Experimental Gerontology 112 (2018) 119–126

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Immunological alterations in frail older adults: A cross sectional study

Vanessa Valdiglesias[a,b,*], Diego Marcos-Pérez[a,c], Maria Lorenzi[d], Graziano Onder[d], Johanna M. Gostner[e], Barbara Strasser[f,g], Dietmar Fuchs[e], Stefano Bonassi[h,i]

[a] Universidade da Coruña, DICOMOSA Group, Department of Psychology, Area of Psychobiology, A Coruña, Spain
[b] ISPUP-EPIUnit, Universidade do Porto, Porto, Portugal
[c] Universidade da Coruña, Department of Cell and Molecular Biology, A Coruña, Spain
[d] Department of Geriatrics - IRCCS Fondazione Policlinico Universitario A. Gemelli, Università Cattolica del Sacro Cuore, Rome, Italy
[e] Biocenter, Innsbruck Medical University, Innsbruck, Austria
[f] Department of Epidemiology and Preventive Medicine, Regensburg University, Regensburg, Germany
[g] Sigmund Freud University, Medical School, Vienna, Austria
[h] Department of Human Sciences and Quality of Life Promotion, San Raffaele University, Rome, Italy
[i] Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Pisana, Roma

ARTICLE INFO

Section Editor: Richard Aspinall

Keywords:
Frailty
Immune activation
Kynurenine/tryptophan ratio
Neopterin
Phenylalanine/tyrosine ratio
C-reactive protein

ABSTRACT

Frailty is a progressive physiologic decline in multiple body systems, characterized by loss of function, loss of physiologic reserve, and increased vulnerability to disease and death. This condition is induced by a complex and multifactorial interaction between genetic, biological, physical, psychological and environmental factors. To understand the interplay between the age-related decline of the immune response, and the upregulation of the inflammatory response, the so called inflammaging, we investigated the role of different inflammatory mediators on frailty status in the elderly. The study was performed in a population of 180 older adults (≥65 years), who were classified according to Fried's frailty phenotype. Plasma concentrations of neopterin, tryptophan, kynurenine, phenylalanine, tyrosine as well as kynurenine/tryptophan (Kyn/Trp) and phenylalanine/tyrosine (Phe/Tyr) ratios were analyzed as immune stimulation biomarkers. In addition, nitrite and C-reactive protein levels were measured as indicators of nitric oxide production and acute inflammation, respectively. Significant increases in neopterin, C-reactive protein and Kyn/Trp ratio, and decreases in tryptophan and nitrite concentrations in frail individuals compared with *non-frail* group were found. Both Kyn/Trp and Phe/Tyr ratios were significantly and positively correlated with neopterin. A positive correlation between kynurenine and tryptophan was also observed. Four parameters, i.e., neopterin, tryptophan, nitrite and C-reactive protein, were found to be strongly related to frailty status, although only nitrite confirmed its role of predictor after multiple regression analysis, supporting its use as a potential biomarker of frailty. Further investigation is required to strengthen the consistence and reproducibility of these findings, and to establish this parameter as a clinical biomarker of frailty.

1. Introduction

The world population is rapidly aging and this trend is evident in almost all countries and areas of the world (United Nations, 2015). This new situation requires a realigning of social and health systems of older people, in order to reduce sanitary and socioeconomic costs for the societies. Over the last decade, one of the most important conceptual advances in geriatrics has been the establishment of the concept of frailty, a progressive physiologic decline in multiple body systems, which is marked by loss of function, loss of physiologic reserve, and

Abbreviations: Kyn, kynurenine; Trp, tryptophan; Phe, phenylalanine; Tyr, tyrosine; GCH-1, guanosine triphosphate cyclohydrolase I; IDO-1, indolamine 2,3-dioxygenase; PAH, phenylalanine 4-hydroxylase; NOS, nitric oxide synthases; NO, nitric oxide; CGA, comprehensive geriatric assessment; ADL, activities of the daily living; IADL, instrumental activities of the daily living; MMSE, mini-mental state examination; GDS, geriatric depression scale; CIRS, cumulative illness rating scale; MNA, mini nutritional assessment; ESS, Exton Smith scale; HPLC, high performance liquid chromatography; MN, micronucleus; HtrA1, high-temperature requirement serine protease A1; sTNF-RII, soluble tumor necrosis factor receptor II; PASE, physical activity scale for elderly; yrs, years; BMI, body mass index; SMI, skeletal muscle index

* Corresponding author at: Department of Psychology, University of A Coruña, Faculty of Education Sciences, Campus Elviña s/n, 15071 A Coruña, Spain.
E-mail addresses: vvaldiglesias@udc.es (V. Valdiglesias), diego.marcos@udc.es (D. Marcos-Pérez), graziano.onder@unicatt.it (G. Onder), johanna.gostner@i-med.ac.at (J.M. Gostner), Barbara.Strasser@i-med.ac.at (B. Strasser), Dietmar.Fuchs@i-med.ac.at (D. Fuchs), stefano.bonassi@sanraffaele.it (S. Bonassi).

https://doi.org/10.1016/j.exger.2018.09.010
Received 6 June 2018; Received in revised form 25 August 2018; Accepted 15 September 2018
Available online 18 September 2018
0531-5565/

increased vulnerability to disease and death (Fulop et al., 2015). Frailty increases susceptibility to stressors and acute illness, falls, disability, institutionalization, and death (reviewed in Espinoza and Walston, 2005). The estimated prevalence of this syndrome among community-dwelling men and women age 65 and older is 7–10%, and up to one-third of those aged 80 or above (reviewed in Li et al., 2011). Although frailty is known to be induced by a complex and multifactorial interaction between genetic, biological (hormonal, metabolic and immune-inflammatory systems), physical, psychological and environmental factors, the current measurements of frailty are based mainly on phenotypical signs.

The alterations that occur with aging, and that compromise the competence of immune system to generate specific responses to internal and external damaging agents, are defined as "immunosenescence" (Castelo-Branco and Soveral, 2014; de Araújo et al., 2013). The chronic antigenic load provoked by aging process may overstimulate the immune system which becomes inefficient over time. Accordingly, immunological alterations have been found in a number of neurodegenerative disorders and age-related syndromes including type II diabetes, dementia and cardiovascular diseases (reviewed in Fulop et al., 2016). Likewise, inflammation, an acute and efficient response to harmful conditions, becomes chronic and usually have detrimental effects in old age. A new name, "inflammaging" (Franceschi et al., 2007) has been proposed for this process which also contributes to the development of a number of age-related chronic diseases such as atherosclerosis (Hunt et al., 2010), type-2 diabetes (Pradhan et al., 2001), Alzheimer's disease (Griffin, 2006) and osteoporosis (Ginaldi et al., 2005). Both processes, chronic immune system activation and inflammation, seem to contribute to the development of frailty (Leng et al., 2011).

As a result of immune activation, the enzymes indolamine 2,3-dioxygenase 1 (IDO-1) and guanosine triphosphate cyclohydrolase I (GCH-1) are expressed under induction of inflammatory factors. On one side IDO-1 converts tryptophan into kynurenine, and the kynurenine/tryptophan (Kyn/Trp) ratio reflects tryptophan breakdown, and estimates IDO-1 enzyme activity. On the other side, GCH-1 is involved in the production of neopterin, its concentration in body fluids is considered as a marker of immune activation. The association between increased neopterin concentrations and enhanced tryptophan breakdown has been well documented in older adults (Pertovaara et al., 2006; Theofylaktopoulou et al., 2013).

Other important enzymes involved in the immune-inflammatory response are phenylalanine 4-hydroxylase (PAH), which converts the essential amino acid phenylalanine to tyrosine, and the nitric oxide synthases (NOS), which are involved in the conversion of arginine to nitric oxide (NO·) (Neurauter et al., 2008). Inflammation and immune activation may reduce both PAH and NOS activities. Eventually, phenylalanine/tyrosine (Phe/Tyr) ratio is considered to be an estimate of PAH activity (Anderson et al., 1994). Similarly, C-reactive protein is also commonly used as an inflammatory marker, since its level rises in response to inflammation.

On this basis, the objective of the present study was to assess the presence of association between frailty status and the chronic low-grade immune stimulation in the elderly, by analysing a set of inflammatory mediators. For this purpose, immune stimulation biomarkers involved in IDO-1 and GCH-1 enzymatic pathways (namely neopterin, tryptophan and phenylalanine breakdown as indicators of IDO and PHA activities, respectively, nitrite as an indicator of NO production, and C-reactive protein as an indicator of acute inflammation) were determined in a population of Italian older adults.

2. Material and methods

2.1. Study population

A population of 180 older adults (≥65 years) was selected among individuals referred to the geriatric outpatient clinic at the Centro di Medicina dell'Invecchiamento (Ce.M.I), Policlinico Agostino Gemelli hospital (Rome, Italy). A clinical evaluation was carried out by trained geriatricians. Exclusion criteria included an estimated life expectancy < 6 months, inability to walk for 4 m, and unwillingness or inability to provide informed consent. Individuals who agreed to participate in the study were clearly informed about the aim of the study and detailed information about their participation was provided. All participants gave informed consent to be included in this study. The study protocol followed the principles embodied in the Declaration of Helsinki and was approved by the Institutional Ethical Committees of the institutions involved, i.e., Catholic University of the Sacred Heart (UCSC), and IRCCS San Raffaele Pisana.

Peripheral blood samples from subjects were obtained by venipuncture, collected in vacutainer tubes containing sodium heparin, and then transported to the laboratory in cold (4 °C), where they were immediately processed (with a maximum time limit of 2 h from the time of collection at any case). Plasma samples were obtained by centrifugation at 950*g* for 10 min, aliquoted and stored at −80 °C until analysis. All laboratory measurements were performed in a blinded manner since all samples were coded at the moment of collection.

2.2. Frailty status

All participants included in the study were classified as frail (93 subjects) and non-frail (87 subjects) according to Fried's criteria (Fried et al., 2001). These criteria classify as frail those individuals presenting 3 or more out of five specific phenotypic parameters, whereas subjects present 2, 1 or none of them were classified as non-frail. The following parameters were considered: (i) unintentional weight loss in the previous 12 months; (ii) poor endurance and energy; (iii) weakness, defined by poor grip strength; (iv) slowness, assessed via timed 4-m speed; and low physical activity level according to the physical scale for elderly (PASE) (Washburn and Smith, 1993).

2.3. Clinical assessment

Clinical assessment of study subjects was performed as previously described (Valdiglesias et al., 2015). In brief, all participants completed a questionnaire about socio-epidemiological and clinical features to collect information on demographic and lifestyle factors (diet, alcohol, smoking habit, drugs prescribing at admission, co-habitation status, etc.). In addition, each patient received a comprehensive geriatric assessment (CGA), and a prognostic index was estimated to evaluate their life expectancy. Information on different clinical parameters was also obtained by means of proper questionnaires and/or validated scales. In particular, disability status was evaluated by the Katz's Activities of the Daily Living (ADL) (Katz and Akpom, 1976). Cognitive performance was assessed using the mini-mental state examination (MMSE) (Folstein et al., 1975), mood was evaluated by the 15-item Geriatric Depression Scale (GDS) (Yesavage and Sheikh, 1986), comorbidity was assessed by study physicians using the Cumulative Illness Rating Scale (CIRS) (Linn et al., 1968); nutritional status according to the Mini Nutritional Assessment (MNA); the risk of developing pressure sores was assessed by the Exton Smith Scale (ESS).

2.4. Kynurenine and tryptophan analysis

Concentrations of kynurenine and tryptophan in plasma were determined simultaneously by high performance liquid chromatography (HPLC) using an external albumin-based calibrator (serum pool) and an internal calibrator (3-nitro-L-tyrosine), following the general protocol proposed by Laich et al., 2002. The HPLC pump was a Model 210 (Varian ProStar, Palo Alto, CA). Sample collection was performed by an autosampler Model 400 (Varian ProStar) with a 20 μL sample loop and a cooling unit (4 °C). For separation, reversed-phase cartridges LiChroCART RP-18e columns (55 mm length, 3 μm grain size) from Merck