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Micronucleus frequency in peripheral blood lymphocytes and frailty status in elderly. A lack of association with clinical features

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ABSTRACT

Frailty is a condition of vulnerability that carries an increased risk of poor outcome in elder adults. Frail individuals show fatigue, weight loss, muscle weakness, and a reduced physical function, and are known to frequently experience disability, social isolation, and institutionalization. Identifying frail people is a critical step for geriatricians to provide timely geriatric care and, eventually, to improve the quality of life in elderly. The aim of the present study is to investigate the association between frailty status and micronucleus (MN) frequency, a known marker of genomic instability, in a sample of elder adults. Several clinical features were evaluated and their possible association with MN frequency was tested. Criteria proposed by Fried were used to identify frail subjects. Overall, 180 elder adults entered the study, 93 of them (51.7%) frail. No association between MN frequency and frailty status was found under the specific conditions tested in this study (mean ratio = 1.06; 95% CI 0.96–1.18). The inclusion of MN frequency in the Fried's frailty scale minimally improved the classification of study subjects according to the multidimensional prognostic index (MPI). The presence of genomic instability in the ageing process and in most chronic diseases, demands further investigation on this issue.

1. Introduction

Frailty is a condition of vulnerability that carries an increased risk of poor outcome in elder adults. Common signs and symptoms are fatigue, weight loss, muscle weakness, and progressive decline in functions [1]. People with these characteristics are described as 'frail' and are known to experience more frequently poor health outcomes, including disability, social isolation, and institutionalization [1,2]. Two different pathways to frailty have recently been hypothesized: the first one reflects a multisystem physiological change associated to ageing, not necessarily disease-based; the second one considers frailty as a final and common condition in patients affected by severe diseases or comorbidity [1]. The estimated prevalence of frailty in the elder population is around 15%, although in some settings this proportion may increase up to 38% [3].

Identifying frail people is a critical step for geriatricians to develop and implement an interdisciplinary treatment plan, aimed at improving physical and psychological function, decreasing the need of admission to nursing homes and hospitals, reducing the risk of death, and, eventually, improving patient satisfaction with health care [4]. The interplay between physical, psychological, and social factors determines the needs of frail elder people. In this frame, a number of conceptual definitions and criteria to identify frailty, mainly based on clinical features, have been reported.

Even though no clear consensus on the definition of frailty has been reached so far, all definitions can be included into two groups, based on phenotypic criteria or including accumulation of deficit models [5]. The phenotypic frailty index most commonly used has been proposed by Fried et al. [1], and is based on a battery of symptoms and clinical signs, i.e., weight loss, exhaustion, physical activity, walking time, and grip strength. On the other hand, frailty index based on cumulative deficit models considers a larger

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pattern of symptoms, diseases, conditions and disabilities that can include up to 92 components [6]. The main advantage of the latter approach is the quantitative estimate of frailty rather than be classified as present or absent.

Despite the fact that frailty prevalence can vary substantially depending on definition, and that the prevalence estimated by accumulation model is usually higher, an important association between frailty and mortality was reported regardless of the criteria used [reviewed in Ref. [5]]. Indeed, a considerable convergence between these two approaches to recognize frailty has been reported [7].

In clinical practice, it is crucial to identify frail elder subjects as early as possible in order to implement preventive actions and to provide specialized geriatric care, which would lead to improve the quality of life in old age and reduce social and healthcare costs. However, the current methods used to identify frailty are mainly based on clinical symptoms, and a specific physiological basis to the geriatric syndrome of frailty has not been completely established yet, although extensive literature is available [8–10]. A multidisciplinary assessment of elder subjects and events associated to frailty is currently the focus of extensive research, with the priority of identifying new biomarkers capable of recognize frailty before clinical signs are manifested.

Most age-related diseases and ageing signs are associate with genomic instability and with unrepaired or erroneously repaired genome damage [11]. Genomic instability refers to a set of genetic events capable of causing temporary or permanent unscheduled alterations within the genome [12,13]. This term encompasses diverse cellular changes, including accelerated rate of chromosomal alterations, which result in gains or losses of whole chromosomes as well as inversions, deletions, duplications, and translocations of large chromosomal segments. This kind of genomic instability is a hallmark of several age-related diseases, including cancer, and is associated to the ageing phenotype, varying from individual to individual and depending on the interaction between genetic profile and environmental conditions. Hence, genome instability could – directly or indirectly – be a primary cause of the ageing phenotype [12,13]. Therefore, use of biomarkers of genetic instability may be a valuable addition to the process of prospective diagnostics of subjects with high risk of frailty and could lead to improvement in their clinical care. The use of these markers to identify frail subjects may improve therapeutic strategies, allowing to recover biological balance with personalized treatments.

Several molecular and cellular events have been proposed as biomarkers of genomic instability; however, the frequency of micronuclei (MN), originated either from chromosome breakage or chromosome malsegregation events, is an ideal biomarker to investigate genomic instability [14]. MN frequency in peripheral blood lymphocytes is extensively used in molecular epidemiology and cytogenetics to evaluate the presence and the extent of chromosomal damage in human populations [15]. The high reliability and low cost of the MN technique, has contributed to the worldwide success and adoption of this biomarker for *in vitro* and *in vivo* studies [16,17]. Furthermore, this biomarker has been associated with the risk of age-related diseases, and to characteristics features of the ageing phenotype, including loss of physical functions, mental retardation, disability, death [14,18], neurodegenerative disorders [14,19–20], and premature ageing syndromes [21,22].

The MN assay was performed in peripheral blood lymphocytes since these cells present a number of biological and practical advantages that make them particularly suitable for epidemiological studies: considerable amounts of cells can be easily obtained within a short time frame, their use avoids cell cycle effects, since unstimulated lymphocytes are non-cycling, minimizes the possibility of intra-individual heterogeneity and a limited amount of blood is necessary. Furthermore, the level of genetic damage in lymphocytes is usually considered to reflect the level of damage in other different cells and tissues, mainly cancer-prone tissues and cells undergoing carcinogenesis [23,24]. Indeed, there are a number of studies relating the presence of genomic instability in lymphocytes to high cancer risk on different tissues and organs [25–27] or human reproductive failure [28]. This would explain, at least in part, that despite the fact that lymphocytes are fundamentally different than cells that compose solid organs and therefore present limitations as a surrogate tissue model, they are the most used cells in research of genomic instability [29]. Finally, MN frequency was determined by automatic scoring because this method offers several interesting advantages with regard to common visual scoring, including the fast acquisition of results, which allows the analysis of large numbers of slides, the exclusion of subjective judgement and individual scoring skills, and the possibility of repeated scoring of the same slide [30].

The use of genomic instability biomarkers associated with ageing and ageing-related phenotype may contribute to anticipate the recognition of frail individuals and to improve the sensitivity and specificity of their classification. On this basis, the association between frailty status and a genomic instability biomarker such as MN has been evaluated in this study through a cross sectional design, comparing a group of 'frail' elder subjects with a group of 'non-frail' elder subjects.

2. Materials and methods

2.1. Study design and population

A population of 180 elder adults (age >65) 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) between October 2012 and January 2013. Exclusion criteria were: presence of disease conditions with 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. The study was approved by the Institutional Review Boards of the institutions involved, i.e., Catholic University of the Sacred Heart (UCSC), and IRCCS San Raffaele Pisana. All participants signed a written consent.

2.2. Clinical assessment

All individuals included in the study were classified as 'frail' (93 subjects) or 'non frail' (87 subjects) according to Fried's frailty criteria [1]. The following parameters were considered: (1) unintentional weight loss in the previous 12 months; (2) poor endurance and energy; (3) weakness, defined by poor grip strength; (4) slowness, assessed via timed 4-m walk speed; and (5) low physical activity level according to the physical activity scale for the elderly (PASE) [31]. Patients positive for three or more Fried's criteria were classified as 'frail'. A detailed report of cut-off values used to assess frailty criteria can be found in Marzetti et al. [32].

A questionnaire about socio-epidemiological and clinical features including detailed information on demographic and lifestyle factors (diet, alcohol, smoking habit, etc.) was administered at the time of enrollment. In addition, each patient received a comprehensive geriatric assessment (CGA), and a multidimensional prognostic index was estimated to evaluate their life expectancy.

Disability status was evaluated by the Katz's scale: activities of the daily living (ADL) [33]. Cognition was assessed using the mini-mental state examination (MMSE) [34], while mood was evaluated