Experimental Gerontology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Experimental Gerontology

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

Assessment of health status by molecular measures in adults ranging from middle-aged to old: Ready for clinical use?

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ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form 25 March 2016
Accepted 30 March 2016
Available online xxxx

Keywords:
Functional capacity
p16INK4a
CD57
Aging
Senescence
Immunosenescence

ABSTRACT

In addition to measures already used in clinical practice, molecular measures have been proposed to assess health status, but these have not yet been introduced into clinical practice. We aimed to test the association of functional capacity measures used in current practice and molecular measures with age and health status.
The cohort consisted of 178 middle-aged to old participants of the Leiden Longevity Study (range 42–82 years). We tested associations between functional capacity measures (physical tests: grip strength, 4-meter walk, chair stand test; cognitive tests: Stroop test, digit symbol substitution test and 15-picture learning test) with age and with cardiovascular or metabolic disease as a measure of the health status. These associations with age and health status were also tested for molecular measures (C reactive protein (CRP), numbers of senescent p16INK4a positive cells in the epidermis and dermis and putative immunosenescence (presence of CD57+ T cells)).
All functional capacity measures were associated with age. CRP and epidermal p16INK4a positivity were also associated with age, but with smaller estimates. Grip strength and the Stroop test were associated with cardiovascular or metabolic disease, as was epidermal p16INK4a positivity. All associations with cardiovascular or metabolic disease attenuated when adjusting for age.
In conclusion, in middle-aged to old persons, the molecular measures tested here were more weakly associated with age and health status than functional capacity measures. Whether these molecular measures associate more closely with health status in the elderly or in specific groups of patients needs to be explored further.

1. Introduction

Markers that characterize the rate of aging in humans are important in an era of increasing lifespan and emerging novel opportunities for interventions to potentially prolong lifespan. Ideally, these measures should associate with age and with prevalence of age-related disease as a measure of an individual's health status. In clinical practice, functional capacity can be assessed by testing e.g. muscle strength, balance and walking speed. Performance on physical performance tests are associated with age (Beenakker et al., 2010; Stijntjes et al., 2015; Verlinden et al., 2013), as well as health outcomes such as disability (Guralnik et al., 1995), disease (Abellan van et al., 2009) and mortality (Ling et al., 2010; Studenski et al., 2011), even at middle age (Stijntjes et al., 2015). The cognitive domain is evaluated by making use of the Mini Mental State Examination (MMSE) assessing global cognitive functioning but also testing specific cognitive domains such as executive functioning, recall and attention. Performance on cognitive tests is associated with age (Stijntjes et al., 2013), as well as disease (Reis et al., 2013; Lee et al., 2014) and mortality (Gussekloo et al., 1997). These functional capacity measures have proven their use in clinical practice.

Molecular mechanisms which are causally related to the aging process have also been reported to provide insights into an individual's health (Lopez-Otin et al., 2013). Molecular measures associated with age and health status include telomere length, transcriptomic and epigenetic parameters, inflammatory markers and cellular senescence (Pallis et al., 2014; Peters et al., 2015; Horvath et al., 2015). Low grade systemic inflammation, measured by e.g. C-reactive protein (CRP) has been associated with age (Bruunsgaard, 2006), disease (De et al.,

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http://dx.doi.org/10.1016/j.exger.2016.03.020
0531-5565/

2006; Akbaraly et al., 2013) and mortality (De et al., 2006; Akbaraly et al., 2013; Bruunsgaard et al., 2003; Bruunsgaard et al., 2003). Cellular senescence, the phenomenon of permanent cell cycle arrest of somatic cells after a certain number of cell divisions or particular insults such as DNA damage, is found to be more prevalent at higher age in many tissues (Bhat et al., 2012; Liu et al., 2009; Melk et al., 2004; Ressler et al., 2006). Immunosenescence, which may involve cellular senescence of T lymphocytes, has been linked to mortality (Wikby et al., 1994; Ferguson et al., 1995). Furthermore, cellular senescence has been related to many age-related diseases such as diabetes (Verzola et al., 2008), glomerular disease (Sis et al., 2007) and chronic obstructive pulmonary disease (Tsuji et al., 2006), which strengthens the rationale to use senescence as a potential marker for the human aging process. A small number of studies has used such senescence measures to predict clinical outcome, but with inconsistent results (Koppelstaetter et al., 2008; Gingell-Littlejohn et al., 2013; Pustavoitau et al., 2016). Overall, molecular measures have not yet found their way into clinical practice.

We aimed to evaluate the associations between functional capacity measures and molecular measures (focussing specifically on CRP, skin senescence and immunosenescence) from middle age onwards (age range 42–82 years), and to associate these with the presence of cardiovascular or metabolic disease as a measure for health status.

2. Methods

2.1. Study design and participants

In the Leiden Longevity Study, factors contributing to familial longevity are studied in long-lived families; the study design has been described previously (Schoenmaker et al., 2006). Offspring of nonagenarian siblings as well as their partners, who act as environmentally-matched controls, participated in this study. Subjects were recruited from July 2002 to May 2006. Over several years (November 2006 to May 2008, and September 2009 to December 2010) data on functional capacity and molecular measures were acquired from these participants, many of which have been published previously (Stijntjes et al., 2015; Stijntjes et al., 2013; Ling et al., 2012; Waaijer et al., 2012; Derhovanessian et al., 2010). The study was approved by the Medical Ethics Committee of Leiden University Medical Center and all participants gave their informed consent.

2.2. Medical history

Medical history on myocardial infarction, cerebrovascular accident, hypertension, diabetes mellitus, malignancy, chronic obstructive pulmonary disease and rheumatoid arthritis was obtained from general practitioners. Presence of any of these diseases was defined as one or more disease in the medical history. Presence of a cardiovascular or metabolic disease was defined as one or more of four cardiovascular or metabolic diseases (presence of myocardial infarction, cerebrovascular accident, hypertension or diabetes mellitus).

2.3. Functional capacity measures

Functional capacity measures were based on measures used in clinical practice, and included measures of physical and cognitive domains. Grip strength of the dominant hand was measured in the upright position with maximal force using a hand dynamometer (Jamar, Sammons Preston Inc., Bolingbrook, IL, USA) (Ling et al., 2012). The best of three attempts, expressed in kilograms, was used for analysis. Four meter walking speed was measured twice across a 4-meter course starting from a standing position. Participants were asked to walk at usual pace. The fastest time in seconds was used for analyses. The time to stand up and sit down on a chair as fast as possible five times formed the chair stand test (Stijntjes et al., 2015). Cognition was assessed by neuropsychological testing (Stijntjes et al., 2013). For this study, part 3 of the Stroop test (time in seconds needed to read colored words printed in an incongruous ink color), the digit symbol substitution test (correct number of symbols) and delayed recall (correct number after 20 min) of the 15-picture learning test were used because of their known associations with age in larger cohorts (Stijntjes et al., 2013).

2.4. Molecular measures

From the diverse set of existing molecular measures, we here studied specific measures of inflammation, skin senescence and immunosenescence.

High sensitive C-reactive protein (hsCRP) was measured in serum (Hitachi Modular P 800 from Roche, Almere, the Netherlands) (Rozing et al., 2011). Participants with a hsCRP > 10 mg/L were excluded from the analyses due to possible acute infection.

Skin biopsies from the upper inner arm were taken and stained for senescence-associated p16INK4a expression by immunohistochemistry, as described previously (Waaijer et al., 2012). p16INK4a-positive cells were counted separately in the epidermis (positive staining cells per mm length of the epidermal-dermal junction) and in the dermis (positive staining cells per $1mm^2$ dermis).

Peripheral blood mononuclear cells (PBMCs) were analyzed by flow cytometry for T-cell differentiation phenotypes (Derhovanessian et al., 2010) to study immunosenescence. The frequency of T cells bearing the 'senescence-associated' marker CD57 was previously found to be higher in elderly than in young individuals (McNerlan et al., 1998; Di Benedetto et al., 2015). In the present study, we therefore selected the proportion of CD4+CD57+ and CD8+CD57+ T-cells for analysis. Cytomegalovirus (CMV) serostatus was taken into account as a possible confounder, and measured by ELISA using the CMV-IgG-ELISA PKS assay (Medac GmbH, Wedel, Germany), as per the manufacturer's instructions (Derhovanessian et al., 2010).

2.5. Analyses

2.5.1. Datasets

Analysis of associations of the measures of the functional capacity and molecular measures with age and cardiovascular/metabolic disease was conducted using data from 178 participants. The number of included participants was limited by the measure with the lowest number of available data (epidermal and dermal p16INK4a positivity). Not all measures were available in this exact same group of 178 participants, and therefore data from randomly selected participants of the cohort in whom the measures were available were added to complement the dataset (4-meter walk test and chair stand test random subset N = 95, Stroop test, digit symbol substitution test and 15-picture learning test random subset N = 57, CRP random subset N = 15, proportion of CD8+CD57+ and CD4+CD57+ T-cells random subset N = 98). The functional capacity and molecular measures were divided into tertiles of worst, average and best test results. Values of these tertiles are shown in Supplementary Table 1.

2.5.2. Statistics

Statistical analyses were performed using IBM SPSS Statistics 20. Graphs were drawn with Prism GraphPad version 5. First we tested whether functional capacity and molecular measures were associated with age using linear regression (with estimated means via linear mixed models). The first model was adjusted for sex (grip strength analyses used sex-specific tertiles) and the immunosenescence associations for CMV serostatus. The second model additionally adjusted for the presence of one or more diseases. Next, we tested whether functional capacity and molecular measures associate with health status, determined by history of one or more cardiovascular or metabolic diseases using logistic regression. The first model was adjusted for sex (except grip strength analysis) and for CMV serostatus for immunosenescence associations. The second model additionally adjusted for age.

Please cite this article as: Waaijer, M.E.C., et al., Assessment of health status by molecular measures in adults ranging from middle-aged to old: Ready for clinical use?, Exp. Gerontol. (2016), http://dx.doi.org/10.1016/j.exger.2016.03.020