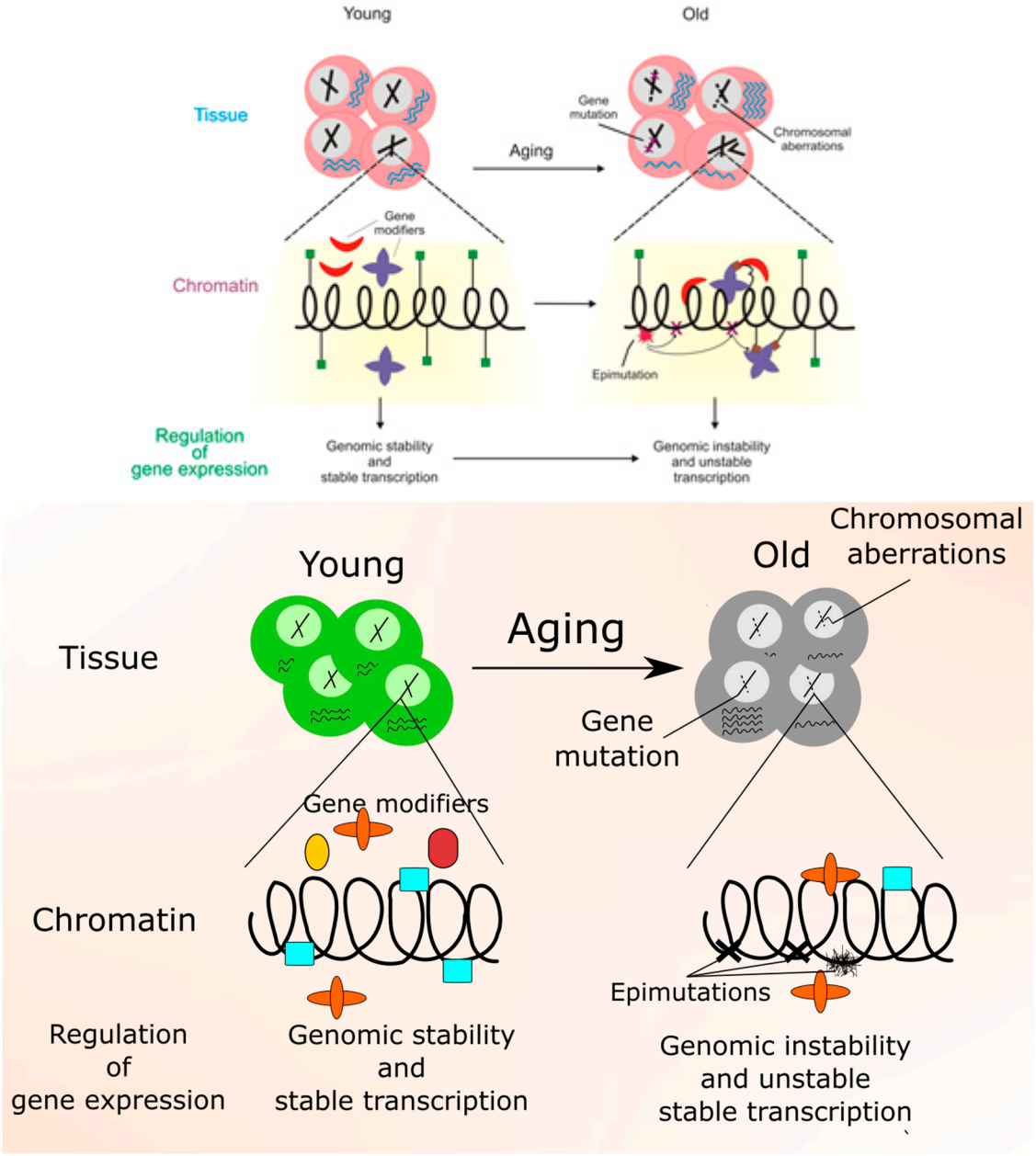

Figure 4. The crosstalk between epigenetic changes, transcription, and genomic instability. In a young organism, transcription is fully controlled and produced the same amount of mRNA in the cells that activate the same genes. In these cells, normal chromatin state and genomic stability are maintained. With increased age, genomic instability increases, resulting in gene mutations and chromosomal aberrations and unstable transcription. Increased DNA damage may result in DNA damage response inducing the recruitment of epigenetic modifiers of chromatin structure and locally resuming its conformation, which may partly stabilize the transcription of neighboring genes. Epimutations, which accumulate in later life, may hamper this process.

It became evident that the extent of DNA methylation decreases with aging and that alterations might induce the abnormal expression of genes important for the aging process [56,57]. Early interest in the association of aging and loss of 5-metC, an indicator of DNA methylation, focused mainly on hypomethylation of some genes important in aging, similarly to tumorigenesis [58]. However, it was shown later that aging mice transcriptionally activated alleles that were epigenetically silenced in their younger age [59]. Issa et al. were the first to observe the age-associated hypermethylation of

CpG (cytosine-guanine dinucleotide) islands in age-related genes in different human tissues [60–64]. Several mechanisms may be responsible for increased methylation in CpG islands in the promoters of aging-related genes (reviewed in [65]).

DNA methylation, a primary epigenetic event, is characterized by a high inter-individual variability that is underlined by different environmental and lifestyle factors, including the diet (reviewed in [66]). However, it is mostly unknown how dietary compounds affect the DNA methylation pattern. The only exception is the tea polyphenol, epigallocatechin-3-gallate, which is known to act as a competitive inhibitor located in a pocket in the active center of an enzyme responsible for DNA methylation [67]. Li et al. observed that glucose restriction in cultures of normal human fibroblasts extended their Hayflick limit [68]. This result cannot be directly translated into the extension of the human lifespan, but cellular senescence is considered to be associated with organismal aging (reviewed in [69]).

The importance of the chromatin structure, determined mainly by the covalent modifications of histones, in the process of aging has been confirmed by studies on two human genetic diseases: Hutchinson–Gilford (HGPS) progeria syndrome and Werner syndrome, which are characterized by premature aging phenotypes with a shortened life span and are accepted models for studying the biology of aging in humans (reviewed in [70,71]). Both syndromes are characterized by molecular changes that can be linked with normal human aging. Epigenetic alterations are detected in both syndromes, especially HGPS. These include alterations in the histone distribution, telomere attrition, and the function and biosynthesis of miRNAs.

In general, chromatin structure, which carries much of the epigenetic information, is considered a major element in the epigenetics of the aging process (reviewed in [72]). Packaging DNA into highly organized nucleosomal structure allows for a precise regulation of all genomic processes occurring in the nucleus, including DNA replication, transcription, recombination, and DNA repair through a defined access to DNA. In general, aging is believed to be associated with nucleosomal remodeling increasing the susceptibility to persistent DNA damage [73].

The third main element of epigenetic regulation, non-coding RNAs, with broad two categories, short non-coding RNAs (sncRNA) and long non-coding RNAs (lncRNAs), is reported to display some disrupted functions with aging [74–77]. Mainly, micro RNAs (miRNA) and lncRNAs were studied for their age-related aspects and, in fact, the majority of miRNAs were shown to be downregulated with age [78–80].

Although far from the main subject of this review, the honeybee (*Apis mellifera*) offers likely the most convenient example of the effects of diet on lifespan mediated by epigenetic mechanisms [81]. Honeybee larvae are not genetically predetermined to be a queen, but the queen phenotype, with a lifespan up to 20 times longer than a worker, results from the diet containing royal jelly [82]. This effect is mediated by DNA methyltransferase 3 changing the DNA methylation profile.

6. Epigenetic Link between Nutrition, Aging, and Cancer

Caloric restriction (CR), a 30–40% reduction in the caloric intake while maintaining adequate nutrition, and rapamycin are known to extend lifespan. Although the exact mechanism of their action in this effect is not known, the epigenome is considered to be their target, along with genome stability, protein quality control, telomere attrition and function, mitochondrial function, nutrient sensing, cellular senescence, stem cell exhaustion, cellular stress responses, and intercellular communication [83–85].

McCay et al. were the first to report that a CR diet extended the lifespan of mice [86]. Since then, several works have shown a positive correlation between CR and lifespan in various organisms, including yeast, worms, flies, fish, and primates (reviewed in [87]). Two of these works are worth mentioning, as they reported apparently contrasting results on CR and longevity. Colman et al. showed that CR resulted in the extension of lifespan and a reduction in overall mortality of Rhesus monkeys as compared with controls fed an ad libitum diet [88,89]. On the other hand, Mattison et al. demonstrated that Rhesus monkeys fed with a CR diet did not show any lifespan extension, although these animals displayed a reduction in some age-related diseases, including cancer [90]. Contrasting results obtained

in these two studies may be underlined by differences in diet composition, which might differentially affect the epigenetic profile, but also the profile itself could be different in the animals in these two experiments, as they were not conducted in the same environmental conditions. Environment and lifestyle have been shown to have a profound effect on the epigenetic profile [5].

Several mechanisms behind the effect of CR on longevity can be considered. The direct consequence of CR is a reduced energy status in the organism and related decrease in blood glucose, insulin, insulin-like growth factor (IGF-1), growth hormones, and other hormones (reviewed in [91]). Diminished status of cellular energy leads to lower mitochondrial activity and consequently lower aerobic respiration, increased adenosine monophosphate / adenosine triphosphate (AMP/ATP) ratio, and increased nicotinamide adenine dinucleotide (NAD+) levels. Further, two cellular nutrient and energy sensors, adenosine monophosphate kinase (AMPK) and sirtuin 1 deacetylase (SIRT1) are activated [92–94]. Activated AMPK induces a series of events resulting in reduced fatty acid synthesis, oxidation, and cholesterol synthesis, but active SIRT1 may increase ketogenesis and lipolysis, and decrease glycolysis. Other proteins can be involved in these processes [95].

The impact of CR on the epigenome was initially associated with an increasing stability of the genome by reduction in the loss of DNA methylation [68]. However, later, the Issa's lab showed that epigenetic drift, including both gains and losses of DNA methylation at various genome sites, was conserved among species and was correlated with lifespan and CR [96]. Recently, Hernando-Herraez showed that mouse stem cells acquire epigenetic drift by the accumulation of stochastic changes of DNA methylation in the promoters of many genes, which leads to altered transcriptional control and the aging of stem cells [97]. Epigenetic mechanisms of anti-aging effects resulting from CR were then postulated and shown in several works [5,91,94,95,98–100].

Some tumors, including brain, head and neck, and lung cancers are glucose dependent, so patients with these tumors may benefit from a diet limiting glucose (e.g., a ketogenic diet), but in general, CR is not documented to have an anticancer effect [101]. CR and ketogenic diet result in increased fatty acid oxidation and acetyl-CoA (acetyl-coenzyme A) production, which, in turn, leads to the enhanced production of β-hydroxybutyrate, which is a source of energy for the brain and an inhibitor of glycolysis [102]. Therefore, a CR diet may increase the antioxidant capacity of normal tissues, but this is not the case in cancer cells [103,104].

There is not a strong rationale for a CR diet in malnourished cancer patients. It is even postulated that a high fat and protein diet better fulfills the nutritional requirements of cancer patients than restrictive diets [105]. For obvious reasons, research performed on obese subjects and experimental animals should not be directly related to cancer patients, especially those with advanced age. On the other hand, elderly cancer patients are often malnourished, and it is rather a risky decision for a doctor to recommend any restrictive diet. Currently, only tumors with a strong dependence on glucose should be considered for such dietary intervention, but each case should be treated individually considering other circumstances, especially those associated with the aging-related features of a patient.

It has been shown that the introduction of certain foods, including grapes (resveratrol), soy (genistein), cruciferous vegetables, and green tea, might have a protective effect against aging and cancer [106]. Moreover, several studies showed that a diet containing these substances (an "epigenetic diet") reduced the incidence of some diseases and is similar in this regard to a CR diet [107,108].

7. Summary, Conclusions, and Perspectives

Most cancers occur in older adults, and many factors other than chronologic age determine morbidity and mortality and contribute to the strategies surrounding cancer care. Nutritional studies among older adults with cancer are scarce, but the extension in the life expectancy and new therapeutic strategies imply the need for nutritional support and interventions for this group of patients, as recommended by the American Cancer Society and the National Comprehensive Cancer Network [23,109].

Considering research performed so far, any restrictive diet, including a CR diet, is not generally recommended for older adults with cancer. This conclusion does not, however, preclude a beneficial effect of such a diet in cancer prevention. Several nutrients included in a CR diet show epigenetic mechanisms of action, modulating DNA methylation, histone modification, and non-coding RNA functions. However, anticancer-preventive action should be clearly distinguished from beneficial effects in cancer, especially in its advanced form. The mechanism of metastasis, the primary cause of cancer-related death, is poorly known, and it involves different molecular events than cancer initiation, promotion, or even invasion, the initial step of metastasis [110]. This problem is complex, as both aging and cancer significantly affect global gene expression at transcriptome, proteome, and metabolome levels, and it is challenging to predict how these changes would be modulated by nutrition. At present, epigenetics seems to be the most promising link between aging, cancer, and nutrition.

One important feature of epigenetic modifications is that they may be modulated or even reversed by the diet, which is not the case of genetic alterations, first gene mutations, or chromosomal aberrations [111]. Studies performed so far indicate that not only the kind (quality) of the diet but also the amount of energy (calories) may be important for this modulation. So, what is the kind of diet recommended for older adults undergoing cancer therapy? Of course, it is not easy to give a general answer to this question, as it depends on the cancer type and the kind of therapy. Is a so-called "epigenetic diet" a solution? Does such a diet really exist? Although an "epigenetic diet" is sometimes defined as a diet affecting the epigenome, in fact, a diet that would not affect the epigenetic profile would be very sophisticated, if possible, at all. As we concluded in our previous work, an "epigenetic diet" is a rather misleading term, as it is hardly possible to find a diet that would not affect the epigenome [112]. Instead, three kinds of diet can be considered to amend the needs of elderly patients undergoing cancer therapy. Firstly, there is the basal diet, which is adjusted to the general state of a patient's health and kind of cancer. Considering the possibility of epigenome modulation, some compounds should be eliminated from this diet (elimination diet) and/or some should be added (supplementary diet). This is the basal diet, which is adjusted to the specificity of this group of patients. Caloric restriction, which is considered to be a direct way to increase lifespan, should not be recommended in general in older adults undergoing cancer therapy, as such groups of patients face age- and cancer-associated anorexia and cathepsia.

Further research is needed to identify which elements of the diet most effectively decrease morbidity and mortality among older adults with cancer. Molecular studies on changes in the epigenetic profile in these subjects may provide information on the use of drugs modulating that profile, which may contribute to evidence-based practice. No one expects that any kind of diet will result in cancer regression—the goal should be to optimize cancer therapy and to improve quality of life impaired by advanced age, cancer, and cancer therapy. Studies that integrate geriatric and oncology care with nutrition and the modulation of the epigenome seem to be at present a rationale means in which to provide information on appropriate nutritional support nutrition in older adults with cancer.

Author Contributions: Conceptualization: J.B., C.C., E.P., and J.C.; writing—original draft preparation: J.B.; writing—review and editing: J.B., C.C., J.C., E.P., and J.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors thank M. Kicinska of the University of Lodz for her help in figure preparation.

Conflicts of Interest: The authors declare no conflict of interest.

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Review

Diet to Reduce the Metabolic Syndrome Associated with Menopause. The Logic for Olive Oil

Juan José Hidalgo-Mora [1], Laura Cortés-Sierra [1], Miguel-Ángel García-Pérez [2], Juan J. Tarín [3] and Antonio Cano [1,4,*]

1 Service of Obstetrics and Gynecology, Hospital Clínico Universitario—INCLIVA, Av Blasco Ibáñez 17, 46010 Valencia, Spain; hidalgomorajj@gmail.com (J.J.H.-M.); cortes_lausie@gva.es (L.C.-S.)
2 Department of Genetics, Faculty of Biological Sciences, University of Valencia, Burjassot, and INCLIVA, Av Blasco Ibáñez 17, 46010 Valencia, Spain; Miguel.Garcia@uv.es
3 Department of Cellular Biology, Functional Biology and Physical Anthropology, Faculty of Biological Sciences, University of Valencia, Burjassot, 46100 Valencia, Spain; Juan.J.Tarin@uv.es
4 Department of Pediatrics, Obstetrics and Gynecology, University of Valencia, Av Blasco Ibáñez 15, 46010 Valencia, Spain
* Correspondence: antonio.cano@uv.es; Tel.: +34-96-983087

Received: 14 September 2020; Accepted: 14 October 2020; Published: 18 October 2020

Abstract: The rates of metabolic syndrome are increasing in parallel with the increasing prevalence of obesity, primarily due to its concomitant insulin resistance. This is particularly concerning for women, as the years around menopause are accompanied by an increase in visceral obesity, a strong determinant of insulin resistance. A fall in estrogens and increase in the androgen/estrogen ratio is attributed a determining role in this process, which has been confirmed in other physiological models, such as polycystic ovary syndrome. A healthy lifestyle, with special emphasis on nutrition, has been recommended as a first-line strategy in consensuses and guidelines. A consistent body of evidence has accumulated suggesting that the Mediterranean diet, with olive oil as a vital component, has both health benefits and acceptable adherence. Herein, we provide an updated overview of current knowledge on the benefits of olive oil most relevant to menopause-associated metabolic syndrome, including an analysis of the components with the greatest health impact, their effect on basic mechanisms of disease, and the state of the art regarding their action on the main features of metabolic syndrome.

Keywords: olive oil; metabolic syndrome; obesity; women; menopause; healthy ageing

1. Introduction

The metabolic syndrome (MetS) consists of a cluster of risk factors that increase the risk of type 2 diabetes and cardiovascular disease (CVD) [1]. This cluster includes dysglycemia, increased blood pressure, lipid abnormalities as defined by hypertriglyceridemia and low high-density lipoprotein (HDL) cholesterol, and central obesity [2]. A conservative estimate is that around 100 million people may be affected worldwide, but the figure might be higher [3]. This makes the MetS a public health issue with a definitive impact on any healthy ageing strategy.

The prevalence of this syndrome is distributed heterogeneously worldwide, with geographic region, ethnicity, sex, age, socio-economic status, and education among the factors playing a role. It is believed that insulin resistance is the link underpinning the clustering of these risk factors [1]. Obesity, particularly central obesity, is also understood as a trigger because of its predisposing effect on insulin resistance [4].

2. Literature Search

We conducted a PubMed database search for publications between 1 January 2000 and 1 October 2020, pairing the term "olive oil" with "metabolic OR metabolic syndrome OR obesity OR central obesity OR weight OR waist OR blood pressure OR cholesterol OR triglycerides OR lipids OR insulin resistance OR diabetes OR menopause". Only papers written in English or Spanish were considered, yielding a total of 7885 titles. The initial search considered the title, or title and abstract when the title raised uncertainty about the content of the paper, reducing the list to 251 articles. Systematic reviews and meta-analyses were included in the selection. We manually searched the reference lists of selected review papers to retrieve other citations of potential interest. Studies based on special populations (adolescents, transplant patients, pregnant women, etc.) were excluded. After cross-cleaning the lists, a total of 120 papers were chosen. (Figure 1).

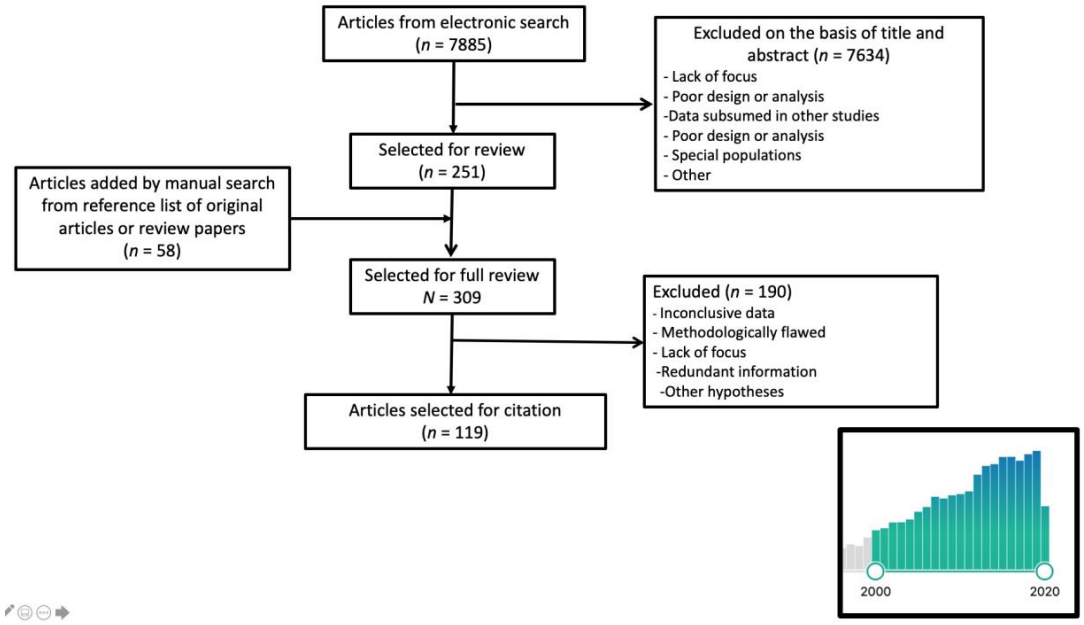

Figure 1. Literature search flowchart. The bars in the insert represent the trend in the numbers of papers published per year between 2000 and 2020. The year 2020 is incomplete because the search only included papers published until 1 October.

3. Insulin Resistance in Menopausal Women

The rates of all forms of obesity are rising rapidly worldwide, and the problem is expected to worsen [5]. The association between excessive calorie intake and inactive lifestyle from an early age onwards has led to a global epidemic affecting both poor and rich countries. Women are affected by this obesity epidemic equally as [5], if not more so than, men [6].

Central obesity is defined by the abdominal accumulation of fat, which can be located mainly at the subcutaneous or visceral level, or both. Central visceral rather than subcutaneous obesity is a major determinant of insulin resistance, which has been considered a driver of detrimental outcomes in the MetS [7]. As with men, central obesity also confers increased risk in women, as shown in the 10-year follow-up of 156,624 postmenopausal women enrolled in the Women's Health Initiative (WHI) cohort [8]. This finding is significant because unlike men, women have specific risk factors for central obesity. Indeed, women undergo dramatic hormonal changes at midlife, arising during the perimenopausal period, in which there is a significant decline in circulating estrogen levels [9]. Experimental and clinical studies concur that the fall in estrogens is associated with an increase in visceral fat [10–12]. Likewise, longitudinal population studies such as the Study of Women Across the Nation (SWAN) have confirmed that the odds of suffering from metabolic syndrome more than double

in the years around the menopause [13]. This effect of menopause can undergo slight modifications according to ethnicity as a result of the different patterns of hormonal changes among women of different racial origins [14].

The potential contribution of hormonal changes other than those in estrogens has been studied in depth, with the case of polycystic ovary syndrome (PCOS) providing a good model [15]. Proof of the specific potential of hormones has been found in non-obese women presenting with PCOS, who also show an increased risk of MetS [16]. A relative increase in androgens vs. estrogens has been attributed a central role, although the issue is still a matter of some debate [17]. For example, androgen concentrations did not increase the risk for diabetes among overweight women who were already glucose intolerant in a secondary analysis of the Diabetes Prevention Program (DPP) and the Diabetes Prevention Program Outcomes Study (DPPOS) [18]. It might be that androgens distinct from testosterone, such as dehydroepiandrosterone-sulfate (DHEAS), could have a compensatory effect [19,20].

The androgen/estrogen imbalance also occurs at the time of menopause because of the fall in estrogens in the presence of a much lower decline in androgens (Figure 2). Confirmatory evidence has been obtained in a group of postmenopausal women whose testosterone levels were measured with an ultrasensitive method and their body composition and abdominal deposits with dual energy X-ray absorptiometry (DXA) and magnetic resonance imaging, respectively [21].

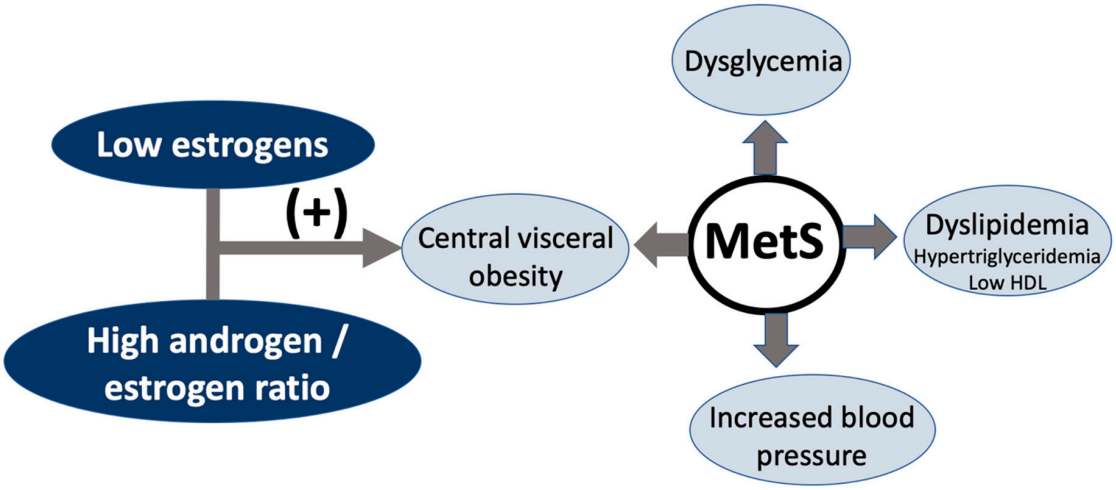

Figure 2. The metabolic syndrome (MetS) is defined as a cluster of four different risk factors, namely, dysglycemia, dyslipidemia, increased blood pressure, and central visceral obesity. While these affect both sexes, at midlife, women go through the menopause, which involves a rapid fall in estrogens and a very slow decline in androgens. Both the reduction in estrogens and the increase in the androgen/estrogen ratio have been attributed to promoting central obesity. Increased insulin resistance may then affect the other three factors in the cluster. HDL: high-density lipoprotein.

The potential influence of other factors cannot be excluded. For example, low levels of sex hormone binding globulin (SHBG) occurring when estrogen levels are low have been shown to be associated with the MetS and type 2 diabetes in both men and women [22,23]. More recent studies have gone further and have confirmed an increased risk for cardiovascular events. This was the conclusion from the observational cohort of 161,108 postmenopausal women enrolled in the Women's Health Initiative (WHI) study, in which an inverse association between the serum levels of SHBG and the incidence of ischemic stroke was found [24].

It seems that the hormonal regulation of fat distribution is a strong variable affecting the differing fat distribution patterns between sexes, but much of the detail is still unknown. The possibility that a healthy diet may limit these menopause-dependent changes is an attractive hypothesis. The PCOS model is again illustrative, as recent clinical studies have shown that diet can worsen, when unhealthy [25], or improve, when healthy [26], hormonal and metabolic changes in the PCOS phenotype.

4. The Role of Healthy Nutrition

Lifestyle has been recommended as a first-line measure against MetS. Physical activity [27] and healthy nutrition are the two most widely promoted interventions [28]. A notable recent initiative is the EAT-Lancet Commission, which has underlined the need to foment healthy diet patterns that are respectful to local traditions and the environment. A healthy reference diet has been defined, with a high consumption of fruits and vegetables together with a reduction of processed meat or refined sugar as the main features [29]. The Mediterranean diet (MedDiet) has been thoroughly investigated and received worldwide recognition as one of the healthiest options [30]. Interestingly, the MedDiet has recently been recommended as a useful ally to manage women's health needs during the menopause transition and after menopause [31].

Olive oil (OO) is among the most widely researched MedDiet components in both experimental models and clinical studies. Furthermore, indications in the literature suggest a role of OO in improving insulin sensitivity [32]. There is a dearth of studies specifically addressing the impact of OO on MetS disorders associated with menopause. However, there is considerable information on the action of OO on the mechanisms and the clinical features associated with the MetS. This information can be taken to better understand the effect of OO in limiting the development of MetS during menopause, as supported by a recent expert consensus [31]. In the coming sections, we will analyze the OO components with the greatest health impact, their effect on basic mechanisms of disease, and the state of the art regarding their action on the main components of MetS. Although there are four main OO subtypes (extra virgin, virgin, refined and pomace) [33], few studies discriminate by subtype, precluding us from considering them separately here.

5. Components in Olive Oil with a Health Impact

Olive oil includes a wealth of compounds, or compound families, in which unsaturated fat far exceeds saturated fats. Polyphenols form another group of compounds that contribute substantially to the health impact of OO.

5.1. Unsaturated Fat

Olive oil conforms to the recommendation put forth since the Seven Countries study [34] that saturated fat should be replaced by unsaturated fat of vegetable origin. The main OO components are oleic acid (70%), classed as a monounsaturated fatty acid (MUFA), and linoleic acid (15%), a polyunsaturated fatty acid (PUFA). Other unsaponifiable fatty acids may also be present in OO, depending on whether the variant is refined, virgin or extra virgin [35] (Figure 3).

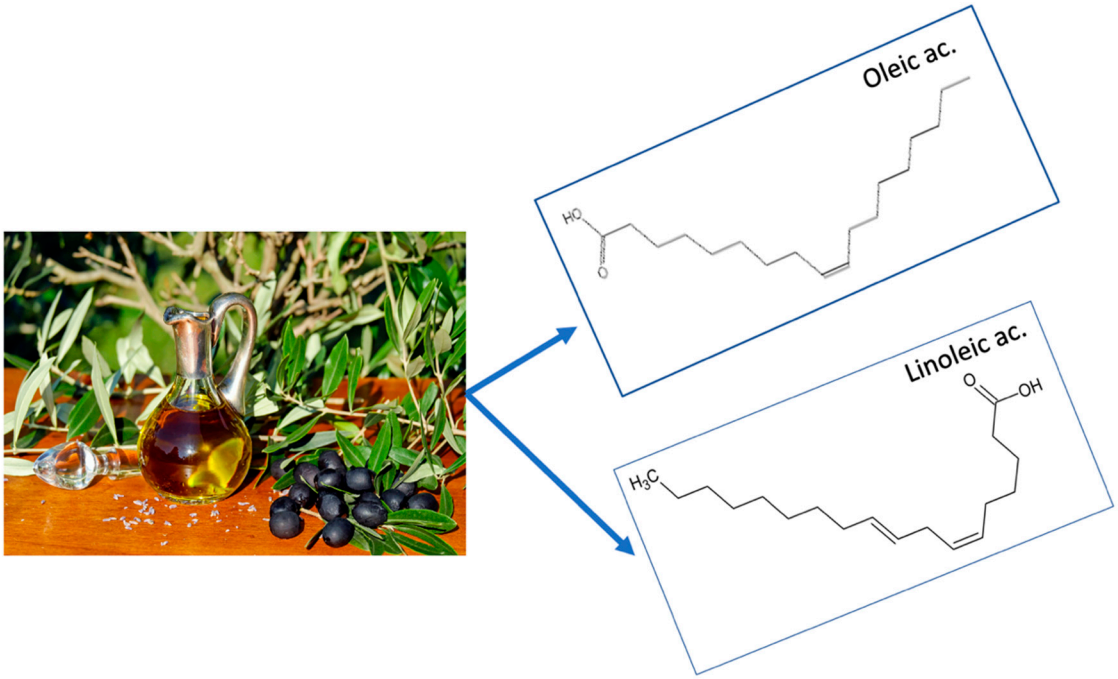

Figure 3. Olive oil is a source of unsaturated fat, whose two main components are oleic acid, a monounsaturated fat making up 70% of the total fat, and linoleic acid, a polyunsaturated fat representing 15% of the total fat content.

5.2. Polyphenols

Polyphenols are a family of phytochemicals with a molecular structure containing phenol rings. Present in a wide variety of food sources, the members of this large family include flavonoids, phenolic acids, lignans and stilbenes, all of which exhibit both antioxidant and anti-inflammatory properties [36,37].

One predominant characteristic of polyphenols is their metabolism in the intestine, where a huge number (ranging between 100,000 and 200,000) of secondary compounds are generated with the intervention of local microbiota. The concentration of secondary metabolites falls sharply, from the mM to μM range in the original source to the nM range in the plasma [38].

The metabolic impact is rapid, as shown in a crossover study in which a reduction in foods containing polyphenols was already reflected in a change in biomarkers, such as the ratio of thromboxane A_2 and prostaglandin I_2, in the urine at the first control at 2 weeks [39].

The interest in polyphenols stems from findings of studies in other foods, for example, in several types of fruits, cocoa, etc., in which those compounds conferred a health protective effect [40,41]. Studies with OO have also supported this benefit.

Polyphenols in Olive Oil

The benefits of the polyphenols in OO have been shown in studies assessing either the effect of the whole family or the specific roles played by particular components.

Total polyphenol excretion in the urine was analyzed in an ancillary sub-study of the PREvención con DIeta MEDiterránea (PREDIMED) trial aimed at testing the efficacy of a MedDiet supplemented with extra-virgin olive oil (EVOO) or nuts, versus a control diet consisting of a recommended low-fat diet for primary CVD prevention [42]. Those of the 1139 participating individuals in the highest tercile of total urinary polyphenol excretion exhibited a lower plasma concentration of inflammatory biomarkers and significant improvement in cardiovascular indicators, namely, blood pressure and lipidograms [36], as reviewed in [43]. A meta-analysis has confirmed that polyphenol content is associated with an improved profile of several cardiovascular risk factors [44].

The specific role of certain polyphenols in OO, such as hydroxytyrosol (HxT) [45] and oleocanthal [46], have attracted particular attention (Figure 4).

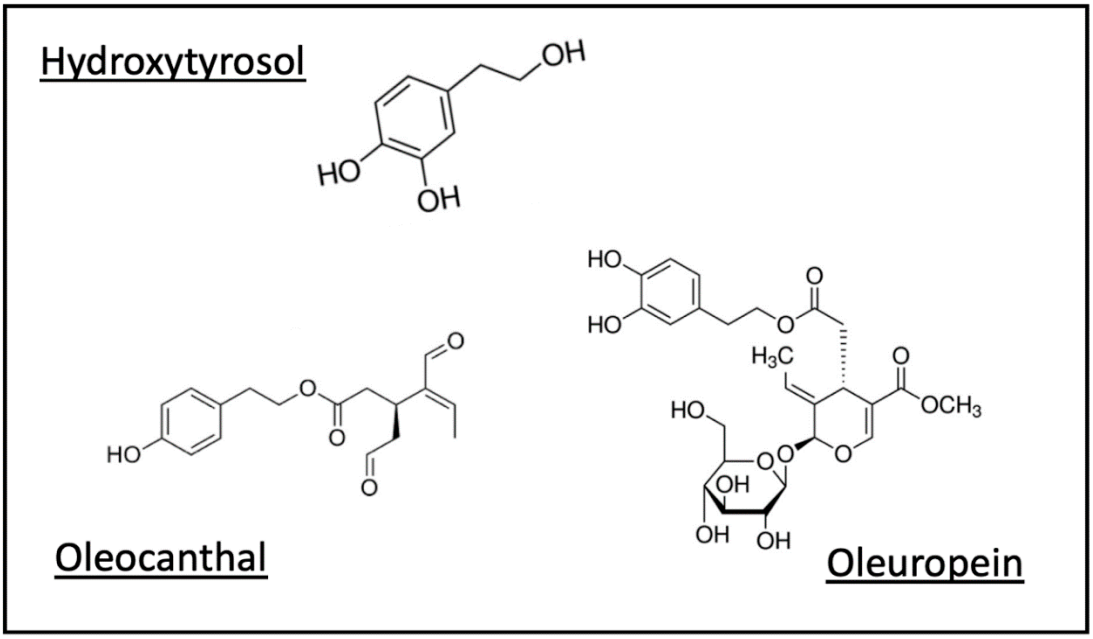

Figure 4. Polyphenols are a family of vegetal compounds characterized by phenol rings as part of their molecular structure. While the family includes a long list of compounds, current data can be found on the health benefits of some, with most available information centered on hydroxytyrosol, oleocanthal and oleuropein.

The levels of 3-*O*-methyl-hydroxytyrosol, a urinary metabolite of HxT, have been reported as inversely correlated with the risk of CVD and overall mortality in elderly subjects [47]. As shown in this study, one important feature of HxT concerns its good bioavailability, in contrast with resveratrol, another molecule that has been ascribed healthy properties based only upon benefits in experimental terms, since in vivo bioavailability is poor [48].

Oleuropein is an ester of HxT that in experimental models has shown preventive effects in early-stage cancer [49]. Work on breast, thyroid and colorectal cell lines has shown anti-proliferative potential and pro-apoptotic effects [49].

6. Effect on General Mechanisms of Disease

Homeostasis in the human body is a direct reflection of functional status at the cellular level. Cellular damage may be the result of various mechanisms, including defects in elementary cellular functions such as respiration or nutrition, and the noxious actions of external agents.

Inflammation and oxidative stress are two intertwined basic mechanisms that play key roles in cellular damage during processes such as ageing [50,51] and disease [52]. Nutrition, which provides micronutrients, metal ions, and other cofactors, is considered to regulate oxidative stress and inflammation [53,54]. Additionally, microbiota have been recognized as a mechanism strongly sensitive to diet.

6.1. Inflammation and Oxidative Stress

Inflammation is an important factor in the onset and progression of sub-clinical phases, as well as in the occurrence of clinical events, in several diseases including CVD [52,55], osteoporosis [56], cancer [57], neurodegenerative disorders and Alzheimer's disease, among others [58,59].

Oxidative stress, in turn, results from an imbalance between oxidant and antioxidant mechanisms. Reactive oxygen species (ROS) are small reactive molecules that regulate crucial biological processes. An excess of ROS generates reactions leading to DNA damage, the modification of proteins and the peroxidation of lipids. As has been noted for inflammation, these biological processes are involved in many diseases, such as atherosclerosis [60], type 2 diabetes [61] and others [62]. For example, a recognized effect of lipid peroxidation is an increase in oxidized LDL (oxLDL), a well-known pro-atherosclerotic factor [63]; this has led to proposed approaches to modulate oxidative stress (redox medicine) [53].

A close relationship exists between inflammation and oxidative stress [62]. For example, oxidative stress has been shown to intervene in the development and perpetuation of inflammation [64]. However, the opposite also occurs, as in the example of atherosclerosis, which is considered an oxidative response to inflammation [65].

The Impact of Olive Oil

The phenolic compounds in OO have shown a well-supported anti-inflammatory capacity [66–68], and clinical studies are confirmatory. The anti-inflammatory properties of the MedDiet have been demonstrated to be owing at least partly to OO [69]. Moreover, a study on high cardiovascular risk subjects showed that a higher intake of OO and nuts was associated with a reduction in several inflammatory markers, as exemplified by C-reactive protein, interleukin-6 and certain adhesion molecules [70]. A more recent randomized controlled trial (RCT) confirmed a differential impact of OO triterpenes [71].

There is also abundant information on the antioxidant effects of OO, which have been investigated using different experimental models [72]. The main MUFA in OO, oleic acid, is more resistant to oxidation than PUFAs. Phenolic compounds also show substantial antioxidant capacity [73–75], as is the case of HxT, which has demonstrated remarkable antioxidant potential in both in vitro and animal experiments—for a review, see [67,76,77]—as well as in healthy volunteers [78]. The free radical scavenging potential of HxT has been recognized by the European Food Safety Authority [79]. The EUROLIVE study has provided additional clinical support in finding that the phenolic content in OO was directly related to a reduction in heart disease risk factors [80].

Considerable antioxidant and anti-inflammatory potential has also been exhibited by oleocanthal, a phenolic compound responsible for the burning sensation at the back of the throat when consuming EVOO [81,82], and by other phenolic compounds [83,84].

6.2. Microbiota

The development of metagenomics technology has advanced the genomic study of microbes in the body. There is growing evidence linking obesity and type 2 diabetes with dysbiosis, a term describing alteration in the composition of intestinal microbiota. Dysbiosis is associated with changes in the intestinal barrier, which facilitate metabolite access to crucial organs such as the liver or fat. An overload of molecules such as lipopolysaccharides (LPS) and other endotoxins results in inflammatory processes, leading to clinically relevant conditions such as the aforementioned obesity [85].

Diet has an important effect on microbiota profiles and turnover [86,87]; for example, it has been shown that the MedDiet may change the gut microbiota, which has a knock-on clinical impact [88]. With regard to OO, an association of the intake of this oil with an increase in the biodiversity of the intestinal microbiota has been shown in rodent models [69]. As a general conclusion, the fatty acids in OO favor composition patterns with a higher prevalence of species that hinder dysbiosis. Polyphenols, by contrast, act as prebiotics and favor, among others, the genus *Bacteroidetes*, which are attributed a protective role against atherosclerosis [89]. Other microbiota modifications have been linked with changes in MetS features or other beneficial outcomes [90–92].

Clinical studies are still sparse and limited by the difficulty of establishing a causative role for the observed microbiota changes in the investigated outcomes. One small-scale RCT researching the effect

of an OO-enriched biscuit found an increase in the output of the gut microbiota and metabolic changes suggestive of reduced oxLDL, although no real change in oxLDL could be detected [93]. Another small-sized RCT found that taking polyphenol-enriched OO for 3 weeks decreased oxLDL levels while increasing bifidobacteria and phenolic metabolite populations [94].

7. Impact on Metabolic Syndrome and Its Components

The protective role of OO against disease was addressed in a meeting of the International Olive Council [95], which highlighted mechanistic studies related to the action of polyphenols and fatty acids. The effect of OO on the MetS has been directly addressed in studies assessing the impact either on the MetS itself, or separately on each MetS component.

7.1. Metabolic Syndrome

There are a wealth of experimental studies, principally with cell cultures or rodent models, showing a role for polyphenols, mainly HxT, in improving MetS features [96,97], including some key ones such as adiposity and insulin resistance [77].

Intervention clinical studies have also yielded some evidence in this area. Supplementing a diet with EVOO, at least 4 tablespoons per day as in the PREDIMED study, was followed by a reversion of the MetS (control vs. EVOO hazard ratio (HR) = 1.35; 95% confidence intervals (CI): 1.15, 1.58) [98]. Some studies have used OO enriched with polyphenols, with mixed or inconclusive results [99] or with a reduction in certain features of the MetS, specifically, glycemia, blood pressure and LDL oxidation [100].

The current consensus is that more long-term RCTs are required to reach consistent conclusions [101]. Despite this, the international panel recommendation for MetS prevention and management through lifestyle included OO consumption at doses of 20–50 g/d along with the MedDiet [102].

7.2. Lipids

The EUROLIVE randomized trial found that the polyphenol content in OO was inversely associated with the total cholesterol/HDL ratio and triglyceride levels [80]. Other studies with virgin OO (30 mL/d) enriched with phenolic compounds and triterpenes have found increased HDL levels [103] and HDL functionality [104]. Further effects have been shown in smaller studies, including oxLDL reduction with polyphenol-enriched OO [94].

The differential impact of the polyphenols in OO has been analyzed in a meta-analysis including papers published up to December 2018. EVOO with a high phenolic content slightly reduced LDL cholesterol when compared with EVOO with low phenolic content (mean difference −0.14 mmol/L; 95% CI: −0.28, −0.01). Additional benefits were found for oxLDL, which showed an inverse dose–response relationship with the intake of phenolic compounds [105].

EVOO at the low dose of 10 g was also associated with a reduction in postprandial triglycerides in subjects with impaired fasting glucose levels [106]. Additionally related to this OO variant, PREDIMED has generated a list of sub-studies that have reported lipid changes associated with the EVOO-supplemented arm. Among these are a reduction in LDL atherogenicity, including resistance against oxidation, particle size, composition and cytotoxicity [107,108] and an increased cholesterol efflux capacity of HDL [108].

7.3. Blood Pressure

Some studies have associated the MUFA in OO with a reduction in vascular tone [109], so a decrease in blood pressure may be expected. Polyphenols are also involved in vascular dilation, according to data obtained from experiments with rodents [110]. Some peptides in OO have also been confirmed to exhibit anti-hypertensive activity through an angiotensin-converting enzyme inhibitory activity [111].

Clinical studies are still sparse, as was acknowledged in a systematic review that, based on data from only 69 subjects, concluded that systolic, but not diastolic, blood pressure was reduced by OO [112]. This review did not include the PREDIMED sub-study, which found a small reduction (mean: −2.3 mm Hg; 95% CI: −4.0, −0.5 for systolic, and mean: −1.2 mm Hg; 95% CI: −2.2, −0.2 for diastolic) in the EVOO-supplemented arm after 1 year in a subset of 235 subjects, with a mean age of 66.5 years, at high cardiovascular risk (85.4% with hypertension) [113]. It is unclear whether the effect may differ in individuals who are normotensive or free of other cardiovascular risk factors.

A more recent meta-analysis on the differential effects of distinct types of OO found an inverse, dose–response association between the phenolic compounds from OO and systolic blood pressure in a secondary analysis [105].

7.4. Body Weight and Waist Circumference

There is experimental evidence supporting a protective effect of the HxT in OO against adiposity [77,97], as reviewed in [96], an action that has also been shown for other polyphenols such as europein [114].

Zamora et al. conducted a systematic review and meta-analysis of RCTs with at least 12 weeks' intervention in adults without CVD, analyzing papers published up to December 2016. Diets enriched in OO were more effective than control diets in weight reduction (−0.92 kg; 95% CI: −1.16–0.67), waist circumference reduction (−0.60 cm; 95% CI: −1.17, −0.04) and lowering body mass index (BMI) (−0.90 kg/m^2; 95% CI: −0.91, −0.88) [115]. PREDIMED was included in the analysis, and the authors acknowledged that the large scale of that study influenced the conclusion.

A subsequent RCT in which OO was compared with coconut oil and butter could not detect any change in weight or waist circumference, but both the sample size (91 subjects) and the intervention duration (4 weeks) were limited [116].

7.5. Dysglycemia and Diabetes

As for the previous MetS components, the data in this area derive from work focused on the impact of polyphenols on different experimental models. There is a general consensus in that OO components, and particularly polyphenols, improve glycemic control [117], as reviewed in [118].

Clinical studies have been included in a meta-analysis of prospective cohort studies and trials. The findings show that the risk of diabetes in individuals in the highest OO intake category was lower than in the lowest one (relative risk (RR) = 0.84; 95% CI: 0.77, 0.92), and OO supplementation in subjects with type 2 diabetes was associated with a more pronounced reduction of HbA1c and fasting glycemia than in control groups [119].

8. Conclusions

The incidence of MetS is growing rapidly in women. The trend is probably influenced by the increase in the rates of obesity as a result of the presence of comorbid insulin resistance. These difficulties are exacerbated in women around the time of the menopause, when hormonal changes begin to promote central obesity. Given the association of metabolic syndrome with disease, this is a vital issue in any strategy focused on healthy ageing.

Guidance is therefore needed to overcome the problem, particularly during the menopause, given that this occurs at midlife, a crucial moment during which the sub-clinical phases of many non-communicable diseases often emerge. A healthy lifestyle, with nutrition as a vital component, needs to be implemented as a primary measure. For successful adoption and adherence, a healthy diet needs to be easy to follow and effective, two conditions successfully met by the MedDiet. The results of the above-presented data indicate that OO is a key food in the MedDiet that may prove especially helpful for women, particularly during this life stage. Experimental and clinical studies in the literature have been used as support. The clinical evidence, however, is limited by the observational nature of most studies.

Author Contributions: Conceptualization, J.J.H.-M., L.C.-S. and A.C.; methodology, J.J.H.-M., L.C.-S., M.-Á.G.-P. and J.J.T.; software, M.-Á.G.-P.; validation, M.-Á.G.-P., J.J.T. and A.C.; formal analysis, J.J.H.-M., L.C.-S., M.-Á.G.-P. and J.J.T.; resources, J.J.H.-M., L.C.-S., M.-Á.G.-P. and J.J.T.; data curation, M.-Á.G.-P. and J.J.T.; writing—original draft preparation, A.C.; writing—review and editing, A.C., J.J.H.-M. and L.C.-S.; visualization, J.J.H.-M., L.C.-S., M.-Á.G.-P., J.J.T. and A.C.; supervision, A.C.; project administration, A.C.; funding acquisition, M.-Á.G.-P., J.J.T. and A.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received funding from grant number 664367 FOCUS, from the Consumers, Health, Agriculture and Food Executive Agency (CHAFEA) of the European Commission, under the European Union Health Programme (2014–2020).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Review

Application of Citicoline in Neurological Disorders: A Systematic Review

Patryk Jasielski *, Faustyna Pięd el, Mikołaj Piwek, Agata Rocka, Véronique Petit and Konrad Rejdak

Department of Neurology, Medical University of Lublin, 20-059 Lublin, Poland; faustyna.piedel@gmail.com (F.P.); miki2398@gmail.com (M.P.); agatarocka2@gmail.com (A.R.); vpetit@onet.pl (V.P.); konrad.rejdak@umlub.pl (K.R.)

* Correspondence: patryk.jasielski111@gmail.com

Received: 5 August 2020; Accepted: 9 October 2020; Published: 12 October 2020

Abstract: Citicoline is a chemical compound involved in the synthesis of cell membranes. It also has other, not yet explained functions. Research on the use of citicoline is conducted in neurology, ophthalmology, and psychiatry. Citicoline is widely available as a dietary supplement. It is often used to enhance cognitive functions. In our article, accessible databases were searched for articles regarding citicoline use in neurological diseases. This article has a systemic review form. After rejecting non-eligible reports, 47 remaining articles were reviewed. The review found that citicoline has been proven to be a useful compound in preventing dementia progression. It also enhances cognitive functions among healthy individuals and improves prognosis after stroke. In an animal model of nerve damage and neuropathy, citicoline stimulated regeneration and lessened pain. Among patients who underwent brain trauma, citicoline has an unclear clinical effect. Citicoline has a wide range of effects and could be an essential substance in the treatment of many neurological diseases. Its positive impact on learning and cognitive functions among the healthy population is also worth noting.

Keywords: citicoline; neurology; supplementation; treatment

1. Introduction

Citicoline is an abbreviation of cytidine-5′-diphosphocholine (CDP-choline). It is an endogenous chemical compound. Citicoline is globally available as a dietary supplement and in many countries as a drug. It can be bought as a tablet or an injection. In the human body, citicoline is degraded to cytidine and choline during hydrolysis and dephosphorylation. Subsequently, cytidine and choline are substrates for phosphatidylcholine and CDP-choline synthesis in neurons. However, the detailed mechanism of citicoline functioning is not well understood [1,2]. Citicoline has minimal toxicity and is rapidly metabolized. Products of metabolization are eliminated as carbon dioxide. Citicoline safety has been repeatedly proven in research based on animals [3].

Citicoline has comprehensive neuroprotective properties. One such mechanisms is an increase in sirtuin-1 level (silent information regulator 1, SIRT1). SIRT1 belongs to the histone deacetylase family. SIRT1 regulates metabolic homeostasis and neuronal aging [4]. It may also have neuroprotective effects and have a beneficial effect on neurodegenerative diseases such as Parkinson's and Alzheimer's diseases [5–7]. Citicoline raises the level and increases SIRT1 activity in the rat brain, cultured neurons, and in peripheral blood mononuclear cells [8].

Another mechanism is related to the influence on the levels of neurotransmitters in synapses. Citicoline increases the level of dopamine and norepinephrine in the central nervous system, which contributes to neuroprotection in hypoxia [9]. Choline, one of the breakdown products of citicoline, serves as a substrate for the synthesis of acetylcholine. This neurotransmitter also has a

neuroprotective effect [10,11]. Citicoline raises the level of serotonin, which is also supposed to promote neuroprotective effects [12]. Citicoline lowers glutamate levels. This neurotransmitter, mainly through the action of the N-methyl-d-aspartate (NMDA) receptor, is responsible for damage to the brain during ischemia [13]. Citicoline is an intermediary for the synthesis of phosphatidylcholine, which is composed of the neuronal cell membrane. Thus, it has neuroprotective properties because a greater availability of phosphatidylcholine may stimulate the repair and regeneration of damaged cell membranes of neurons [12,14,15]. Moreover, when choline is depleted, phospholipids are hydrolyzed to restore choline levels. Acetylcholine synthesis is favored when the available amount of choline is limited. Therefore, citicoline is a source of choline, avoiding phosphatidylcholine hydrolysis [16].

Another likely mechanism of action is to block inflammation (caused by, e.g., ischemia) by inhibiting phospholipase A2. This enzyme is involved in the breakdown of membrane phospholipids into arachidonic acid. The oxidative metabolism of arachidonic acid contributes to the generation of neuroinflammation and reactive oxygen species (ROS). By blocking phospholipase A2, citicoline may contribute to the reduction of inflammation, ROS formation, and neuronal damage [17]. Citicoline may also show anti-apoptotic effects [18]. Citicoline is also beneficial in glaucoma and amblyopia [1,2]. Research on animals and humans demonstrated that citicoline improves brain functions and stunts cognitive deficits [19].

Concluding, in research based on animals and humans, it was proved that citicoline is beneficial in the regeneration of neurons, can increase levels of neurotransmitters, and has a positive impact on cognitive functions. Moreover, it can be an additional drug in the therapy of depression and mood regulation.

2. Materials and Methods

A search for articles about the usage of citicoline in the treatment of neurological disorders was performed. The following databases were analyzed: PubMed, Scopus, Web of Science, Cochrane Library, and Clinicaltrials.gov. The search was undertaken in April 2020. To find articles, the following keywords were used: "citicoline", "neurology disorders", "CDP-choline", and "cytidine-5′-diphosphocholine". A three-step analysis of found articles—title, abstract, and entire text—was undertaken. Two independent scholars conducted the analysis. Inclusion and exclusion criteria are summarized in the table (Table 1). Research based on animals is included in only one subsection about neuropathic pain and neuronal regeneration.

Table 1. Inclusion and exclusion criteria of articles.

Inclusion Criteria	Exclusion Criteria
Written in English	Articles written in a language other than English
Clinical trial, multicenter study, meta-analysis	Review, case report
Studies on animals and humans	Studies other than on animals and humans

The summary of articles analyzed is presented on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart (Figure 1).

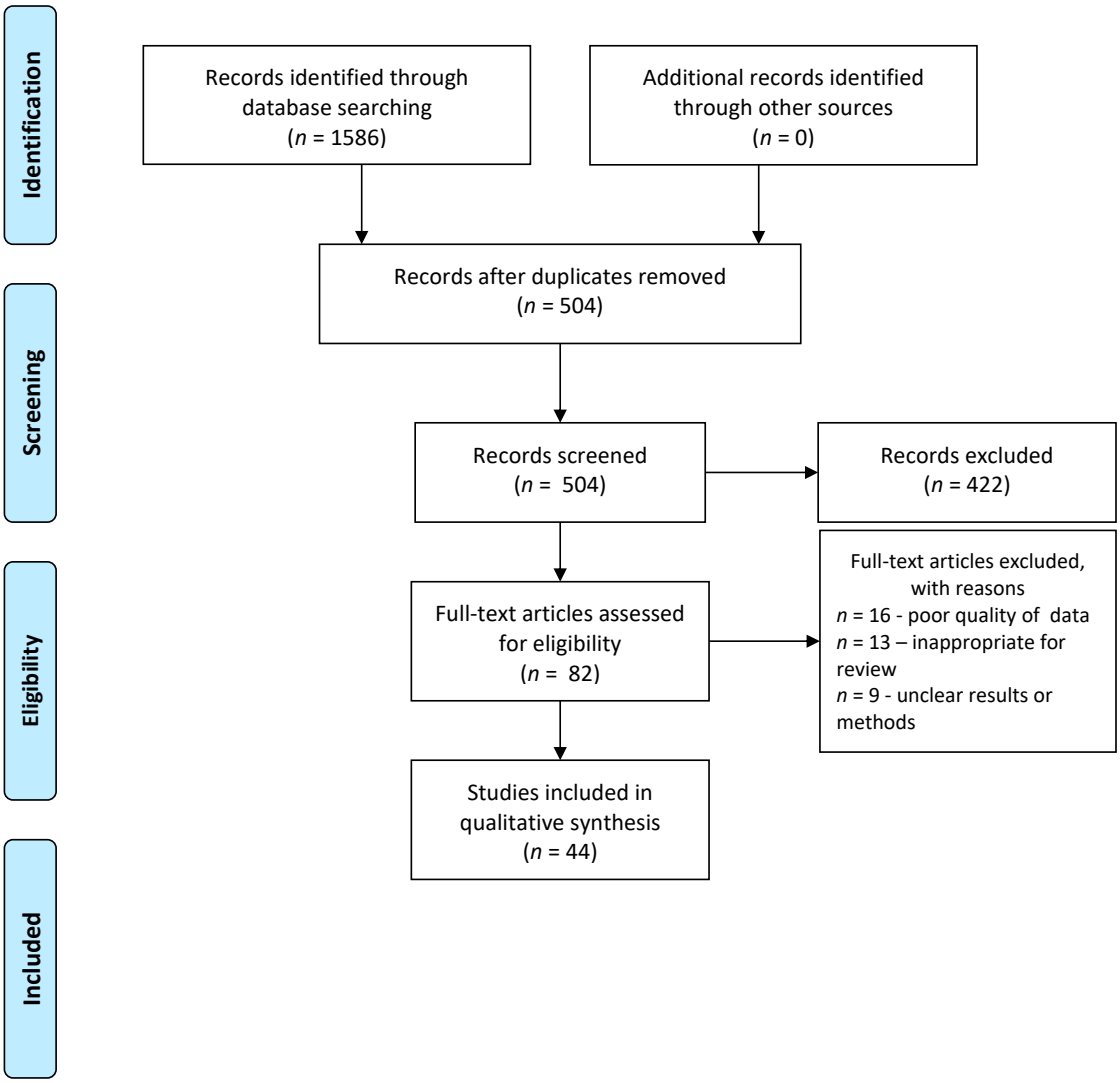

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart.

3. Results

A total of 504 records were screened after the implementation of inclusion criteria. After assessment for eligibility, 47 studies were included in the qualitative synthesis.

3.1. Application of Citicoline in Brain Stroke

Stroke is one of the most common neurological conditions. Depending on its location and size, it can lead to severe neurological disorders or be practically asymptomatic. The application of appropriate rehabilitation, support, and neuroprotective treatment may reduce its harmful effects and facilitate recovery, or at least amend the functioning of the patient [20]. The following scales are used to assess the effectiveness of the applied therapy: Barthel index (BI), National Institute of Health Stroke Scale (NIHSS), and modified Rankin Scale (mRS). The NIHSS and mRS scales are used to assess body dysfunction in patients who have suffered a stroke. The higher the score on both scales, the greater the assessed organism dysfunction and worse functioning [21,22]. The BI scale is used to assess everyday functioning, and ability to cope with basic tasks (including eating, dressing, taking care of hygiene). The higher the result on the BI scale, the better the respondent's functioning [23].

Mehta et al. administrated neuroprotective drugs—citicoline, edaravone, minocycline, and cerebrolysin—in patients with ischemic stroke of the middle cerebral artery. In the citicoline

group, the NIHSS score at baseline was 14.00 ± 4.34, whereas after 11 days, it was 8.90, and after 90 days, it was 3.53. In both measurements, these results were significant compared to the control group ($p < 0.001$). In the BI scale, significant results were also obtained ($p < 0.001$): at the beginning of the therapy, the value was 36.0; on day 11, it was 64.0; and at 90 days, it was 86.0 [24]. Other researchers obtained similar positive results of citicoline. During the 3-month follow-up, stroke patients were divided into the citicoline group and the control group. Patients treated with citicoline achieved significantly higher BI scores both in the 1st and 3rd month ($p < 0.001$ in 1st month, $p = 0.002$ in 3rd month). The obtained results were more favorable in the study group, both in patients after ischemic and hemorrhagic stroke [25]. The advantage of the following study is its longer period of stroke patient observation. Patients received citicoline for one year, 6 weeks after a diagnosed stroke. In the clinical evaluation, both after 6 and 12 months, no significant differences were noticed in the results obtained on the mRS scale by the control group and the group receiving citicoline ($p = 0.186$). However, patients receiving citicoline achieved better results in terms of cognitive functions: attention–executive functions (odds ratio (OR) 1.721, 95% confidence interval (CI) 1.065–2.781, $p = 0.027$ at 6 months; OR 2.379, 95% CI 1.269–4.462, $p = 0.007$ at 12 months) and temporal orientation (OR 1.780, 95% CI 1.020–3.104, $p = 0.042$ at 6 months; OR 2.155, 95% CI 1.017–4.566, $p = 0.045$ at 12 months), even if taking into account risk factors and the severity of the stroke [26]. In addition to clinical improvement, another study examined the effect of citicoline on the risk of death 90 days after stroke. The frequency of incidences of non-neurological complications during hospitalization was also analyzed. In the mRS assessment, patients receiving citicoline after 30 days had significantly lower mean scores and lower median scores ($p = 0.03$ for both). After 90 days, the difference between the groups was no longer statistically significant, while the trend of better results continued in the citicoline group. Adjusting for age, gender, NIHSS at hospital admittance, hospital arrival in <24 h, and relevant risk factors, citicoline treatment was independently associated with a lower 30- and 90-day mortality risk (OR 0.30, 95% CI 0.10–0.88, $p = 0.03$; OR 0.33, 95% CI 0.12–0.87, $p = 0.03$, respectively), and with a lower rate of non-neurological complications acquired during hospitalization (OR 0.20, 95% CI 0.08–0.22, $p = 0.001$) [27]. In the study by Alvarez-Sabín et al. [28], the scholars examined the effect of citicoline on the quality of life of people after ischemic stroke. To assess the quality of life, the EuroQoL-5D scale was used. Patients who received citicoline had a significantly better quality of life compared to the control group ($p = 0.041$). Citicoline use, regardless of the age of the respondents, was an independent factor improving the quality of life and was beneficial for patients after stroke. Moreover, the absence of citicoline treatment (OR 2.321, 95% CI 1.057–5.100, $p = 0.036$) was an independent predictor of poor or very poor quality of life. Additionally, the patients' cognitive functions were examined at 1 month, 6 months, 1 year, and 2 years. After 2 years, patients receiving citicoline had fewer cognitive impairments, but this did not reach statistical significance. However, this group achieved a significant improvement in cognitive functions ($p = 0.005$). The control group did not obtain a significant amendment.

Cho et al. [29] conducted a study on a group of 4191 people. A total of 3736 patients received citicoline up to 24 h after stroke (early group) and 455 received treatment later than 24 h (late group). For clinical evaluation, a short form of the National Institute of Health Stroke Scale (s-NIHSS), a short form of the Barthel Index (s-BI), and mRS were used. S-NIHSS and s-BI at week 6 of citicoline administration were significantly better than at baseline. S-NIHSS improved from 9.8 ± 2.9 at baseline to 6.9 ± 2.4 at 6 weeks ($p < 0.001$), and s-BI improved from 6.0 ± 1.9 to 4.2 ± 1.7 ($p < 0.001$). In 125 patients who received citicoline for more than 12 weeks, further improvements were noted in s-NIHSS at the end of therapy compared to at week 6 (6.4 ± 1.6, $p < 0.001$). The early group showed significantly more improvement in s-NIHSS and s-BI at week 6 (mean s-NIHSS changes between baseline and 6 weeks: early group, 3.0 ± 2.2; late group, 2.1 ± 2.4; $p < 0.001$). After adjustment for age, sex, and risk factors, these results were significant for the s-NIHSS at week 6 ($p < 0.001$). Efficiency of the therapy was dose dependent. Improvements were more significant in the higher dose group (≥2000 mg/day) ($p < 0.001$).

Another study analyzed the effect of different citicoline doses on changes in the BI score using logistic regression analysis with the baseline NIHSS score used as a covariate. There was a significant

treatment effect seen with the logistic regression analysis comparing citicoline treatments to placebo at 12 weeks ($p \leq 0.05$). The OR for improvement in BI on citicoline 500 mg treatment was 2.0, and on 2000 mg, it was 2.1. The total mean BI scores for the four groups at 12 weeks were 56 for placebo, 71 for 500 mg, 55 for 1000 mg, and 65 for 2000 mg. The mean differences in the total BI scores were statistically significant ($p \leq 0.05$) for the 500 mg group compared with the placebo group. Full recovery (BI ≥ 95) after 12 weeks was completed in 33% of patients from the placebo group, 53% from 500 mg, 29% from 1000 mg, and 45% from the 2000 mg group. There was a significant difference between placebo and 500 mg groups ($p = 0.01$). The mean mRS scores for the four groups were 3.1 for placebo, 2.5 for the 500 mg citicoline group, 3.1 for the 1000 mg citicoline group, and 2.6 for the 2000 mg citicoline group [30].

The next study assessed the effect of citicoline on the percentage of independent patients at 3 months using the mRS (independent when mRS score ≤ 2) scale in people after hemorrhagic stroke. Secondary efficacy endpoints included the evolution of neurological deficits assessed with the NIHSS. After 3 months, 5 patients from the citicoline group attained mRS ≤ 2; in the control group, only 1 patient attained this standard (OR 5.38, 95% CI 0.55–52.4). The evolution of NIHSS scores from baseline to 12 weeks were similar and reached statistical significance in both groups ($p < 0.01$) [31].

In another analyzed study, the Scandinavian Stroke Scale (SSS) was used to assess neurological deficits in patients receiving and not receiving citicoline. This scale includes both the prognostic score (which assesses, among other aspects, awareness) and also the long-term score (which determines, among other factors, limbs muscle strength, speech, and gait). A higher score on this scale indicates lower neurological deficits. In the conducted study, differences were noticed on the 3rd day after starting citicoline therapy. In terms of orientation and awareness, 90.1% of the respondents receiving citicoline were fully aware and oriented; while in the control group, only 74.5% were fully aware and oriented (OR 3.12, 95% CI 1.12–8.77, $p = 0.026$). Patients treated with citicoline also had faster improvement in speech. On day 7, no significant differences were noticed in terms of neurological deficits between the groups in the SSS scale (results 41.1 in the study group and 39.1 in the control group). However, on day 21 of follow-up, patients in the citicoline group achieved significantly better SSS scores (49.2 and 44.7 points, respectively, $p = 0.017$). The improvement was noticeable, especially in terms of mobility of the lower limbs ($p = 0.036$) and in gait ($p = 0.002$). The results obtained in the BI and mRS scale on the day of discharge from the hospital (day 21–24) were also better in the research group. The effects on the BI scale were 89.9, while patients in the control group were 82.3, $p = 0.039$. Moreover, in the group treated with citicoline, a significantly higher percentage of patients obtained the result of a complete return to the ability to perform daily activities (BI > 90) (OR 4.01, 95% CI 1.73–9.37, $p = 0.006$). The reference group included more patients with unsatisfactory recovery, i.e., with total points scores of ≤60 (OR 3.48, 95% CI 1.37–8.95, $p = 0.013$). In addition, on the mRS scale, patients from the research group obtained better results: 54 did not have significant symptoms and were able to perform everyday duties, whereas such an outcome was obtained by 17 people from the control group (OR 4.01, 95% CI 1.73–9.37, $p = 0.0006$) [32].

Iranmanesh et al. [33] assessed the effect of citicoline on muscle strength in patients after hemorrhagic stroke. Muscular strength was assessed using the Lovett scale. The mean muscular strength before intervention in all patients was 2.5 (1–4). After treatment, in the citicoline group, the mean muscle force increased to 4 (1–5), and in the placebo group, it increased to 3.12 (1.5–5) (Mann–Whitney test, $p = 0.019$). Citicoline helped to restore muscle strength to a greater extent than placebo.

The Japanese Coma Scale (JCS) and Global Improvement Rating (GIR) were used in another study. GIR was assessed as six categories based on changes in the level of consciousness, individual neurologic signs, and the patient's general condition. Improvements in the level of consciousness were similar between groups on days 1, 2, and 3, but they were significantly improved for the citicoline group on days 7 and 14. The rates of improvement were calculated at the final assessment: 51% for the citicoline group compared with 33% in control group ($p < 0.05$). On Days 2, 7, and 14, the GIR of the citicoline

group was significantly better than that of the placebo group. Improvement was noted in 32% and 18% on Day 7, and 54% and 29% on Day 14, for the citicoline and placebo groups, respectively. Rates of improvement at the final assessment were 52% for patients receiving citicoline and 26% for those receiving placebo ($p < 0.01$) [34].

In a study by Sobrino et al. [35], the influence of citicoline on the number of circulating endothelial progenitor cells (EPCs) was assessed in correlation with the improvement of the health assessed by NIHSS and mRS scales. The level of EPCs is associated with cardiovascular risk. Higher concentrations of these cells indicate a better prognosis. Some of the patients receiving citicoline were also treated with recombinant plasminogen activator (14 patients, 12 without such treatment). An increase in the number of colony-forming unit–endothelial cell (CFU-EC) units 1 week after the start of treatment was considered an indicator of a high probability of good treatment outcomes. Such an increase was significantly more frequent ($p < 0.0001$) among those receiving citicoline alone as compared to in combination with thrombolytic therapy. In the model, after taking into account the size of the stroke and the time from the onset of stroke, the administration of citicoline (OR 17.6, 95% CI 2.3–137.5, $p = 0.006$) and citicoline with thrombolytic drugs (OR 108.5, 95% CI 2.9–1094.2, $p = 0.001$) were independently associated with an increase in CFU-EC ≥ 4. After 3 months, the results obtained in the NIHSS ($p = 0.003$) and mRS ($p = 0.012$) scales were significantly better in the group receiving citicoline, both alone and with a thrombolytic drug. In addition, the amount of CFU-EC in this group after 3 months was significantly higher than that in the control group ($p < 0.0001$).

Another study investigated the effect of citicoline administration on the levels of angiostatin, neurofilaments, and acid fibril protein in people after an ischemic stroke associated with atrial fibrillation. Acid fibril protein is a sensitive marker of brain tissue damage and the effectiveness of neuroprotection. In humans, the concentration of this protein correlates with the formation of the astrocytic scar after trauma or stroke. Neurofilaments are considered a marker of neuronal death and may be useful in determining the prognosis after stroke. The last substance to be tested was angiostatin, which is an anti-angiogenic substance. However, the role of this substance in ischemic stroke is still unclear. In the citicoline group, the levels of both neurofilaments and acid fibril protein were significantly lower ($p < 0.05$ and $p < 0.01$, respectively). In addition, the level of angiostatin decreased to a significant extent (by 40% to the baseline value) ($p < 0.05$). In the control group, there was no change in the level of the tested substances in relation to the base concentration. On this basis, the authors concluded that citicoline has a protective effect on astrocytes and neurons. Moreover, it has a beneficial impact on the regulation of angiogenesis in an ischemically damaged brain [36].

In the next study, patients with hemorrhagic stroke took part. The influence of citicoline on the level of acid fibril protein, and on the level of copeptin, was also assessed. Copeptin is a glycoprotein that is thought to be a prognostic factor in stroke. After 28 days of treatment, both the levels of fibril acid and copeptin in the citicoline group were significantly lower than in the control group (both $p < 0.05$). Patients were also assessed using the NIHSS and BI scales. After 28 days, the NIHSS result in the research group was 9.43, while in the control group, it was 14.56 ($p < 0.05$). Similarly, on the BI scale, people from the research group obtained better results (69.28 vs. 51.57) ($p < 0.05$). In both groups, all the parameters tested had a similar value at the beginning of the experiment [37].

Another potential effect of citicoline in ischemic stroke may be its effect on cerebral perfusion. At the beginning of the treatment, patients underwent transcranial and extracranial Doppler ultrasound examinations of cerebral circulation. The flow in the following arteries was assessed: common carotid, internal carotid, anterior, middle and posterior brain, ocular, vertebral, and basilar arteries. There were significant differences between the groups in the maximum systolic velocity and the mean flow velocity. The maximum systolic velocity differed significantly in the following arteries: right common carotid ($p = 0.008$), internal carotid ($p = 0.031$), right ($p = 0.008$) and left ($p = 0.002$) vertebral arteries; in all of these arteries, the velocities measured were lower in the citicoline group. The mean flow velocity was significantly different between the right internal carotid ($p = 0.031$) and left anterior cerebral artery groups ($p = 0.033$); the measured speeds were also lower in the citicoline group. The study groups,

with the exception of the supply of citicoline, did not differ in clinical features. This study was the first of its kind to investigate the effects of citicoline on cerebral perfusion. The clinical significance of these differences is not fully understood. Further research is recommended [38].

In a meta-analysis by Secades et al. [39], ten studies were included, in which a total of 4420 patients participated. The primary efficacy measure was patient independence at the end of the scheduled follow-up. For this purpose, the mRS scale was used (mRS score of 0–2 indicates independence of patient). In the studies where mRS was not available, the most comprehensive measure of disability from trial was used. The administration of citicoline was associated with a significant higher rate of independence, regardless of the method of evaluation (OR 1.56, 95% CI 1.12–2.16 under random effects; OR 1.20, 95% CI 1.06–1.36 under fixed effects). The time gap between studies is 32 years. Hence, a significant level of heterogeneity was observed ($p = 0.0002$).

All of the analyzed articles noted thus far indicated that citicoline is an effective drug in the improvement of the clinical condition of patients after stroke. However, based on subsequent articles, it can be concluded that the decisive role of citicoline is uncertain.

In a multicenter study called ICTUS, in which over 2000 patients participated, more than 1000 received citicoline for 6 weeks after an ischemic stroke event. A total of 873 were included in the protocol (the rest died or did not meet the requirements). Ninety days after the start of treatment, patients enrolled in the protocol were re-assessed using the NIHSS, mRS, and BI scales. After this period, the general state of health was similar in both groups (OR 1.03, 95% CI 0.86–1.25; $p = 0.364$). After 90 days, mRS ≤ 1 was achieved by 169 (19.4%) subjects in the citicoline group and 163 (19.2%) subjects in the placebo group (OR 1.04, 95% CI 0.79–1.36). On the NIHSS scale, the result of ≤1 in the study group was obtained by 209 (24.1%) patients, and in the control group, the result of ≤1 was achieved by 190 patients (22.3%) (OR 1.17, 95% CI 0.91–1.51). In BI, the result of ≥95 was obtained in the group receiving citicoline by 250 (28.8%) of the patients, and in the control group, it was obtained by 246 patients (28.9%) (OR 1.01, 95% CI 0.79–1.28). The obtained mRS results were similar for both groups. In the group covered by the protocol, the mean raw improvement in the NIHSS scale in the research group was 2.18, and in the control group, it was 0.91 ($p = 0.051$). However, in the group of people over 70 years of age, citicoline was found to have more beneficial properties for functioning than in the younger group of patients (OR 1.17, 95% CI 0.92–1.50, $p = 0.001$). The same relationship was observed for patients with an NIHSS score < 14 (OR 1.08, 95% CI 0.86–1.35, $p = 0.021$) and for patients not treated with thrombolytic therapy (OR 1.11, 95% CI 0.85–1.46, $p = 0.041$). Authors included an interpretation of their study and an updated meta-analysis consisting of six clinical trials. When the best treatment was applied, citicoline did not display any clinical improvement. Nevertheless, the effect of the drug remains significant (OR 1.14, 95% CI 1.00–1.30), which is based on an updated fixed-effects meta-analysis. Moreover, in the meta-analysis, a significant heterogeneity of effects ($p = 0.0029$) between the previous studies and the ICTUS trial was observed. Beneficial properties of citicoline for patients over 70 years of age, who did not receive thrombolytic therapy and with an NIHSS score < 14, and a lack of these positive outcomes in other groups, require further analyses. Mortality and the frequency of side effects were similar in both groups [40]. Another study tested the effectiveness of citicoline and edaravone in patients after ischemic stroke. Three months after starting treatment, the patients' condition was assessed using the NIHSS and mRS scales. Patients receiving citicoline had slightly better results than the control group on the mRS scale (1.95 vs. 2.08) and also on the NIHSS scale (6.41 vs. 7.08). However, these differences were not significant [41].

The meta-analyses mentioned before [39,40] were analyzed by Yu et al. [42]. The authors used a parametric analysis of meta-analyses, maximum likelihood estimate (MLE), which can be interpreted as a weighted average of the study-specific estimate of the effect size with a shrinkage and Bayes estimator for standardized mean difference. The authors presented also a method to account for publication bias. Using their methods, the researchers obtained slightly lower OR values than in previous meta-analyses. However, the obtained OR values still remained statistically significant. OR for the meta-analysis by Secades et al. was 1.10, 95% CI, 1.02–1.17, and for study ICTUS, it was OR 1.08, 95% CI, 1.01–1.16.

Clark et al. [43] used BI and NIHSS to assess the effectiveness of citicoline. Unfortunately, after 12 weeks, no significant differences between the control and citicoline group were observed ($p > 0.05$). In patients with mild baseline strokes (NIHSS < 8), no differences were seen between groups. However, for patients with moderate-to-severe strokes (NIHSS ≥ 8), citicoline treatment was beneficial. In this group, BI ≥ 95 was attained by 33% vs. 21% in the control ($p = 0.05$), mRS ≤ 1; 19% vs. 11% ($p = 0.07$), and NIHSS ≤ 1; 19% vs. 11% ($p = 0.08$). In this group, citicoline treatment appears to be an overall benefit (OR 1.9, $p = 0.04$). Moreover, the percentage of patients who had large improvements in their NIHSS scores (≥7) at 12 weeks compared with the baseline was evaluated. A higher percentage (42%) of citicoline-treated patients had improvement compared with patients receiving placebo (30%) ($p = 0.01$). Similar results were obtained in the next study [44]. After 90 days, no significant differences were observed between the citicoline and placebo groups (52% vs. 51% patients attained NIHSS ≥ 7). Furthermore, there were no between-group differences in mortality.

Another study assessed changes in the volume of ischemic lesions using magnetic resonance imaging (MRI) and changes in clinical condition. The primary statistical analysis of the difference in the distribution of changes in lesion volume between placebo and citicoline groups from baseline to week 12 by the Smirnov test was not significant ($p = 0.18$). In patients who were treated 12 h or less from stroke onset, a larger growth of the lesion (mean ± SE) between baseline and week 12 was observed in the placebo group than in the citicoline group (30.3 ± 11.4 craniocaudal (cc) vs. 10.5 ± 6.0 cc planes, respectively). However, there were no significant differences between treatment groups in any of the clinical outcome measures. Citicoline did not significantly improve either clinical outcome or brain lesion in MRI [45].

Based on the above analysis, it is not possible to unequivocally state whether citicoline is an effective drug improving the clinical condition of people after stroke. In the vast majority of studies, citicoline had a significant beneficial effect both on the clinical situation and on molecular changes. However, one sizeable multicenter study undermined the efficacy of citicoline and showed its uncertain effectiveness. Further research is required to determine the effectiveness and potential mechanisms of the action of citicoline. The most important features of the research cited above are summarized in the table (Table 2).

3.2. Citicoline Usage in Cognitive Functions Betterment, Dementia Prevention, and Treatment

Cognitive functions are understood as being memory, attention, speech, awareness, and other, more complex functions, e.g., abstract thinking, judging, and calculation. These are essential functions in everyday situations. The decline of those skills due to aging, dementia, or brain damage is linked with increased hindrance in work and a self-reliant life. Due to an aging society, the problem of dementia will become increasingly prevalent and begin to impact society and its finances. Hence, it is crucial to research substances that can impede the negative effects of such processes. Another important aspect is the usage of such compounds to enhance natural abilities in school and work. The application of safe drugs that can improve learning and the ability to work could potentially impact society and contribute to its development.

During cognitive functions assessment, many scales and questionnaires are used. The Mini-Mental State Examination (MMSE) is a screen test to assess mental functions and dementia: the higher the score, the better preserved are cognitive functions. The correct results range from 27 to 30. The next scales are Activities of Daily Living (ADL) and Instrumental Activity of Daily Living (IADL). ADL is used to assess daily activities crucial for survival, such as hygiene and nutrition. IADL encompasses activities that are not essential for survival (finances, transportation), but they greatly enhance the self-reliance of individuals and raise the quality of life. A higher score in both scales attests to the better functioning of a person. The Neuropsychiatric Inventory (NPI) is used in the evaluation of typical neuropsychiatric symptoms, such as mood and behavior alteration, and stimuli perception. Higher scores in this scale are linked with a higher intensity of symptoms. The Geriatric Depression Scale (GDS) is used in depression assessment. The higher the score, the more severe the depression.

Table 2. The most important features of research based on patients after a stroke event.

References	Number of Patients Received Citicoline	Mean Age (Years)	Dose of Citicoline (mg/Daily)	Period of Citicoline Administration (Days)	Used Methods to Assessment Effectiveness of Citicoline	Frequency of Examination by Scales (Days after Start Treatment)
Mehta et al. [24]	20	59.5	500 × 2	42	NIHSS, BI	0, 11, 90
Ghosh et al. [25]	50	64.02	1000 × 2500 × 2	5 and 25	BI	30, 90
Alvarez-Sabín et al. [26]	172	67.2	1000	365	mRS	30, 180, 365
Jiménez et al. [27]	86	68.6	1000, 500	9	NIHSS, mRS	30, 90
Alvarez-Sabín et al. [28]	86	67.5	1000	365	EuroQol-5D	30, 180, 365, 2 years
Cho et al. [29]	4191	67.04	500–2000	42 and 84	s-NIHSS, s-BI, mRS	42, 84
Clark et al. [30]	195	67.5	500, 1000, 2000	42	BI, NIHSS, mRS	7, 21, 42, 84
Secades et al. [31]	19	74.5	2000	14	mRS, NIHSS	14, 90
Martynov et al. [32]	89	62.7	1000	21	SSS, BI, mRS	1, 7 and 21 (SSS); 21–24 (BI, mRS)
Iranmanesh et al. [33]	16	61.15	500	14	Lovett scale	90
Tazaki et al. [34]	133	-	1000	14	JCS, GIR	1, 2, 3, 7, 14
Sobrino et al. [35]	26	71.4	2000	42	NIHSS, mRS	7, 90
Tykhomyrov et al. [36]	33	76	1000	14	-	-
Zang et al. [37]	52	57.53	500	28	NIHSS, BI	28
Seifaddini et al. [38]	32	-	500	7	-	-
Dávalos et al. [40]	1148	72.9	2000 × 21,000 × 2	3 and 39 (42 overall)	NIHSS, BI, mRS	90
Mittall et al. [41]	24	54.83	500 × 2	42	NIHSS, mRS	90
Clark et al. [43]	267	70	500	42	NIHSS, BI, mRS	7, 21, 42, 84
Clark et al. [44]	453	68	2000	42	NIHSS, mRS	90
Warach et al. [45]	41	68.5	500	42	BI, NIHSS, mRS, MRI	7, 42, 84

Barthel Index (BI); National Institute of Health Stroke Scale (NIHSS); modified Rankin Scale (mRS); Scandinavian Stroke Scale (SSS); The Japanese Coma Scale (JCS); Global Improvement Rating(GIR); magnetic resonance imaging (MRI).

In the first cited paper, citicoline was combined with rivastigmine, and the effect of the combination was measured among mixed dementia (MD) and Alzheimer's disease (AD) patients. Patients were tested at onset as well as in the 3rd and 9th month of the study using many scales: MMSE, ADL, IADL, NPI, and GDS. At the beginning of the study, there were no noticeable differences between groups on every scale. In the 3rd ($p = 0.001$) and 9th ($p = 0.000$) month, the MMSE results of citicoline users were significantly better (2 points on average) compared to the control group. NPI results proved to be better in the research group (7.12 vs. 9.51; $p = 0.000$). Results achieved in ADL, IADL, and GDS were not noticeably different between control and research groups [46]. Similar research was conducted by Controneo et al. [47]. In this research, patients had mild vascular dementia (MMSE ≥ 21). Significant differences in MMSE were not observed between the groups. However, there was a steep decline in the control group in comparison to citicoline users in the 3rd and 9th month of the study (in both, $p = 0.0001$). In the test group, there was no drop in MMSE results; the control group noticed a significant decline. ADL and IADL results were similar in both groups. However, GDS results were slightly better among citicoline users, albeit these results were not statistically significant ($p = 0.06$). Citicoline, despite not enhancing cognitive functions in this research, prevented dementia development.

In another study, citicoline was combined with an acetylcholinesterase inhibitor in Alzheimer's disease patients. In this study, the group receiving citicoline achieved higher results in MMSE both in the 3rd month (16.88 vs. 17.62; $p = 0.000$) and in the 9th month (17.62 vs. 17.89; $p = 0.000$) of treatment. This research also did not record significant differences in ADL, IADL, NPI, and GDS between groups [48]. The next analyzed research regarded the citicoline effect on mild cognitive impairment in Parkinson's disease. To assess the function level of patients, two scales were employed: Montreal Cognitive Assessment (MoCA) and Scales for Outcomes in Parkinson's Disease-Cognition (SCOPA-COG). These are the scales used in the diagnosis of mild cognitive function impairment; the higher the score, the better functioning of the individual. At the onset of the study, there was no difference in MoCA and SCOPA-COG scores between the control and research groups. After 12 months of study, the results of the citicoline group were not significantly better than the control group (23.65 vs. 22.53; $p > 0.05$). However, in the 18th month, the discrepancy between results was bigger (23.12 vs. 21.49; $p < 0.05$). On the SCOPA-COG scale, results achieved by citicoline users were visibly better both at 12 months (21.55 vs. 20.73, $p < 0.05$) and 18 months (21.09 vs. 19.25, $p < 0.01$) of study. Citicoline did not improve results on both scales. Starting results in MoCA and SCOPA-COG were 24.03 and 23.79, respectively. However, citicoline did inhibit the progression of cognitive function impairment [49].

The pro-cognitive effects of citicoline and the slowing of the development of age-related brain lesions may be due to its action on the increase in phosphodiesters (PDE) levels in the brain. Healthy older patients participated in the study. After 6 weeks of taking the drug, an average 7.3% increase in brain PDE (including glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE)) was observed ($p = 0.008$). After 12 weeks, no significant increase in PDE levels was observed compared to those taking citicoline. Interestingly, the increase in PDE levels correlated with the improvement in verbal learning on the California Verbal Learning Test (CVLT) scale [15].

Alvarez et al. [50] assessed the effect of citicoline on cognitive functions and cerebral flow in the middle cerebral artery (MCA) in patients with Alzheimer's disease. The Alzheimer's Disease Assessment Scale (ADAS) and the Clinical Interview Based Impression of Change (CIBIC) were used to assess cognitive functions. ADAS assesses basic cognitive functions; the higher the score, the worse the functioning. After 12 weeks, the difference between the groups in the CIBIS scores did not reach statistical significance. In contrast in the ADAS scale, the citicoline group achieved significantly better results (difference between groups 3.23 ± 1.8, $p < 0.05$). In cerebral flows in the citicoline group, a significant increase in mean systolic and diastolic velocities in MCA compared to the placebo group was observed ($p < 0.05$)

In a study by Spiers et al. [51], the effect of the drug on memory was assessed in healthy volunteers. The Logical Memory subtest stories of the Wechsler Memory Scale (WMS) and the Wechsler Memory Scale-Revised (WMSR) were used to assess memory. After 3 months, patients from the citicoline group

with relatively inefficient memories had significantly better results in delayed logical memory test compared to baseline results ($p < 0.05$). Citicoline has not been shown to improve memory in people with efficient memory.

Cohen et al. [52] assessed the effectiveness of citicoline in people with vascular dementia. The drug was taken for a year. A battery of neuropsychological tests was used to assess neuropsychological functioning, consisting of a series of tests assessing various cognitive functions. Participants also had the MRI. There were no significant differences in neurocognitive change scores between baseline and either the 6- or 12-month assessments across any of the cognitive domains between the placebo and citicoline group (in all tests $p > 0.05$). The changes observed in the MRI also did not differ significantly between the groups ($p = 0.17$). Citicoline in this study proved unsuccessful.

Another study focused on citicoline impact on motor functions and attention among teenagers (13–18 years old). To assess motor functions, the Finger Tap Test was conducted. The Finger Tap Test (FTT) consists of the Finger Tap Total Dominant Hand (FTDH) and Finger Tap Total Non-Dominant Hand (FTNDH). In this test, the subject has to tap the button with a selected finger as fast as possible during a specific time. In attention evaluation, the Ruff 2&7 Selective Attention Test (RSAT), which consists of RSAT speed and RSAT accuracy, the Computerized Performance Test, Second Edition (CPT-II), divided into CPT-II detectability (ability to focus on computer tasks) and CPT-II commission errors (impulsiveness test), were used to evaluate attention. At the start of the study, no difference was observed in FTT, RSAT, and CPT-II between the control and test groups. Results in FTDH were higher in the test group in comparison to the control group after 28 days of citicoline supplementation ($p = 0.03$); however, differences in FTNDH did not reach statistically significant values ($p = 0.62$). In the RSAT speed test, the citicoline supplemented group also reached higher scores than the control group ($p = 0.02$), but in RSAT accuracy, the difference was small ($p = 0.86$). Compared to the start of the research, the citicoline users achieved significantly better results both in CPT-II detectability and CPT-II commission error ($p = 0.03$ and $p = 0.01$, respectively). Citicoline supplementation was linked with motor function enhancement, attention betterment, and impulsiveness reduction among test subjects [53].

The next study focused on citicoline combined with the effect of drinking on behavior control and executive functions. The Continuous Performance Task (CPT), which was used in the evaluation of concentration, memory, and impulse control, was used in the study. Other examinations used were the Austin Maze (hand–eye coordination and memory), Go/No-Go task (concentration, behavioral inhibition), and Digit Symbol Substitution test (attention, spatial coordination, and information processing). The last performed test was the Trail Making Test, which assessed information processing speed, brain plasticity, and executive functions. Subjects had an electroencephalography (EEG) taken in repose and during an examination. Thirty minutes after the drink was consumed, EEG results were taken. People who drank citicoline with caffeine solved the Austin Maze significantly faster than the control group ($p = 0.008$), and learned faster how to solve it ($p = 0.008$) (people from the test group solved the labyrinth in 134 s on average, whereas the control group averaged 186 s). The number of trials needed to solve the test perfectly was also significantly lower in the test group ($p = 0.028$). In CPT, people under the effect of citicoline had a faster reaction time ($p = 0.001$) and made fewer mistakes ($p = 0.001$) in comparison to the placebo group. In Digit Symbol Substitution tests, those solved by supplemented people had more positive answers ($p = 0.008$), and Go/No-Go had significantly fewer errors ($p = 0.006$) than the control group. However, there was no significant difference in the Trail Making Test between groups. Those results show that citicoline increases the capability to remember and improves concentration and perceptivity. There was increased brain activity in the frontal lobe and prefrontal cortex in the EEG measured by event-related potentials (P450) ($p < 0.05$). Patients who received citicoline had higher amplitude-measured potentials [54].

Bruce assessed the effect of a citicoline-containing drink on changes in the potentials recorded in the EEG during rest and performing an intellectual task (event-related potentials, ERPs). The alpha and gamma waves visible in the EEG were considered to be modifiable by attention and were therefore measured. The Auditory Oddball test was used as a task to assess awareness, in which the subjects

were to ignore low-pitched sounds (500 Hz) and respond to high-pitched sounds (1000 Hz). The test was performed 30 min after drinking the citicoline drink. In ERPs, waves with two potentials were distinguished: N100 and N200. N100 waves are related to focus and arousal, whereas N200 waves are considered to be related to cognitive processes. People who received the citicoline drink had significantly higher alpha wave amplitudes ($p < 0.05$). In terms of gamma waves, no significant differences were found. In an event-related potential (ERP), a significant increase in N100 waves was noted compared to the placebo ($p < 0.05$). There were no significant differences in the N200 wave range. Citicoline, in this study, raised attention among healthy people [55].

Another study assessed the effects of citicoline on cognition with the CogState Battery. This test assesses, among others, memory, attention, and decision-making abilities. Based on the results obtained, 24 volunteers were divided into three groups: those with low results, average results, and high achievements, each consisting of eight people. Then, in each group, some people received a placebo, and some received citicoline, in either 500 or 1000 mg doses. In terms of psychomotor skills, in the group with low results, both 500 mg ($p < 0.006$) and 1000 mg ($p < 0.001$) of citicoline significantly improved the results compared to those receiving placebo. Citicoline did not affect the results obtained by the other groups. In terms of attention, citicoline did not significantly improve the results in any of the groups. Working memory was enhanced considerably in the low-score group, both at 500 mg ($p < 0.008$) and 1000 mg ($p < 0.021$); citicoline did not affect the other groups. In terms of problem-solving, citicoline also proved to be significantly beneficial in the low-score group, both at 500 mg ($p < 0.005$) and 1000 mg ($p < 0.037$). In terms of delayed memory, only in the group with low results did citicoline significantly improve the results obtained at the dose of 1000 mg ($p < 0.042$). In verbal memorization, the 1000 mg dose significantly improved the results of the group with a low initial result ($p < 0.0001$). In delayed oral memorization, citicoline at doses of both 500 and 1000 mg also improved the results of the group with low initial scores ($p < 0.033$ and $p < 0.042$, respectively). Interestingly, citicoline reduced the results obtained by the group with high initial scores significantly ($p < 0.05$) in all of the examined aspects. This study demonstrated that citicoline improved cognitive functions in people with low initial functioning levels, but it decreased these functions in people with high initial levels of functioning [56].

Another study examined the effect of citicoline on cognitive functions in people with somatoform disorders. Cognitive functions were assessed using the Cognitive Emotional Regulation Questionnaire (CERQ), and impulsivity and attention were assessed using the Test of Variables of Attention (TOVA). The tests were performed initially, after 30 days (at the end of citicoline administration), and after 60 days. On day 60 of treatment initiation, patients receiving citicoline achieved significantly better TOVA scores ($p < 0.05$) than those receiving placebo. They also outperformed the baseline ($p < 0.05$).

Similarly, on the CERQ scale, the results of the test group were significantly better than those of the control group ($p < 0.05$) in terms of long-term thinking, planning, and the ability to focus. Most of the aspects examined also showed significant improvement compared to the start of treatment ($p < 0.05$). To summarize, citicoline has been proven to be useful as a means of improving the cognitive skills and functioning of patients with somatoform disorders [57].

Citicoline, in almost all of the studies, has been found to be an effective drug in terms of its influence on cognitive functions. In patients with dementia of various origins, it inhibited the progression of the disease during observation and improved their daily functioning. It has also proved useful in improving the results achieved in tests assessing cognitive functions in healthy people. The most important features of research cited above are summarized in the table (Table 3).

Table 3. Key clinical features of studies in dementia patients and healthy volunteers.

References	Number of Patients Received Citicoline	Mean Age (Years)	Dose of Citicoline (mg/Daily)	Period of Citicoline Administration (Days)	Used Methods to Assessment Effectiveness of Citicoline	Frequency of Examination by Scales (Days after Start Treatment)
Castagna et al. [46]	92	81.3	1000	270	MMSE, ADL, IADL, NPI, GDS	90, 270
Cotroneo et al. [47]	265	79.9	500 × 2	270	MMSE, ADL, IADL, GDS	90, 270
Gareri et al. [48]	251	-	1000	270	MMSE, ADL, IADL, NPI, GDS	90, 270
Zhenguang et al. [49]	41	61.7	200 × 3	18 months	MoCA, SCOPA-COG	12, 18 months
Alvarez et al. [50]	13	73	1000	84	ADAS	84
Babb et al. [15]	19	70.3	500	42, 84	CVLT	42, 84
Spiers et al. [51]	46	67.2	1000	90	WMS, WMSR	30, 90
Cohen et al. [52]	15	78.1	1000			180, 360
McGlade et al. [53]	51	15.41	250/500	28	FTT, RSAT, CPT-II	28
Bruce et al. [54]	30	24.2	250	-		30 min
Bruce et al. [55]	10	28.1	250	-	EEG	30 min
Knott et al. [56]	24	21.3	500/1000	-	Cogstate	12-14
Chutko et al. [57]	46	32.3	1000	30	TOVA, CERQ,	30, 60

Mini-Mental State Examination (MMSE); Activities of Daily Living (ADL); Instrumental Activity of Daily Living (IADL); The Neuropsychiatric Inventory (NPI); Geriatric Depression Scale (GDS); Montreal Cognitive Assessment (MoCA); Scales for Outcomes in Parkinson's Disease-Cognition (SCOPA-COG); The Alzheimer's Disease Assessment Scale (ADAS); the California Verbal Learning Test (CVLT); the Wechsler Memory Scale (WMS); the Wechsler Memory Scale-Revised (WMSR); The Finger Tap Test (FTT); Ruff 2&7 Selective Attention Test (RSAT); Computerized Performance Test, Second Edition (CPT-II); electroencephalography (EEG); Test of Variables of Attention (TOVA); Cognitive Emotional Regulation Questionnaire (CERQ).

3.3. Application of Citicoline among Patients after Traumatic Brain Injury (TBI)

Head injuries continue to be a significant cause of disability and mortality. In the United States, they are the leading cause of disability in people under 45 and account for 30.5% of all injury-related deaths [58]. The search for a treatment that would reduce the negative consequences of brain injuries poses a significant problem.

In the study by Trimmel et al. [59], 67 patients with brain trauma were administered citicoline during their stay in the intensive care unit (ICU). The Rotterdam CT score, Glasgow Coma Scale (GCS), and the Injury Severity Score (ISS) were used to initially assess the condition of patients after trauma. The state of patients at the start of treatment did not differ significantly in the two groups ($p > 0.05$). Mortality during the stay in ICU was 5% for the research group and 24% for the control group (OR 6.7, $p < 0.01$), and the mortality during hospitalization was 9% and 24% ($p = 0.035$), respectively. The 6-month mortality in the citicoline group was 13%, and it was 28% in the placebo group ($p = 0.031$). A share of 34% of patients in the study group had unfavorable results after 6 months, and 57% in the control group had unfavorable results after 6 months ($p = 0.015$). Patients from the citicoline group had significantly higher odds of ICU survival (OR 6.7, $p < 0.01$), hospital survival (OR 3.2, $p = 0.024$), favorable outcome after 6 months (OR 2.5, $p < 0.01$), and survival after 6 months (OR 2.6, $p = 0.037$). After adjustment for age, the first available GCS, and ISS, adjusted ORs still disclosed significantly better odds for ICU survivals (OR 6.7, 95% CI 1.6–28.8, $p = 0.014$) and favorable outcome after 6 months (OR 2.6, 95% CI 1.1–6.0, $p = 0.022$) in the citicoline group. Citicoline contributed to the reduction of both per-accident and long-term mortality. Moreover, it helped to achieve more favorable long-term treatment outcomes.

In another study, the influence of citicoline on the GCS score was assessed daily, and the levels of fetuin-A and Matrix Gla protein (MGP) were examined. Fetuin-A is an anti-inflammatory protein that inhibits the production of pro-inflammatory cytokines and the development of atherosclerosis. MGP also prevents the formation of atherosclerosis. During the observation, no significant differences in the obtained results in GCS were observed between the groups ($p > 0.05$). The level of fetuin-A did not differ significantly between the groups ($p = 0.08$ on the 6th and 12th days of the study). A significantly higher level of MGP was observed in the study group ($p = 0.01$) on the 12th day of observation. Citicoline in this study showed moderate effectiveness—it had no significant effect on GCS. It only influenced the level of MGP [60].

In the multicenter study, the Citicoline Brain Injury Treatment Trial (COBRIT), 607 patients were administered citicoline for 90 days. The TBI Clinical Trials Network Core Battery was used to assess patient function. This is a complex test, consisting of nine components, in which the overall level of functioning, including intelligence and memory, is determined. The two groups did not differ in the 90-day evaluation with respect to the TBI Clinical Trials Network Core Battery (OR 0.98, 95% CI 0.83–1.15). Moreover, the study included the Glasgow Outcome Scale–Extended (GOS-E) to assess the degree of recovery of head injury victims. After 90 days from the start of treatment, the mean improvement in the GOS-E score in the research group was 35.4%, and in the control group, it was 35.6%. On the other scales, the improvement ranged from 37.3% to 86.5% in the citicoline group and from 42.7% to 84.0% in the control group ($p > 0.05$ for all). Citicoline results were not significantly different compared to those of the control group. Similarly, in the study at 180 days after the start of treatment, the results in both groups did not differ significantly (OR 0.87, 95% CI 0.72–1.04, $p = 0.13$). Moreover, among patients with complicated mild brain trauma, patients in the placebo group achieved substantially better results in the studies than in the research group (OR 0.72, 95% CI 0.56–0.91, $p = 0.004$). In patients with moderate/severe TBI, no statistically significant difference was observed between groups (OR 1.26, 95% CI 0.92–1.70, $p = 0.14$). In addition, the survival rates in the first 30 days did not differ between groups ($p = 0.17$) [61].

A total of 2706 patients from twelve controlled-trials were included in the meta-analysis, which was performed by Secades [62]. To be included, the trials must assess the effect of citicoline in the acute phase of TBI, be comparative studies, and have independence outcomes evaluated with the Glasgow

Outcome Scale (GOS) or similar scales. Articles from the last four decades were analyzed. The primary efficacy measure was patient independence at the end of a follow-up period, which was evaluated as a score GOS 4–5 indicating a perfect outcome or with mild sequelae. According to the formal meta-analysis, based on the random effects model, the use of citicoline is associated with a significant increase in the rates of independence with an OR of 1.815 (95% CI 1.302–2.530). Due to the time gap of 34 years between studies, a significant heterogeneity ($p = 0.001$) was detected. Under the fixed-effects model, the meta-analysis attains an OR of 1.451 (95% CI 1.224–1.721), reinforcing the results obtained. Importantly, the effectiveness of citicoline has decreased over the years due to improvements in the overall quality of healthcare. However, citicoline should still be added to treatment in people after TBI.

The effectiveness of citicoline in TBI treatment is not entirely clear. In a large, multicenter study, its effectiveness was comparable to that of a placebo. However, the clinical effectiveness of citicoline has been proven in performed meta-analysis. Hence, continued exploration of the use of citicoline and other drugs in the treatment of brain trauma is advisable. The most important features of the research cited above are summarized in the table (Table 4).

Table 4. Key clinical features of studies in patients after traumatic brain injury (TBI).

References	Numer of Patients Received Citicoline	Mean Age (Years)	Dose of Citicoline (mg/Daily)	Period of Citicoline Administration (Days)	Used Methods to Assessment Effectiveness of Citicoline	Frequency of Examination (Days after Start Treatment
Trimmel et al. [59]	67	54.6	3000	Treatment in ICU, maximally 21 days	Mortality, unfavorable outcome	Hospital discharge and 180 days
Shokouhi et al. [60]	29	30.94	2000	15	GCS, level of fetuin-A, MGP	6, 12, 15
Zafonte et al. [61]	607	-	2000	90	TBI Clinical Trials Network Core Battery	30, 90, 180

Glasgow Coma Scale (GCS); Matrix Gla protein (MGP).

3.4. The Application of Citicoline in the Regeneration of Peripheral Nerves after Injury and the Treatment of Neuropathic Pain: Animal Models

Various pathological conditions can lead to damage to peripheral nerves and the occurrence of neuropathic pain. One of the most common causes is degenerative changes leading to injury, e.g., the sciatic nerve, diabetes-related neuropathy, and drug-induced nerve damage. Many polyneuropathies of various etiologies also lead to nerve damage. With the aging of the population, the problem of nerve damage will become more frequent and require new treatments. To date, the use of citicoline has been studied in this neurological condition only in animal models.

In the study by Emril et al., the effect of a gelatin sponge soaked with various concentrations of citicoline on the regeneration of the damaged sciatic nerve was investigated. Different amounts of citicoline (100 µmol/L) were used in two groups: 0.4 and 0.8 mL. The Sciatic Functional Index (SFI) and Extensor Postural Thrust (EPT) were used to assess the motor functions of the nerve. Von Frey filaments (threshold 100 g) were used to assess neuropathic pain. After 4 weeks, neuropathic pain only occurred in 2 of 10 rats in the 0.4 mL citicoline group and 8/10 in the control group ($p < 0.05$). In the 0.8 mL group, 4/10 rats suffered from neuropathic pain ($p = 0.18$). There were no significant differences between the groups in the SFI test ($p = 0.26$). In the EPT test, the groups receiving citicoline had a significantly lower percentage of a motor deficit than in the control group: for 0.4 and 0.8 mL, respectively, 14.28% and 20.6% ($p = 0.00$). Citicoline in this test was effective in alleviating neuropathic pain and reducing motor deficit [63].

In another study, rats with injured sciatic nerves received citicoline at three different doses: 300, 600, and 900 mmol/kg. The SFI was used to assess the improvement in function, and the assessment was made at 4, 8, and 12 weeks. An electromyographic examination (EMG) was also performed at week 12. Thereafter, the nerves were analyzed histologically. In the SFI test, citicoline at a dose of

900 mmol/kg resulted in a significant improvement in the function of the sciatic nerve at both 8 and 12 weeks compared to the control group and at week 8 compared to the other study groups ($p < 0.05$). Citicoline at a dose of 600 mmol/kg caused a significant improvement at 12 weeks of follow-up ($p < 0.05$). In addition, in EMG at week 12, citicoline at doses of 600 and 900 mmol/kg caused a significantly smaller delay compared to the control group. In the microscopic examination, rats in the 900 mmol/kg citicoline group had a considerably denser axonal network and significantly more myelinated axons compared to the other groups ($p < 0.001$). In the study groups, the scar after injury was substantially smaller than in the control group ($p < 0.05$) [64].

In the next study, rats after sciatic nerve injury were divided into five groups: control, receiving citicoline, cytidine, choline, and cytidine + choline. A portion of the rats in each group had sutured nerves on day 1 and some on day 3 post-cut. The SFI examination was performed 4, 8, and 12 weeks after the operation. After 12 weeks, the macro and microscopic examination of the nerve was performed. The citicoline group at weeks 4, 8, and 12 had significantly better results in the SFI test than the control group, and results were comparable to the cytidine + choline group ($p < 0.001$). In the macroscopic examination, rats with nerve repairs on day 1 receiving citicoline had significantly better nerve regeneration. The nerve repaired rats achieved similar results in all groups after 3 days. In histology, rats receiving citicoline had significantly more axons, which were better organized and larger in diameter than those of rats in the control group. Histological observations were related to the SFI score ($p < 0.01$) [65].

In a study by Caner et al., in which the same substances were tested, similar results were obtained in the SFI test, and in the macro and microscopic examination of the sciatic nerve. In addition, EMG was performed in this experiment. The citicoline group had a significantly higher amplitude of the muscle response than the control group ($p < 0.001$) [66].

In a study by Özay et al. [67], the effectiveness of citicoline in accelerating the regeneration of the damaged sciatic nerve was also assessed. For this purpose, SFI and EMG were used, and the macro and microscopic evaluation of the nerve was performed. Rats received 0.4 mL (100 µmol/L) citicoline or 0.4 mL placebo. The SFI values as an indicator of functional recovery were significantly better in rats treated with citicoline than those in rats treated with saline. A statistical difference between the two groups was found in every assessment starting from 4 weeks post surgery (in 4, 8, and 12 weeks $p < 0.001$). In EMG, no significant difference between groups was observed 4 weeks after surgery. However, after 12 weeks, nerve action potentials in the citicoline group were significantly higher ($p < 0.05$). In microscopic evaluation, nerves treated with citicoline had significantly higher axon counts and mean axon diameters than nerves treated with saline ($p < 0.05$). Citicoline in this study also improved nerve regeneration. Kanat et al. studied the influence of citicoline on the occurrence of neuropathic pain induced by oxaliplatin (OXA). To assess the neuropathic pain threshold, the Randall–Sellito test was used, which consisted of compressing the animal's paw with increasing weight. After OXA administration, the pain threshold decreased from 144.3 to 66 g on the first day and 47.5 g on the second day after administration. Citicoline in the dose of 2 µmol increased the pain threshold to 150 g, and this effect lasted for several hours ($p < 0.001$). Lower doses also increased the pain threshold but showed a shorter duration of action [68].

In another experiment, the influence of citicoline on the inflammatory pain process induced by Carrageenan injection and on neuropathic pain caused by damage to the sciatic nerve was investigated. The Randall–Sellito test was used to assess the pain threshold. In inflammatory pain, the dose of 2 µmol increased the sensory threshold pain from 50 to 300 g ($p < 0.001$). In neuropathic pain, the pain threshold changed from 75 to 250 g ($p < 0.001$) [69].

Another experiment investigated the effect of citicoline on the level of metalloproteinases after sciatic nerve injury. Metalloproteinases are enzymes responsible for the destruction of Schwann cells, which are responsible for the regeneration of neuronal axons after nerve damage. Their level correlates with the ability to regenerate damaged nerves. Sciatic nerve samples for testing the level of metalloproteinases type 2 and 9 were taken on days 1, 3, and 7 after the nerve injury. It was observed that on day 1 after surgery, the activity of metalloproteinases 2 and 9 increased to a similar extent in the

control group and that receiving citicoline. However, on the 3rd and 7th day after surgery, the activity of metalloproteinase 2 and 9 in the group receiving citicoline decreased by 36% and 23%, respectively, and by 15% and 12% in the control group, respectively. Moreover, histological examination showed significantly more myelinated axons in the citicoline group than in the control group: 34.5%, 36%, and 90.4% more, respectively, on days 1, 3, and 7 ($p < 0.001$). The total number of myelinated axons in the citicoline group was 75%, 183%, and 146% greater on days 1, 3, and 7, respectively ($p < 0.001$). The influence of citicoline on the level of metalloproteinases may explain its beneficial effect in the regeneration of damaged peripheral nerves [70].

Citicoline is beneficial in nerve regeneration and the reduction of neuropathic and inflammatory pain in most of the studies cited above. It resulted in faster and more intense nerve regeneration, which is visible both in the macroscopic and microscopic images. It also increased the density of the axonal network. Its beneficial effect may be related to including the reduction of the concentration and activity of metalloproteinases.

4. Strengths and Limitations

The main strength of this article is a comprehensive look at the use of citicoline in neurology. Articles exploring the use of citicoline in various neurological conditions were included. Articles from different years and databases were reviewed.

The main limitation/bias of our review is to include only articles in the English language. Another limitation is the lack of access to the full content of some articles.

5. Conclusions

This systematic review showed that citicoline has a wide range of uses in neurological conditions. In dementia, it is useful primarily in inhibiting disease progression, and, according to the results of some studies, reversing adverse changes. Citicoline also improved memory and other cognitive functions among healthy volunteers. For this purpose, they were assessed with various tests, which adds credibility to these studies. Citicoline has also been shown to be a promising drug in reducing neuropathic pain and accelerating nerve regeneration. Unfortunately, these studies were only conducted in animal models. Citicoline may prove to be a potentially beneficial adjunct in the treatment of stroke. However, citicoline has unclear effects in the treatment of brain injuries. Citicoline, depending on its application, can be considered both as a dietary supplement and as a medicine. Further research on this substance should be carried out, including other neurological and non-neurological diseases.

Author Contributions: Conceptualization, P.J. and V.P.; methodology P.J. and F.P.; software, P.J.; validation, P.J., F.P., M.P. and A.R.; formal analysis, M.P. and A.R.; investigation, P.J. and F.P.; resources, F.P. and P.J.; data curation, P.J. and F.P.; writing—original draft preparation, P.J. and A.R.; writing—review and editing, P.J., V.P. and K.R.; visualization, M.P.; supervision, K.R.; project administration, K.R.; funding acquisition, K.R. Please turn to the CRediT taxonomy for the term explanation. All authors have read and agreed to the published version of the manuscript

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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Association of Lower Nutritional Status and Education Level with the Severity of Depression Symptoms in Older Adults—A Cross Sectional Survey

Zuzanna Chrzastek [1], Agnieszka Guligowska [1], Bartlomiej Soltysik [1], Malgorzata Piglowska [1], Ewa Borowiak [2], Joanna Kostka [3] and Tomasz Kostka [1,*]

[1] Department of Geriatrics, Healthy Ageing Research Centre, Medical University of Lodz, 92-115 Lodz, Poland; zuzanna.chrzastek@umed.lodz.pl (Z.C.); agnieszka.guligowska@umed.lodz.pl (A.G.); bartlomiej.soltysik@umed.lodz.pl (B.S.); malgorzata.piglowska@umed.lodz.pl (M.P.)
[2] Department of Conservative Nursing, Medical University of Lodz, 90-251 Lodz, Poland; ewa.borowiak@umed.lodz.pl
[3] Department of Gerontology, Medical University of Lodz, 93-113 Lodz, Poland; joanna.kostka@umed.lodz.pl
* Correspondence: tomasz.kostka@umed.lodz.pl; Tel.: +48-42-679-31-44

Abstract: The study analyzes the relationship between nutritional status and depression symptoms severity in the older population. A total of 1975 older outpatients (1457 women and 518 men, median age 75) were included in the study. Depression symptoms severity was assessed using the Geriatric Depression Scale (GDS). Participants were divided into two subgroups according to GDS score. Group A: 0–5 points—without depression symptoms (1237, W:898, M:339), and group B: 6–15 points—with depression symptoms (738, W:559, M:179). The nutritional status of the patients was assessed with Mini Nutritional Assessment (MNA) and basic anthropometric variables (waist, hips, calf circumferences, body mass index (BMI), waist to hip ratio (WHR), and waist to height ratio (WHtR)). Education years and chronic diseases were also noted. Women with higher depression symptoms severity had significantly lower MNA scores [A: 26.5 (24–28) (median (25%−75% quartiles)) vs. B:23 (20.5–26)], shorter education time [A:12 (8–16) vs. B:7 (7–12)], smaller calf circumference [A:36 (33–38) vs. B: 34 (32–37)], and higher WHtR score [A:57.4 (52.3–62.9) vs. B:58.8 (52.1–65.6)]. Men with depression symptoms had lower MNA scores [A:26.5 (24.5–28) vs. B:24 (20.5–26.5)], shorter education [A:12 (9.5–16), B:10 (7–12)], and smaller calf circumference [A:37 (34–39), B:36 (33–38)]. In the model of stepwise multiple regression including age, years of education, anthropometric variables, MNA and concomitant diseases nutritional assessment, and education years were the only independent variables predicting severity of depression symptoms both in women and men. Additionally, in the female group, odds were higher with higher WHtR. Results obtained in the study indicate a strong relationship between proper nutritional status and education level with depression symptoms severity in older women and men.

Keywords: depression; nutritional status; education level; older people

Citation: Chrzastek, Z.; Guligowska, A.; Soltysik, B.; Piglowska, M.; Borowiak, E.; Kostka, J.; Kostka, T. Association of Lower Nutritional Status and Education Level with the Severity of Depression Symptoms in Older Adults—A Cross Sectional Survey. *Nutrients* **2021**, *13*, 515. https://doi.org/10.3390/nu13020515

Academic Editor: Antoni Sureda
Received: 15 December 2020
Accepted: 1 February 2021
Published: 4 February 2021

1. Introduction

Recent decades have shown an increase in the proportion of older adults in general population due to extended lifespans. The World Health Organization (WHO) estimates that the population of people aged 60 + will double to 22% from 2015 to 2050 [1]. Ageing is characterized by multiple diseases and health issues and a reduction in the functionality of the organism as a whole, alongside a visible and progressive decline in cognitive functions. Mental or neurological disorders affect up to 20% of people over 60 worldwide. According to the WHO, one of the most common chronic psychological conditions is depression affecting as many as 264 million people worldwide and as much as 7% of the population over 60 [1,2].

Deterioration of physical and mental health in older patients is often associated with inadequate diet and lifestyle [3]. The impact of diet and its individual nutrients on the risk of depression and the severity of its symptoms has been noted in previous studies [4,5]. Older people are particularly vulnerable to improper nutritional status, which may result in deterioration of bodily functions caused by deficiency of nutritional agents [6]. Malnutrition can aggravate existing problems and contribute to development of new ones [7,8]. Nutritional status is considered to be one of the predictors of survival in the older population [9]. Malnutrition is a very common problem in older patients: it is estimated that over 50% of older adults are malnourished or at risk of malnutrition; however, these calculations depend on the tool used for assessment, studied population, and healthcare quality [10,11]. In addition, seniors from all backgrounds, i.e., home, hospital, and nursing home, are at risk of malnutrition [12].

It seems that malnutrition can lead to development of depression symptoms and, conversely, existing depression may affect nutritional problems, reluctance to eat, and thus, lead to development of malnutrition [13,14]. Older, malnourished persons were 31% more likely to present symptoms of depression than people with normal nutritional status [15]. Introduction of an appropriate intervention addressing each of those disorders appears to improve the effectiveness of treatment [16].

Previous studies have examined small groups or have failed to assess a connection between basic anthropometric variables and indicators of nutritional status in the context of depressive disorders. Few studies concern older people population. Likewise, education level and commonly found in older patients' concomitant diseases may have an impact on the nutritional status-depression relationship. Therefore, the aim of this study was to indicate which variables: education years, anthropometric indicators, nutritional status, and concomitant diseases may have the strongest relation with depression symptoms. So far, no large research has been carried out to clarify the relationship between these variables and depression symptoms severity.

2. Materials and Methods

2.1. Design of the Study and Participants

Study participants were community-dwelling older adults. Their visits to the Geriatric Clinic were conditioned not only by health check-up visits but also for sole participation in the research projects conducted in the clinic. Predominance of women in the studied group results from the demographic profile of the Polish population. The ratio of males to females is 0.66 in age group $\geq$65, reflecting a higher mortality rate among older men [17,18]. Another factor influencing superior number of women over men included in the study may be the fact that women view their quality of life and health as worse as shown in the PolSenior - Study on Ageing and Longevity, therefore, are keener to participate in general health checkup studies [19]. The inclusion criteria were as follows: 60 years of age or older, ability to intake food orally, lack of communication, and comprehension problems as well as informed consent for participation in the study. Patients with severe dementia or those receiving enteral nutrition were excluded from the study. Depression symptoms severity was assessed with the 15-item Geriatric Depression Scale (GDS) questionnaire. The GDS is one of the most popular self-reporting tests used to assess the presence of depression symptoms in the older population [20]. It includes 15 questions with simple yes/no alternative answers. A higher GDS score indicates greater severity of depression symptoms: the highest possible score is 15 and obtaining five points or less indicates absence of depression [21]. In our study, patients were divided into two subgroups according to GDS score. Group A included those with a lack of depression symptoms (0–5 points): 1237 patients (898 women and 339 men). Group B included patients with depression symptoms (> 5 points): 738 patients (559 women and 179 men).

2.2. Procedure

A comprehensive geriatric assessment was conducted during face-to-face interviews by qualified researchers including medical doctors, nutritionists, and PhD students from the Geriatric Clinic of the Medical University of Lodz. All participants underwent physical and mental examinations. Nutritional status was assessed using the Mini Nutritional Assessment (MNA) questionnaire, which contains 18 questions relating to important elements of nutritional status including food intake, weight loss, mobility, presence of acute stress or disease, neurological problems, intake of medications, body mass index (BMI), and arm and calf circumferences (CC). The maximum score is 30 points. A score higher than 23.5 points indicates satisfactory nutritional status, while a lower score indicates malnutrition [22]. Patients were measured and weighed on RADWAG personal weight scales (WPT60 150OW) (RADWAG Balances and Scales, Radom, Poland), waist (WC), hip (HC), arm (AC) and calf (CC) circumferences were measured using SECA measuring tape (SECA Deutschland, Hamburg, Germany). Body mass index (BMI) was calculated by dividing body weight by height in meters squared. Waist to hip ratio (WHR) was calculated as WC divided by hip circumference in centimeters. Waist to height ratio (WHtR) was calculated by the formula: (WC [cm]/height [cm]) $\times$ 100. Age, years of education, and chronic diseases of study participants were also recorded.

2.3. Statistical Analysis

To detect MNA score difference of 2 points (26 vs. 24) between GDS score groups, with standard deviation of MNA equal to 3 points, 49 subjects are required in each group. To detect an MNA score difference of 2 points (26 vs. 24) between GDS score groups, with standard deviation of MNA equal to 4 points, 86 subjects are required in each group. To detect a correlation coefficient of 0.15 with an alpha of 0.05 and test power of 90.0%, a sample size of 462 individuals is needed. The sample was selected on the base of inclusion and exclusion criteria, and it was a convenience sampling. The normality of data distribution was confirmed by the Shapiro-Wilk test. As the data were non-normally distributed, variables were presented as median values and interquartile ranges, they were compared using the Mann–Whitney U test. The Chi^2 test was used to compare qualitative values. GDS score and sex were used as grouping variables. Spearman's and Pearson's rank correlation coefficients were calculated. For both women and men, independent variables that predicted inclusion to each group with the presence of depression symptoms (GDS > 5) were selected using logistic stepwise regression based on odds ratios and corresponding 95% confidence intervals (95%CI). Variables included in the model were age; years of education; BMI; WC; CC; WHR; WHtR; MNA; presence of hypertension, stroke, cancer, osteoporosis, chronic obstructive pulmonary disease (COPD), congestive heart failure, diabetes, and suffered myocardial infarction. The limit of statistical significance was set at a *p*-value of less than 0.05. Analyses were carried out using Statistica 13.1 software (StatSoft Polska, Cracow, Poland).

2.4. Ethical Certification

The study was approved by the Ethics Committee of the Medical University of Lodz (approval number: RNN/73/15/KE), and written informed consent was obtained from all subjects.

3. Results

In the study, a total of 2189 patient were examined. Two hundred and fourteen participants were excluded from the study because of missing essential data such as GDS, MNA, education years, or anthropometric variables. Ultimately, 1975 outpatients from the Department of Geriatrics of Medical University in Lodz were enrolled in the study (1457 women and 518 men) (Figure 1).

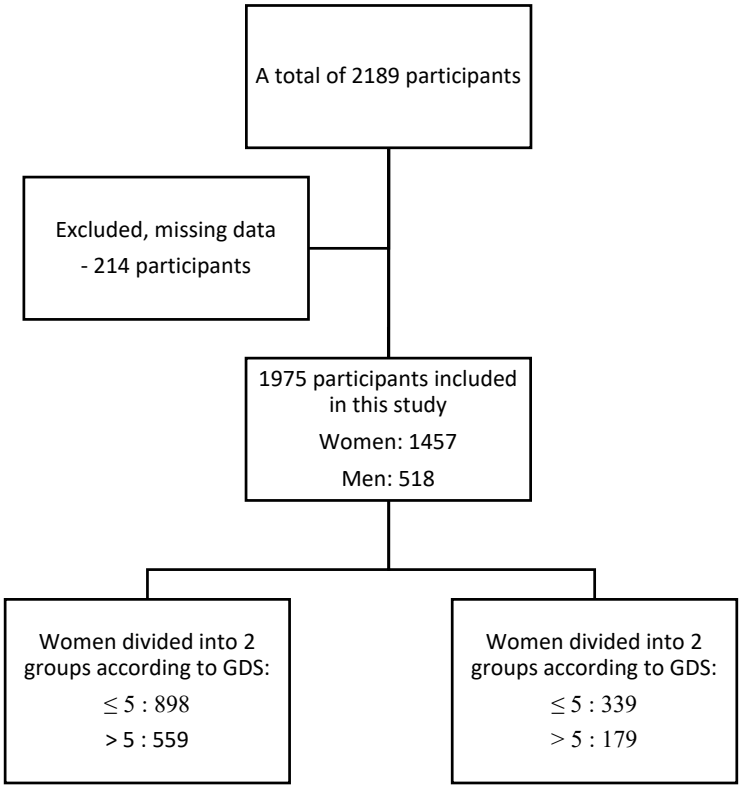

Figure 1. Flowchart of the study.

Table 1 summarizes the general characteristics of the study population according to sex. Statistical analysis revealed no differences in BMI, WHtR, and MNA between women and men. Age, education years, WC, HC, CC, and WHR differed according to sex. The prevalence of depression, osteoporosis, and myocardial infarction differed between women and men (Table 1).

Table 1. General characteristic of the study population (*n* = 1975) according to sex.

Variable	All (*n* = 1975)	Women (*n* = 1457)	Men (*n* = 518)	*p*-Value
Age [years]	75 (67–80)	75 (67–81)	73 (66–78)	<0.001 [a]
Education [years]	11 (7–14)	11 (7–13)	11 (7–15)	0.023 [a]
BMI [kg/m^2]	26.9 (24.1–30.1)	26.9 (23.9–30.5)	26.7 (24.5–29.5)	ns [a]
Waist circumference [cm]	93 (84–102)	91 (82–100)	99 (92–107)	<0.001 [a]
Hips circumference [cm]	104 (98–111)	105 (98–112)	102 (98–107)	<0.001 [a]
Calf circumference [cm]	36 (33–38)	35 (33–38)	36 (34–39)	<0.001 [a]
WHtR	57.9 (52.6–63.6)	58.1 (52.2–63.7)	57.7 (53.9–62.9)	ns [a]
WHR	0.89 (0.83–0.94)	0.86 (0.81–0.91)	0.97 (0.93–1.01)	<0.001 [a]
MNA	25.5 (22.5–27.5)	25.5 (22.5–27.5)	25.7 (23.5–27.5)	ns [a]
Depression [*n* (%)]	241 (12.2)	196 (13.4)	45 (8.7)	0.004 [b]
Hypertension [*n* (%)]	1330 (67.3)	995 (68.2)	335 (64.7)	ns [b]
Stroke [*n* (%)]	233 (11.8)	167 (11.5)	66 (12.7)	ns [b]
Cancer [*n* (%)]	165 (8.4)	118 (8.1)	47 (9.1)	ns [b]
Osteoporosis [*n* (%)]	485 (24.6)	403 (27.7)	82 (15.8)	<0.001 [b]
COPD [*n* (%)]	114 (5.8)	86 (5.9)	28 (5.4)	ns [b]
Congestive heart failure [*n* (%)]	745 (37.7)	533 (36.6)	212 (40.9)	ns [b]
Diabetes [*n* (%)]	376 (19.1)	266 (18.3)	110 (21.3)	ns [b]
Myocardial infarction [*n* (%)]	214 (10.8)	134 (9.2)	80 (15.4)	<0.001 [b]

The quantitative values are presented as median and interquartile difference, qualitative values as number and percentage. [a] Mann–Whitney U-test; [b] Chi^2-test. Abbreviations: BMI—body mass index, WHtR—waist to height ratio, WHR—waist to hip ratio, MNA—Mini Nutritional Assessment, GDS—Geriatric Depression Scale, COPD—chronic obstructive pulmonary disease.

Table 2 shows a comparison of the major anthropometric variables in women and men divided according to the GDS score. In the female group, no differences were found in BMI, WC, HC, and WHR. Women with depression symptoms were older, had received a significantly shorter education, and had a smaller CC and higher WHtR compared to women with a lower GDS score. Significant differences in nutritional status according to the MNA scale were observed. The prevalence of chronic diseases such as depression, hypertension, stroke, COPD, congestive heart failure, diabetes, and myocardial infarction differed according to GDS score. In the male group, there were no differences in age, BMI, WC, HC, WHtR, and WHR between men divided according to GDS score. In the group with depression symptoms, men spent noticeably less time on education, had smaller CC, and scored fewer points on the MNA scale. The prevalence of depression, stroke, and congestive heart failure differed between the GDS groups (Table 2).

Table 2. Comparison of anthropometric variables, MNA, and prevalence of chronic diseases in female and male groups divided according to GDS score (GDS $\leq$ 5 vs. GDS > 5 separately in women and men).

Variable	Women GDS $\leq$ 5 (n = 898) Median (Quartiles)	Women GDS > 5 (n = 559) Median (Quartiles)	Men GDS $\leq$ 5 (n = 339) Median (Quartiles)	Men GDS > 5 (n = 179) Median (Quartiles)
Age [years] [a]	74 (66–79)	77 (70–83) ***	74 (66–79)	72 (68–77)
Education [years] [a]	12 (8–16)	7 (7–12) ***	12 (9.5–16)	10 (7–12) ***
BMI [kg/m2] [a]	27 (24.2–30.1)	26.7 (23.4–30.1)	27 (24.6–29.7)	26.5 (24.1–29.4)
Waist circumference [cm] [a]	90 (82–99)	91 (82–102)	99 (93–106)	100 (90–108)
Hips circumference [cm] [a]	105 (99–112)	105 (98–114)	102 (98–107)	102 (96–107)
Calf circumference [cm] [a]	36 (33–38)	34 (32–37) ***	57.2 (54–62)	36 (33–38) *
WHtR [a]	57.4 (52.3–62.9)	58.8 (52.1–65.6) *	57.2 (54–62)	58.7 (53–63.8)
WHR [a]	0.86 (0.81–0.91)	0.87 (0.82–0.91)	0.97 (0.93–1)	0.96 (0.93–1)
MNA [a]	26.5 (24–28)	23 (20.5–26) ***	26.5 (24.5–28)	24 (20.5–26.5) ***
Depression [n (%)] [b]	107 (11.9)	89 (15.9) *	17 (5)	28 (15.6) ***
Hypertension [n (%)] [b]	583 (64.9)	412 (73.7) ***	218 (64.3)	117 (65.4)
Stroke [n (%)] [b]	70 (7.8)	97 (17.3) ***	31 (9.1)	35 (19.5) ***
Cancer [n (%)] [b]	76 (8.5)	42 (7.5)	29 (8.5)	18 (10.1)
Osteoporosis [n (%)] [b]	243 (27.1)	160 (28.6)	57 (16.8)	25 (14)
COPD [n (%)] [b]	62 (6.9)	24 (4.3) *	17 (5)	11 (6.2)
Congestive heart failure [n (%)] [b]	291 (32.4)	242 (43.3) ***	122 (36)	90 (50.3) **
Diabetes [n (%)] [b]	141 (15.7)	125 (22.4) ***	72 (21.2)	38 (21.3)
Myocardial infarction [n (%)] [b]	71 (7.9)	63 (11.3) *	50 (14.7)	30 (16.8)

The quantitative values are presented as median and interquartile difference, qualitative values as number and percentage. [a] Mann-Whitney U-test; [b] Chi^2-test; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; Abbreviations: GDS—Geriatric Depression Scale, BMI—body mass index, WHtR—waist to height ratio, WHR—waist to hip ratio, MNA—Mini Nutritional Assessment, COPD—chronic obstructive pulmonary disease.

Table 3 shows the Spearman's and Pearson's rank correlation coefficients of GDS score and major anthropometric variables. Among women education years, CC and MNA negatively correlated with the severity of depression symptoms, and a positive correlation was observed between WHtR and GDS. Among men, GDS negatively correlated with education years, CC, and MNA (Table 3).

Table 4 presents the results of logistic stepwise regression. For both women and men, fewer education years and a lower MNA score were associated with an increased risk of depression symptoms. Additionally, among women, the odds ratio increased with WHtR. Other variables, viz., age, BMI, WC, HC, CC, and WHR were statistically insignificant, as well as chronic diseases. In order to improve visualization of cumulative effects of education years and MNA on GDS score, the results are presented on surface charts (Figures 2 and 3). After excluding 12.2% of people diagnosed with depression, the regression analysis did not change significantly. MNA and level of education remained the only independent significant predictors of depression symptoms. In the women's group, the relationship with WHtR ceased to be statistically significant, while the relationship with MNA became even stronger.

Table 3. Spearman's and Pearson's rank correlation coefficients between GDS score and education years, high, weight, BMI, waist, hip and calf circumferences, WHR, WHtR, and MNA in women and men.

Parameters	Women rS (rP)	Men rS (rP)
Age [years]	0.25 *** (0.22 ***)	−0.02 (−0.04)
Education [years]	−0.37 *** (−0.36 ***)	−0.31 *** (−0.30 ***)
BMI [kg/m^2]	−0.03 (0.00)	−0.06 (−0.06)
Waist circumference [cm]	0.04 (0.04)	−0.001 (−0.04)
Hips circumference [cm]	0.04 (0.05)	−0.004 (0.003)
Calf circumference [cm]	−0.17 *** (−0.13 ***)	−0.13 ** (−0.11 **)
WHR	0.03 (−0.01)	−0.03 (−0.07)
WHtR	0.06 * (0.05 *)	−0.02 (0.002)
MNA	−0.42 *** (−0.38 ***)	−0.40 *** (−0.45 ***)

rS—Spearman's rank correlation, rP—Pearson's rank correlation, WHtR—waist to height ratio, MNA—Mini Nutritional Assessment * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Table 4. Odds ratios (95% confidence interval) for the risk of belonging to the group with higher score of depression symptoms (GDS > 5) for education years, MNA score, and WHtR.

Variable	Women			Men		
	OR	95%CI	*p*	OR	95%CI	*p*
Education [years]	0.87	0.84–0.90	<0.001	0.9	0.85–0.95	<0.001
MNA	0.83	0.80–0.86	<0.001	0.81	0.75–0.87	<0.001
WHtR	1.02	1.002–1.03	0.03	-	-	-

OR—odds ratio, 95%CI—95% confidence interval.

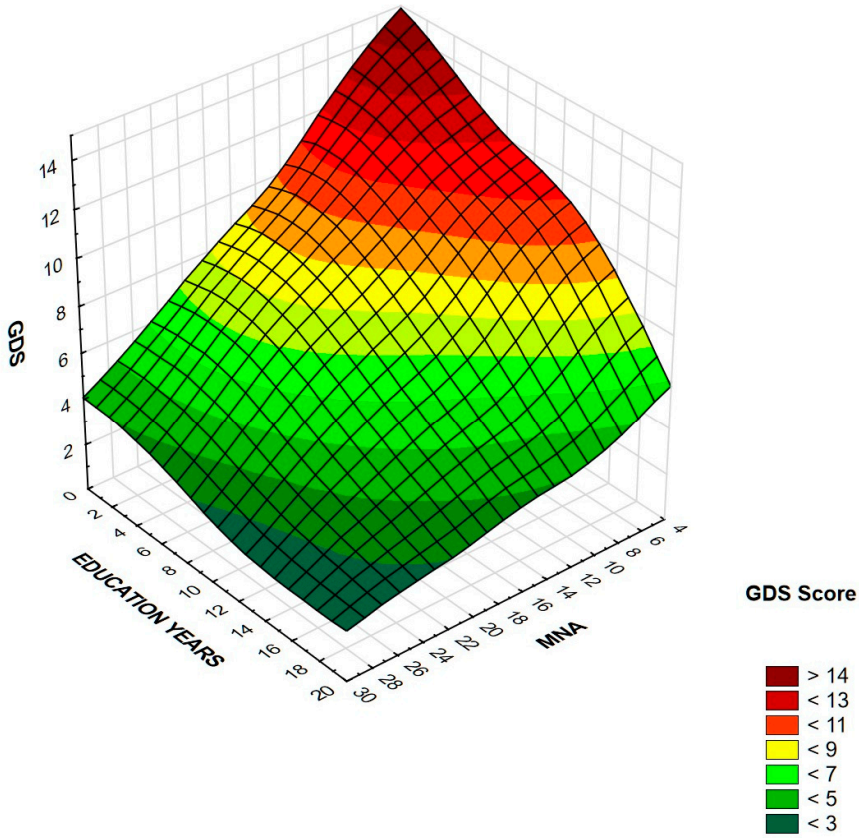

Figure 2. Association between GDS score, education years, and MNA in women. Higher GDS is associated with lower education level and lower MNA score in this group.

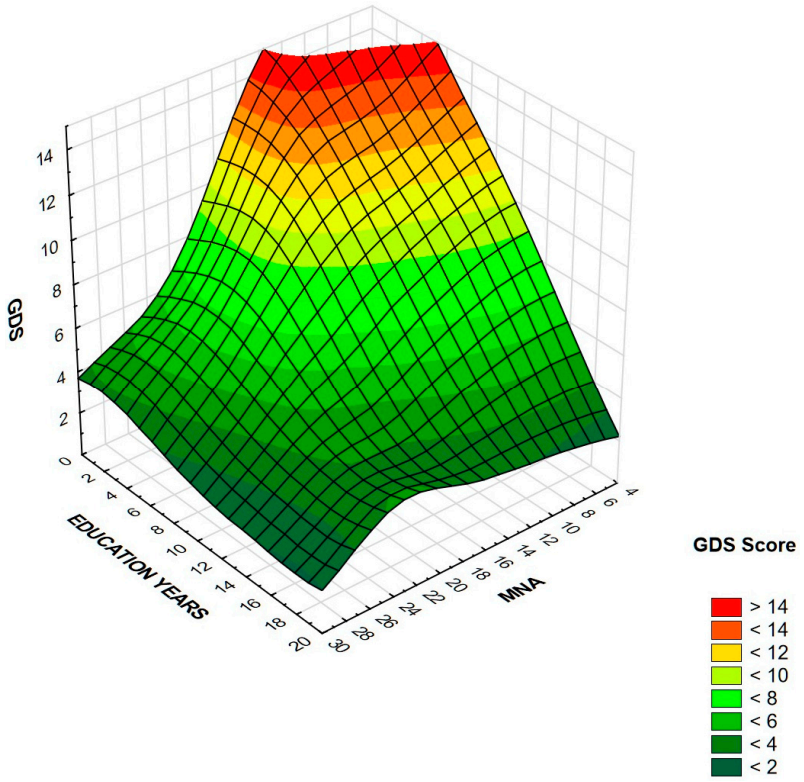

Figure 3. Association between GDS score, education years, and MNA in men. Higher GDS is associated with lower education level and lower MNA score in this group.

4. Discussion

Obtained results clearly indicate that nutritional status is associated with risk of depression symptoms in the older population. Education level is the second main protective factor, even more important than presence of concomitant diseases. The study included a large representative group of community-dwelling older adults from Central-Eastern Europe. The differences in numbers of women and their age in relation to men reflects demographic situation in Poland and a greater willingness of women to participate in research [17,19]. The difference between the age in women from groups with and without depression symptoms results from the fact that the risk of depression increases with age, which has been noted in many studies [19,23]. Age was included as a disturbing factor in the stepwise regression analysis model without affecting the significance of the analyzed variables.

Previous studies have shown a link between diet and nutritional status assessed with MNA and the risk and severity of depression [24]. However, few of these studies have been conducted among the older population [25,26]. In a recent study conducted in a Greek older population, nutritional status was independently associated with cognitive and psychological status [27]. The combination of an improper diet with low physical activity can lead to sarcopenic obesity through a reduction in muscle mass and an increase in fatty tissue [28,29]. This and other conditions common among older people, such as oedema [30], may interfere with a correct assessment of nutritional status. Diseases that can affect body composition/volume, such as congestive heart failure or immobilization caused by, for example osteoarthritis, merit particular consideration when choosing a suitable nutritional status assessment method in older people groups [31,32]. In such cases, commonly used anthropometric indicators such as BMI, body weight, and circumferences will not be useful [33]. The present study also takes chronic diseases into consideration. Women with depression symptoms are more likely to suffer from hypertension, stroke, congestive heart failure, diabetes, and myocardial infarctions, while men with depression are more susceptible to stroke and congestive heart failure. All statistically significant

diseases were included in the regression analysis; however, none of them influenced the significance of educational years and MNA. Only when MNA was excluded from the analysis did the relationship between depression symptoms and congestive heart failure become significant (results not shown in the tables), indicating how strongly nutritional status affects the severity of depression symptoms. The addition of MNA to analysis reduced the significance of other potentially important parameters such as the presence of chronic diseases, which were previously recognized as important risk factors of developing depression symptoms [34]. Another possible interpretation of obtained data may be the fact that depression may reduce appetite and, consequently, increase the risk of malnutrition in older people, which was noted in previous studies [35].

Our findings did not identify any significant correlation between GDS and WHR. The latter should be used to assess the distribution of adipose tissue in obese individuals, not as an indicator of obesity [36]. This finding may suggest that the WHR has minor relevance when assessing older populations. However, in women, a correlation was observed between GDS and WHtR: an anthropometric index for measuring abdominal obesity regardless of sex. WHtR seems to be a better predictor of cardiovascular risk than other indicators [37]. Obtained statistically significant results indicate greater validity of WHtR as an indicator that can be used in older women. However, more research needs to be conducted in order to confirm such a correlation in men. Other commonly used anthropometric variables are circumferences. Previous studies have found larger WC to be associated with increased abdominal adiposity and elevated risk of metabolic syndrome development as well as its consequences such as hypertension or diabetes [38]. WC has been also associated with prevalence of depression symptoms [39]. In the present study, no correlation was found between WC and GDS. As abdominal obesity is associated with a higher prevalence of chronic diseases including depression, there is a need to find the best tool to properly assess fat distribution in older people [40,41]. Smaller CC is associated with frailty syndrome, sarcopenia [42,43], and nutritional status [44]. Our results indicate a significant correlation between CC and GDS in both sexes. Nevertheless, a much stronger correlation was found between MNA and GDS. CC appears to be an important anthropometric variable that may be used to assess nutritional status or decreased muscle mass in the older population, but it is more accurate when combined with other variables and indicators of the MNA scale. It may be especially important given the recently demonstrated high sensitivity of MNA scale for identifying nutritional risk in older adults with COVID−19 [45].

Our results indicate that the severity of depression symptoms in older adults is also associated with lower education level, similarly, as confirmed in previous studies [46]. This highlights the need to focus attention on older persons with lower education level [47]. Likewise, in low-income older adults, increased self-reported depressive symptoms were related to less favorable nutritional status [48]. The stepwise regression analysis confirmed a strong relationship between the severity of depression symptoms, nutritional status assessed with MNA, and number of years of education in the older population. These two predictors are stronger than the accompanying diseases, both in women and men. Worse nutritional status can lead to many disorders, which can promote development of depression symptoms. Mood deterioration can also be associated with improper eating, which in turn, can aggravate nutritional problems and increase the chance of improper dietary choices [49]. This observed relationship between nutritional status and the risk and severity of depression underlines the need for a dietician to participate in the therapeutic process of seniors. Appropriate malnutrition screening can reduce the burden of the healthcare system, increase the possibility of faster and more effective treatment introduction, and improve the quality of life of older people. Implementing proper nutrition and compensating for nutritional deficiencies could increase the effectiveness of depression treatment and reduce the severity of its symptoms. Previous studies confirm positive clinical outcomes of dietary supplementation in depressive patients [50]. However, more research is needed to confirm these results worldwide.

The main advantages of our study are careful recruitment of patients and large group size. So far, no study has been conducted to bind nutritional status assessment tools and depression symptoms in such a big population of older adults. It also uses internationally recognized tests for assessing nutritional status (MNA) and severity of depression symptoms (GDS), both of which have been properly validated in Poland. Furthermore, anthropometric measurements were performed with great care with the use of validated professional medical equipment. However, there are some limitations. Firstly, the study group was restricted to Central-European community-dwelling older individuals therefore results may be different in other cultures. Correlations of nutritional status and depression may also be different in the institutionalized environments [51,52]. Secondly, no tests were conducted among people with severe dementia or those who were not able to present at the clinic due to mobility problems. Thirdly, seasonal variations of mood could also be important. Finally, the most important aim of this study was to analyze the most common anthropometric variables, which are easy to use and widely available in healthcare premises. Nevertheless, future research should corroborate present findings with more exact body composition analyses such as bioimpedance or DEXA.

Further research should be considered to evaluate the impact of diet and nutritional status on depression among older patients and to confirm the need for comprehensive nutritional assessments performed by medical professionals in routine treatment and follow up. Intervention and prospective cohort studies should also be considered to verify whether appropriate nutritional intervention and improvement in nutritional status influence the severity of depression symptoms in the older populations.

5. Conclusions

Our findings indicate a strong relationship between nutritional status assessed with the MNA scale, education years, and the severity of depression symptoms in both women and men. WHtR was also associated with the presence of depression symptoms in women.

Author Contributions: Conceptualization, Z.C.; methodology, Z.C., A.G., and T.K.; validation, Z.C.; formal analysis, Z.C.; investigation, Z.C., A.G., B.S., M.P., E.B., J.K., and T.K.; resources, Z.C., A.G., B.S., M.P., E.B., J.K., and T.K.; data curation, Z.C.; writing—original draft preparation, Z.C.; writing—review and editing, Z.C., A.G., B.S., M.P., E.B., J.K., and T.K.; visualization, Z.C.; supervision, T.K.; funding acquisition, T.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Medical University of Lodz [grant number 503/6−077-01/503 61-001].

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Medical University of Lodz (approval number: RNN/73/15/KE).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: Non declared.

Conflicts of Interest: No potential conflict of interest was reported by the authors.

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Tryptophan Intake and Metabolism in Older Adults with Mood Disorders

Cezary Chojnacki [1,*], Tomasz Popławski [2], Jan Chojnacki [1], Michał Fila [3], Paulina Konrad [1] and Janusz Blasiak [2]

[1] Department of Clinical Nutrition and Gastroenterological Diagnostics, Medical University of Lodz, 90-647 Lodz, Poland; jan.chojnacki@umed.lodz.pl (J.C.); paulina.konrad@umed.lodz.pl (P.K.)

[2] Department of Molecular Genetics, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland; tomasz.poplawski@biol.uni.lodz.pl (T.P.); janusz.blasiak@biol.uni.lodz.pl (J.B.)

[3] Department of Neurology, Polish Mother Memorial Hospital Research Institute, 93-338 Lodz, Poland; michalfila@poczta.onet.pl

* Correspondence: cezary.chojnacki@umed.lodz.pl; Tel.: +48-42-6393040

Received: 15 September 2020; Accepted: 16 October 2020; Published: 18 October 2020

Abstract: The role of serotonin in the pathogenesis of depression is well-documented, while the involvement of other tryptophan (TRP) metabolites generated in the kynurenine pathway is less known. The aim of this study was to assess the intake and metabolism of TRP in elderly patients with mood disorders. Ninety subjects in three groups, 30 subjects each, were enrolled in this study: controls (healthy young adults, group I) and elderly individuals without (group II) or with (group III) symptoms of mild and moderate depression, as assessed by the Hamilton Depression Rating Scale (HAM-D) and further referred to as mood disorders. The average TRP intake was evaluated with the nutrition calculator. Urinary levels of TRP, 5-hydroxyindoleacetic acid (5-HIAA), L-kynurenine (KYN), kynurenic acid (KynA), xanthurenic acid (XA), and quinolinic acid (QA) were determined by liquid chromatography with tandem mass spectrometry and related to creatinine level. The average daily intake of TRP was significantly lower in group III than the remaining two groups, but group III was also characterized by higher urinary levels of KYN, KynA, XA, and QA as compared with younger adult individuals and elderly patients without mood disorders. Therefore, mild and moderate depression in the elderly may be associated with a lower intake of TRP and changes in its kynurenine metabolic pathway, which suggests a potential dietary TRP-based intervention in this group of patients.

Keywords: tryptophan; diet in the elderly; depression; mood disorders; serotonin and kynurenine pathways of tryptophan metabolism

1. Introduction

The process of aging influences structural, functional, biochemical, molecular, and genetic characteristics in many cells and tissues, affecting several individual somatic and behavioral features [1]. The elderly often have sleep disorders and the pathogenesis of these syndromes can be related to various factors, including the use of caffeine, tobacco, and alcohol, sleep habits, and comorbid diseases [2]. Older adults also often suffer from mood disorders and possible causes include sociopsychological factors, such as weakening of family and social ties and feeling of loneliness [3]. However, aging may affect all internal organs, including the brain. Multi-causal, accumulating organic damage could also contribute to behavioral and mood disorders and a range of affective problems in elderly patients. Furthermore, the activity of digestive and endocrine glands decreases with aging [4].

It is commonly agreed that a decreased serotonergic function is involved in the onset and progression of depression [5]. However, serotonin is involved in many physiological functions and

behavioral processes, including mood, appetite, sleep, activity, suicide, sexual behavior, and cognition; and all of them can be affected in depression [6]. The great majority of serotonin (up to 90%) is produced and metabolized in the digestive tract and the rest in the nervous system [7]. It is synthesized in the gastrointestinal tract by enterochromaffin cells, mast cells, lymphocytes, and intestinal bacteria with the involvement of tryptophan (TRP) hydroxylase 1 (TPH-1) and in the central nervous system, with the involvement of TPH-2. Approximately 5% of TRP is metabolized in the serotonin pathway and the rest in the kynurenine pathway with the involvement of indole 2,3-dioxygenases (IDO-1 and IDO-2) [8]. Although TPH and IDO enzymes compete for access to tryptophan, healthy subjects with a balanced diet have the amount of TRP high enough for both enzymes [9]. Whereas usual TRP intake is approximately 900–1000 mg daily, the recommended daily allowance for adults is projected between 250 mg/day and 425 mg/day, which corresponds to a dietary intake of 3.5–6.0 mg/kg of body weight per day [10]. Another enzyme, tryptophan 2,3-dioxygenase (TDO), is found mainly in the liver, but also in other organs and metabolizes TRP to carbon dioxide, water, and ATP, limiting its level in the blood.

Many factors affect TRP metabolism. The expression of TPH-1 in the digestive tract is potentiated by some nutrients, bacteria, and pro-inflammatory cytokines and reduced by stress hormones [11]. Cytokines, such as interferon-α (IFN-α), IFN-β, tumor necrosis factor-α (TNF-α), and IFN-γ may upregulate IDO expression [12]. Kynurenine metabolites, including 3-hydroxy-kynurenine (3-OH-KYN) and quinolinic acid (QA), may affect brain function [13]. 3-OH-KYN may induce oxidative stress by increased production of reactive oxygen species (ROS), and QA may overstimulate hippocampal *N*-methyl-D-aspartate (NMDA) receptors, leading to apoptosis and hippocampal atrophy and both these effects have been associated with depression [12].

The intake of different amounts of TRP may differentially affect its metabolism and older adults may be characterized by a diverse consumption of this amino acid than average. Moreover, in the elderly, the activity of the enzymes metabolizing TRP may change in comparison to younger individuals. These features may lead to serious consequences resulting from different TRP metabolic pathways in older adults than others. In the present work, we investigated the association of the occurrence of mood disorders with tryptophan intake and metabolism in the elderly in comparison with younger and older adults without such disorders.

2. Materials and Methods

2.1. Patients

Ninety subjects, 60 women and 30 men, aged 36–85 years, were enrolled in this study. The study was performed in 2016–2020 in the Department of Clinical Nutrition and Gastroenterological Diagnostics and in the Department of Gastroenterology, Medical University of Lodz, Lodz, Poland. Initially, each subject was assessed for mental condition using the Hamilton Depression Rating Scale (HAM-D). The following score criteria were adopted: 0–7, no mental disorder; 8–12, mild depression; 13–18, moderate depression; 19–29, severe depression; over 30, very severe depression. Then, three groups were selected: group I, subjects without mood disorders and other ailments, aged 36–52 years; group II, subjects without mood disorders, aged 65–82 years; group III, patients with symptoms of mild and moderate depression (depressive mood disorders), aged 69–85 years.

In order to determine the occurrence of somatic diseases, clinical tests were carried out in all subjects to assess the condition of the circulatory, digestive, and nervous systems. Exclusion criteria were: circulatory or respiratory failure, advanced diabetes, liver diseases, renal failure, inflammatory bowel diseases, cancer, and use of psychotropic and sleeping pills.

2.2. Nutrition Procedures

All individuals were recommended to record the type and quantity of products consumed per day for 21 days prior to investigations in the nutrition diary. The average TRP intake was then calculated

using the nutrition calculator with the application Kcalmar.pro—Premium (Hermex, Lublin, Poland). After 21 days, biochemical testing of blood and urine was performed.

2.3. Laboratory Tests

The following fasting blood tests were performed: blood cell count, C-reactive protein, glucose, bilirubin, urea, creatinine, profile of lipids, thyroid-stimulating hormone, free thyroxine, free triiodothyronine, vitamins D3 and B12, alanine and asparagine aminotransferases, gamma-glutamyl transpeptidase, alkaline phosphatase, amylase, and lipase. Urine samples for the analysis of TRP metabolites were collected in the morning on an empty stomach into a special container with a solution of 0.1% hydrochloric acid as a stabilizer. Using liquid chromatography with tandem mass spectrometry (LC-MS/MS, Ganzimmun Diagnostics AG, Mainz, Germany), we determined the concentration of L-tryptophan and its following metabolites: 5-hydroxyindoleacetic acid (5-HIAA), L-kynurenine (KYN), kynurenic acid (KynA), xanthurenic acid (XA), and quinolinic acid (QA). The levels of these metabolites were expressed in mg per gram of creatinine (mg/gCr). The ratios of the levels of 5-hydroxyindoleacetic acid and tryptophan as well as kynurenine and tryptophan were also calculated. The 5-HIAA/TRP ratio was considered as an indicator of TPH-1 activity and the KYN/TRP ratio reflected the activity of DOI-1.

2.4. Ethical Issues

This study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. Written consent was obtained from each subject enrolled in the study and the study protocol was approved by The Bioethics Committee of Medical University of Lodz (RNN/176/18/KE).

2.5. Data Analysis

Normality of data distribution was checked using Shapiro–Wilk *W* test. The homogeneity of variance was tested by Brown–Forsythe modification of Levene test. One-way ANOVA and Kruskal–Wallis tests were used to compare difference between groups. Then, post hoc procedures were applied to determine significance of differences between specific groups: pairwise comparisons of group II or III against group I were performed by multiple comparisons using the two-sided Dunnett's *t*-test and contrasts were applied to compare groups II and III. The Bonferroni–Dunn test was used for post hoc analysis after Kruskal–Wallis test. All statistical analyses were performed with STATISTICA 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results

General characteristics of the individuals enrolled in this study and the results of routine laboratory tests are presented in Table 1.

The average daily intake of L-tryptophan in group III was significantly ($p < 0.001$) lower than that in groups I and II. Patients in group III showed significantly ($p < 0.001$) higher scores of the Hamilton Depression Rating Scale than those in groups I and II.

A substantial proportion of elderly patients (groups II and III) suffered from somatic diseases that are presented in Table 2.

Table 1. Characteristics of the subjects enrolled in this study: group I, controls; group II, elderly subjects; group III, elderly patients with mood disorders.

Feature [a]	Group I (n = 30)	Group II (n = 30)	Group III (n = 30)	p
Age (years)	42.1 ± 4.2	75.3 ± 4.7	74.6 ± 5.1	ns
Gender				
M	12	8	10	ns
F	18	22	20	ns
BMI (kg/m^2)	24.8 ± 3.2	26.1 ± 2.4	25.4 ± 3.1	ns
GFR (mL/min)	92 ± 11.2	78.2 ± 14.3	82.6 ± 20.1	ns
ALT (μ/L)	16.5 ± 3.4)	16.4 ± 1.9	17.2 ± 5.4	ns
AST (μ/L)	12.1 ± 2.6	18.2 ± 3.3	20.9 ± 6.1	ns
CRP (μ/g)	0.99 ± 0.63	1.14 ± 0.86	1.65 ± 1.02	ns
TRP daily intake (mg)	1446 ± 201	1370 ± 242	826 ± 106	<0.01 *,#
HAM-D score	5.1 ± 1.2	4.9 ± 0.9	13.6 ± 3.7	<0.001 *,#

[a] average ± SD; SD, standard deviation; M, male; F, female; BMI, body mass index; GFR, glomerular filtration rate; ALT, alanine aminotransferase; AST, asparagine aminotransferase; CRP, C-reactive protein; TRP, L-tryptophan; HAM-D, Hamilton Depression Rating Scale; ns (non-significant), $p > 0.05$; * group I vs. group III; # group II vs. group III.

Table 2. Somatic diseases in elderly patients without (group II) and with (group III) mood disorders.

Disease	Group II (n (%))	Group III (n (%))
Hypertension	12 (40.0)	16 (53.3)
Coronary disease	7 (23.3)	6 (20.0)
Diabetes	8 (26.6)	9 (30.0)
Dyslipidemia	11 (37.8)	17 (56.6)
Bowel disorders	16 (53.3)	16 (53.3)

No significant differences were observed in the occurrence of somatic diseases between groups II and III. Due to these diseases, elderly patients took appropriate medications according to pre-established recommendations (Table 3).

Table 3. Drugs used by elderly patients without (group II) and with (group III) depressive mood disorders.

Drugs	Group II (n (%))	Group III (n (%))
Beta-blockers	11 (36.6)	9 (30.0)
Calcium channel blockers	6 (20.0)	10 (33.3)
Angiotensin inhibitors	12 (40.0)	8 (26.6)
Sartans	6 (20.0)	4 (13.3)
Statins	11 (36.6)	12 (40.0)
Anticoagulant drugs	9 (30.0)	11 (36.6)
Antidiabetic drugs	8 (26.6)	9 (30.0)
Other	19 (63.3)	18 (60.0)

No significant differences were observed in the drug usage between these two groups.

During the 21-day follow-up, patients measured their blood pressure twice a day and diabetic patients measured their blood glucose levels. These values were stable and no dose adjustment was necessary.

The urinary levels of TRP in group III was lower than that in younger adults: 10.4 ± 1.18 vs. 13.3 ± 2.31 mg/gCr ($p < 0.05$, Figure 1A), but there was no difference in the levels of urinary 5-hydroxyindoleacetic acid (5-HIAA, Figure 1B) and TRP/5-HIAA ratio (Figure 1C) between these groups. As 5-HIAA is a main TRP metabolite in its serotonin metabolic pathway, we speculated that

this pathway was not affected in older adults with mood disorders as these subjects presented the same 5-HIAA/TRP ratio as the remaining two groups.

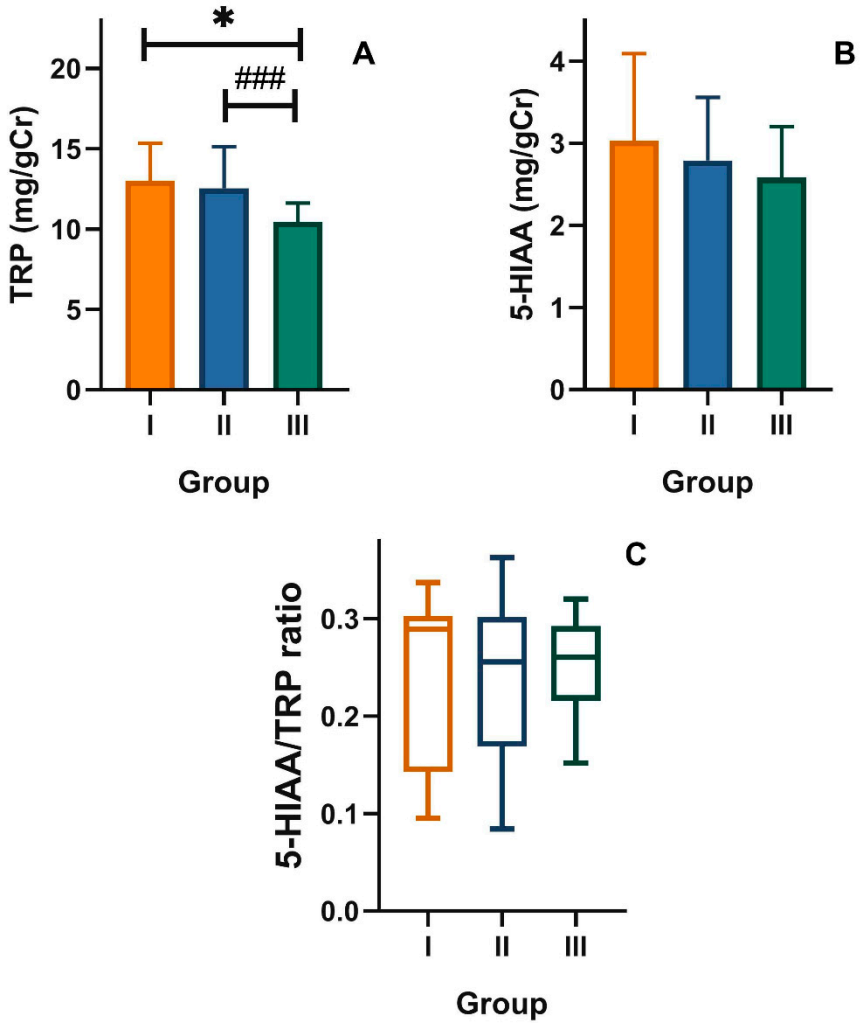

Figure 1. Urinary levels of (**A**) tryptophan (TRP) and (**B**) 5-hydroxyaminoacetic acid (5-HIAA) expressed in milligram per gram of creatinine (mg/gCr), and (**C**) 5-HIAA/TRP ratio in healthy young adult individuals (group I) and in the elderly without (group II) and with mood disorders (group III); mean ± SD (**A**,**B**) or median with boxes represent I and III quartiles, and error bars represent 1.5 times the interquartile distance. Differences between groups were analyzed by ANOVA (**A**,**B**) with Dunnett's multiple comparison method (group III vs. I) or contrast (III vs. II); the differences between groups in C were assessed by Kruskal–Wallis test; $n = 30$ in each group; * $p < 0.05$; ### $p < 0.001$.

The level of L-kynurenine (KYN) in group III was higher than that in group I: 0.85 ± 0.21 vs. 0.45 ± 0.09 mg/gCr ($p < 0.001$, Figure 2B). However, the KYN/TRP ratio in group III was significantly higher than that in the control group: 0.08 ± 0.02 vs. 0.03 ± 0.01 mg/gCr ($p < 0.001$, Figure 2C). As KYN is the main metabolite of TRP in its kynurenine pathway, we reasoned that this pathway was potentiated in older adults with mood disorders. This was confirmed by the increased ratio of KYN to TRP in these patients (Figure 2C).

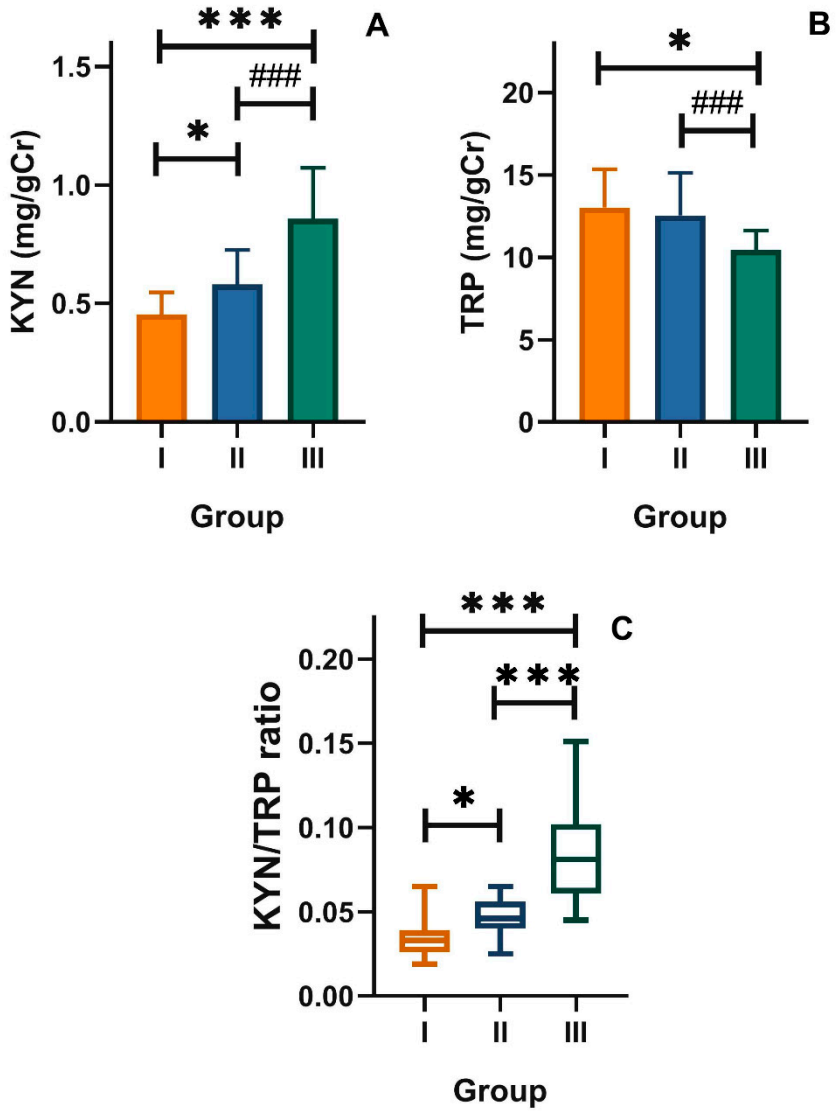

Figure 2. Urinary levels of (**A**) L-kynurenine (KYN) and (**B**) tryptophan (TRP) expressed in milligram per gram of creatinine (mg/gCr), and (**C**) kynurenine/tryptophan ratio (KYN/TRP) in healthy young adult individuals (group I) and in the elderly without (group II) and with mood disorders (group III); mean ± SD (**A**,**B**) or median with boxes represent I and III quartiles, and error bars represent 1.5 times the interquartile distance. Differences between groups were analyzed by ANOVA (**A**,**B**) with Dunnett's multiple comparison method (group III vs. I and group II vs. I) or contrast (group III vs. II); the differences between groups in C were assessed by Kruskal–Wallis test; $n = 30$ in each group; * $p < 0.05$, *** $p < 0.001$; ### $p < 0.001$.

Elderly individuals with mood disorders showed a higher level of kynurenic acid in urine than subjects in groups II and I (2.93 ± 0.92 mg/gCr vs. 2.20 ± 0.63 mg/gCr and 2.08 ± 0.47 mg/gCr, respectively, $p < 0.001$, Figure 3A). Similar differences occurred in the levels of xanthurenic acid: group I, 0.73 ± 0.27 mg/gCr; group II, 0.84 ± 0.28 mg/gCr; group III, 1.00 ± 0.32 mg/gCr ($p < 0.001$, Figure 3B). Older adult individuals showed a significantly increased ($p < 0.001$) level of quinolinic acid (7.13 ± 1.03 mg/gCr), whereas the level of QA in group II was 4.18 ± 1.19 mg/gCr and in group I was 3.10 ± 1.05 mg/gCr (Figure 3C). These results supported those presented in Figure 2 (the kynurenine pathway of TRP metabolism was potentiated in elderly with mood disorders as the concentration of products of this pathway was increased in these subjects. The ratio of KynA, XA, and QA to TRP was also increased in that group.

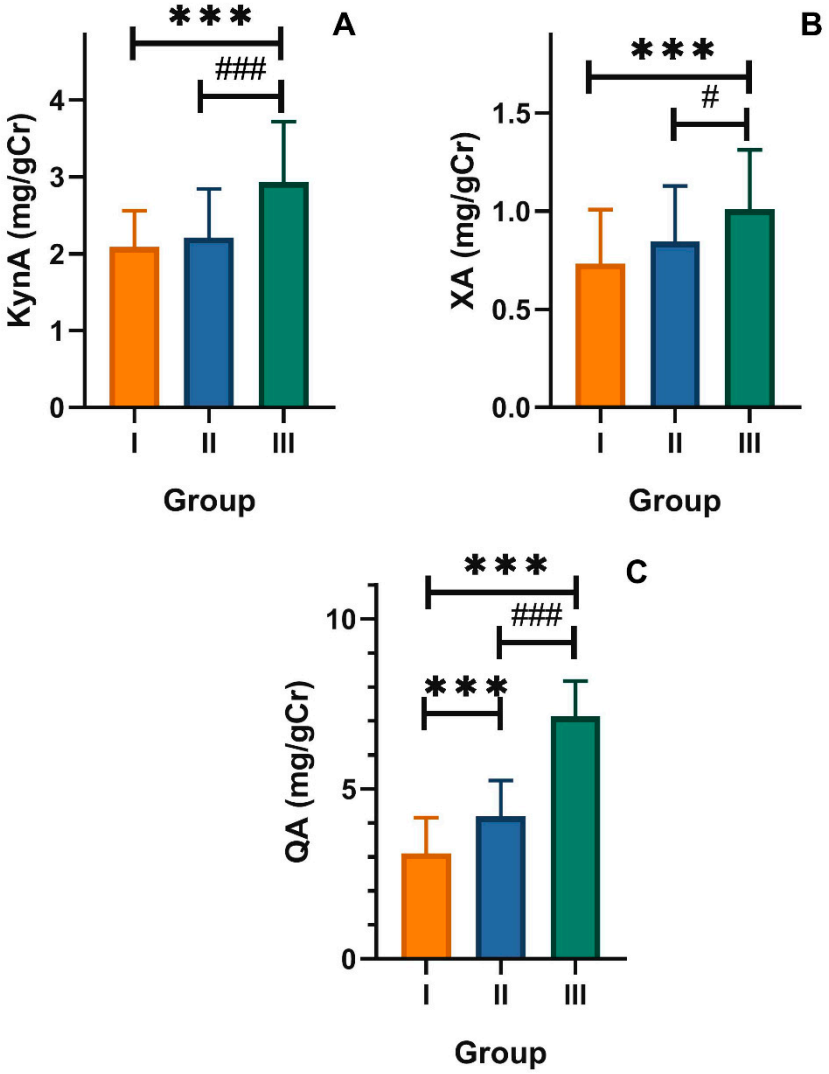

Figure 3. Urinary levels of (**A**) kynurenic acid (KynA), (**B**) xanthurenic acid (XA), and (**C**) quinolinic acid (QA) expressed in milligram per gram of creatinine (mg/gCr) in healthy young adult individuals (group I) and in the elderly without (group II) and with mood disorders (group III); mean ± SD differences between groups were analyzed by ANOVA with Dunnett's multiple comparison method (group III vs. I and group II vs. I) or contrast (group III vs. II); $n = 30$ in each group; *** $p < 0.001$; # $p < 0.05$; ### $p < 0.001$.

Finally, we compared the concentration of tryptophan and its metabolites as well as the metabolite ratios in the three groups with dependence on gender, as the total number of women enrolled in our study ($n = 60$) was significantly higher than men ($n = 30$).

In group II, women presented a higher KYN concentration and 5-HIAA/KYN ratio than men (Table 4, $p < 0.05$ in both cases). In group III, women had lower XA concentration than men ($p < 0.05$). No difference was observed between women and men in the remaining parameters in any group.

Table 4. Urinary levels of tryptophan (TRP) and its metabolites expressed in milligram per gram of creatinine and their ratios in healthy young adult individuals (group I) and in the elderly without (group II) and with mood disorders (group III) [1].

Group	I		II		III	
Gender	**M**	**F**	**M**	**F**	**M**	**F**
TRP	13.4 ± 2.54	12.78 ± 2.18	12.29 ± 1.96	12.64 ± (2.81	10.26 ± 1.48	10.54 ± 1.04
5-HIAA	2.68 ± 0.86	3.26 ± 1.12	2.86 ± 0.98	2.76 ± 0.7	2.37 ± 0.67	2.68 ± 0.59
KYN	0.43 ± 0.09	0.47 ± 0.09	0.49 ± 0.11	0.61 ± 0.14 *	0.91 ± 0.25	0.83 ± 0.2
5-HIAA/TRP	0.19 (0.13–0.30)	0.29 (0.16–0.3)	0.26 (0.19–0.3)	0.25 (0.17–0.3)	0.24 (0.2–0.28)	0.27 (0.22–0.29)
5-HIAA/KYN	0.03 (0.02–0.04)	0.03 (0.03–0.04)	0.04 (0.03–0.04)	0.05 (0.04–0.06) *	0.09 (0.07–0.13)	0.07 (0.06–0.1)
KynA	2.01 ± 0.5	2.13 ± 0.46	2.36 ± 0.72	2.15 ± 0.61	2.97 ± 0.9	2.91 ± 0.74
XA	0.85 ± 0.23	0.65 ± 0.28	0.68 ± 0.17	0.9 ± 0.29	1.17 ± 0.20	0.92 ± 0.31 *
QA	3.15 ± 0.79	3.07 ± 1.22	4.22 ± 0.45	4.18 ± 1.21	6.70 ± 0.64	7.34 ± 1.14

[1] Mean ± SD or median and the range of I and III quartiles; 5-HIA, 5-hydroxyaminoacetic acid; KYN, L-kynurenine; KynA, kynurenic acid; XA, xanthurenic acid; QA, quinolinic acid; M, men; F, women; * $p < 0.05$ as compared with men.

4. Discussion

The process of aging is associated with a loss of functional reserve of multiple organ systems, increased prevalence of chronic diseases, and enhanced susceptibility to stress [14]. These consequences of aging occur at a different step and with a different rate in individuals, resulting in a great heterogeneity within the elderly. It is still a challenging task to answer the question on the reasons for the difference between chronological and biological aging in various individuals [15].

Mood disorders are relatively frequent in older adults and become a major public health problem [16]. Depression in the elderly may be associated with medical comorbidities and cognitive decline, in addition to increased risk of dementia, suicide attempts, and overall mortality [17].

There are differences in clinical presentation and pathogenesis of mood disorders in older adults and nutritional neuroscience is an emerging branch indicating that nutrition is related to cognition, behavior, and emotions [18]. Therefore, diet may have clinical implications and is important for the effective treatment of mood disorders in the elderly.

In the present work, we found that older adults with mood disorders had a lower intake of tryptophan than their peers and younger individuals without psychiatric problems. As tryptophan cannot be synthesized by humans, its administration is one of the main determinants of its fate in human body. The other is the activity of enzymes involved in TRP metabolism. We showed that individuals with mood disorders consumed less TRP with their diet than their peers without mood disorders and younger individuals. Further, we showed that the concentration of products of a major TRP metabolic pathway was increased in the elderly subjects with mood disorders, independently of whether it was expressed directly or related to TRP amount. Therefore, the subjects with mood disorders might display increased activity of enzymes of the kynurenine pathway of TRP metabolism.

It was not surprising that subjects with mood disorders had lower levels of TRP as compared with individuals of the two remaining groups, as they were characterized by lower intake of TRP. However, such diminished administration of tryptophan in individuals with mood disorders did not hamper the increase in the production of metabolites of the kynurenine pathway of TRP degradation. In fact, over 95% of free TRP is a substrate for this pathway in normal subjects [19]. TRP metabolism through this pathway is mainly involved in the regulation of immunological response, intestinal homeostasis, and neuronal functions [20].

Subjects with depression had lower urine levels of TRP and 5-HIAA and lower 5-HIAA/TRP ratio, which suggested lower tryptophan hydroxylase activity in these subjects. On the other hand, the levels of KYN and the KYN/TRP ratio were increased, suggesting an increased IDO activity.

Decreased levels of TRP was observed in the blood of patients with depression in several clinical trials [21–23]. Although clinical trials mainly concentrate on supplementing or depriving TRP or its metabolites for the treatment of neuropsychiatric diseases, current preclinical efforts in drug development for these diseases have mainly focused on altering the rheostat of neuroactive metabolites

of the kynurenine pathway [24]. In humans, the KYN/TRP ratio, revealing the involvement of the KYN pathway, increases with age [25]. Such increase was also reported in patients with depression, but other studies showed no association or even a decrease [26–28]. Despite these somehow controversial results, TRP supplementation is considered potentially beneficial in many neurological and psychiatric diseases [24]. However, the optimal dose of tryptophan in the prevention or treatment of specific diseases is yet to be established. It is accepted that a daily dose of tryptophan at 5.0 mg/kg of body weight is sufficient for the basic needs of the normal organism, but aging may increase this dose.

Although we enrolled a significantly higher number of women ($n = 60$) than men ($n = 30$) (Table 1), there were no differences between the women to men ratio in each group. That is why our analysis did not include gender as a confounder. However, we made some calculations to check whether the parameters we investigated differed in women and men (Table 4). For seven parameters in three groups, making a total of twenty-one quantities, we observed a gender-specific difference only in three cases. Therefore, the kynurenine tryptophan metabolic pathways may not be strongly gender-dependent.

Our study had several limitations, which point at important elements of further research. Firstly, the number of subjects enrolled was not very impressive, but we had relatively homogenous and well characterized groups. The mood disorders were diagnosed on the basis of Hamilton Depression Scale and no further psychiatric characteristics of the subject were determined. The data on tryptophan intake were taken from information provided by subjects, who recorded the kind and amount of food they consumed. We assumed that the provided information was honest and reliable. It was the input for food calculator that gave data on the TRP content in the meals that the subjects had. We performed our analysis in urine adding at least one step to metabolic changes of TRP in blood.

In conclusion, older adults with mood disorders consumed less tryptophan than their peers without mental disturbances. The elderly with mood disorders were also characterized by a potentiated kynurenine pathway of the tryptophan metabolism. Therefore, further research should determine whether diet supplementation with tryptophan may be beneficial in the prevention and treatment of mood disorders in the elderly. Further studies on the role of enzymes of the kynurenine pathway in the pathogenesis of mood disorders may assess their potential as a target in the treatment of such disorders in the elderly.

Author Contributions: Conceptualization, C.C. and P.K.; methodology, C.C., T.P. and P.K.; investigation, C.C., P.K., T.P., M.F.; writing—original draft preparation, C.C., P.K., J.C., T.P., J.B., M.F.; writing—revised version and proofreading, J.B. and T.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Medical University of Lodz, grant number 503/6-006-05.

Acknowledgments: The authors thank the lab technicians from ALAB Laboratoria for participating in diagnostic procedures.

Conflicts of Interest: The authors declare no conflict of interest.

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Identification of Mortality Risks in the Advancement of Old Age: Application of Proportional Hazard Models Based on the Stepwise Variable Selection and the Bayesian Model Averaging Approach

Ewelina Łukaszyk [1,2,*,†], Katarzyna Bień-Barkowska [3,†] and Barbara Bień [1,2]

[1] Department of Geriatrics, Medical University of Bialystok, Fabryczna 27, 15-471 Bialystok, Poland; bien@umb.edu.pl
[2] Geriatric Ward, Hospital of the Ministry of the Interior and Administration in Bialystok, 15-471 Białystok, Poland
[3] Institute of Econometrics, Warsaw School of Economics, Madalińskiego 6/8, 02-513 Warsaw, Poland; katarzyna.bien@sgh.waw.pl
* Correspondence: ewelina.lukaszyk@umb.edu.pl; Tel.: +48-47-710-40-23
† These authors contributed equally to this work.

Abstract: Identifying factors that affect mortality requires a robust statistical approach. This study's objective is to assess an optimal set of variables that are independently associated with the mortality risk of 433 older comorbid adults that have been discharged from the geriatric ward. We used both the stepwise backward variable selection and the iterative Bayesian model averaging (BMA) approaches to the Cox proportional hazards models. Potential predictors of the mortality rate were based on a broad range of clinical data; functional and laboratory tests, including geriatric nutritional risk index (GNRI); lymphocyte count; vitamin D, and the age-weighted Charlson comorbidity index. The results of the multivariable analysis identified seven explanatory variables that are independently associated with the length of survival. The mortality rate was higher in males than in females; it increased with the comorbidity level and C-reactive proteins plasma level but was negatively affected by a person's mobility, GNRI and lymphocyte count, as well as the vitamin D plasma level.

Keywords: survival; geriatrics; vitamin D; TUG; lymphocytes; GNRI; Charlson Comorbidity Index; Bayesian model averaging

Citation: Łukaszyk, E.; Bień-Barkowska, K.; Bień, B. Identification of Mortality Risks in the Advancement of Old Age: Application of Proportional Hazard Models Based on the Stepwise Variable Selection and the Bayesian Model Averaging Approach. *Nutrients* **2021**, *13*, 1098. https://doi.org/10.3390/nu13041098

Academic Editor: Tomasz Kostka

Received: 7 February 2021
Accepted: 24 March 2021
Published: 27 March 2021

1. Introduction

Older adults are often the most complex and complicated medical patients and, therefore, at high risk for morbidity and mortality. Although advanced age remains the most important risk factor for death [1], it should not be forgotten that chronological age usually is not an equivalent of biological age [2]. Moreover, the importance of conventional risk factors, such as obesity [3,4], high cholesterol concentration, and cardiovascular diseases (including hypertension), tends to decrease in the oldest decades of age [5]. Therefore, there is still a need to look for other possible factors that might affect mortality in the oldest populations.

It is well known that malnutrition is associated with morbidity, and mortality; increased frequency of hospital admissions, prolonged hospital stays, and immune dysfunctions [6,7]. Screening tools for malnutrition, such as the Mini Nutritional Assessment (MNA), are routinely used in comprehensive geriatric assessment [8]. A novel and more precise scoring system has been recently proposed: The Geriatric Nutritional Risk Index (GNRI) [9]. It has been validated in institutionalized patients [10], dialysis patients [11], and patients with heart failure [12]. GNRI scoring includes serum albumin; therefore, it may be better correlated with systemic inflammation in the elderly.

It has been shown that malnutrition affects the immune status, which is manifested by a decrease in the total lymphocyte count [13]. Moreover, low lymphocyte count has been considered as an indicator of immunosenescence in geriatric patients and could be associated with increased mortality risk in the elderly population [14,15]. Together, both GNRI and total lymphocyte count might also improve the evaluation of nutritional risk and could predict short-term health complications [16].

Vitamin D deficiency is a global health problem, especially in elderly populations with poor nutritional status. The optimal 25-OH-vitamin D level revealed beneficial effects for patients with numerous illnesses; these include diabetes mellitus, cancer, autoimmune diseases, cognitive function, and even COVID-19 [17]. The vitamin D receptors are expressed by various types of immune cells that include lymphocytes [18]. The research has shown that decreased total lymphocyte count was associated with low levels of 25(OH) vitamin D [19]. However, the evidence of a relationship between vitamin D status and mortality is still inconsistent.

There is some evidence that the Comprehensive Geriatric Assessment (CGA) in older populations predicts an overall survival rate in cancer patients [20,21]. Physical performance measured by standardized tools, such as Timed Up and Go test (TUG) seems to predict adverse cardiovascular outcomes and mortality in the elderly [22]. Multiple comorbid medical conditions are observed much more often in older adults [23]. Comorbidity is associated with significant health complications, including mortality [24], and is most often assessed using the Charlson Comorbidity Index (ChCI) [25].

Attempting to combine easily identified and accessible indicators of health, functional, and nutritional status, this study's main objective is to identify an optimal set of predictors that are independently associated with the mortality risk of older comorbid adults formerly hospitalized in a geriatric ward just before the COVID-19 pandemic. As the standard stepwise variable selection procedures that chose one subset of predictors are often criticized for neglecting the model uncertainty, and a p-value near 0.05 can offer only weak evidence against the null hypothesis of no effect [26], we additionally used the more statistically robust method of Bayesian model averaging (BMA) [27–30]. The BMA method appropriately averages over all non-negligible probable models and leads to statistically sound inferences about risk factors for mortality rates.

2. Materials and Methods

2.1. Study Design

The design of this study was based on survival analysis of 433 patients that were discharged from the hospital between the end of 2016 and the end of 2018 (298 women and 135 men, aged 62–102, with an average age of 82.4, SD 6.5). The retrospective data were collected from the geriatric teaching unit (17 beds) in a medium-sized hospital—drawing on a local population of over 0.3 million. Patients were admitted to the geriatric ward due to multidimensional treatment needs and a recent aggravation of multifaceted health problems. An average patient presented 6.7 (SD of 2.3) chronic conditions out of 21 defined (ischemic heart disease, hypertension, atrial fibrillation, heart failure, cerebrovascular disease, arthritis, Parkinson disease, depression, dementia, delirium, anemia, diabetes, infection, liver disease, chronic kidney disease, ulcer, thyroid disorder, cancer, benign prostatic hyperplasia, connective tissue disease, chronic obstructive pulmonary disease). The mean length of hospital stays was 7.0 days (SD of 4.0). No exclusion criteria were applied to the study. The hospital records on different sociodemographic and health-related characteristics of geriatric patients were combined with the information about the length of survival time for each person after the hospital discharge. The exact dates of deaths were obtained from the Ministry of Digital Affairs. The censoring date for the survival was 3 March, 2020, i.e., just before the COVID-19 pandemic; up to that date, 132 persons (30.5%) died. The time of survival variable ranged from 1 day to 1594 days (4.4 years), with the median survival time equal to 893 days (about 2.5 years). The study was conducted in accordance with the Declaration of

Helsinki, and the protocol was approved by the Ethics Committee of Medical University of Bialystok (Project identification code: R-I-002/602/2018).

2.2. Potential Predictors of Mortality Rate

Geriatric patients are persons of advanced old age with complex morbidity; they are in need of comprehensive geriatric assessment due to a recent deterioration of their physical and/or psychical health [31]. A highly qualified team including geriatricians, physiotherapists, psychologists, and nurses diagnosed the patients in line with the CGA guidelines [32]. The hospital records were completed based on the thorough interviews with patients, physical and functional assessment, and laboratory findings.

The potential explanatory variables (predictors) for the mortality risk of hospitalized older adults included sociodemographic and health-related characteristics (Table 1). These variables included basic sociodemographic features, such as age; gender; mode of living (alone or in an institution versus with their family); number of years spent in education; anthropologic measures, such as weight (kg), and height (in meters); as well as the outcomes of CGA procedures routinely performed on the 1st or 2nd day after admission. More specifically, the functional status, being defined as the ability to complete basic activities of daily living (ADL), was evaluated using the Barthel Index [33]. The total score of the basic ADL ranged from a minimum of 0 (complete dependence) to a maximum of 100 (complete independence). Instrumental ADL (I-ADL) were assessed using the Duke OARS (Older Americans Resources and Services) Assessment [34]. Six I-ADL domains were included: Housework (cleaning floors and other domestic tasks), preparing their own meals, everyday shopping, using the telephone, handling their own money, and taking their own medicines. The summary I-ADL score ranged from 0 (lowest function) to 12 (highest function). The risk of bedsores was assessed with the Norton scale [35] (the lesser the score, the lower the risk) and the undernutrition with the MNA short form [36] (the lesser the score, the higher the risk). Emotional status was evaluated using the 15-item Geriatric Depression Scale [37], with a range of 0–5 showing no depression and 6–15 indicating a rising risk of depression. Cognitive status was assessed using the Modified Short Blessed Test [38], with a range of 0–7 indicating normal or mild cognitive impairment and 8–28 indicating a rising risk for dementia. Lastly, the Mini Mental State Examination [39] with a range 0–30 (the lesser the score, the worse the level of cognitive status is).

In order to determine a patient's mobility, the TUG test was performed [40]. The TUG test measured the time (in seconds) needed to rise from a chair and walk 3 meters, turn around, walk back to the chair, and sit down (the use of an assistive device was allowed—if needed). The speed of the TUG performance (in m/second) was recalculated for all patients. To this end, the distance of 6 m was divided by the time of TUG test performance in seconds. In order to not exclude from the study most disabled bedridden patients (42 cases), those persons were assigned the value of 0 m/second.

Major biochemical measurements included: Plasma hemoglobin in g/dL, the total lymphocyte count in K/μL, plasma albumin in g/dL, plasma vitamin B12 in pG/mL, plasma 25(OH)vitamin D in ng/mL, blood level of total cholesterol in mg/dL, C-reactive protein level in mg/L, fasting glucose in mg/dL, creatinine level in mg/dL, and the glomerular filtration rate according to the CKD-EPI formula in mL/minute/1.73 m^2. Hematological measurements were performed using fresh venous blood with EDTA, as well as clotted blood.

Apart from the laboratory findings, the GNRI score was calculated according to the Lorentz formula [9]. GNRI is a well-recognized and complex measure of the geriatric nutritional status summarizing information on a patient's height (in cm) and weight (in kg), as well as the albumin level in g/L [41].

Table 1. Descriptive statistics of geriatric patients.

	All Patients ***N* = 433**	**Died** ***N* = 132**	**Survived** ***N* = 301**	***p*-Value** [a]
Male, n (%)	135 (31.18)	53 (40.15)	82 (27.24)	0.007
Living Alone, n (%)	148 (34.18)	42 (31.82)	106 (35.22)	0.492
Age, mean (SD [b])	82.38 (6.54)	84.17 (6.31)	81.59 (6.49)	<0.001
Years of education, mean (SD)	9.28 (4.21)	8.55 (4.13)	9.60 (4.21)	0.009
No. of hospitalization days (SD)	7.02 (4.01)	7.95 (5.46)	6.61 (3.1)	0.0263
Barthel Index (0–100), mean (SD)	79.88 (23.88)	68.33 (28.50)	84.95 (19.54)	<0.001
I-ADL Index (0–12), mean (SD)	6.53 (3.88)	4.65 (3.82)	7.36 (3.61)	<0.001
Norton Index (1–20), mean (SD)	16.79 (2.86)	15.47 (3.19)	17.36 (2.49)	<0.001
MNA Score (0–14), mean (SD)	10.95 (2.76)	10.21 (2.88)	11.27 (2.64)	<0.001
Geriatric Depression Scale Score (0–15), mean (SD)	6.02 (3.84)	6.33 (4.01)	5.88 (3.76)	0.330
Blessed Score (0–28), mean (SD)	11.03 (9.13)	14.70 (9.81)	9.43 (8.34)	<0.001
MMSE Score (0–30), mean (SD)	20.70 (7.33)	17.57 (8.44)	22.07 (6.33)	<0.001
Speed of TUG test (m/s), mean (SD)	0.34 (0.24)	0.24 (0.19)	0.39 (0.24)	<0.001
Hemoglobin (g/dL), mean (SD)	12.61 (1.66)	12.24 (1.78)	12.77 (1.59)	0.006
Total Lymphocyte Count (K/μL), mean (SD)	1.75 (0.80)	1.56 (0.59)	1.83 (0.85)	<0.001
Fasting Glucose (mg/dL), mean (SD)	107.62 (30.33)	108.78 (26.42)	107.12 (31.93)	0.526
Vitamin B12 (pG/mL), mean (SD)	415.58 (298.61)	427.04 (315.66)	410.55 (291.22)	0.642
Vitamin D (ng/mL), mean (SD)	22.94 (15.34)	18.66 (13.83)	24.82 (15.61)	<0.001
Total Cholesterol (mg/dL), mean (SD)	174.78 (45.80)	164.23 (46.29)	179.40 (44.88)	<0.001
CRP (mg/L), mean (SD)	9.36 (28.08)	15.71 (42.89)	6.57 (17.50)	<0.001
Creatinine (mg/dL), mean (SD)	0.92 (0.45)	1.02 (0.69)	0.88 (0.28)	0.031
GFR (ml/min/1.73 m^2), mean (SD)	67.38 (18.75)	64.23 (19.88)	68.76 (18.09)	<0.001
Geriatric Nutritional Risk Index, mean (SD)	113.56 (13.28)	108.75 (13.15)	115.67 (12.79)	<0.001
Age-weighted Charlson Comorbidity Index (1–31), mean (SD)	8.30 (2.92)	9.65 (3.18)	7.71 (2.59)	<0.001

[a] Chi-square test or Mann–Whitney test for no difference between the two distributions (for died and survived), as appropriate; [b] SD denotes standard deviation.

Due to a significant number of coexisting conditions in one geriatric patient, comorbidity was measured with the age-weighted ChCI [25]. It ranged from a minimum of 0 to a maximum of 31, depending on the age and presence of selected diseases (including inter alia cardiovascular diseases, diabetes mellitus, dementia, pulmonary disease, cancer) with assigned weights.

2.3. Statistical Analysis

A Chi-squared or Mann–Whitney test was used to assess whether there was a significant difference between the distributions of potential explanatory variables that described the patients who died and the patients who survived over the course of the follow-up period.

The univariable Cox proportional hazards models (Cox PH) were estimated to investigate the statistical significance of the association between each of the 24 sociodemographic or health-oriented characteristics of geriatric patients and the mortality rate. Selected continuous variables were log-transformed if such a nonlinear functional form allowed for a better goodness-of-fit—according to the model log-likelihood value. The level of statistical significance was set as $p < 0.05$.

Next, multivariable Cox PH models were estimated. As the initial set of potential predictors for the mortality rate included up to 24 explanatory variables, we applied and

compared 2 alternative methods of variable selection; namely, the standard stepwise backward variable selection procedure and the more computation-intensive iterative BMA method [27–30,42–44]. Contrary to the stepwise variable selection methods, the latter approach represents a coherent procedure that improves upon the uncertainty of the single model [27,42]—especially if there are multiple predictor choices for the mortality rate that might potentially include confounding variables [28]. Therefore, the BMA can allow us to assess whether any redundant variable was inappropriately identified as a significant predictor of the mortality rate. Instead of using one Cox PH model, we implemented an approach proposed by Volinsky et al. (1997) and used a set of Cox PH models for making inferences [28]. The BMA mechanism is based on the appropriate weighted averaging over all such candidate models, wherein each of them includes a different set of explanatory variables. The BMA approach is becoming increasingly popular and, for example, was also used for both gene selection and the classification of microarray data [43] or in medical applications based on logistic regression setup [44,45]. Specifically, the BMA results are usually reported using the mean of parameter estimates from models that are characterized by a sufficiently high posterior probability—given observational data. This mean coefficient corresponding to a given explanatory variable captures the direction and strength of the effect that this predictor exerted on the mortality rate. Another important result is the posterior probability that the parameter corresponding to an explanatory variable is nonzero, $P(\beta \neq 0|D)$, where D denotes the data. The descriptive interpretation of obtained values can be the following [28]. If $P(\beta \neq 0|D) < 0.5$, *there is evidence against an effect* of a given predictor on the mortality rate of geriatric inpatients; if $0.5 < P(\beta \neq 0|D) < 0.75$, there is *weak evidence of an effect*; if $0.75 < P(\beta \neq 0|D) < 0.95$, *there is positive evidence*; if $0.95 < P(\beta \neq 0|D) < 0.99$, *there is strong evidence*; and if $0.99 < P(\beta \neq 0|D)$, *there is very strong evidence* that a given predictor exerts an effect on the mortality rate of hospitalized comorbid older adults. The final set of best predictors in our study was obtained using the iterative version of BMA [43], in which only these risk factors with the posterior probability greater than 0.3 were retained in the final Cox PH model.

The predictive performance of the identified set of best predictors was tested with 10-fold cross-validation [46]. To this end, the whole sample, which comprised of 433 observations, was randomly split into 10 nearly equal non-overlapping portions of data (one subset containing 46 observations and each of the other subsets containing 43 observations). In each of the 10 iterations of the cross-validation procedure, nine subsets were jointly used as a training set, whereas the remaining smaller amount of data served as a testing set. Once the models were estimated on a training set, their predictive performance was checked on the remaining testing set of observations. This process was repeated 10 times, and in each of these 10 iterations, different training and testing samples were used.

The predictive performance of the variables, identified as statistically significant using the backward variable selection method, was evaluated based on the *predictive discrimination ability*; this examined how well this set of variables sorted and classified the subjects in the testing block into the risk categories (lower, medium, and higher mortality risk) [28]. To this end, in each of the 10 iterations of the cross-validation procedure, the model was estimated on the training set to obtain the vector of parameter estimates $\hat{\beta}$ and to calculate *the risk scores*, *i.e.*, $x_i^T\hat{\beta}$, where x_i^T denotes the row vector of covariates for the ith subject (i.e., a geriatric patient). The lower-mortality-risk group, the medium-mortality-risk group, and the higher-mortality-risk group of patients were identified based on the 0.33-quantile (z_1) and 0.66-quantile (z_2) of the risk scores calculated for all the subjects in the training set. Next, the risk scores were recalculated for the patients from the testing set, and each subject was assigned to the particular risk group based on z_1 and z_2 values. More specifically, a patient, characterized by a risk score $x_i^T\hat{\beta} < z_1$ was classified as the one having a lower mortality risk. A patient, for whom $z_1 \leq x_i^T\hat{\beta} < z_2$, was assigned to a group with a medium mortality risk, and a patient for whom $z_2 \leq x_i^T\hat{\beta}$, was classified as one having a higher mortality risk.

The predictive discrimination ability of variables, retained as significant using the backward variable selection method, was also compared with the predictive discrimination ability of iterative BMA. In short, in every single replication of the cross-validation procedure, BMA requires fitting to the training set K candidate models: $M_1, M_2, \ldots, M_K$ to obtain the corresponding parameter estimates: $\hat{\beta}_1, \hat{\beta}_2, \ldots, \hat{\beta}_K$, and the corresponding risk scores $x_i^T\hat{\beta}_1, x_i^T\hat{\beta}_2, \ldots, x_i^T\hat{\beta}_K$. The mean risk score for a geriatric patient was calculated as a weighted average from K competing models: $\sum_{k=1}^{K} x_i^T\hat{\beta}_K P(M_k|D)$, where $P(M_k|D)$ denotes the posterior probability of the kth model. The 0.33-quantile and the 0.66-quantile of the mean risk scores can be used to classify the patients in the testing set to appropriate risk groups.

Statistical analyses were performed with STATA software version 15.0 (StataCorp LP, College Station, TX, USA) and R software (version 3.6.3, R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org (accessed on 15 April 2020)) with the BMA package for the Bayesian model-averaging (authors: Raftery, A., Hoeting, J., Volinsky, C., Painter, I., Yeung, K.Y.; https://cran.r-project.org/web/packages/BMA (accessed on 15 April 2020)) [47].

3. Results

Estimation results for the univariable Cox PH models are illustrated in Figure 1 (panel A). Accordingly, the mortality risk of former geriatric patients was significantly higher in males than in females (HR = 1.56; $p = 0.012$; 95% CI: [1.10, 2.21]), increased with the number of hospitalization days (HR = 1.1; $p < 0.001$; 95% CI: [1.06, 1.14]), patient's age (HR = 1.06; $p < 0.001$; 95% CI: [1.03, 1.09]), the Modified Short Blessed Test score (HR = 1.06; $p < 0.001$; 95% CI: [1.04, 1.08]), log CRP level (HR = 1.32; $p < 0.001$; 95% CI: [1.18, 1.48]), plasma creatinine level (HR = 1.84; $p < 0.001$; 95% CI: [1.47, 2.32]), and patient's comorbidity measured with the age-weighted ChCI (HR = 1.25; $p < 0.001$; 95% CI: [1.18, 1.32]). On the other hand, the scores on the Barthel Index (HR = 0.98; $p < 0.001$; 95% CI: [0.97, 0.98]) or the I-ADL index (HR = 0.85; $p < 0.001$; 95% CI: [0.81, 0.89]) were significantly negatively related to the patient's mortality rate. Moreover, the mortality risk decreased with a patient's Norton score (HR = 0.85; $p < 0.001$; 95% CI: [0.82, 0.89]), the MNA (HR = 0.89; $p < 0.001$; 95% CI: [0.84, 0.94]), MMSE score (HR = 0.93; $p < 0.001$; 95% CI: [0.91, 0.95]), the GNRI (HR = 0.96; $p < 0.001$; 95% CI: [0.95, 0.98]), the mobility level measured by the TUG speed (HR = 0.05; $p < 0.001$; 95% CI: [0.02, 0.12]), and selected biochemical measurements: the log hemoglobin level (HR = 0.13; $p < 0.001$; 95% CI: [0.04, 0.40]), log total lymphocyte count (HR = 0.65; $p < 0.001$; 95% CI: [0.57, 0.76]), the log total cholesterol level (HR = 0.29; $p < 0.001$; 95% CI: [0.15, 0.55]), log GFR (HR = 0.43; $p = 0.001$; 95% CI: [0.27, 0.71]), and the log vitamin D level (HR = 0.63; $p < 0.001$; 95% CI: [0.51, 0.78]). All other explanatory variables were not significantly related to the patient's mortality rate.

The stepwise backward variable selection method that was applied to the multivariable Cox PH model retained seven explanatory variables that were independently and significantly associated with the roughly 2.5-year survival of older adults (Figure 1, panel B). We can see that the mortality rate was significantly higher in males than in females (HR = 1.91; $p < 0.001$; 95% CI: [1.33, 2.76]); it increased with the comorbidity status measured with the age-weighted ChCI (HR = 1.14; $p < 0.001$; 95% CI: [1.07, 1.21]) and, besides this, it also increased with the log C-reactive protein—indicating an inflammatory condition (HR = 1.16; $p = 0.014$; 95% CI: [1.03, 1.30]). The nutritional status of an older adult that was evaluated using GNRI (HR = 0.98; $p = 0.003$; 95% CI: [0.97, 0.99]) and log total lymphocyte count (HR = 0.65; $p < 0.001$; 95% CI: [0.53, 0.80]) also had an independent beneficial effect on survival, as they both were negatively associated with the mortality rate. Additionally, the mortality risk turned out to decrease with the increasing speed of performing the TUG test (HR = 0.11; $p < 0.001$; 95% CI: [0.04, 0.29]) and the rising log vitamin D level (HR = 0.71; $p = 0.002$; 95% CI: [0.58, 0.88]), whereas the critically low vitamin D level was especially dangerous (Figure 2, panel E). Figure 2 illustrates the obtained

association between each of these variables and the length of survival for the men and women, separately. The individual survival curves were computed from the estimated multivariable Cox models at the selected value of one predictor, whereas the remaining covariates were set at their average levels.

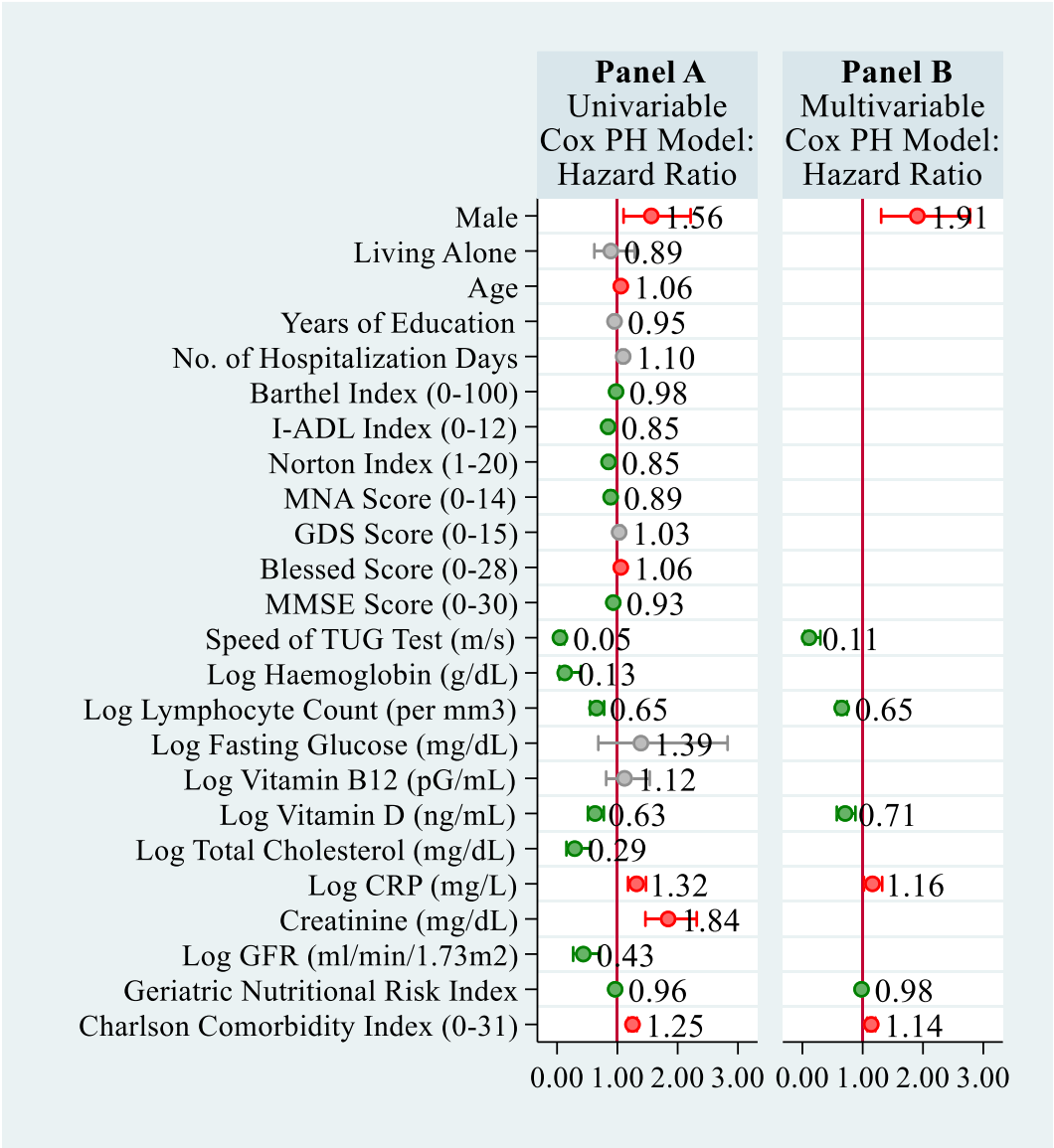

Figure 1. Association between the sociodemographic and health-related characteristics of former geriatric patients and their mortality rate: Results from the univariable Cox PH model (panel **A**) and the multivariable Cox PH model obtained with the stepwise backward variable selection method with the significance level set as $p < 0.05$ (panel **B**).

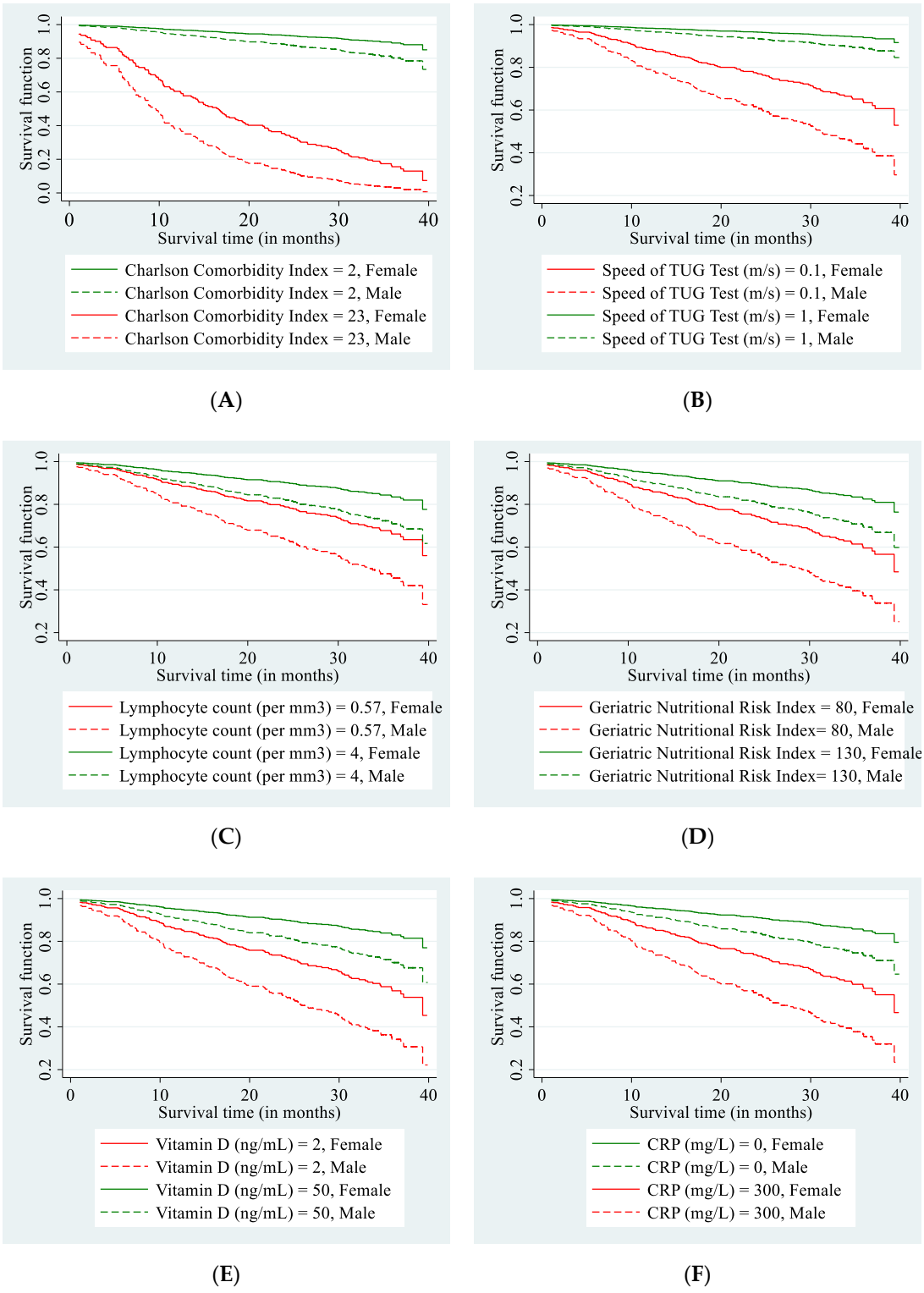

Figure 2. Survival curves for a geriatric patient depending on his or her sex and the selected health-related covariates identified using the stepwise backward variable selection approach (with $p < 0.05$): Age-weighted Charlson Comorbidity Index (panel **A**), the speed of performing the Timed Up and Go (TUG) test (panel **B**), total lymphocyte count (panel **C**), Geriatric Nutritional Risk Index (panel **D**), the vitamin D level (panel **E**), and CRP level (panel **F**). For each panel, the survival curves were derived from the multivariable Cox PH model for given sex and for selected very low or very high values of one explanatory variable, whereas the values of remaining covariates in the model were set at their average level.

According to the iterative BMA method, the multivariable Cox PH model that achieved the highest posterior probability, given the observational data, was the same as the Cox PH model obtained with the backward stepwise variable selection method. The application of the iterative BMA also resulted in the same combination of mortality predictors (Table 2). Accordingly, there was *very strong evidence* that being a male was associated with a higher mortality, i.e., $P(\beta \neq 0|D) = 1$. There was also *very strong evidence* that scale of comorbidity measured with the age-weighted ChCI increased mortality risk, i.e., $P(\beta \neq 0|D) = 1$. The BMA results also clearly confirmed that the higher the (log) speed of performing the TUG test, the lesser the mortality risk was, i.e., *there is very strong evidence of an effect*, $P(\beta \neq 0|D) = 1$. Controlling for the impact of other covariates, the nutritional status of an older comorbid adult, which could be captured by the (log) total lymphocyte count and the GNRI, turned out to have a beneficial effect on survival, i.e., there was *very strong evidence of an effect* for (log) total lymphocyte count, $P(\beta \neq 0|D) = 1$, and there was *positive evidence of an impact* for the GNRI, $P(\beta \neq 0|D) = 0.923$, correspondingly. Having adjusted for other factors, there was also *positive evidence* that a rising level of (log) vitamin D had an additional independent beneficial impact on alleviating mortality risk, i.e., $P(\beta \neq 0|D) = 0.916$). *Only weak evidence of an effect* had been obtained with respect to an impact of a rising (log) CRP level on mortality, i.e., $P(\beta \neq 0|D) = 0.689$.

Table 2. Results from the iterated Bayesian model averaging of the multivariable Cox PH regressions. Posterior parameter estimates (averages), their standard deviations, and probabilities that coefficients are nonzero for the seven identified variables affecting mortality rate.

Predictors	Average Coefficient	Standard Deviation of Coefficients	$P(\beta \neq 0 \mid D)$
Male	0.632	0.189	1.000
Age-weighted Charlson Comorbidity Index	0.139	0.032	1.000
Speed of TUG Test	−2.255	0.502	1.000
Log Total Lymphocyte Count	−0.423	0.105	1.000
Geriatric Nutritional Risk Index	−0.020	0.009	0.923
Log Vitamin D	−0.309	0.140	0.916
Log CRP	0.106	0.088	0.689

The predictive performance of the final multivariable Cox PH model (including seven previously identified variables: Being male, age-weighted Charlson Comorbidity Index, speed of TUG test, log total lymphocyte count, Geriatric Nutritional Risk index, log vitamin D, and log CRP) was remarkably good. Out of patients assigned to a lower mortality risk, only 15 (10.6%) patients died, whereas out of patients assigned to a higher mortality risk category, 74 (50%) died. The discrimination accuracy for this set of predictors was only slightly worse than if using the BMA approach that weights the risk scores from competing models. For BMA, out of the 142 patients assigned to a lower risk category, only 15 (10.6%) died; and out of the 148 patients in the higher risk category, 76 died (51.4%). Hence, in two patients, the weighted average of different candidate models improved the predictive accuracy of classification. The predicted classification of former geriatric patients into the lower and higher categories of mortality risk was illustrated in Figure 3, where the individual scatterplots present the impact of two paired continuous or polytomous predictors (out of six identified as most important) for men or women separately. Identification of subjects at higher mortality risk is most visible for patients with low speed of performing the TUG test and high comorbidity level. These less mobile and more comorbid patients were much more often assigned to a higher-mortality-risk group, and indeed, they more frequently died during the 2.5-year period after being discharged from the hospital. The summary of the predictive performance for the Cox PH model with

the seven most important covariates is presented in Figure 4. A combination of seven identified predictors for mortality rate successfully discriminates between patients at the lower and higher risk of death over the next 2.5 years.

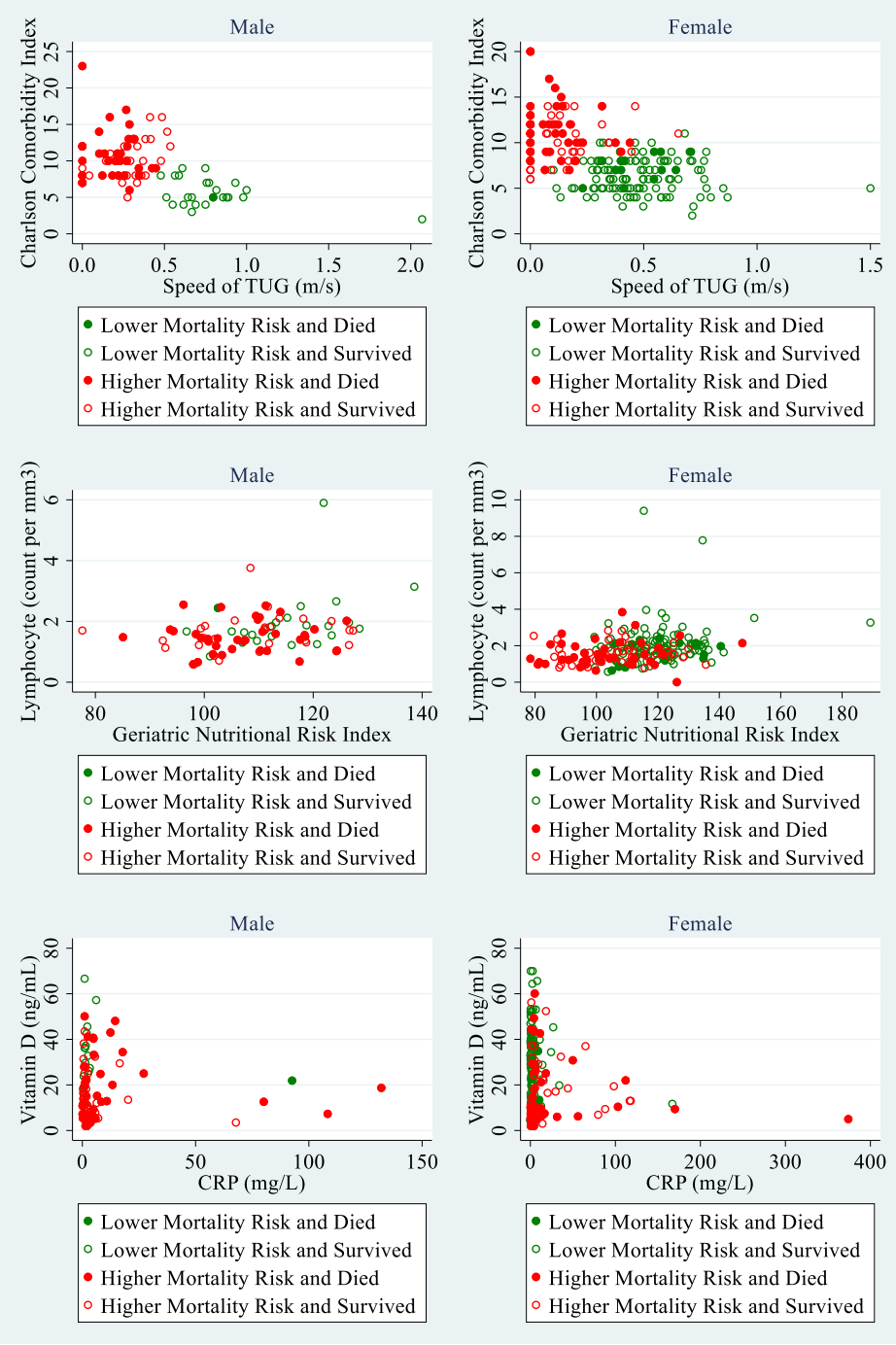

Figure 3. Classification of geriatric patients into the category of lower mortality risk and the category of higher mortality risk based on seven most important predictors identified by both: The stepwise backward selection method and the iterative BMA approach. Results from the 10-fold cross-validation of the multivariable Cox PH model.

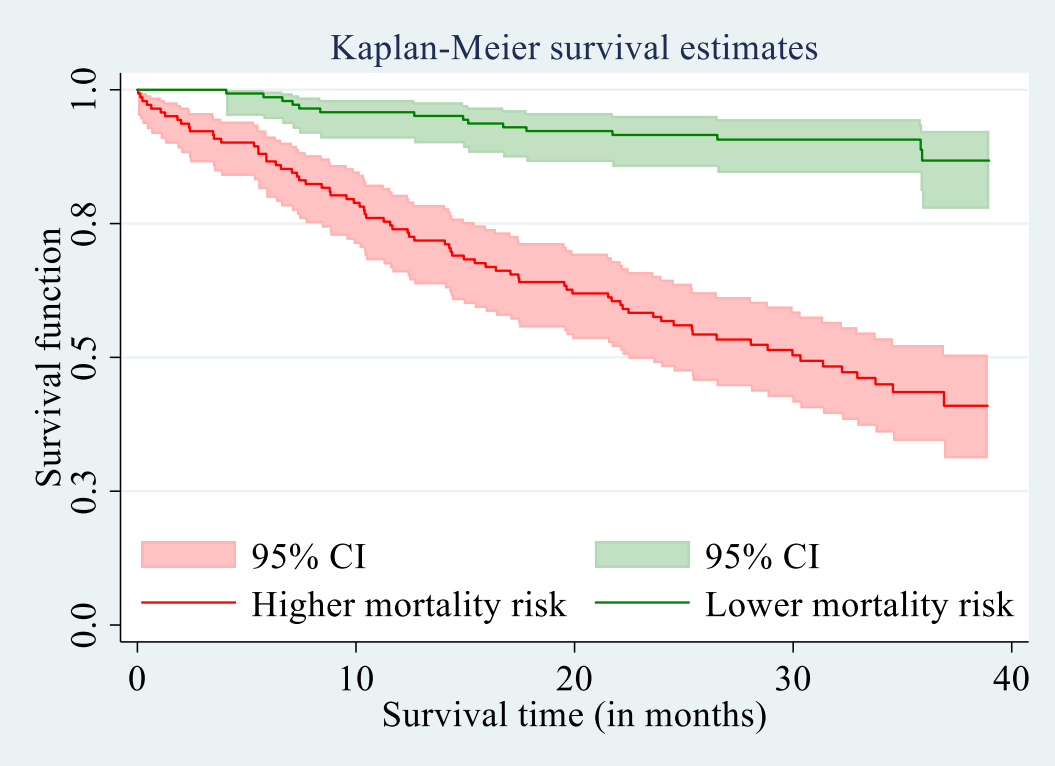

Figure 4. Kaplan–Meier survival curves for patients assigned to a higher and a lower mortality risk category based on a 10-fold cross-validation. Results of the multivariable Cox PH model with the same seven most important risk factors for mortality rate identified by both: The stepwise backward variable selection and the iterative BMA approach.

4. Discussion

The extension of life expectancy is driven inter alia by a decline in death rates amongst older people [48]. In recent decades, older people have lived longer and less disabled, despite the higher rate of morbidity controlled by more and more effective treatments [49]. Therefore, we have undertaken an assessment of the factors that determine the respectively longer or shorter survival time in older and comorbid adults.

The presented study identified seven significant factors independently associated with the mortality rate in community-dwelling older adults with high comorbidity. They included: Gender, age-related comorbidity measured with the age-weighted Charlson Comorbidity Index, physical performance that was assessed by the TUG test, immunity state (total lymphocyte count and vitamin D plasma level), protein-energy status assessed by the Geriatric Nutritional Risk Index, and inflammation measured with C-reactive proteins. Beyond gender, the set of contributors covers numerous potentials for survival modification. Although each of the relevant factors interplays with others, they independently affect the organism's resources. These include: Body structure and its functionality, nutrition as a protein-energetic reserve, and both immunologic and anti-inflammatory capacity [50]. According to the current knowledge about mechanisms of aging [51], they also contribute to the stability of the homeostasis in an aging individual.

According to the BMA method, only four out of seven factors, namely male gender, age-weighted comorbidity, speed of the TUG performance, and a total lymphocyte count achieved the highest probability unambiguously in explaining the risk of mortality over 2.5 years in multi-morbid older adults.

Gender and age are widely known unmodifiable contributors to the mortality rate. Women outlive men at all ages; however, they show much lower health indices than men of the same age in all industrialized countries, which creates the so-called "male-female-health-survival paradox" [52]. It has also been confirmed in our analysis (Table 2).

Other strong predictors of mortality, such as comorbidity [53] and reduced mobility [54], have long been known and evident. Older adults present an enormous amount of phenotypic diversity [55], huge complexity, and heterogeneity of clinical pictures [56]. Moreover, the geriatric patients are, to some extent, a selected population due to attrition rates resulting from the age-independent mortality before reaching old age [57]. Our previous results [58], as well as those presented in this study, confirm this pragmatic statement about the shorter survival rate of comorbid older adults. The age-weighted Charlson Comorbidity index combines typical age-related morbidity that appears with the advancement of chronological age [25]. This single indicator covers a wide range of age-related morbidities; these include cardiovascular and cerebrovascular chronic conditions, dementias of any origin, diabetes mellitus at different stages, chronic kidney disease, chronic obstructive pulmonary disease, cancer, and many others. Can comorbidity be potentially considered a modifiable target for prolonging patient's survival? According to a well-known maxim '*what has happened cannot be undone*', the answer is pessimistic. However, from a different perspective, many of these age-related disorders could be potentially preventable. One can find such data in relation to the main killers of older adults, namely cardiovascular diseases [59], as well as dementia [60]. If their risk factors were identified early in life and eliminated, then the survival time should increase, and the overall quality of life in older age should improve.

The speed of the TUG test performance used in this study was found to be as important as the aforementioned factors for explaining survival in the oldest and comorbid populations. The speed of completing any task appears to be a universal indicator of biological aging—regardless of the cause. It combines the outcomes of normal aging like age-related sarcopenia, osteopenia, and immunosenescence, as well as the accumulation in life course pathology like multimorbidities and the deficits of nutritional status. It is worth emphasizing that the positive relationship between walking speed and vitamin D levels was found in the meta-analysis of older adults [61].

Total lymphocyte count is an easily obtained nutrition maker [62] and is, at the same time, the indicator of the efficiency of the immune system—even in younger adults [63]. Lymphopenia was associated with a shorter survival that is independent of traditional risk factors in the 12-year follow-up observational study covering over 31,000 participants [64]. In our study, low total lymphocyte count was also strongly associated with a higher risk of mortality that is independent of other factors, which is in line with previous studies [14,15].

Undernutrition is one of the potentially modifiable factors directly associated with life expectancy [65]. It is often an under-recognized condition in hospitalized older adults [66]. GNRI combines albumin's plasma level (protein-energy status) with body weight and, for this reason, was developed for the geriatric population to indicate nutrition-related complications [9]. Moreover, GNRI was revealed as a good predictor of muscle strength in institutionalized older patients [67], as well as a specific and sensitive tool used in detecting frailty and sarcopenia [68]. According to a recent study by Yuan Y. et al. [69], the low GNRI not only prognoses disease-related complications and mortality but also shows worse exercise tolerance in patients with COPD [70]. The iterative BMA method used in this study showed that there is positive evidence of an independent GNRI effect on the mortality risk in old and comorbid adults. It would be worth considering to evaluate the utility of using GNRI and total lymphocyte count as complex and predictive measures for assessing the risks of health complications [16].

Nowadays, hypovitaminosis D is a global health problem [71]. In many observational studies, 25(OH)vitamin D deficiency was correlated with diabetes, cancer, obesity, hypertension, cardiovascular diseases, and cognitive dysfunction [72]. Vitamin D also plays an important role in immune system activity [73]. Mao X. et al. [19] have also elucidated the correlation between lymphocyte subsets and 25 (OH)vitamin D in older adults with age-related diseases. In our research, an insufficient (i.e., very low) concentration of 25(OH)vitamin D turned out to be an independent explanatory variable for the increased

mortality in elderly comorbid adults. However, despite the great interest and numerous studies on the effect of vitamin D on survival, the results are still inconclusive [74,75].

Low-grade inflammation, defined as elevated CRP concentration, appears to be associated with an increased risk of mortality in older adults [76]. Moreover, higher inflammatory parameters were observed in older people with frailty [77]. These findings stay in line with our results, although according to the iterative BMA method, we have obtained only weak evidence of the association between the CRP and the mortality risk.

It is remarkable that the same set of factors, including male gender and comorbidity, is associated with the risk of severe outbreak or death in patients with the COVID-19 disease [78]. There is also some evidence that adequate vitamin D concentration has shown beneficial effects in the SARS-Cov-2 infection [79,80]. Therefore, medical trials are currently being conducted in older adults diagnosed with COVID-19 [81]. GNRI may also be used as a predictive tool for high-risk elderly patients with COVID-19, as well as other nutritional scales [82]. Moreover, in older adults infected with SARS-Cov2, lymphopenia occurs more frequently—especially in severe cases [83,84].

Hence, the question arises: Can the accumulation of factors identified in our study be considered a kind of universal sign of multi-organ failure and impending death? Although almost all identified predictors of the over 2-year survival are independently associated with the mortality rate, they interact with each other. Comorbidity usually deteriorates physical activities, nutrition, vitamin D status, immunity, and vice versa. This might suggest that supplementing older people with a diet containing protein-energy ingredients and vitamin D would have a positive effect on immunity and physical activity, and, as a result, would equate to longer survival rates in older adults.

The limitations of this analysis lie in the relatively small sample and the short time of observation. It should also be added that many other measures and indices not included in this study might determine mortality. Moreover, because the study design is not experimental but observational, the dependencies investigated in this research should be confirmed in randomized controlled trials based on the optimization of the nutritional and functional status of geriatric patients. However, the application of the iterative BMA approach, which accounts for the model uncertainty neglected by the solely stepwise variable selection methods, reinforces the reliability of answers posed to the question under study.

5. Conclusions

This study identifies the best combination of risk factors for all-cause mortality in older comorbid adults formerly hospitalized in the geriatric ward due to recent deterioration of their physical and/or psychical health. The mortality rate turned out to be independently associated with gender, age, comorbidity, mobility, as well as nutritional and immunologic body's reserves. 25(OH)vitamin D plasma level is also shown to play a protective role for the oldest and comorbid people. Therefore, the recommendations for older adults should focus on optimal nutrition, maintenance or enrichment immunity with better vitamin D status, physical activity, and the prevention of chronic (specifically inflammatory) diseases [85]. However, further research into these factors should still be actively pursued. There is a need for further prospective studies based on the population of the oldest comorbid adults, thus far usually excluded from clinical randomized control trials.

Author Contributions: E.Ł. and K.B.-B. contributed equally to this study (co-shared first authorship). Conceptualization, B.B.; methodology, K.B.-B.; software, K.B.-B.; validation, E.Ł. and K.B.-B.; formal analysis, K.B.-B.; investigation, E.Ł. and B.B.; resources, E.Ł.; data curation, B.B., and E.Ł.; writing—original draft preparation, E.Ł. and K.B.-B.; writing—review and editing, E.Ł., K.B.-B., B.B.; visualization, K.B.-B.; supervision, B.B.; project administration, E.Ł.; funding acquisition, E.Ł. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Medical University of Bialystok—grant number: SUB/1/DN/20/001/3301.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Medical University of Bialystok (Project identification code: R-I-002/602/2018).

Data Availability Statement: The data presented in this study are not publicly available due to thefact of confidentiality reasons. These data are available on request from the Prof. Barbara Bien (bien@umb.edu.pl).

Acknowledgments: The research required the collaboration of interdisciplinary geriatric team employed in the Geriatric Word of the Hospital of the Ministry of Interior and Administration in Bialystok, where the Department of Geriatrics of Medical University of Bialystok is seated. We thank all of them as well as the hospital authorities for agreement and cooperation.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; and in the decision to publish the results.

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Comparison of Nutrition Risk Screening 2002 and Subjective Global Assessment Form as Short Nutrition Assessment Tools in Older Hospitalized Adults

Łukasz Kroc, Elizaveta Fife, Edyta Piechocka-Wochniak, Bartłomiej Sołtysik and Tomasz Kostka *

Healthy Ageing Research Centre (HARC), Department of Geriatrics, Medical University of Lodz, Pieniny 30, 92-003 Lodz, Poland; lukasz.kroc@umed.lodz.pl (Ł.K.); elizaveta.fife@umed.lodz.pl (E.F.); edyta.piechocka-wochniak@umed.lodz.pl (E.P.-W.); bartlomiej.soltysik@umed.lodz.pl (B.S.)
* Correspondence: tomasz.kostka@umed.lodz.pl

Abstract: The aim of the present study was to compare two widely recommended short nutrition assessment tools—Nutrition Risk Screening 2002 (NRS-2002) and Subjective Global Assessment Form (SGA)—with other Comprehensive Geriatric Assessment (CGA) measurements. The study included 622 consecutively hospitalized older subjects, aged 81.7 ± 7.8 years. The criteria to participate were the ability to communicate and given consent. Both NRS-2002 and SGA were inversely related to anthropometric measurements, functional assessment tests, Mini-Mental State Examination (MMSE) and positively associated with the Vulnerable Elders Survey-13 (VES-13) score. Results of SGA and NRS-2002 were not related to sex and 15-item Geriatric Depression Scale (GDS) score. Comparison of well-nourished subjects and patients with suggested problems with nutrition according to NRS-2002 (0–2 vs. 3–7) and SGA (A vs. B + C) gave comparable results. Both nutritional scales at given cut-off points similarly discriminated anthropometric data and other CGA tools in the populations of well-nourished vs. malnourished hospitalized older subjects. In conclusion, we can recommend using both NRS-2002 and SGA to detect malnutrition or risk of malnutrition in a routine clinical practice of the geriatric department ward.

Keywords: malnutrition; NRS-2002; SGA; VES-13; Comprehensive Geriatric Assessment

Citation: Kroc, Ł.; Fife, E.; Piechocka-Wochniak, E.; Sołtysik, B.; Kostka, T. Comparison of Nutrition Risk Screening 2002 and Subjective Global Assessment Form as Short Nutrition Assessment Tools in Older Hospitalized Adults. *Nutrients* **2021**, *13*, 225. https://doi.org/10.3390/nu13010225

Received: 22 December 2020
Accepted: 12 January 2021
Published: 14 January 2021

1. Introduction

Malnutrition (undernutrition) is one of the most common problems in aging societies. In Europe, an estimated 33 million people are at risk of malnutrition [1]. Studies show that up to one third of patients in hospitals and nursing homes are at risk of undernutrition, as are 10% of individuals over the age of 65 in the European Union (EU) [2,3]. Malnutrition is associated with impaired muscle function, decreased bone mass, immune dysfunction, reduced cognitive functioning, anemia, prolonged hospitalization, and increased risk of frailty, falls, morbidity and mortality [4,5].

The prevalence of malnutrition is even higher in geriatric hospitalized population—between 30% and 60% [6–9]. Therefore, valid and quick detection of malnutrition is of utmost importance in hospitalized elderly and several short nutritional tests have been proposed to check for malnutrition in that population. Nutrition Risk Screening 2002 (NRS-2002) [10,11] and Subjective Global Assessment Form (SGA) [12] are among the most widely used [13].

Although both tools have become commonly used in hospitalized patients in various clinical settings, current literature shows a relatively small amount of data about the validation of NRS-2002 and SGA scales in hospitalized older patients and its relationship with other widely used geriatric measures–especially in large population studies. In older adults with multiple deficiencies and comorbidities, the routine format of medical examination and other common tests and procedures is usually not sufficient. Therefore, the Comprehensive Geriatric Assessment (CGA) has been developed to address patients' problems

with medical comorbidities, functional status and psychosocial capacities [14]. The aim of this study was to assess concurrent validity and compare NRS-2002 and SGA with other tools commonly used in the CGA in a large population of hospitalized older subjects.

2. Materials and Methods

2.1. Design of the Study and Participants

The study initially included 963 older people, aged 60 and above years old, who were hospitalized in the acute care Geriatric University Clinic, Central Veterans' Hospital in Lodz (Poland), between January 2018 and November 2019. The criteria for the participation in this study were efficient verbal communication and given consent. Out of the 963 hospitalized patients, 341 were further excluded due to incomplete data (one or more of validation tests were incomplete), severe dementia or terminal illness. Therefore, 622 patients with completed data were finally included to the study (Figure 1). The following tests were conducted in all subjects: the NRS-2002 and SGA to measure nutritional status, Activities of Daily Living (ADL) [15] and Instrumental Activities of Daily Living (IADL) [16] to measure functional status, Mini Mental State Examination (MMSE) [17] to measure cognitive status, Geriatric Depression Scale (GDS) [18] to measure depression status and Vulnerable Elders Survey -13 (VES-13) [19] was used as a screening tool for frailty. All the tests were conducted by the physicians of the geriatric ward at admission.

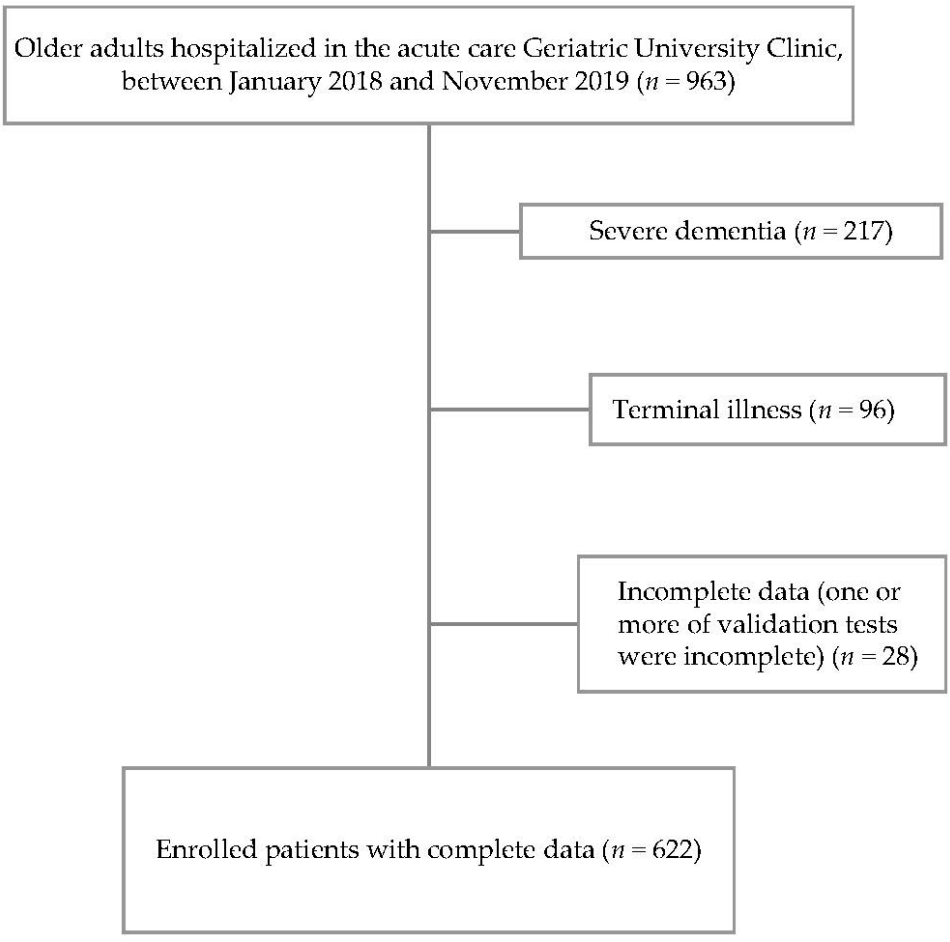

Figure 1. Flow chart of enrollment for the study.

2.2. Nutritional Questionnaires

NRS-2002 was designed as a tool to identify patients at nutritional risk [10,11]. Nutritional risk was assessed through two criteria: impaired nutritional status and disease

severity. A score between 0 and 3 was given for each criterion. Nutritional status was determined by three variables: BMI, recent body mass loss, and food intake during the week before hospital admission. Disease severity was analyzed by assessing increased nutritional requirements caused by recent medical history (falls, fractures, operations, oncologic and intensive care therapy) and concomitant chronic diseases. For people aged 70 and above years old, an additional extra point was added. The NRS-2002 score is a sum of the total of the nutritional score, severity of disease score and the age adjustment score. The total number of points ranges from 0 to 7. Patients with a score of 3 and more are suggested to have problems with nutrition [20].

SGA is determined on the basis of medical history about changes in nutrients intake, body mass loss, symptoms affecting oral intake (diarrhea, vomiting, nausea, dysphagia, oral problems), functional capacity (fatigue and progressive loss of function), and on physical examination findings such as subcutaneous fat, muscle wasting, presence of edema and ascites. Patients with severe malnutrition were classified as C (or 3 points), moderate malnutrition as B (or 2 points), and normal nutrition as A (or 1 point). The information necessary to fulfill the SGA was collected directly from the patients, or if this was not possible, the data were provided by accompanying family members [12].

2.3. Other Tools

ADL scale (Katz scale) evaluates such parameters as for example, the ability to maintain hygiene or to feed him/herself. Low scores on this scale indicate an inability to function independently. Patients score 1 point for positive responses of the type: "I do not have any problem with this ability". The total number of points ranges from 0 to 6, with scores of 5 and 6 indicating patients in good condition [15].

IADL scale (Lawton scale) examines the ability of seniors to manage their life in the modern environment. The IADL takes into account for example, the ability to use the phone or managing money. Patients receive 1 point for positive responses indicating "I do not have any problem with this ability". The total number of points ranges from 0 to 8, with scores of 7 and 8 indicating good condition [16].

MMSE is the most commonly used test for problems with memory or other mental abilities. It can be used to help diagnose dementia. This test consist of questions about orientation concerning time and place, attention and calculation, recall, language and praxis. The maximum possible score is 30 points, with a score of 24 points or more indicating that patients do not have problems with memory loss [17].

GDS has 15 questions describing the well-being of the patient. The maximum possible is 15, with scores of 5 or less indicating no problems with depression [18].

VES-13 includes questions about age (<75 years = 0 points, 75–84 years = 1 point, age $\geq$ 85 years = 3 points), self-rated health status (poor or fair = 1 point, good or average = 0 points) and two main sections: one about physical functioning and the other about the need for assistance with daily activities. The whole VES-13 consists of 13 questions, with a maximum score of 10 points for the worst prognosis [19].

2.4. Statistical Analysis

Data was verified for normality of distribution (Kolmogorov-Smirnov test) and equality of variances (Levene's test). Pearson's and Spearman's correlation coefficients were used to measure the strength and direction of the relationship between two variables. Values of NRS-2002 and SGA were further dichotomized to compare well-nourished subjects with patients suspected of malnutrition (NRS-2002 0-2 vs. NRS-2002 3-7 and SGA A vs. SGA B + C). The sensitivity (the proportion of SGA B + C cases correctly identified as NRS-2002 3-7 cases) and specificity (the proportion of SGA A correctly identified as NRS-2002 0-2 cases) of NRS-2002 to detect malnutrition as compared to SGA was calculated. The one-way analysis of variance (ANOVA), Mann-Whitney test and chi-square test (with Yates' correction for 2 $\times$ 2 tables) were used to test for differences between the sex and nutritional status groups. Statistical analysis was carried out using Statistica 13.1

software (StatSoft Polska, Cracow, Poland). The quantitative data were expressed as mean ± standard deviation. The limit of significance was set at $p = 0.05$.

2.5. Ethical Certification

The study was approved by the Ethics Committee of the Medical University of Lodz (approval number: RNN/300/17/KE) and written informed consent was obtained from all subjects.

3. Results

Patient characteristics is presented in Table 1. The reasons for hospitalization were very diverse, ranging from anemia and pneumonia, to gastrointestinal bleeding, loss of body mass, diagnosis of physical or cognitive function decline, stroke or diabetes mellitus. The majority of patients had several concomitant diseases. Mean age of the whole population was 81.7 ± 7.8 years. Women had lower body mass, all the circumferences, ADL and IADL, and higher GDS and VES-13 than men. NRS-2002 and SGA were virtually the same in women and men.

Table 1. Characteristics of the patients—summary statistics for age, anthropometric measurements, ADL, IADL, MMSE, GDS, VES-13, NRS-2002 and SGA.

	All	**Women**	**Men**
	(*n* = 622)	**(*n* = 431)**	**(*n* = 191)**
Age	81.7 ± 7.78	81.9 ± 7.70	81.4 ± 7.97
Body mass (kg)	65.9 ± 15.5	62.3 ± 14.2	74.3 ± 15.3 ***
Waist circumference (cm)	93.1 ± 13.8	91.1 ± 13.3	97.5 ± 13.8 ***
Calf circumference (cm)	34.6 ± 5.98	34.1 ± 5.78	35.6 ± 6.29 **
Arm circumference (cm)	27.4 ± 4.83	27.1 ± 4.90	28.1 ± 4.63 *
BMI (kg/m^2)	25.6 ± 4.96	25.5 ± 5.14	25.7 ± 4.55
ADL	4.73 ± 1.77	4.64 ± 1.83	4.94 ± 1.62 *
IADL	5.04 ± 2.86	4.89 ± 2.90	5.38 ± 2.76 *
MMSE	21.6 ± 7.88	21.25 ± 8.00	22.3 ± 7.56
GDS	5.07 ± 3.57	5.27 ± 3.59	4.62 ± 3.51 *
VES-13	6.54 ± 2.92	6.75 ± 2.87	6.06 ± 2.96 **
NRS-2002	1.61 ± 1.25	1.61 ± 1.21	1.62 ± 1.33
SGA	1.16 ± 0.42	1.17 ± 0.42	1.15 ± 0.41

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. BMI, Body Mass Index; ADL, Activities of Daily Living; IADL, Instrumental Activities of Daily Living; MMSE, Mini-Mental State Examination; GDS, Geriatric Depression Scale; VES-13, Vulnerable Elders Survey-13; NRS-2002, Nutrition Risk Screening 2002; SGA, Subjective Global Assessment Form.

Tables 2 and 3 show distribution of scores of the two nutritional scales–NRS-2002 and SGA. The majority of the population was not malnourished according to both scales. The distribution of both NRS-2002 and SGA was very similar in women and men.

Table 4 shows Spearman correlation coefficients of NRS-2002 (0–7 points) and SGA (1–3 points) with age, anthropometric data and other CGA tools. NRS-2002 score correlated directly with age while SGA did not. Both NRS-2002 and SGA negatively correlated with anthropometric data, ADL, IADL and MMSE. Significant positive correlations were found between nutritional scales and VES-13. For anthropometric data these associations were similar while for ADL, IADL, MMSE and VES-13 were higher for NRS-2002 than for the SGA. These correlations were generally similar in women and men. There were no relationship between GDS and nutritional scales. The results of Pearson's correlations were very similar.

Table 2. NRS-2002 distribution.

NRS-2002	All (n = 622)		Women (n = 431)		Men (n = 191)	
0	52	8.4%	35	8.1%	17	8.9%
1	363	58.4%	248	57.5%	115	60.2%
2	70	11.3%	55	12.8%	15	7.9%
3	83	13.3%	56	13%	27	14.1%
4	30	4.8%	23	5.3%	7	3.7%
5	15	2.4%	10	2.3%	5	2.6%
6	8	1.3%	3	0.7%	5	2.6%
7	1	0.2%	1	0.2%	0	0%

Table 3. SGA distribution.

SGA	All (n = 622)		Women (n =431)		Men (n = 191)	
A	529	85%	363	84.2%	166	86.90%
B	82	13.2%	61	14.2%	21	11%
C	11	1.8%	7	1.6%	4	2.10%

Table 4. Spearman correlation coefficients of NRS-2002 and SGA with age, anthropometric data and other Comprehensive Geriatric Assessment measurements.

	All		Women		Men	
	NRS-2002	SGA	NRS-2002	SGA	NRS-2002	SGA
Age	0.30 *	0.03	0.29 *	0.03	0.33 *	0.04
Body mass (kg)	−0.34 *	−0.40 *	−0.37 *	−0.45 *	−0.32 *	−0.33 *
Waist circumference (cm)	−0.32 *	−0.38 *	−0.34 *	−0.43 *	−0.27 *	−0.30 *
Calf circumference (cm)	−0.26 *	−0.34 *	−0.26 *	−0.36 *	−0.25 *	−0.32 *
Arm circumference (cm)	−0.30 *	−0.38 *	−0.31 *	−0.37 *	−0.27 *	−0.39 *
BMI	−0.38 *	−0.43 *	−0.40 *	−0.46 *	−0.33 *	−0.36 *
ADL	−0.28 *	−0.19 *	−0.31 *	−0.20 *	−0.22 *	−0.14
IADL	−0.28 *	−0.14 *	−0.29 *	−0.16 *	−0.24 *	−0.10
MMSE	−0.26 *	−0.13 *	−0.28 *	−0.15 *	−0.21 *	−0.10
GDS	0.06	0.07	0.05	0.04	0.06	0.12
VES-13	0.26 *	0.11 *	0.25 *	0.10 *	0.27 *	0.13

* $p < 0.05$.

Table 5 presents the comparison of well-nourished subjects and patients with suggested problems with nutrition according to NRS-2002 (0-2 vs. 3-7) and between the group without problems with nutrition (SGA A) and the group of subjects suspected of malnutrition or malnourished (SGA B + C). Concerning age, nutritional-different subgroups were better discriminated by NRS-2002. Both nutritional scales at given cut-off points similarly discriminated anthropometric data and other CGA tools in the populations of well-nourished vs. malnourished hospitalized older subjects. The sensitivity of NRS-2002 to detect malnutrition was 77.4% and specificity was 87.7% as compared to SGA.

Table 5. Comparison of the subjects with different nutritional status according to NRS-2002 (NRS 0+1+2 vs. NRS 3-7) and SGA (SGA A vs. SGA B + C).

	NRS 0+1+2 (*n* = 485)	**NRS 3-7 (*n* = 137)**	**SGA A (*n* = 529)**	**SGA B + C (*n* = 93)**
Age	81.2 ± 8.03	83.6 ± 6.51 **	81.6 ± 7.90	82.5 ± 7.03
Men (%)	30.3%	32.1%	31.4%	26.9%
Body mass (kg)	68.1 ± 15.3	58.3 ± 14.2 ***	68.4 ± 15.04	52.03 ± 10.1 ***
Waist circumference (cm)	94.97 ± 13.2	85.7 ± 13.5 ***	95.3 ± 12.98	80.4 ± 11.3 ***
Calf circumference (cm)	35.3 ± 5.82	32.02 ± 5.87 **	35.4 ± 5.87	30.3 ± 4.58 ***
Arm circumference (cm)	27.8 ± 4.69	25.7 ± 5.11 ***	28.1 ± 4.69	23.5 ± 3.67 ***
BMI (kg/m^2)	26.4 ± 4.80	22.6 ± 4.44 ***	26.4 ± 4.76	20.8 ± 3.11 ***
ADL	4.96 ± 1.59	3.90 ± 2.11 ***	4.88 ± 1.67	3.92 ± 2.09 ***
IADL	5.37 ± 2.73	3.82 ± 3.004 ***	5.22 ± 2.78	4.01 ± 3.14 ***
MMSE	22.3 ± 7.44	19.1 ± 8.901 ***	22.02 ± 7.56	18.9 ± 9.11 ***
GDS	4.99 ± 3.48	5.37 ± 3.89	4.96 ± 3.50	5.73 ± 3.92
VES-13	6.31 ± 2.97	7.35 ± 2.55 ***	6.41 ± 2.93	7.26 ± 2.74 **
SGA	1.04 ± 0.20	1.61 ± 0.63 ***	-	-
NRS-2002	-	-	1.28 ± 0.89	3.45 ± 1.37 ***

** $p < 0.01$; *** $p < 0.001$.

4. Discussion

This report is one of the first studies comparing NRS-2002 and SGA with tools from CGA. Both nutritional approaches are widely used in screening and assessment of malnutrition [13]. CGA is a multidisciplinary set of procedures that identifies medical, psychosocial, and functional capabilities of an older adult. CGA is a standard assessment methodology at geriatric wards. Our data indicates that both short nutrition assessment tools are similarly but moderately related to physical and mental function of hospitalized older adults.

There is a variety of tests for screening and assessment of malnutrition like Mini Nutritional Assessment (MNA), Malnutrition Universal Screening Tool (MUST), NRS-2002 and SGA [13,21]. Nevertheless, there is no single tool that can be considered as the universal gold standard for the diagnosis of nutritional status in hospitalized patients [22,23]. SGA and NRS-2002 are among the most widely validated and recommended for older patients [13]. Several studies proved the usefulness of those tools to predict the length of hospital stay or clinical outcome [24,25]. In 124 critically ill patients the SGA rating correlated significantly with percentage of body mass loss, serum albumin level, health status scores and mortality [26]. Malnutrition assessed with SGA in 66 consecutive patients prior to peripheral blood stem cell transplantation was associated with increased length of hospital stay [27]. Both SGA and MNA predicted 3-year mortality in 83 consecutive acute geriatric patients [28]. NRS-2002 and Mini Nutrition Assessment-Short Form (MNA-SF) had similar performance to predict unfavourable clinical outcomes in 705 patients admitted to a Brazilian public university hospital [29]. NRS-2002 was a valuable prognostic tool in 750 adults admitted to the emergency service [30]. In a large multicentre prospective cohort study NRS-2002 was an independent predictor of poor clinical outcome in 5051 patients [31]. In a prospective analysis of 536 hospitalized Chinese patients both NRS-2002 and MNA scores could predict mortality [32].

Several studies compared malnutrition short assessment tools, some of those studies used SGA as a reference method or a "gold standard" [25,33–35]. Both MUST and NRS-2002 showed good agreement with SGA in identification of nutritional risk in 577 adult patients admitted to a public emergency room [34]. Comparison of four short nutrition assessment tools (NRS-2002, MUST, SGA and MNA) in 400 patients admitted to the hospital revealed significant differences between the four nutritional assessment tools. The best agreement

between the tools was for NRS-2002 with SGA and MUST with SGA. The authors concluded that at admission, NRS-2002 and MUST should be used to screen for nutritional status [36]. On the other hand, in 995 patients assessed at hospital admission NRS-2002 had higher sensitivity and specificity than MUST and Nutritional Risk Index (NRI), as compared to SGA [25]. The sensitivity was 62% and specificity was 93% with the NRS-2002 [25]. The criterion validity of the Malnutrition Screening Tool (MST), MUST, NRS-2002, MNA-SF, modified MST (MST combined with low BMI), and BMI as independent tools was assessed in 693 patients from Vietnam using SGA or low BMI (<18.5 kg/m2) as the reference method. Based on specificity and sensitivity, the first choice for the most appropriate assessment tool for use was the NRS-2002 [37]. Zhang et al. compared SGA and NRS-2002 in 312 oncologic patients [38]. The SGA-A had a higher sensitivity (93.73%) but a poorer specificity (2.30%) than the NRS-2002 <3 points (69.30% and 25.00%, respectively) after comparison with albumin. A high similarity between the SGA and NRS-2002 for evaluating nutritional status was found [38,39]. A systematic review including 111 studies representing 52,911 participants showed that NRS-2002 and SGA had a significant correlation with BMI and several biomarkers of malnutrition. Those results were similar for SGA and NRS-2002 [40]. On the other hand, Ozkalkanli et al. compared NRS-2002 and SGA in predicting the development of complications in patients undergoing orthopaedic surgery. Sensitivity was 50% with the SGA and 69% with the NRS-2002; specificity was 77% with the SGA and 80% with the NRS-2002. The authors concluded that NRS-2002 predicted the development of complications better than the SGA [41].

In the present study, the agreement between the two short nutrition assessment tools was very high. The sensitivity of NRS-2002 to detect malnutrition was 77.4% and specificity was 87.7% as compared to SGA. Interestingly, though several studies linked clinical outcome measures to malnutrition assessment tools and compared different tools, very few studies assessed malnutrition measures in relation to the CGA measurements in older subjects. In one available study the prevalence of malnutrition was 53.6% according to the SGA and 44.6% according to the NRS-2002 in 815 hospitalized patients with an average age of 62.2 years [39]. The prevalence of malnutrition was strongly correlated with the severity of depression and dementia [39]. In another study an important correlation was found between SGA and several cognitive/functional geriatric tests in 81 elderly dialysis patients [42].

In our study, the prevalence of malnutrition was 22% according to NRS-2002 and 15% according to SGA. Both NRS-2002 and SGA showed correlation with anthropometric data and CGA measurements concerning physical and cognitive functioning. The fact that the distribution of both NRS-2002 and SGA was very similar in women and men, and correlations of NRS-2002 and SGA with age, anthropometric data and other CGA tools were generally similar in both sexes provides important practical information about usefulness of both nutritional tools equally in older women and men. Significant association of both tools was also observed with VES-13. VES-13 was used as a measure of frailty, as it is one of the most commonly used instruments [43] with a high sensitivity for predicting the occurrence of disability, mortality and institutionalization [44]. Lack of correlation with age and weaker correlations with physical, cognitive and frailty data for SGA may suggest that NRS-2002 might be more suitable for hospitalized older adults. This potential disparity should be corroborated in future prospective studies. Especially, given recently demonstrated high sensitivity of NRS-2002 for identifying nutritional risk and predictive validity for prolonged hospitalization in older adults with COVID-19 [45]. Adding those physical, cognitive and frailty data to phenotypic and etiologic criteria of malnutrition proposed by the Global Leadership Initiative on Malnutrition (GLIM) might also have enriched diagnosis and severity grading of malnutrition [13].

While this study shows several advantages it also has some limitations. The study was conducted in the "real world" geriatric hospitalized population–in patients with multiple medical problems but being able to respond and perform basic geriatric tests. Therefore, the group of patients was relatively heterogenic and many patients were excluded due

to the terminal status or incapacity to perform all tests. Relationship of nutritional status to functional correlates may also be different during long-term hospitalization or in an institutional environment [46]. Secondly, we used only two short nutritional assessment tests–NRS-2002 and SGA. Other nutritional assessment tools like MNA or MUST might have performed better, but they are more difficult to apply in everyday screening practice. Finally, an important aspect of prevention of malnutrition is not only checking the state of nutrition on admission, but also monitoring the nutritional status and its predictive value during and after the hospitalization. Future prospective studies are needed to assess the best and feasible short assessment procedure to predict future outcomes in hospitalized older subjects.

5. Conclusions

We can recommend using both NRS-2002 and SGA to detect malnutrition or risk of malnutrition in a routine clinical practice of the geriatric department ward. These tests similarly discriminate the two groups of well-nourished vs. malnourished/at risk older hospitalized patients. Nevertheless, the relationship of both tests to other measures of routine geriatric assessment is moderate and future research should search for further optimisation of nutritional assessment in a geriatric hospital setting.

Author Contributions: Conceptualization, Ł.K. and T.K.; Methodology, Ł.K., E.F. and T.K.; Validation, Ł.K. and T.K.; Formal Analysis, Ł.K. and T.K.; Investigation, Ł.K., E.F., E.P.-W., B.S. and T.K.; Resources, Ł.K., E.F., E.P.-W., B.S. and T.K.; Data Curation, Ł.K.; Writing—Original Draft Preparation, Ł.K.; Writing—Review & Editing, Ł.K., E.F., E.P.-W., B.S. and T.K.; Visualization, Ł.K.; Supervision, T.K.; Funding Acquisition, T.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Medical University of Lodz [grant number 503/6-077-01/503 61-001].

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Medical University of Lodz (approval number: RNN/300/17/KE). Informed consent was obtained from all subjects involved in the study.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Conflicts of Interest: No potential conflict of interest was reported by the authors.

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Epidemiology of Hypoalbuminemia in Hospitalized Patients: A Clinical Matter or an Emerging Public Health Problem?

Stefania Moramarco [1,*], Laura Morciano [1], Luca Morucci [1], Mario Messinese [1], Paola Gualtieri [2], Mariachiara Carestia [1], Fausto Ciccacci [3], Stefano Orlando [1], Ersilia Buonomo [1], Jacopo Maria Legramante [4], Antonino De Lorenzo [2] and Leonardo Palombi [1,3]

1 Section of Hygiene and Public Health, Department of Biomedicine and Prevention, University of Rome Tor Vergata, Via Montpellier, 1-00133 Rome, Italy; laura.morciano@gmail.com (L.M.); lucamorucci88@gmail.com (L.M.); mario.messinese@students.uniroma2.eu (M.M.); mariachiara.carestia@uniroma2.it (M.C.); stefano.orlando@uniroma2.it (S.O.); buonome@uniroma2.it (E.B.); palombi@uniroma2.it (L.P.)

2 Section of Clinical Nutrition and Nutrigenomics, Department of Biomedicine and Prevention, University of Rome Tor Vergata, Via Montpellier, 1-00133 Rome, Italy; paola.gualtieri@uniroma2.it (P.G.); delorenzo@uniroma2.it (A.D.L.)

3 Unicamillus, International Medical University in Rome, Via di Sant'Alessandro, 8-00131 Rome, Italy; fausto.ciccacci@gmail.com

4 Department of Systems Medicine, University of Rome Tor Vergata, Via Montpellier, 1-00133 Rome, Italy; legramante@med.uniroma2.it

* Correspondence: stefania.moramarco@gmail.com

Received: 21 October 2020; Accepted: 25 November 2020; Published: 27 November 2020

Abstract: Serum albumin levels are strongly associated with the morbidity, prognosis, and mortality rates of patients with hypoalbuminemia, which is a frequent problem during hospitalization. An observational retrospective study was carried out to analyze changes in albumin levels in hospitalized patients at the "Fondazione Policlinico Tor Vergata—PTV" in 2018. The prevalence of preexisting hypoalbuminemia at the time of discharge from hospital was investigated using a sample of 9428 patients. Information was collected from the discharge files recorded in the central informatics system of the hospital. Analysis of albumin levels at admission and at discharge was conducted by classes of albuminemia and then stratified by age. At the time of admission, hypoalbuminemia was found to be present in more than half of the sample, with no sex differences. The serum albumin level tended to decrease with age, with pathologic levels appearing from 50 years and progressive worsening thereafter. The condition of marked and mild hypoalbuminemia was more prevalent in patients over 65 years of age. Our findings suggest that hypoalbuminemia should be considered a dangerous condition in itself and a serious public health problem. We aimed to emphasize the role of albumin as useful marker of the in-hospital malnutrition and frailty, to be integrated in the routinely assessment of patients for reconsidering ad hoc healthcare pathways after discharge from hospital, especially when dealing with fragile populations.

Keywords: elderly; fragile populations; hospitalization; hypoalbuminemia; public health; serum albumin

1. Introduction

Human serum albumin is an important parameter for the routine assessment of the nutritional status of patients with acute and chronic conditions [1]. Additionally, it is a recognized valuable

biomarker of many diseases, such as cancer [2], ischemia [3], and obesity [4], and is used for monitoring inflammatory activity. Inflammation is a well-known cause of hypoalbuminemia in a number of diseases, including rheumatoid arthritis [5,6]. Albumin is also associated with diseases related to the control of glycemia and adipose tissue [7,8].

In clinical practice, hypoalbuminemia [9] is commonly discovered in association with nutritional deterioration and disease-related inflammatory response [10]. Along with the evolution of the disease itself, this condition might be a result of the aging process, with levels of albumin decreasing with advancing age [11]. However, the relationship between hypoalbuminemia and age has not been fully elucidated; therefore, the association should also take into account diseases and other age-related conditions rather than age alone [12].

Serum albumin levels are strongly associated with morbidity, prognosis, and mortality in both acute and chronic disease patients [13,14], and hypoalbuminemia is a frequent problem in hospitalized patients. Hypoalbuminemia is directly associated with the likelihood of developing frailty conditions [15] and can predict outcome in critically ill patients [16,17] and mortality regardless of comorbidity factors in emergency medical patients [18]. This condition leads to prolonged or recurrent hospitalization, with additional medical costs derived from consequently more expensive treatments for a more efficient management of patients, including the need of extra medical resources [19,20].

In addition, albumin is used in some prognostic indices, such as the Prognostic Nutritional Index and the Prognostic Inflammatory Nutritional Index. Thanks to this, it is possible to evaluate the relationship of albumin with some solid tumors (colorectal, gastric, pancreas, etc.) and the state of inflammation and malnutrition [21,22].

The present study analyzed changes in albumin levels in hospitalized patients by assessing the prevalence of preexisting hypoalbuminemia at the time of discharge from hospital. The main goal was to support the evidence that low albumin levels still need to be regarded as a dangerous condition in itself and a serious public health problem. By reconsidering the importance of the inclusion of hypoalbuminemia as a specific diagnosis in hospital discharge files, we aimed to propose albumin and its related factors as a reliable biomarker of socio-economic disadvantage for reconsidering ad hoc healthcare pathways for patients after hospital discharge, especially when dealing with fragile populations.

2. Materials and Methods

An observational retrospective study was carried out on the entire hospital population of the "Fondazione Policlinico Tor Vergata—PTV" in 2018. Hospital discharge files of admitted patients were collected from the central informatics system of the hospital. Data were extracted using the informatics system AREAS-ADT, with diagnosis coded using the ICD-9 classification (2007 version). All ordinary hospitalizations were included in the analysis (code 1), while all extraordinary hospitalizations (code 2-Day Hospital, code 3-Home treatment, code 4-Day-Surgery with overnight stay) were not considered. Only data of patients above 18 years of age were included in the analysis.

Levels of albumin for the same period of reference were obtained from the informatics system of the Unit of Laboratory Medicine—U.O.C. Medicina di Laboratorio (Modulab)—of the hospital, which stores all the information of samples collected from the admitted patients. Information was collected on the day of the blood sample and a nosology code was assigned to every inpatient event.

A database associating the hospital discharge files with the nosology code was generated. Information included personal data, principal diagnosis, secondary diagnosis (from 1° to 5°), procedures applied during hospitalization, and date on blood sample. In case of multiple admissions, only the first and last values of albumin were analyzed. No anthropometric measurements were available in the database.

To ensure privacy, all data were coded, without personal names.

Consent for data management, analysis, and publication was obtained from the Ethical Committee of the "Fondazione Policlinico Tor Vergata—PTV" (identification number 33/19). The study was

conducted in compliance with the Ethical Principles for Medical Research Involving Human subjects of the World Medical Association Declaration of Helsinki (1975).

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences—SPSS version 24 (IBM, Somers, NY, USA).

A descriptive analysis was performed for the entire study population and subgroups according to age and serum albumin levels.

Four categories of albuminemia were presented in accordance with the classification of previous studies: marked hypoalbuminemia (<2.5 mg/dL), mild hypoalbuminemia (2.5–3.5 mg/dL), normal albumin (3.5–4.5 mg/dL), and hyperalbuminemia (>4.5 mg/dL) [16,23–25].

Analysis of albumin levels at admission and at discharge was conducted by dividing the sample into patients below and above 65 years of age. In addition, age was stratified into six classes according to the classification of Akirov et al. [25]: <40, 40–50, 50–60, 60–70, 70–80, and >80 years. The hospitalization diagnoses (ICD-9 coded) were grouped according to the Major Diagnostic Categories (MDC24), then to the Diagnosis Related Groups (DRG24).

Continuous variables were presented as means with standard deviations (SD), while categorical variables were presented as percentages. The statistical significance of differences between albumin levels among groups were assessed with Student's t-test or ANOVA with Bonferroni post-hoc test for differences among groups, while the chi-squared test was used to compare categorical variables.

Odds ratios and 95% confidence intervals (OR; 95% CI) were calculated to assess the relationship of the risk of mortality with pathologic serum albumin and old age.

Binary logistic regressions were performed to investigate socio-demographic aspects and individual factors that affected different types of outcomes. Because there were multiple independent variables, a stepwise forward regression approach was used.

3. Results

A total of 9428 ordinary hospitalizations were recorded in 2018. Of the patients, 55.6% were male. The mean age was 65.2 years ± 16.8 SD, and the median age was 68 years. More than half of the events (57.5%) were recorded for people above 65 years of age. Information on citizenship, marital status, education level, and respective levels of albumin is presented in Table 1. Over 90% of the patients were Italian. As regards marital status, nearly 77% of the participants were married, followed by singles (over 17%). The most frequent educational level among the patients was intermediate school (65.9%), followed by secondary school (16.7%), and primary school (13.2%). A small percentage of patients had the highest educational level (3.4%), while the percentage of illiterate patients was negligible (less than 1%).

Albumin dosage at admission was available for 9367 records, with an overall mean level of 3.389 mg/dL ± 0.634 SD. Albuminemia decreased with increasing age and was below the level of normality (<3.5 mg/dL) in the 50–60 years age group. All the values of albumin were significantly different, with the exception of the 40–50 years age group compared with the 50–60 years age group.

As regards socio-demographic information, albumin levels were statistically lower in Italians ($p < 0.001$), in married and widowed patients compared with single patients ($p < 0.001$ and $p = 0.035$, respectively), and in patients with lower educational levels (primary and intermediate) compared with higher levels (secondary and higher).

Table 1. General characteristics of the sample at the baseline.

	Variable	Frequency, N. (%)	Albumin Level, Mean (mg/dL) ± SD	*p* Values (ANOVA Test)
Gender	Males	5238 (55.6)	3.392 ± 0.683	NS
	Females	4190 (44.4)	3.378 ± 0.614	
AGE (years)	<40	886 (9.4)	3.734 ± 0.604	All statistically significant $p < 0.001$ except 40–50 vs. 50–60 NS
	40-50	879 (9.3)	3.558 ± 0.635	
	50-60	1502 (15.9)	3.481 ± 0.641	
	60-70	2067 (21.9)	3.393 ± 0.629	
	70-80	2340 (24.8)	3.306 ± 0.584	
	>80	1754 (18.6)	3.143 ± 0.568	
CITIZENSHIP	Italian	8674 (92)	3.376 ± 0.630	<0.001
	Foreign	754 (8)	3.501 ± 0.652	
MARITAL STATUS	Single	1638 (17.4)	3.494 ± 0.650	Single vs. Married <0.001
	Married	7245 (76.8)	3.360 ± 0.628	
	Separated/Divorced	218 (2.3)	3.420 ± 0.638	
	Widow	327 (3.5)	3.382 ± 0.597	Single vs. Widow = 0.035
EDUCATION	Illiterate	83 (0.9)	3.385 ± 0.593	Prim. vs. Int. <0.001
	Primary School	1242 (13.2)	3.270 ± 0.592	Prim. vs. Sec. <0.001
	Intermediate School	6211 (65.9)	3.357 ± 0.626	Prim. vs. High <0.001
	Secondary School	1570 (16.7)	3.551 ± 0.654	Int. vs. Sec. <0.001
	Higher Education	322 (3.4)	3.578 ± 0.630	Int. vs. High = 0.044

The results of the stratification of albuminemia into categories at the time of admission are reported in Table 2. Mean values for all categories were all statistically significant (ANOVA test; $p < 0.001$). Nearly half of the sample already had hypoalbuminemia at their first visit to the hospital (42.9% mild and 9.6% marked), while only slightly less than 45% of patients had a normal level of albumin. The condition of insufficiency albuminemia was significantly more prevalent in elderly patients than in their younger counterparts (67.4% vs. 32.6% for marked; 67.1% vs. 32.9% for mild). Conversely, normal or even highest levels of albumin were more prevalent in patients aged <65 years than in patients aged >65 years (52.2% vs. 47.8% for normal albuminemia; 78.5% vs. 21.5% for hyperalbuminemia; $p < 0.001$) (Figure 1).

Table 2. Categories of albuminemia at time of admission (baseline).

Albuminemia at Baseline	Frequency n, (%)	Value, Mean Value ± SD	Prevalence in >65 Years Old, n, (%)	Prevalence in <65 Years Old, n, (%)
Marked hypoalbuminemia (<2.5 mg/dL), and	909 (9.6)	2.177 ± 0.288	613 (67.4) $p < 0.001$	296 (32.6)
Mild hypoalbuminemia (2.5–3.5 mg/dL)	4045 (42.9)	3.073 ± 0.267	2714 (67.1) $p < 0.001$	1331 (32.9)
Normal albuminemia (3.5–4.5 mg/dL)	4199 (44.5)	3.884 ± 0.259	2009 (47.8) $p < 0.001$	2190 (52.2)
Hyperalbuminemia (>4.5 mg/dL)	214 (2.3)	4.645 ± 0.127	46 (21.5) $p < 0.001$	168 (78.5)

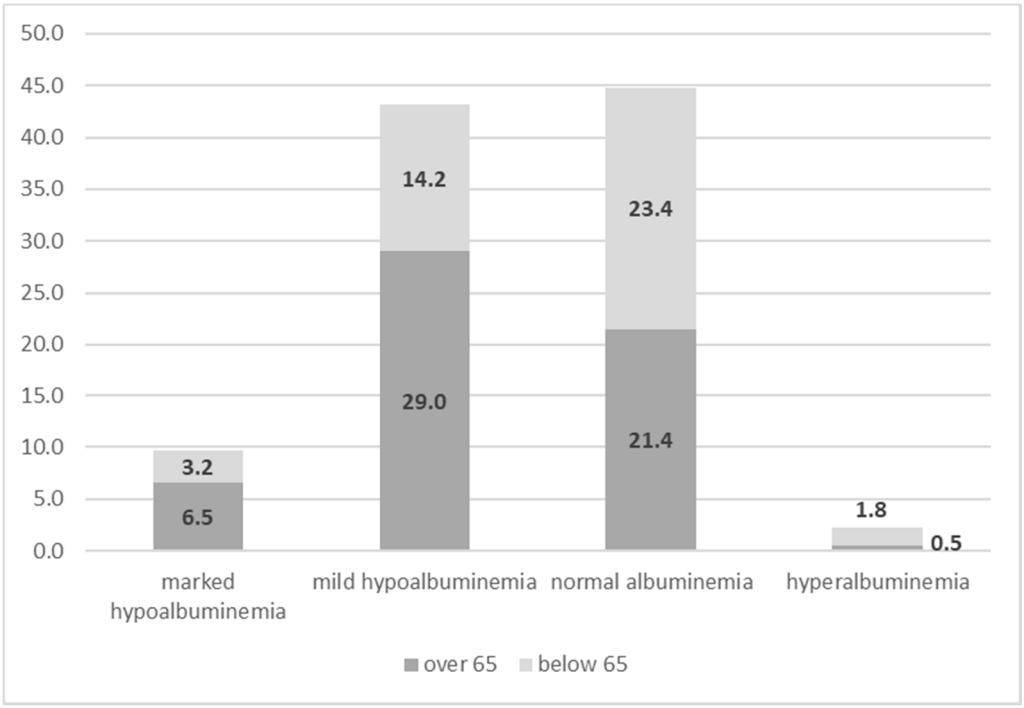

Figure 1. Different levels of albumin at baseline by dichotomized age.

The reasons for hospitalization, classified according to the MDC24 categories, are reported in Table 3. The Table shows the numbers and percentages of events recorded by Major Diagnosis Categories. Mean values of albumin by MDC24 at baseline have also been reported. Patients were hospitalized primarily for diseases and disorders of the nervous system (MDC1)—accounting for nearly the twenty percent of all the events, followed by those of the respiratory system (MDC4)—14%-, circulatory system (MDC5)—nearly thirteen percent—those for musculoskeletal system (MDC8)—12.2%, and those of digestive system (MDC6)—slightly more than ten percent. When considering albumin level at baseline, the lowest mean value was found in patients admitted for infectious and parasitic -MCD18 (2.861 mg/dL ± 0.591 SD), while the highest was found in patients admitted for diseases and disorders of the eye-MCD2 (3.991 mg/dL ± 0.402 SD).

Table 3. Hospitalizations by MDC24.

Major Diagnostic Categories (MDC24)	Frequency	Percentage	Albumin at Baseline (mg/dL), Mean ± SD
diseases and disorders of the nervous system **(1)**	1830	19.8	3.663 ± 0.481
diseases and disorders of the respiratory system **(4)**	1299	14.1	3.254 ± 0.621
diseases and disorders of the circulatory system **(5)**	1194	12.9	3.134 ± 0.621
diseases and disorders of the musculoskeletal system and connective tissue **(8)**	1128	12.2	3.471 ± 0.535
diseases and disorders of the digestive system **(6)**	945	10.2	3.324 ± 0.667
diseases and disorders of the hepatobiliary system and pancreas **(7)**	831	9.0	3.291 ± 0.651
myeloproliferative DDs (poorly differentiated neoplasms) **(17)**	429	4.6	3.485 ± 0.616
diseases and disorders of the kidney and urinary tract **(11)**	404	4.4	3.297 ± 0.692
infectious and parasitic DDs (Systemic or unspecified sites) **(18)**	178	1.9	2.861 ± 0.591
factors influencing health status and other contacts with health services **(23)**	177	1.9	3.578 ± 0.674
diseases and disorders of the endocrine, nutritional and metabolic system **(10)**	169	1.8	3.472 ± 0.578
diseases and disorders of the blood and blood forming organs and immunological disorders **(16)**	144	1.6	3.372 ± 0.646
diseases and disorders of the skin, subcutaneous tissue and breast **(9)**	102	1.1	3.328 ± 0.645
diseases and disorders of the ear, nose, mouth and throat **(3)**	96	1.0	3.897 ± 0.56
mental diseases and disorders **(19)**	95	1.0	3.552 ± 0.531
injuries, poison and toxic effect of drugs **(21)**	70	0.8	3.014 ± 0.761
diseases and disorders of the eye **(2)**	51	0.6	3.991 ± 0.402
multiple significant trauma **(24)**	46	0.5	3.027 ± 0.689
human Immunodeficiency virus infection **(25)**	21	0.2	3.235 ±0.845
diseases and disorders of the male reproductive system **(12)**	13	0.1	3.243 ± 0.669
diseases and disorders of the female reproductive system **(13)**	13	0.1	3.328 ± 0.507
alcohol/drug use or induced mental disorders **(20)**	6	0.1	3.418 ± 0.623
newborn and other neonates (perinatal period) **(15)**	3	0	3.483 ± 0.317
burns **(22)**	1	0	3.030
Total	9245	100	3.385 ± 0.629

The hospital does not have a department of pregnancy, childbirth, and puerperium (14).

In order to better understand the specific diagnosis recorded in the hospital by the period of observation, Table 4 presents the events with a frequency of more than 100 in ascending order, coded by Diagnosis Related Groups (DRG) version 24. Levels of albumin at baseline by DRG24 have also been reported, with significant differences among mean values been investigated. The lowest albumin levels were found for patients admitted because of major cardiothoracic surgeries (MCD5–DRG104;

2.800 mg/dL ± 0.562 SD), while the highest values were found for patients with other factors affecting the state of health (MCD23, DRG467; 3.900 mg/dL ± 0.624 SD).

Table 4. Main diagnosis coded by DRG24 of hospitalization.

MDC24	Diagnosis of Hospitalization (DRG24)	Frequencies	Percentages	Albumin at Baseline (Mg/Dl), Mean ± SD	Significant Differences among Drgs: p Values <0.05 (ANOVA Test)
1	Transient cerebral ischemia **(524)**	101	1.1	3.670 ± 0.442	vs. 87, 89, 104, 127, 179, 202, 210, 544, 569
17	Acute leukemia without major surgery **(473)**	101	1.1	3.570 ± 0.547	vs. 87, 89, 104, 127, 179, 202, 210, 569
6	Inflammatory diseases of intestine **(179)**	102	1.1	3.300 ± 0.584	vs. 2, 12, 14, 75, 104, 219, 467, 473, 543
6	Major surgery of both large and small intestine **(569)**	104	1.1	3.285 ± 0.826	vs. 2, 12, 14, 75, 219, 410, 467, 473, 524, 543, 544
23	Other factors affecting the state of health **(467)**	110	1.2	3.900 ± 0.624	vs. 87, 89, 104, 127, 179, 202, 210, 410, 544, 569
8	Operation on the lower limb and humerus except hip. foot and femur **(219)**	124	1.3	3.600 ± 0.418	vs. 87, 89, 104, 127, 179, 202, 210, 544, 569
17	Chemotherapy not associated with secondary diagnosis of acute leukemia **(410)**	127	1.3	3.440 ±0.545	vs. 2, 12, 75, 87, 89, 104, 202, 210, 467, 569
1	Degenerative diseases of the nervous system **(12)**	136	1.4	3.830 ± 0.436	vs. 87, 89, 104, 127, 179, 202, 210, 410, 544, 569
1	Craniotomy with major device implant or major diagnosis of complex acute pathology of the central nervous system **(543)**	143	1.5	3.570 ± 0.426	vs. 87, 89, 104, 127, 179, 202, 210, 569
8	Hip and femur surgery. except major joints **(210)**	147	1.6	3.090 ± 0.420	vs. 2, 12, 14, 75, 219, 410, 467, 473, 524, 543, 544
5	Heart valve surgery and other major cardiothoracic surgeries with cardiac catheterization **(104)**	155	1.6	2.800 ± 0.562	vs. 2, 12, 14, 75, 87, 127, 179, 202, 410, 467, 473, 524, 543, 544
1	Craniotomy **(2)**	165	1.8	3.800 ± 0.408	vs. 87, 89, 104, 127, 179, 202, 210, 410, 544, 569
7	Cirrhosis and alcoholic hepatitis **(202)**	177	1.9	3.040 ± 0.665	vs. 2, 12, 14, 75, 104, 219, 410, 467, 473, 524, 543, 544
5	Heart failure and shock **(127)**	179	1.9	3.300 ± 0.516	vs. 2, 12, 14, 75, 87, 104, 219, 467, 473, 524, 543
4	Major interventions on the chest **(75)**	180	1.9	3.770 ± 0.540	vs. 87, 89, 104, 127, 179, 202, 210, 410, 544, 569
4	Pulmonary edema and respiratory failure **(87)**	204	2.2	3.090 ± 0.598	vs. 2, 12, 14, 75, 104, 127, 219, 410, 463, 473, 524, 543, 544
8	Replacement of major joints or reimplantation of the lower limbs **(544)**	305	3.2	3.395 ± 0.449	vs. 2, 12, 14, 75, 87, 89, 104, 202, 210, 219, 467, 524, 569
4	Simple pneumonia and pleurisy **(89)**	325	3.4	3.100 ± 0.569	vs. 2, 12, 14, 75, 127, 219, 410, 467, 473, 524, 543, 544
1	Intracranial hemorrhage or cerebral infarction **(14)**	551	5.8	3.700 ± 0.469	vs. 87, 89, 104, 127, 179, 202, 210, 544, 569
	Totals	3,436	36.4	3.403 ± 0.595	

Data corresponding to the second dose of albumin at the time of discharge (6508 cases) were available in the system. The mean albumin level at the second dose was 3.132 mg/dL ± 0.59 SD. Findings on socio-demographic conditions and albumin levels at baseline were also confirmed at the second dose, with lower levels in Italians, as well as in married and widowed patients, compared with single patients. Lower levels of albumin were also observed in patients with lower education (primary and intermediate) compared with those with a higher educational level (secondary and higher), and in illiterate patients compared with those who had attended secondary school ($p = 0.017$).

Albuminemia at discharge stratified by category is reported in Table 5. Mean values for all categories were all statistically significant. Hypoalbuminemia increased in our sample, accounting for more than 70% of the cases (14% marked and 57% mild, respectively). Figure 2 shows that at discharge, hypoalbuminemia was very frequent, with the highest prevalence in elderly patients ($p < 0.001$). Normal levels of albumin, especially hyperalbuminemia, were significantly reduced compared with the measurements at the time of admission (Figure 3).

Table 5. Categories of albuminemia at second dosage (discharge).

Albuminemia at Second Dosage	Frequency n, (%)	Value, Mean Value ± SD	Prevalence in Patients over 65 Years Old, n, (%)	Prevalence in Patients below 65 Years Old, n, (%)
Marked hypoalbuminemia (<2.5 mg/dL), and	912 (14)	2.156 ± 0.277	677 (74.2) $p < 0.001$	235 (25.8)
Mild hypoalbuminemia (2.5–3.5 mg/dL)	3709 (57)	3.019 ± 0.281	2452 (66.1) $p < 0.001$	1257 (33.9)
Normal albuminemia (3.5–4.5 mg/dL)	1847 (28.4)	3.808 ± 0.238	830 (44.9) $p < 0.001$	1017 (55.1)
Hyperalbuminemia (>4.5 mg/dL)	40 (0.6)	4.678 ± 0.142	10 (25) $p < 0.001$	30 (75)

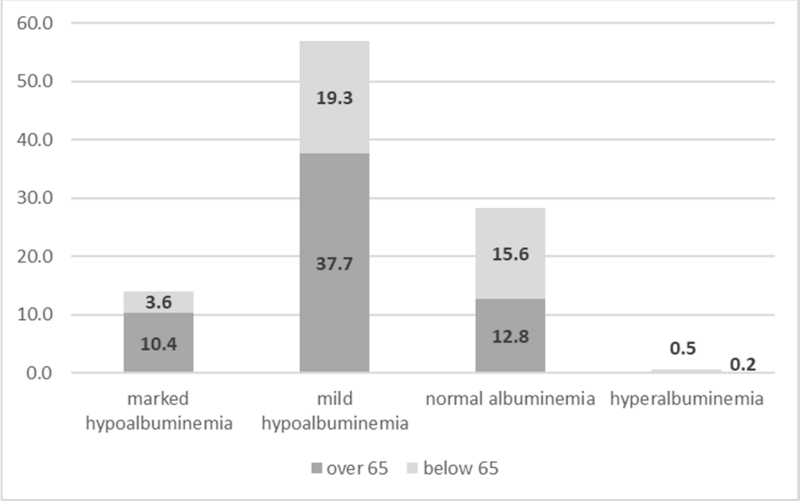

Figure 2. Different levels of albumin at discharge by dichotomized age (below and above 65 years).

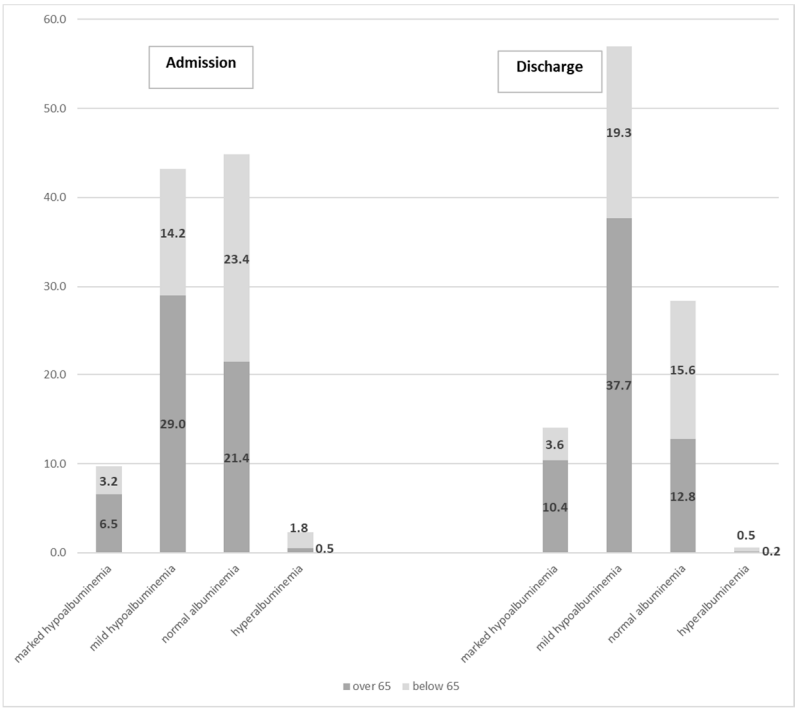

Figure 3. Comparison of prevalence of albumin categories at admission and discharge.

The mean length of hospital stay was 11.38 ± 13.01 (SD) days, ranging from a minimum of 1 to 223 days.

As regards the outcomes (Figure 4), 47.6% of the admissions were discharged to their homes, 18.4% were transferred to another health facility, while 6.5% died. The remaining 27.5% corresponded to other types of discharges.

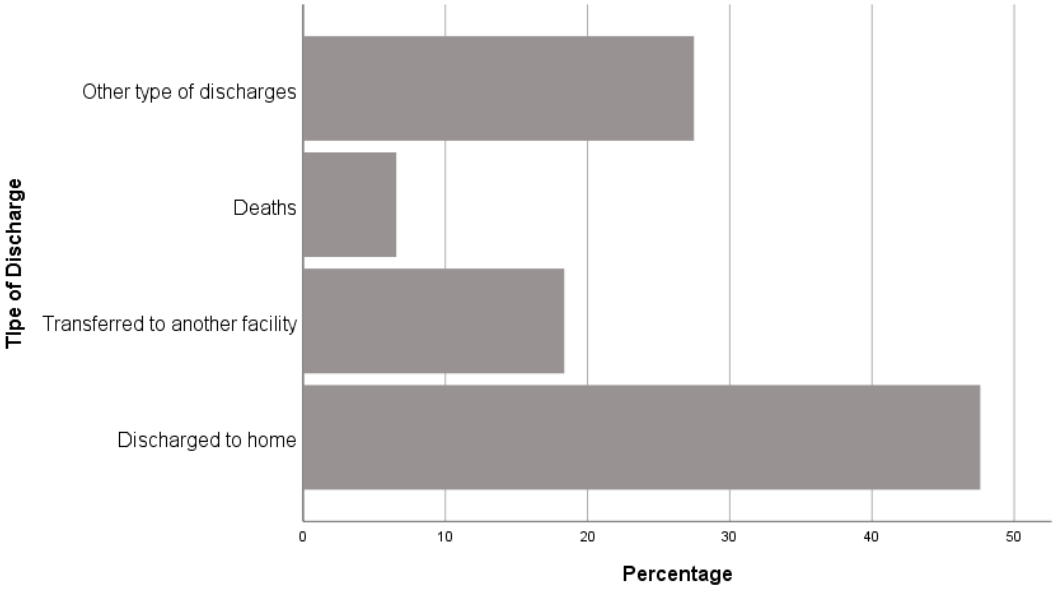

Figure 4. Outcome distribution by categories.

Table 6 presents the mean levels of albumin at admission in relation to outcome at discharge from hospital. Patients who died had the lowest level of albumin (2.839 mg/dL ± 0.669 SD), which was significantly lower than that of patients with all other outcomes ($p < 0.001$), including patients

transferred to their home (3.222 mg/dL ± 0.598 SD; $p < 0.001$). No difference was found in the levels of albumin between patients discharged home and those with other types of outcomes.

Table 6. Albumin level by outcomes.

Outcome	Albumin Level, Mean (mg/dL) ± SD	*p* Value (ANOVA Test)
discharged home	3.455 ± 0.610	Death vs. all <0.001
transferred to long-term health care facilities	3.222 ± 0.598	Transferred vs. all <0.001
death	2.839 ± 0.669	Discharged to home vs.
other type of discharge	3.504 ± 0.597	other type of discharge NS

An increased risk of dying was found in patients with pathologic levels of albumin (<3.5 mg/dL) at the time of admission (OR = 4.720; 95% CI: 3.822–5.830). Elderly patients over 65 years of age were at higher risk of dying (OR = 2.468; 95% CI: 2.042–2.983).

Binary logistic regressions were performed to investigate general characteristics (sex, age), socio-demographic factors (marital status, education, citizenship-Italians vs. foreign), and specific conditions (level of albumin at admission) mainly impacting the different types of outcomes (Table 7).

Table 7. Impact of different outcomes on general characteristics, socio-demographic factors, and specific conditions.

Outcome	Variable	Exp(B)	Significance	95% CI Per Exp(B)
Death	Divorced	2.745	0.029	1.110–6.786
	Widow	2.561	0.004	1.340–4.893
	Sex (male vs. female)	1.376	<0.001	1.153–1.641
	Age	1.029	<0.001	1.023–1.036
	Albumin at admission	0.270	<0.001	0.236–0.310
Discharged home	Single	0.811	<0.001	0.724–0.997
	Higher education	0.765	0.020	0.609–0.959
	Age	0.995	<0.001	0.992–0.997
	Albumin at admission	1.328	<0.001	1.241–1.421
Transferred to long-term health care facilities	Single	1.213	0.019	1.033–1.424
	Secondary	1.637	<0.001	1.358–1.973
	Higher	1.798	0.002	1.237–2.613
	Sex (male vs. female)	0.821	<0.001	0.738–0.914
	Age	1.018	<0.001	1.014–1.022
	Albumin at admission	0.699	<0.001	0.641–0.762
Other types	Single	1.283	<0.001	1.127–1.461
	Secondary	0.825	0.003	0.728–0.935
	Age	0.989	<0.001	0.986–0.992
	Albumin at admission	1.399	<0.001	1.295–1.512

Being divorced or widowed were the two main conditions associated with an increased risk of dying (OR 2.745 and 2.561, respectively). Male patients showed a slightly higher risk than female patients (OR 1.376). With increasing age, there was a tendency for an increase in deaths. Conversely, higher albumin levels at admission were protective factors (OR 0.270; CI 0.236–0.310).

Being single (OR 0.811), having a higher education (OR 0.765), and being young (OR 0.995) were protective factors for being discharged home. The probability of being discharged o home increased with higher levels of albumin at admission.

Being single, female, and older and having a secondary or higher education were all conditions more likely related to the outcome of patients transferred to other health facilities. Conversely, higher albumin levels at admission were protective factors (OR 0.699; CI 0.641–0.762).

Being single and having a higher level of albumin at admission were the main factors associated with an increased risk of other types of discharge. On the other hand, having a secondary school education and being old were protective conditions.

4. Discussion

The present study analyzed data of patients admitted to the hospital "Fondazione Policlinico Tor Vergata—PTV" in 2018. Specifically, we investigated and reported the level of serum albumin according to demographic and socio-economic characteristics of patients, admitted for different causes classified as MDC24.

At the time of admission, hypoalbuminemia was found to be present in more than half of our sample, with no sex differences. The socio-demographic analysis revealed that the serum albumin level was significantly lower in patients who were less educated, with illiterate patients or those having primary or intermediate education presenting hypoalbuminemia. Widow and married patients had a significantly lower level of albumin than single and separated/divorced patients.

As regards Major Diagnostic Categories (MDC24), the lowest level of albumin (≤3.0 mg/dL) was reported, as expected, in patients admitted for infectious and parasitic diseases (MDC18), burns (MDC22), multiple significant trauma (MDC24), injuries, and poison and toxic effect of drugs (MDC21), as result of physiological conditions associated with stress, catabolic state, inflammation [9] and hemorrhage. These findings were confirmed when considering the Diagnosis Related Groups (DRG version 24), when the lowest values of albumin (≤3.0 mg/dL) were found, as might be expected, for main surgeries such as major cardiothoracic surgeries (DRG104) and hip and femur surgery (DRG210), as well as for cirrhosis and alcoholic hepatitis (DRG202). More impressive were the results of low albumin levels (nearly 3.1 mg/dL) when simple pneumonia and pleurisy (DGR89) and pulmonary edema and respiratory failure (DRG87) occurred, underlining a possible correlation between infectious diseases and hypoalbuminemia. Future analyses will allow further investigations of the associations and differences between albuminemia, clinical conditions of patients during hospitalization and outcomes for MDC and DRG. These specific findings have not been the purpose of the current paper.

During hospitalization, the serum albumin level tended to decrease with age, with pathologic levels appearing from 50 years, and then progressively worsening. The condition of marked and mild hypoalbuminemia was more prevalent in elderly patients (over 65 years old); conversely, normal and hyperalbuminemia was more prevalent in patients below 65 years of age. Due to the lack of anthropometric parameters routinely assessed and entered in the database, we can only hypothesize that a relevant fraction of our patients could be in a malnutrition status already at time of admission, even though we are not currently able to exclude clinical causes (inflammation, cancer, etc.) of hypoalbuminemia. We acknowledge that is one of main limit of the current study. Further investigations will be necessary to clarify this point. However, albumin is widely used as a marker of nutritional condition [12] and is known to be related to age and health status [26].

Additionally, we hypothesize that albumin could be an efficient and reliable biomarker of socio-economic disadvantage. Being separated/divorced or widowed increased the risk of dying during hospitalization, as well as being illiterate or with a low education level. On the opposite, being single or having a higher education was associated with a higher probability of other favorable outcomes, especially being discharged at home. After the frailty concept [27] evolved in two different directions—the first was the so-called biomedical model or phenotypic and clinical model, that privileged the physical performance [28,29]; the second was the so-called bio-psychosocial model, which privileged the multidomain vulnerability, strictly associated with higher health services demand and unfavorable outcomes [30]—indirect signs of frailty according to both definitions have been seen in our sample. We can conclude that albumin might be a very useful biomarker to depict frailty in the population, constituting a hinge variable between the biomedical model and the multidimensional bio-psycho-social model. Indeed, from our findings, it seems to be associated with both approaches.

Other than albumin levels at admission, our results emphasize the importance of the change in albumin levels before discharge. Hospitalization worsened the patients' hypoalbuminemia, with more than 70% of the sample having a low level of albumin (either marked or mild) at the time of discharge, with elderly patients accounting for nearly 50% of the sample. A correct evaluation of the nutritional status and personalized nutritional intervention [31] represent important tools for the prognosis and quality of life in hospitalized patients, especially cancer patients. In fact, there are clear mechanisms linking nutritional principles to immune function [32]. Immuno-nutrition treatment improves poor nutritional status or severe malnutrition [33]. The lowering of albumin during the hospital stay could be interpreted not only as a consequence of pathologies and surgical treatments, but also a clear sign of barriers to adequate in-hospital nutrition [34]. Future investigations should be conducted to better understand the specific impact of food access during hospitalizations [35].

Among hospitalized patients, mortality was significantly higher in those with mild and marked hypoalbuminemia, concordant with results from numerous studies reporting hypoalbuminemia as a mortality predictor for different morbidities [25,36–39]. Logistic regression confirmed older age and lower albuminemia to be risk factors for death outcome [40,41].

In addition, we found that even patients transferred to long-term care health facilities had a pathologic level of albumin. Hypoalbuminemia was statistically associated with an increased risk of mortality and of being transferred to long-term health care facilities.

The catchment of patients at risk of malnutrition at an early stage is paramount to undertake proper nutritional treatment, improving their clinical conditions. Given the worrying albumin values obtained during hospitalization, the need to personalize the nutritional approach in the hospital is emphasized as it is an index of malnutrition and wasting of patients. An enhanced recovery after surgery protocol, which has already been validated for the pre- and post-operative period, is a good example of an optimized approach that aims not only to assess and maintain a good state of nutrition but also to obtain optimal recovery, with a reduction in hospital stay. Furthermore, optimizing this protocol reduces the costs related to hospital management and complications. It has been calculated that for 1000 people with colorectal cancer surgery, the total costs would be reduced by about 3.7 million Euros [42].

5. Conclusions

The dosage of serum albumin during hospitalization is a routine practice and an easy-to-access process. From our findings, albumin can be considered as a low-cost marker to stratify patients by risk during hospitalization and as an indicator that, combined with a more exhaustive nutrition evaluation as a best practice, can support decision makers to prescribe nutritional support even after discharging.

Therefore, hypoalbuminemia should be regarded as a dangerous condition in itself to be included as a specific diagnosis in hospital discharge files, especially when a routinely assessment of the malnutrition status is not performed. The need to reconsider ad hoc healthcare pathways for patients after discharge from hospital, especially when fragile populations are involved, warrants further investigations to identify the main procedures associated with albumin levels to lower mortality risk. We emphasize the need to report hypoalbuminemia in a wide range of clinical contexts, from hospital to family doctors and other community facilities, and to consider the relevance of this condition due to its association with hospital admission, intra-hospital mortality, and frailty in a general sense.

The growth in the elderly population demands a transition in healthcare, with a range of modifications in order to maintain the quality of life of the population. In addition to the nutritional status classification, reconsidering albumin as a marker of socio-demographic deprivation during and after hospitalization will support health workers to face this emerging public health problem.

Author Contributions: L.P., A.D.L., E.B. and J.M.L., devised the conceptualization of the work. S.M., M.C., S.O., L.M. (Laura Morciano), L.M. (Luca Morucci), developed the work methodology. S.M., performed the validation of the results. L.M. (Laura Morciano), L.M. (Luca Morucci), L.P, P.G. and F.C. conducted the formal analysis. S.M. and E.B., conducted the investigation. M.M. performed data curation. M.M., S.M., wrote original draft preparation. S.M., wrote original draft, reviewed and edited the manuscript. M.C., L.P., A.D.L., E.B. performed visualization and supervised the work. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors have no conflicts of interest to declare.

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