Guidelines for biomarkers of healthy ageing

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Acknowledgements

We acknowledge Professor Timothy Salthouse (University of Virginia) and Professor Fergus Craik (University of Toronto) for their comments on the development of the cognitive section described in this paper; Sathya Karunananthan (McGill University) and Professor Howard Bergman (McGill University) for their contributions to the physical capability section; Professor Andrew Steptoe for his valuable comments and suggestions on the draft version of the Guidelines; Professor Richard Gershon (Northwestern University), Prof Joe Verghese (Albert Einstein College of Medicine), Prof Christian Drevon (University of Oslo), Prof Rudi Westendorp (University of Leiden), Prof Marion McMurdo (University of Dundee), and Prof Naveed Sattar (Glasgow University, UK) for their contributions to the Workshop on Biomarkers of Healthy Ageing held in Newcastle, UK, on October 22-23, 2012.

Jose Lara and John C. Mathers are members of the LiveWell Programme which is funded by the Lifelong Health and Wellbeing (LLHW) Cross-Council Programme initiative in partnership with the UK Health Departments. John Mathers' research through the Centre for Ageing and Vitality is also funded by the LLHW initiative. The LLHW Funding Partners are: Biotechnology and Biological Sciences Research Council, Engineering and Physical Sciences Research Council, Economic and Social Research Council , MRC, Chief Scientist Office of the Scottish Government Health Directorates, National Institute for Health Research /The Department of Health, The Health and Social Care Research & Development of the Public Health Agency (Northern Ireland), and Wales Office of Research and Development for Health and Social Care, Welsh Assembly Government. Jack Nissan and Ian J. Deary are members of The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the

cross council Lifelong Health and Wellbeing Initiative (MR/K026992/1). Funding from the BBSRC and MRC is gratefully acknowledged. Diana Kuh and Rachel Cooper acknowledge MRC funding (Grant code MC_UU_12019/04) and support in conducting literature reviews on physical capability from Renata Bryce and Sathya Karunananthan.

We acknowledge the support and contribution to discussion of all expert participants of the Workshop on Biomarkers of Healthy Ageing (**Appendix 10**).

Executive summary

There is no gold standard tool for assessing healthy ageing at the individual or population levels. Chronological age is strongly associated with ageing-related functional losses but within an age homogeneous sample there is considerable between-person variation revealing a need for more informative markers of the ageing process. There is growing interest in the identification of biomarkers which capture key features of healthy ageing but, given the biological complexity of the ageing process, there is no single, simple and reliable measure of the rate at which someone is ageing. A cardinal feature of ageing is loss of function which translates into wide-ranging consequences for the individual and for family, carers and society. Here we present our recommendations for a panel of biomarkers which address major areas of function which decline during ageing including physical capability, cognitive function, physiological function, endocrine function, and immune function. Although these biomarkers were identified through population-level studies, the majority are associated with biological mechanisms that have a causative role in the ageing process and therefore might be useful to characterise and to quantify ageing at the individual level.

We reviewed the literature and identified systematic reviews and/or meta-analyses of cohort studies, and other authoritative reports. Our selection criteria for potentially suitable biomarkers included: frequent use in longitudinal studies of ageing; observed to change with age; and evidence for strong association with /prediction of age-related phenotypes such as morbidity, mortality and lifespan. We focussed on studies which assessed these biomarkers and ageing-relevant outcomes in initially healthy populations. Within each domain of function, we identified important sub-domains and have suggested

suitable tools for their measurement. For the latter, we emphasised measures which would be cost effective and practicable for use in large-scale studies. Our proposed biomarkers of healthy ageing were exposed to external critique in an expert Workshop held in Newcastle, UK in October 2012.

In some areas e.g. immune function, the evidence base for selection of appropriate biomarkers of healthy ageing is less well developed and so our recommendations are more tentative. In addition, it is possible that the proposed panel may include some redundancy, i.e. not all measures may be necessary to characterise and quantify ageing of a given individual. Although a sub-set of these markers may explain much of the inter-individual variation, we had insufficient evidence on which to select the minimum, essential biomarker panel. The current evidence on the various biomarkers proposed in this document originates from different populations and while their predictive ability has been tested, these estimates might not necessarily be comparable, since their predictive ability may change with age and baseline characteristics. Studies assessing multiple biomarkers within the same population will be necessary to establish the hierarchy of biomarkers, and to test the ability of sub-sets of the biomarkers to predict healthy ageing.

We hope that our proposed panel of biomarkers will have utility in epidemiological studies of human ageing, in health surveys of older people and as outcome measures in intervention studies intended to improve the likelihood of healthy ageing. In addition, the inclusion of the same common panel of measures of healthy ageing in diverse study designs and populations may enhance the value of those studies by harmonising outcome measures and thus facilitating less equivocal comparisons between studies and the pooling of data across studies.

Introduction

Healthy ageing and wellbeing are common goals in modern societies. The major demographic shift towards higher proportions of older people within the population in many countries worldwide, and the recognition that much of the costs of health and social care in economically-developed countries is concentrated in the last decade or two of life, have sharpened the research focus on ageing.[1]

Research on healthy ageing encompasses: the biological processes contributing to ageing per se; the socio-economic and environmental exposures across life which modulate ageing and the risk of age-related frailty, disability and disease; and the development of interventions which may modulate the ageing trajectory.[2 3] Such research needs measures of biological ageing at the individual level which, in addition to chronological age, can characterise and quantify important functions which are subject to decline at faster, or slower, rates during individual human ageing. Biomarkers of healthy ageing would have utility as outcome measures in trials of interventions designed to extend health span and public health-related population surveys would benefit from reliable, readily-measured indices of healthy ageing.

Over the last 50 years[4-6] there have been several attempts to develop markers of ageing but the complexity of the ageing phenotype[7] brings both conceptual and practical difficulties. Despite earlier efforts,[8-11] there is currently no universally accepted definition of biomarkers of ageing or criteria for their selection, which has resulted in a lack of robust, validated tools for assessing healthy ageing.[4-7] The American Federation for Aging Research proposed that biomarkers of ageing : “1) must predict the rate of aging (it should tell exactly where a

person is in their total lifespan and it must be a better predictor of lifespan than chronological age); 2) it must monitor a basic process that underlies the aging process, not the effects of disease; 3) it must be able to be tested repeatedly without harming the person (for example, a blood test or an imaging technique); 4) it must be something that works in humans and in laboratory animals, such as mice (so that it can be tested in laboratory animals before being validated in humans)".[5] Several candidate biomarkers of ageing have emerged in the past few decades but none has proved universally suitable for, or robust in, measuring or predicting the degree of ageing at either population or individual levels.[12]

Ageing affects all cells, organs and tissues and, in the majority of body systems, is characterised by the gradual loss of function. When extensive, such functional losses have profound effects which impact on the individual and on family members and carers and have wide-ranging consequences for society. Here we aim to identify a panel of objective biomarkers of healthy ageing in humans where healthy ageing is defined as the maintenance of function for the maximal period of time.[3] Having functionality and pragmatism as our guiding principles, this work focused on those biomarkers which characterise and quantify important functions subject to deterioration in mean levels during ageing and for which there are robust, readily applied tools/instruments for their assessment. We focused attention on the domains of physical capability, cognition, physiological and musculoskeletal functions, and endocrine, immune and sensory functions. However, we recognise that there are important subjective features of the healthy ageing phenotype, including psychological and social wellbeing, which are not covered here.[13-15] In addition, there may be important bidirectional relationships between healthy ageing and

wellbeing which are outside the scope of the present work. Our proposed panel of markers was selected from those which are best established, for which there is robust evidence supporting strong associations with ageing phenotypes, and which are likely to be cost-effective and practical for use in larger scale studies. Most literature focuses on morbidity and mortality as ageing phenotypes and there is no independent, gold standard measure of healthy ageing against which existing or novel biomarkers may be assessed.

We aimed to identify objectively assessed biomarkers that are used commonly in population-based studies and applicable in a range of settings (i.e. not limited to use in a laboratory/clinic setting), capable of distinguishing between healthy and unhealthy ageing between individuals at older ages, and which change within individuals over time. Where possible, we sought evidence of replication of the proposed marker in different cohorts and using different study designs. The research base in some domains e.g. measures of age-related immune function proved to be less well developed than in others e.g. measures of physical capability, so that our recommendations in the former domains are more tentative. To help fill the remaining gaps, we also aimed to identify priorities for further research on biomarkers of healthy ageing and these are summarised below in the sections headed "Areas lacking adequate evidence".

Process used to develop recommendations

The process used to develop our recommendations (**Figure 1**) included, 1) comprehensive reviews of the literature relevant to each domain using, where available, existing systematic reviews, meta-analyses (**see Appendix 1**), and other authoritative reports such as the recently-launched NIH Toolbox for Assessment of Neurological and Behavioural Function which includes test batteries for cognitive and motor function (the latter described here as

physical capability);[13] 2) comments from international experts on draft recommendations; and 3) an expert workshop (held in Newcastle, UK on 22-23 October 2012) which aimed to help capture the state-of-the-art in this complex area and to provide an opportunity for the wider ageing research community to critique the proposed panel of biomarkers.

Biomarkers of physical capability

Measures of physical capability, i.e. a person's ability to perform the physical tasks of everyday living, are useful markers of current and future health.[16] Objective, standardised tests of physical capability have been developed and are used increasingly in population-based studies.[17 18] These objective measures complement self-reports, improve validity and reproducibility, capture change over time, and may reduce the influence of cognitive function, culture, language and education that can affect self-reported assessments and so limit comparability across studies.[17 19] From the perspective of healthy ageing, these objective tests have two key advantages. Firstly, they enable the study of variation in functioning across the full spectrum. Secondly, they facilitate identification of those people performing best who cannot be distinguished by self-reported measures which aim to identify people who have difficulty in performing the tasks of everyday living.

Domains of physical capability relevant to ageing and tools for their assessment

Our selection of biomarkers of physical capability was guided by information ascertained in 2008 as part of the Healthy Ageing across the Life Course (HALCyon) research collaboration[3] and the work undertaken in the development of the NIH toolbox motor function domain.[20-22] We established details of their measurement and common variations in protocol, and assessed the extent of evidence supporting these measures as markers of healthy ageing. Four of the 5 sub-domains included in the NIH toolbox,[22] viz. locomotor function, strength, balance and dexterity, were considered to capture the underlying functions that are used most commonly as objective measures of physical capability in longitudinal studies (**Table 1**).

There is considerable variability in the protocols of assessment for these measures.[20 23-27] However, attempts at standardisation across studies are now being made through initiatives such as the NIH toolbox.[22] All tests of physical capability are relatively quick, easy and inexpensive to perform with only grip strength and the pegboard test requiring special instruments.[28] An exception is the use of an accelerometer to measure swaying during balance tests which is recommended by the NIH toolbox.[29] There is strong evidence supporting the validity and reliability of these measures.[22 30 31] However, all the physical capability tests have exclusion criteria and an important consideration, not well addressed in the literature, is how to handle the increasing proportion of people unable to perform these tests at older ages.

Patterns of age-related change in physical capability

Grip strength is the most comprehensively studied physical capability test.[19] Longitudinal studies show that grip strength peaks in the late thirties for both sexes;[32-35] while longitudinal and cross-sectional studies show declines in both sexes from the fifties and sixties.[20 32 34-41] At all ages, grip strength is higher in men than women and there is some evidence for faster decline in men than in women.[34-36 42-45]

Evidence for age-related change in other measures of physical capability is more limited because it is restricted largely to cross-sectional data from relatively small studies. However this limited evidence is consistent in suggesting that physical capabilities decline progressively in later life and that men perform better than women at all ages.[20 24-26 30 46-63]

Associations with health outcomes

A systematic review has shown that weaker grip strength, slower walking speed, longer chair rise time and poorer standing balance performance are associated with higher mortality rates, independent of age in older community-dwelling populations.[16] Meta-analyses of data from several American studies of older people have also revealed a strong association between slower walking speed and higher mortality rates.[64] More recent studies indicate that, in addition to grip strength and walking speed, standing balance and chair rise speed in middle age predict mortality rates over 13 years of follow-up.[65] In another recent systematic review, weaker grip strength was found to be associated with functional decline as assessed by self-reported difficulties performing activities of daily living (ADLs).[66] Three other systematic reviews evaluating risk for subsequent disability (assessed using ADLs) showed that older adults performing poorly in tests of physical capability are more likely to become disabled.[26 67 68] There is also some evidence that poorer performance in grip strength, walking speed, chair rise times and standing balance, is associated with higher risk for cardiovascular disease (CVD), dementia and institutionalisation (as a marker of loss of independence), but none of these associations has been studied sufficiently often to allow definitive conclusions to be drawn.[69]

Areas lacking adequate evidence

Significant variability in the protocols used to assess any one measure of physical capability makes comparisons between, and combination of findings from, different studies difficult. In many cases, these limitations prevent the estimation of the expected rate of decline in a measure with age using data drawn from several sources. In addition, few studies have compared formally the different measures of physical capability and, as with measures of cognitive function (see below), performance in any one measure of physical capability is

likely to be correlated with performance in other such measures. Recent work suggests that there is added value, for the prediction of mortality, in assessing different measures of physical capability in midlife.[65] However, there is currently insufficient evidence, from the perspective of studying healthy ageing, to establish the added value of assessing any one additional specific measure if other measures have been assessed already, to recommend an order of priority for these measures or to define with confidence the minimum number of measures that should be made across the full range of older ages and for different research questions.

Here we have considered each measure of physical capability separately. However, some studies have used a set of tests of several aspects of physical capability interpreted as a total performance score,[70] such as the short physical performance battery (SPPB)[71] or the index of physical fitness age.[56] Further work should establish whether deriving an overall score of physical capability is of greater predictive value than considering each measure separately and the most appropriate approach is likely to depend on the specific research question being addressed.

There is a need for larger longitudinal studies in which these age-related patterns, as well as variations in within-individual changes over time, can be investigated further. Declines in mean levels of physical capability at the population level hide substantial inter-individual variation in rate of decline. For example, being able to identify people who maintain, or improve, their physical capability despite increasing age will be important when studying healthy ageing. More research is needed on the utility of some measures such as dexterity performance in the pegboard test, which was referred to in only one of 22 studies included in a systematic review.[68] In addition, there is a dearth of evidence on the associations of

physical capability with measures of positive aspects of health,[72] such as quality of life, that may be important criteria for healthy ageing.

Biomarkers of physiological function

Ageing encompasses varied and complex changes at the structural, functional, and molecular levels of most cell, tissue and organ systems in the human body and a gradual loss of the homeostatic mechanisms necessary to maintain tissue function and physiological capacity is a hallmark of ageing.[7] Such loss may translate, eventually, into metabolic dysregulation leading to the development of early signs of pre-disease which, if not identified and managed, will result eventually in functional loss, chronic disease and finally death. A well-recognised example is age-related loss of skeletal muscle mass and strength potentially leading to sarcopenia.[73] However, subtle changes in the function of most organs can occur by the third or fourth decades of life.[74]

Domains of age-related physiological (and musculoskeletal) function and tools for their assessment

Here we focused on biomarkers of lung function, bone health, body composition, cardiovascular function and glucose metabolism (**Table 2**) which consist of clinical and research tests predicting physiological and metabolic health and biological ageing. Whilst mortality and multi-morbidity were the ageing-related phenotypes most commonly evaluated for associations with physiological biomarkers, there is some evidence for association with other functional domains considered in this paper.

Patterns of age-related change and associations with health outcomes

Lung function: From age 25 years, lung function assessed through forced expiratory volume (FEV1; the most common measure documented in epidemiologic studies) declines at

approximately 32ml/year in men and 25 ml/year in women.[75-80] Numerous population studies have documented an inverse association between FEV1 and ageing-related endpoints including future total and cardiovascular mortality, cognitive function and fractures.[81-88]

Bone health: Bone mass declines with age in both men and women although whether the decline is greater in women is debated.[89-96] Techniques for measuring bone mass include dual x-ray absorptiometry (DXA), broadband ultrasound attenuation (BUA), and quantitative computed tomography (CT) (**Appendix 2**) and both site specific (hip or spine) DXA and heel BUA have been used extensively in epidemiologic studies. DXA is the most widely used method to assess bone mineral density and is the method of choice to diagnose osteoporosis.[97] Bone mass or density (measured using DXA or BUA) predicts future fracture risk as well as mortality and other age-relevant health outcomes.[98-108] BUA is an attractive alternative to DXA given its portability, lower cost, and no exposure to ionising radiation. A recent meta-analysis showed that BUA predicted fracture risk similarly to DXA.[109]

Body mass and body composition: Ageing is associated with body composition changes including increased body fat, reduced muscle mass and, with exception of the heart, reduced organ mass. Greater abdominal adiposity is a risk factor for ageing and for age-related diseases with the lowest mortality risk for those with waist circumferences (WC) below 94 and 77 cm for men and women, respectively. The relative risk (RR) of mortality is doubled for those with WCs above 132 and 116 cm in men and women, respectively.[110] Body mass index (BMI) is a useful measure of overall adiposity since each 5 kg/m^2 increase in BMI is associated with 30% higher overall mortality, 40% higher vascular mortality, 60–120% higher diabetic, renal, and hepatic mortality.[111] High BMI, independent of gender and

other confounding factors, is a risk factor for cognitive decline.[112] In addition, weight gain in middle age is associated with substantially reduced likelihood of healthy survival after age 70 years in women.[113]

Muscle mass can be assessed using CT, magnetic resonance imaging (MRI), DXA, bioimpedance analysis (BIA), and body potassium. Evidence shows that muscle mass, such as leg muscle mass, declines with age.[44 114-116] Cross sectional and prospective studies that have examined the relationship between regional muscle mass *per se* and health outcomes have reported that low skeletal muscle index (skeletal muscle mass/body mass percent) is associated with increased likelihood of functional impairment and disability.[115 117 118] Recent developments from the FNIH Sarcopenia Project may help to establish universal cut-points for low muscle mass and weakness.[119]

Cardiovascular function: Ageing of the cardiovascular system is associated with ageing of both cardiac muscle and the vascular wall. Although there are many inflammation and haemostasis-related biomarkers of cardiovascular function,[120-125] the classical, widely measured, and well documented physiological markers of risk of cardiovascular-related diseases remain the strongest biomarkers of ageing. Systematic reviews and meta-analyses provide strong evidence that blood pressure[126 127] (BP), lipid profile (including total cholesterol, low- and high-density lipoprotein cholesterol,[128 129] and triglycerides[130] concentrations) are predictors of morbidity and mortality. A difference of 20 mmHg in systolic BP (or 10 mmHg in diastolic BP) is associated with > two fold difference in death from several vascular causes.[126] High BP in midlife is associated with lower cognitive function in later life.[131] Among the components of the Metabolic Syndrome, high-BP and impaired fasting glucose are significant predictors of greater CV-morbidity and mortality.[132]

There is a lack of evidence on the age-related changes in most cardiovascular biomarkers but, using data from eight UK cohorts, a recent study evaluated the life course trajectories in BP and confirmed age-related changes in BP, independent of BMI.[133] Systolic BP increased from childhood, with a markedly midlife acceleration beginning at 40 years of age, and deceleration and reversion of these increases in late adulthood.[133]

Glucose metabolism: Ageing is associated with alterations in several aspects of glucose metabolism, including insulin receptors and glucose transporters, leading to decreased glucose oxidation and increased liver gluconeogenesis.[134] Biomarkers of dysregulated glucose metabolism including fasting blood glucose concentration and glycated haemoglobin (HbA1C) (an indicator of long-term average blood glucose concentration), are associated with age and predict future cardiovascular events and mortality,[125 135-143] cognitive impairment[144] and dementia[145] in non-diabetics. A difference of 1% in HbA1c levels is associated with a 20% and 26% difference in risk of coronary heart disease (CHD) and total mortality, respectively.[142] Favourable glucose metabolism has been identified as a central factor for familial longevity.[146]

Areas lacking adequate evidence

A number of emerging biomarkers have been associated with increased risk of cardiovascular events and mortality e.g. fibrinogen,[120 125] plasma cystatin C (a marker for chronic kidney disease),[125 147] and Brain Natriuretic Peptide (secreted by ventricular myocytes during periods of increased ventricular stretch and wall tension)[148]. However, the advantage, if any, of these newer markers over the well-established biomarkers identified above needs further research.

The long-established age-related physiological biomarkers are usually measured at single points in time and it has been suggested that monitoring some of these biomarkers over longer time periods, e.g. glucose concentration and ambulatory blood pressure over 24h, may improve their predictive value[149-151] but this needs to be tested in appropriate prospective cohort studies. The lack of evidence on the age-related changes of biomarkers could be addressed by mathematical modelling of longitudinal data.[133]

Biomarkers of cognitive function

Some aspects of cognitive function change with age and this domain is commonly investigated in longitudinal studies of ageing.[152] Age-related impairment is observed frequently in functions such as speed of processing, some aspects of memory, attention and visuo-spatial abilities, whereas some abilities e.g. vocabulary may increase with age.[153] Decline in cognitive functions may limit independence and signal dementia,[154 155] and although there is debate about the age of onset of cognitive decline,[156-160] evidence accruing from longitudinal studies indicates that it can be observed in relatively early adulthood e.g. from around 45 years of age or earlier in some functions.[161]

The selection of biomarkers of healthy ageing in the cognitive domain has some special problems and considerations (**see Appendix 3**). We therefore focused on cognitive domains assessed commonly in human ageing studies and on the cognition battery included in the NIH Toolbox.[13 162]

Domains of cognitive function relevant to healthy ageing and tools for their assessment

We identified 62 cohorts assessing ageing which included measures of cognitive functioning described by 115 different cognitive domain names. There was redundancy in the domain names. From these measures we selected the following cognitive domains relevant to ageing: 1) Executive Function; 2) Processing Speed; 3) Verbal Memory and Learning; 4) Attention; 5) Working memory; 6) Crystallised Ability; 7) Reasoning; 8) Visual Memory and 9) Visuo-Spatial Ability (**Table 3**) which can be assessed by the following tests: 1) Verbal Fluency ; 2) Digit Symbol Coding ; 3) Rey Auditory Verbal Learning Test; 4) Stroop Test; 5)

Digit Span Backwards; 6) Boston Naming Test; 7) Raven's Progressive Matrices; 8) Benton Visual Retention Test and 9) Block Design respectively. A description of these subdomains and tests is provided in **Appendix 4**. Note that there are many other possible tests for each domain. In selecting our battery of cognitive tests, we considered a wide range of practical issues (**see Appendix 5**) and we list other tests commonly used to assess these subdomains (**see Appendix 6**). We stress that: none of the single tests will assess a cognitive domain comprehensively; no single test is a pure measure of that domain, because it will engage other cognitive domains for its completion; and the named tests are examples (commonly-used ones) of many that might be used for each domain. A description of other widely used cognitive domains and tests identified in our searches is also provided (**see Appendix 6**).

In addition to the above proposed list of subdomains and tests to assess cognitive function, the recent development of the NIH Toolbox cognitive battery[13 162] offers a comprehensive set of tools to assess cognitive functions. The NIH Toolbox cognitive battery is intended for use across the lifespan (ages 3-85 years), and is distinguished by its brevity and its suitability for repeated administration in longitudinal designs. The NIH Toolbox focuses on the following five cognitive domains: executive function, episodic memory, language, processing speed, and attention. These domains were ranked in that order of importance by a panel of experts involved in the development of the Toolbox. Although some have slightly different names, all of these can be found in the list of nine domains given above. Although the NIH Toolbox is a significant advance, it may have some limitations in the present context. These are related to its briefness (a feature that may also be a strength when other healthy ageing-related domains, e.g. physical capability, need to be assessed and limit the time

available to assess multiple domains), and the focus on only five domains, thus excluding domains that may be of interest to some researchers.

Patterns of age-related change in proposed cognitive biomarkers and associations with health outcomes

Of the nine subdomains identified above, two cognitive domains viz. executive function and processing speed are associated strongly with ageing phenotypes.[14] Executive function is markedly affected by ageing[163] and exhibits inverted U-shape patterns across the lifespan.[164] Processing speed declines progressively with age from young adulthood.[165-168] Decline in cognitive processing speed is associated with greater risk of all-cause mortality,[143] [169-171] CVD, CHD, stroke, and respiratory disease up to 13 years later, even after adjustment for known risk factors.[169] There is also evidence that better processing speed is associated with longevity.[172] In addition, episodic memory, a subdomain included as part of the NIH toolbox cognitive battery, is exquisitely sensitive to brain ageing and functional loss is observed commonly in individuals with mild cognitive impairment, in neurodegenerative diseases such as Alzheimer's, Huntington's, and Parkinson's and also in dementia and psychiatric diseases.[173] Episodic memory significantly predicted later mortality; a standard deviation advantage in memory was associated with 21% reduction in the risk of death in older individuals (HR 0.79; 95%CI 0.66 to 0.95).[174] During the development of the NIH Toolbox, the results of an experts' survey showed that the domains perceived as relevant to health were executive function and episodic memory ranked as the two top cognitive domains, with processing speed in fourth place behind language.[162] Tests to assess episodic memory often measure the learning and reproduction of a list of items such as the Rey Auditory Verbal Learning Test,[175] the California Verbal Learning Test,[176] or the NIH Toolbox

picture sequence memory test involving recalling increasingly lengthy series of illustrated objects and activities that are presented in a particular order on the computer screen.[162] In some studies the maintenance of the learned material due to rehearsal between the encoding and retrieval sessions is prevented by an intervening and distracting task. The Rey auditory verbal learning test[175] is based on the interference induced by the consecutive learning of two word lists and the immediate and delayed recall of the first list. Another commonly-used test is immediate and delayed recall of a paragraph containing a story with about 25 'ideas' in it. The Wechsler Logical Memory test is one such.[177] The 'episodic' memory tests mentioned here might more properly be called 'verbal declarative' memory tests. Based on the current evidence of age-related changes of cognitive function and their associations with health outcomes, these three cognitive subdomains—executive function, processing speed and verbal declarative memory—are a possible minimum set of cognitive domains to be assessed in ageing studies (**Figure 2**). If time allows it would be important to add tests of crystallised cognitive ability (e.g. vocabulary-based tests that afford an estimate of peak prior ability because they are relatively non-age-sensitive) and non-verbal reasoning (e.g. Raven's Progressive Matrices, because such tests are strong indicators of general fluid [relatively age-sensitive] cognitive capability).

Of necessity, our proposed panel of cognition-related biomarkers of healthy ageing is not comprehensive but includes the most frequently used biomarkers of cognitive function during ageing and our panel is intended to be useful to researchers wishing to assess healthy ageing. Those interested in harmonising with major cohort studies should consult the tools listed in **Table 1**. Furthermore harmonisation of these tests is likely to occur in the future with the use cognitive function tests included as part of the NIH Toolbox.[13 162]

Areas lacking adequate evidence

To date, computer-based tests of cognitive function have not been used widely in major cohorts but the increased availability of computerised tools such as those in the NIH toolbox and the imperative to increase cost-effectiveness are likely to drive the migration to digital methodologies. This will require that tests are supported by on-going technical development to ensure that these are 'future-proof' against advances in operating systems and computer hardware. In addition, where the cognitive tests are administered repeatedly in the same individuals, the problems associated with practice and familiarity will need to be addressed.

In addition, the issue of co-variance among tests of cognitive functions needs more attention by researchers. People who score well on one cognitive test tend to score well on all of the others.[178] This issue has been highlighted by researchers such as Salthouse but its implications are not always appreciated; namely, that the causes of cognitive ageing might affect the variance shared by tests or domains or the variance in a specific test or domain.[178]

Biomarkers of endocrine function

Age-related changes in the endocrine system are very well established and many longitudinal studies and intervention studies, often focussing on the sex hormones and health, have helped to establish causal links with health outcomes. The best described age-related endocrine changes include decline in the sex hormones estrogen and testosterone (menopause and andropause),[179-181] changes in the hypothalamic-pituitary-adrenal (HPA)-axis due to reduced synthesis of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) (adrenopause; which occurs in both male and females from age 20-30 years)[182] and the reduced production of growth hormone and IGF-1 (somatopause).[183] The more recently discovered family of hormones, the adipokines, are key regulators of inflammation as well as of central functions such as appetite and altered serum adipokine levels have been linked with risk of obesity and metabolic syndrome.[184] The study of adipokines in relation to ageing is in its infancy, but the concentration of the adipokine adiponectin appears to change with age and is linked with age-related health outcomes.[185-187]

Domains of age-related endocrine biomarkers and tools for their assessment

We focused on sex hormones, the HPA axis, growth hormone-IGF1, melatonin, adipokines and thyroid hormones (**Table 4**).

Patterns of age-related change in endocrine biomarkers and associations with health outcomes

The strongest evidence supporting relationships with ageing emerged for testosterone, estrogen, DHEAS, and GH/IGF-1. For each of these markers there was strong consensual evidence from longitudinal studies that changes were linked with risk of premature mortality and/ or physical frailty.[188-196] For some endocrine biomarkers, the relationship with ageing appears to be non-linear. For example, the limited available evidence has shown that both high and low IGF-1 is related to mortality risk.[197 198] Whilst DHEAS declines with ageing, it is worth noting that a few studies reported no association with mortality[199-201]. However, low serum DHEAS was associated with increased mortality in older women with concurrent frailty and also with frailty in men.[182] Hormone replacement studies add to the evidence for a causal link to age-related physical and psychosocial decline. In this respect, the strongest associations are for both testosterone and estrogen and risk of physical frailty and bone health.[202-205]

There is evidence that circulating concentrations of melatonin and of adiponectin decline with age but these relationships have been investigated in only a few longitudinal studies.[185 206] Adiponectin shows the strongest association with mortality even after controlling for BMI or change in BMI.[186 187]

Cortisol, a stress hormone produced in the adrenal cortex and a component of the HPA axis, has been associated with age-related disease and disability.[207] There is evidence from longitudinal studies that abnormal cortisol secretion patterns are associated with increased BP, impaired glucose metabolism (fasting insulin and insulin/glucose ratio), and increased incidence of CVD and type 2 diabetes in men.[208] Recently, associations between heightened cortisol reactivity to stress and coronary artery calcification have been identified which may

influence the risk of CHD[209 210] and hypertension.[211] A summary of the evidence for each endocrine marker and several age-related outcomes is provided in **Appendix 7**.

Areas lacking adequate evidence

More longitudinal evidence is needed to enhance understanding of the relationships between cortisol, DHEAS and the DHEAS: cortisol ratio, adipokines (adiponectin, leptin, and ghrelin), somatostatin and ageing, frailty and mortality. In particular, given the strong association between adiponectin and mortality, the relationship of adiponectin with frailty and age-related morbidity should be examined more thoroughly.

Biomarkers of immune function

The immune system protects the organism from pathogens and also from damaged or altered tissues and cells (as occurs with cancer or traumatic injury), whilst not damaging the organism's own tissues. In humans, the immune system develops a memory of exposure to a pathogen so that when the threat is encountered a second time the response is rapid and specific to that pathogen. This so-called adaptive immune system, based on lymphocytes, is also the basis of the vaccination response. It is clear that each of these aspects of immune function declines with age e.g. susceptibility to both bacterial and viral pathogens increases with age, the incidence of cancer is age-related as is loss of tolerance to one's own tissues, evidenced by increased autoimmunity.[212] In addition the ability to mount an adequate, protective vaccination response also deteriorates with age. This age-related decline in immunity is termed immunesenescence and, whilst the field of immunology is well developed, the study of immunesenescence is more recent, with papers beginning to appear in the 1980's.[213]

Domains of age-related immunology biomarkers and tools for their assessment

The immune biomarkers of ageing considered in this review are summarised in **Table 4** and additional details are provided in **Appendix 8**.

Twenty-seven separate searches were run in Medline and a recent comprehensive book[214] was consulted. This revealed that the majority of available evidence comes from cross-sectional studies comparing immune markers in young and older subjects. The evidence base in the immune function domain is limited significantly by the very few longitudinal studies that compare immune cell numbers or function with mortality, or with an age-

related functional read-out such as infection rates or vaccination responses. Notable exceptions are two octogenarian and nonagenarian studies which considered various immune markers (T cell phenotype, cytomegalovirus serostatus and pro-inflammatory cytokine status) in 85-plus year olds with subsequent mortality and which were the basis for the development of the Immune Risk Phenotype (IRP)[215]. The key elements of the IRP are a CD4:CD8 T cell ratio of less than 1, raised numbers of CD8 cells lacking the co-stimulatory receptor CD28 and being seropositive for CMV. However the IRP remains to be validated rigorously in younger cohorts. Results from a study on 20-79 year olds[216] suggest that the IRP is associated with mortality in those over 60 years. A further limitation of the IRP is its narrow scope since it does not consider innate immune factors such as Natural Killer (NK) cell function, which was shown to be linked to infection rates and mortality in a small study of over 75 year old Japanese.[217]

The best studied aspect of immunesenescence is the age-related increase in inflammatory cytokines (IL6, IL1β, TNFα and CRP) which is termed inflammageing.[218] Higher plasma concentrations of inflammatory factors such as IL-6 and TNF-α have been associated with lower grip strength and gait speed in older adults.[219] [220] Measurement of inflammatory cytokines has been incorporated into longitudinal studies and have also been studied in centenarians.[221] The latter group shows fewer signs of ageing of the immune system including the IRP and inflammageing is absent or much reduced, being counteracted in part by high levels of anti-inflammatory cytokines such as IL-10.[222]

Telomere length in leukocytes, which includes lymphocytes and monocytes, is another factor that has received much attention. Lymphocytes proliferate rapidly in response to their cognate antigen and unlike most somatic cells have the ability to extend their

telomeres by inducing telomerase expression but, eventually, this is insufficient to prevent lymphocyte telomere length shortening with age. Despite this association with ageing, it is likely that shortened telomeres are also a marker of the frequency of infections so that leukocyte telomere length may not be a reliable index of biological ageing.[223] In the Newcastle 85+ Study, telomere length was uninformative about health status.[224 225]

Areas lacking adequate evidence

Longitudinal studies should examine relationships between number and function of T cells, neutrophils, NK cells, B cells and mortality, risk of age-related disease and wellbeing in later life. Given the switch from lymphoid to myeloid cell production with age, the ratio of lymphocytes to granulocytes ratio is a potentially useful biomarker of healthy ageing. As noted above, the IRP needs validation in younger people and should be expanded to include additional measures of immune function such as infection incidence or vaccination response. Telomere length in leukocytes, including lymphocytes and monocytes, has received much attention. Despite its association with ageing in several cohorts, it is likely that shortened telomeres are also a marker of infection frequency so that leukocyte telomere length may not be a reliable index of biological ageing. Further studies of telomere length and ageing should include investigation of exposure to infections and CMV seropositivity as possible confounders.

Further considerations for the assessment of biochemical biomarkers

Biochemical biomarkers are determined traditionally in blood fractions such as plasma or serum, or in whole blood. However other biological samples including saliva, hair, fingernails, urine and stool are potential additional sources of information on these and other age-related biomarkers. For logistical and cost reasons, there is growing interest in the use of dried blood spots (DBS).[226] Longitudinal ageing studies such as the Health and Retirement Study, the Study of Health and Retirement in Europe, the Longitudinal Ageing Study in India, and the studies in the WHO Study on global AGEing and adult health now obtain DBSs.

Other potential biomarkers of ageing

Sensory functions are critical to maintain normal levels of independence and interaction with others and to facilitate enjoyment of life's experiences. There is strong evidence that loss of these functions is more prevalent in older adults with loss of audition and vision being the most prominent. In the Beaver Dam Study, the prevalence of hearing loss among adults aged 48-92 years was 46%. The likelihood of hearing loss increased with age (odds ratio (OR) = 1.88 for 5 years, 95% CI 1.80-1.97) and was greater for men than for women (OR = 4.42, 95% CI 3.73-5.24), even after adjusting for age, education, noise exposure, and occupation (OR = 3.65).[227] Visual impairment may reduce a person's ability to undertake daily activities such as reading or driving, as well as limiting other important aspects of life including mobility and social interactions. The prevalence of visual impairment increases with age reaching 8.8 % (95%CI 7.6%-10%) among those aged ≥60 years in the USA. There is evidence indicating association between loss of sensory functions and intelligence. [228-230]

Olfactory acuity declines with age and 62.5% of 80- to 97-year-olds had olfactory impairment and this feature was more common in men.[231] Interestingly, age-related olfactory loss is commonly unnoticed and unreported and the frequency of self-reported olfactory impairment was less than half of the measured frequency (9.5% versus 24.5).[231] Olfactory function is a proposed indicator of the integrity of the ageing brain in older people[232] and it is notable that smell dysfunction is among the earliest "preclinical" signs of neurodegenerative diseases such as Alzheimer disease and sporadic Parkinson's disease.[233]

The recently developed NIH Toolbox[13] measures the following sensory functions viz. audition,[234] vision,[235] olfaction,[236] gustation,[237] vestibular,[29] and pain.[238] Most of these

functions, with the exception of pain, decrease across the lifespan and in disease states and changes in function may overlap with changes in cognitive and motor functions. However, the predictive value of measures of sensory function on age-related health outcomes remains uncertain. For example, in the Beaver Dam Offspring Study, the overall prevalence of olfactory impairment was low (3.8%) and there were no associations between olfactory impairment and general health-related quality of life, depressive symptoms, or dietary choices.[239] Further evidence will be needed before sensory measures can be recommended with confidence as reliable markers of healthy ageing.

Concluding remarks

We have proposed a panel of putative measures of healthy ageing which we hope will be of utility to researchers interested in human ageing (**Figure 2**). They have been designed to be of particular use when undertaking research using cross-sectional and longitudinal designs and as potential outcome measures for those developing interventions to enhance healthy ageing. We have based the selection of biomarkers on the concept that, for pragmatic purposes, healthy ageing can be operationalised as preserved physical, cognitive, physiological, endocrine, immune and metabolic functions. The panel of biomarkers of healthy ageing which we have proposed are well established individually, are used commonly in a range of settings and study designs, have analytic and clinical validity, and some have proven value in clinical practice and health-related research. In addition, for some, their predictive value has been replicated in different cohorts. However, we are aware that there is scientific interest in a number of 'emerging' biomarkers of ageing some of which are being explored in research initiatives such as the Europe-wide MARK-AGE consortium.[240] As evidence of their utility becomes available, further biomarkers could be added to our proposed panel or substituted for items in the current panel.

We did not base our selection of proposed biomarkers of healthy ageing on the conventional measures of sensitivity and specificity because there is no agreed measure of healthy ageing which might have been used as the outcome in such calculations. However, we were able to assess the predictive value of some biomarkers by employing the C statistic with a surrogate phenotype e.g. mortality as the outcome. The C statistic estimates the area under the receiver operating characteristic curve (AUC) and takes values between 0.5 (no discrimination ability) to 1.0 (perfect discrimination). Where available, C statistics values for

the biomarkers described in this report fall within the range 0.6 to 0.79 (**see Appendix 9**). However such C statistics should be interpreted with care. In the presence of strong predictors, the additional value of new biomarkers in predicting future events is usually small to moderate as shown recently by members of our group.[65] This problem is well-recognised and has been the subject of debate in the cardiovascular risk prediction area.[241]

From the available evidence it was not possible to rank the domains or sub-domains evaluated nor to suggest how information from the various domains might be aggregated to provide a simple "healthy ageing" score – even assuming that such a score is conceptually valid or of practical utility. Nevertheless, application of the proposed panel of biomarkers of healthy ageing in longitudinal observational and intervention studies may generate the evidence on which decisions about, and the derivation of, healthy ageing scores might be attempted. A further generic limitation of our work is uncertainty about the validity in very old people of putative biomarkers of healthy ageing which appear robust in younger old individuals. Indeed, in some cases the reverse may apply e.g. higher BP in very old people may be protective.[224] We recognise that our approach neglects other aspects of healthy ageing e.g. social and psychological wellbeing, but these have been covered in other reports.[13 14 242 243] Here we have used a restricted canvas to focus on biologically well-understood, objective measures which could be employed globally in a wide range of different types of study. Adoption of this approach may facilitate the comparability, and pooling, of data from a greater number of studies than is possible at present and so enhance research on healthy ageing.

Contributors and their roles

JCM conceived the idea and JCM, IJD, DK, KTK and JML oversaw its implementation.

JL, RC, JN, and AG, performed the literature searches. KTK, IJD, JN, RC and JML drafted and presented preliminary versions of this material during the workshop on biomarkers of healthy ageing. All authors contributed to writing the manuscript and read and approved the final version.

Competing interest

None declared.

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Tables and Figures

Figure 1. **Process for development of guidelines**

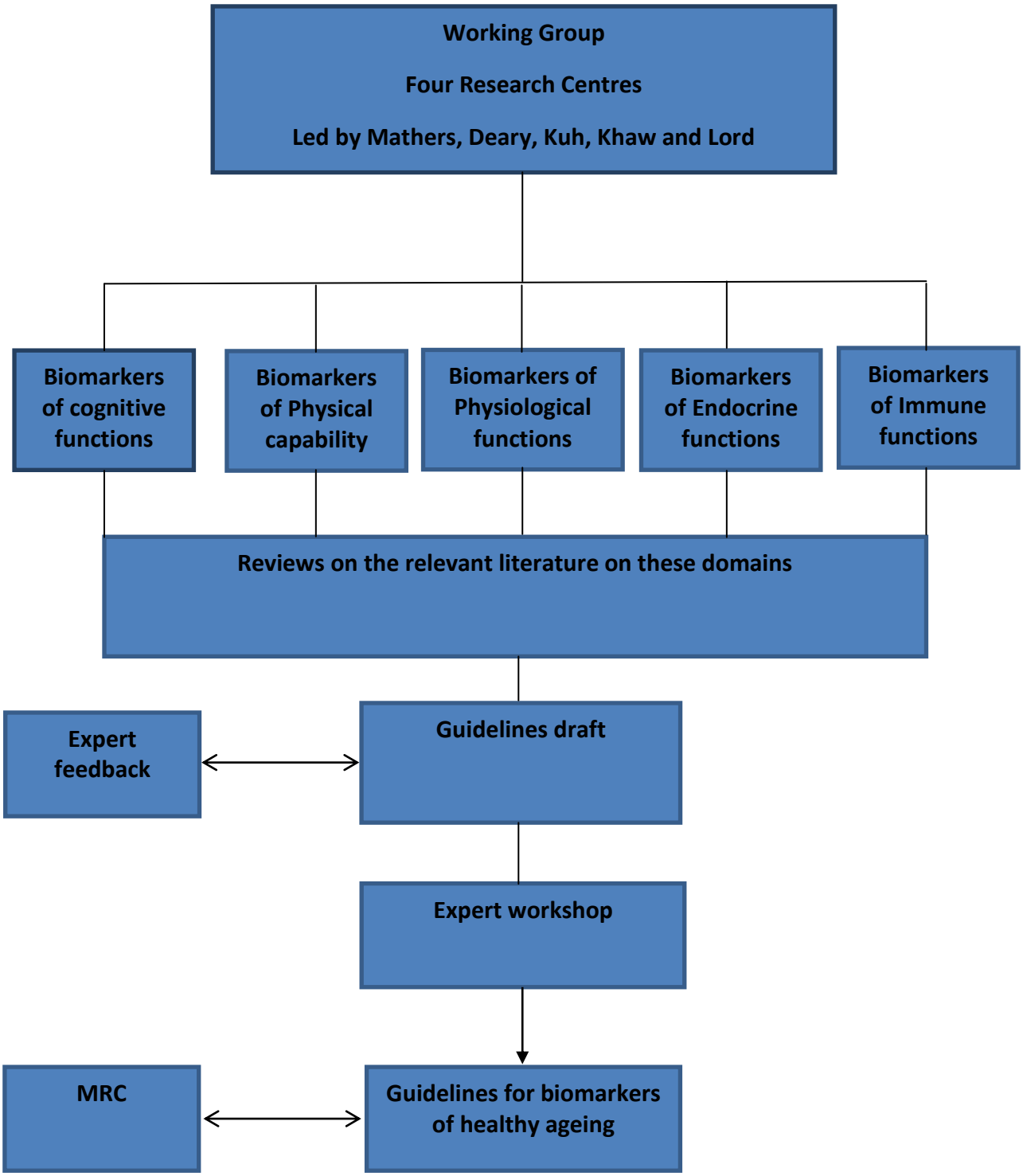


Table 1. Summary of recommended biomarkers in the physical capability domain				
Domain	**Tool/ measure**	**Feasibility of use**	**Prediction of outcome**	**Approximate costs/equipment***
Locomotor function	Gait (Walking) speed	+++	Mortality Falls +++	Stopwatch £6 (US $9.4) Measuring tape £6 (US $9.4) Marker tape £5 (US $7.8) Plastic cones £5 (US $7.8) Clipboard £3 (US $4.7)
	Timed get up and go	+++	Mortality +++	
	Chair rising	+++	Mortality +++	
Strength	Grip strength	+++	Mortality +++	Jamar Hand Dynamometer Hydraulic from £215 (US $335.8) +VAT Digital from £265 (US $413.8) +VAT
Balance	Standing balance (One leg stand, tandem stands)	+++	Mortality +++	***AIREX Balance Pad Elite £90 (US $140.6)* ***Accelerometer £approx £100 (US $156.2)*
Dexterity	Pegboard test	+++	Evidence lacking	Rolyan®9-Hole Peg Test Kit (board+pegs+stopwatch) £72 (US $112.5) (replacement pegs £15 (US $23.4))

Degree of feasibility of use and prediction of outcomes: +++ strong; ++ moderate, + low

*Costs were obtained from a number of online sellers in December 2014.

***Note. Although accelerometers are not yet commonly used to asesss balance in large epidemiological studies, the NIH toolbox balance test requires an AIREX Balance Pad and an accelerometer.*

Table 2 Summary of recommended biomarkers in the physiological domain			
Subdomain	**Tool/test**	**Feasibility of use**	**Prediction of outcome**
Lung function	Spirometry: Forced Expiratory Volume in 1 sec(FEV1)	+++	Mortality, cardiovascular events, fractures, functional health, cognition
Bone health	Bone density, bone mass hip: Dual X ray Absorptiometry	++	Mortality, fractures, CVD
	Ultrasound: broadband ultrasound attenuation (BUA) at heel	+++	
Body composition	Estimated leg muscle mass Dual X ray Absorptiometry	++	Uncertain
	Estimated muscle mass Body impedance	+++	Mortality
	Abdominal fat Waist circumference	+++	Mortality, cardiovascular events,
	Body mass Body Mass Index Body weight	+++	Mortality, cardiovascular events,
Cardiovascular function	Systolic blood pressure Sphygmomanometry	+++	CVD, mortality
	Lipid profile: total cholesterol, LDL-C, HDL-C, Triglycerides Biochemistry assay	++	CHD
Glucose metabolism	Glycated haemoglobin Fasting plasma glucose Biochemistry assay	++	Mortality, CVD

Degree of feasibility of use: +++ strong; ++ moderate, + low

Table 3. Summary of biomarkers in the cognitive domain relevant to ageing						
Subdomain	**Tool/Test**	**No. studies using the test**	**Feasibility of use**	**Time to administer ***	**Age range (norms) ***	**Cost***
Executive Function	Verbal Fluency[a]	41	+++	~5 mins	6-95	Free
Processing Speed	Digit-Symbol Coding[b]	18	+++	~5 mins	16-89	WAIS III = £328 (US $530.3) WAIS IV = £709 (US $1,145)
Working Memory	Digit Span backward	15	+++	~5 mins	16-89	WAIS III = £328 (US $530.3) WAIS IV = £709 (US $1,145)
Crystallised Ability	Boston Naming Test	12	+++	~10-20 mins	20-85	£75 (US$121)
Attention	Stroop[c]	11	+++	~5 mins	8-89	From free to £93 (US $150)
Visuo-Spatial Ability	Block Design	10	++	~10-15 mins	16-89	WAIS III = £328 (US $530.3) WAIS IV = £709 (US $1,145)
Reasoning	Raven's Progressive Matrices	9	+++	~40-60 mins	6.5-70+	SPM = £128 (US $206) APM = £151 (US $243) Comprehensive kit = £541 (US $874)

Verbal Memory & Learning	Rey Auditory Verbal Learning Test[c]	7	+++	~10-15mins	6-89	Free (English version)
Visual Memory	Benton Visual Retention Test	6	+++	~5-20 mins	8-80+	5th Ed = £148) (US $242)

Degree of feasibility of use: +++ strong; ++ moderate, + low

*= Information from several sources including Strauss *et al*. (2006)[175], Lezak *et al*. (2012)[244] Wechsler (1997)[177], Wechsler (1997) and www.pearsonassessments.com.

Key: SPM = Standard Progressive Matrices, APM = Advanced Progressive Matrices, WAIS = Wechsler Adult Intelligence Scale, WMS = Wechsler Memory Scale; we give prices for the whole battery, but note that this covers many tests in addition to those single tests identified in the table.

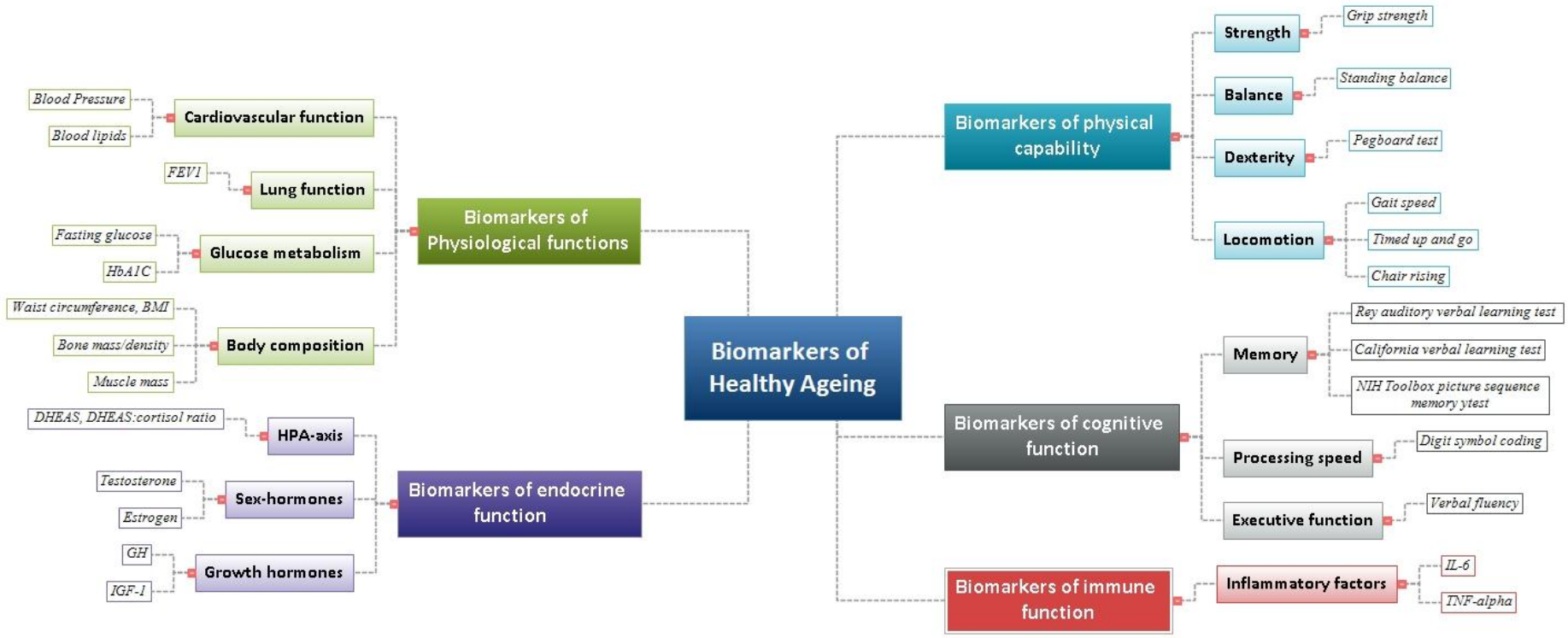
[a]This is often letter fluency or semantic fluency; [b]The reverse—Symbol-Digit Modalities is also used and there are some free versions of this type of test; [c]There are many versions of this type of test;

Table 4 Summary of recommended biomarkers in the endocrine function domain				
Domain	**Tool/Test**	**Relationship to aging**	**Predictor of mortality**	**Association with frailty**
Endocrine function	Adiponectin	+++	+++++	+
	DHEAS:Cortisol ratio	++	+	++
	DHEAS	+++++	+	++
	Growth Hormone/IGF-1	+++++	++	++
	Leptin	++	+	+
	Ghrelin	+	+	+
	Melatonin	++++	+	+
	Estrogen/ Oestrogen	+++++	+	+++++
	Somatostatin	+	+	+
	Testosterone	+++++	+	++++
	Thyroid hormones	++	+	+++
Immune function	B Cells	+++++	+	+
	CMV sero+ve	++++	++	+
	C-reactive protein	+++++	+++++	+
	Dendritic cells	+	+	+
	IL-6	+++++	+++++	+++
	Natural Killer Cells	+++++	+	++
	Neutrophils	+++++	+	+
	Telomere length	+++++	+++++*	+
	T Cell phenotype	+++++	+	+

Supporting evidence: +++++ very strong; ++++ strong, +++ moderate, ++ low, + very low or none; NA not applicable

* large amount of evidence, but mixed results.

Figure 2 Proposed panel of biomarkers of healthy ageing



Appendices to guidelines for biomarkers of healthy ageing

Appendix 1. Literature search strategies	
Physical capability The results of these searches has been reported by Kuh *et al*., elsewhere[28]	Walking speed and timed get up and go (gait speed OR walking speed OR walk speed OR "get up and go" OR TUG OR "timed get up and go") Strength (incl chair rises) Chair rising (chair ris* OR chair stand OR sit to stand) Grip strength (grip strength OR handgrip strength OR hand strength) Balance (One leg stand and tandem stands) (standing balance OR flamingo stand OR tandem stand OR postural control OR postural sway OR postural balance) Pegboard test (pegboard OR peg-board OR “peg test” OR dexterity) Searches were undertaken following this standard format by: Renata Bryce (MRC LHA), Sathya Karunananthan (McGill University) and Rachel Cooper (MRC LHA)
Cognitive function	Relevant chapters of 11 core books on cognitive aging were reviewed. The review of journal articles was carried out by searching Psych Info for keywords relevant to 3 topic headings: Cognition, Ag(e)ing, and Reviews. As well as synonyms of these topic headings, keywords included names of various cognitive domains were used (e.g. memory, executive functioning, processing speed, etc.). Papers which addressed cognitive interventions, medical conditions, or focused on specific tests were not selected. As the search was restricted to reviews, individual empirical studies were not selected either. The search returned 2272 articles, of which 45 were selected for review. Full list of keywords searched for in literature search: exp Aging/ or exp Age Differences/ ag?ing.tw./exp Intelligence/ exp Intelligence/ exp Cognition/ exp Cognitive Ability/exp Cognitive Assessment/exp Cognitive Processes/exp Cognitive Development/exp Cognitive Impairment/exp Memory Trace/ or exp Visuospatial Memory/ or exp Memory/ or exp Explicit Memory/ or exp Short Term Memory/ or exp Wechsler Memory Scale/ or exp Episodic Memory/ or exp Spatial Memory/ or exp Autobiographical Memory/ or exp Prospective Memory/ or exp Semantic Memory/ or exp Memory for Designs Test/ or exp Long Term Memory/ or exp False Memory/ or exp Memory Decay/ or exp Memory Training/ or exp

	Repressed Memory/ or exp Retrospective Memory/ or exp Visual Memory/ or exp Memory Consolidation/ or exp Iconic Memory/ or exp Memory Disorders/ or exp Verbal Memory/ or exp Implicit Memory/ exp Paired Associate Learning/ or exp Verbal Learning/ or exp Perceptual Learning/ or exp Observational Learning/ or exp Spatial Learning/ or exp Implicit Learning/ or exp Learning Ability/ exp Executive Function/ exp Sustained Attention/ or exp Divided Attention/ or exp Attention/ or exp Attention Span/ or exp Visual Attention/ or exp Selective Attention/ exp Creativity Measurement/ or exp Creativity/ exp Cognitive Processing Speed/ exp Cognitive Style/ exp Inductive Deductive Reasoning/ or exp Case Based Reasoning/ or exp Reasoning/ exp Procedural Knowledge/ or exp Declarative Knowledge/ or exp "Knowledge (General)"/ Visuospatial Ability/ Verbal Ability/ Spatial Perception/ or exp Visual Perception/ or exp Speech Perception/ or exp Face Perception/ or exp Self Perception/ or exp Time Perception/ or exp Perception/ or exp Auditory Perception/ or exp Social Perception/ intelligence.tw./IQ.tw./cognit*.tw./memory.tw./executive function.tw./ attention.tw./processing speed.tw./reasoning.tw. / review.tw./ exp "Literature Review"/
Endocrine function (Neuroendocrine)	Literature review of published reports, systematic reviews. Met-analyses and also recent comprehensive text books (e.g. The Neuroimmune biology of ageing). The review of journal articles was carried out by searching the database Medline with the following search terms: ((((Ageing) OR Aging) OR Age differences) AND _____ AND _____. The first blank was filled with one of the biomarkers: DHEAS, DHEAS:Cortisol, testosterone, oestrogen/estrogen, melatonin, somatostatin, growth hormone, IGF, adiponectin, leptin, ghrelin, somatopause. The second blank was filled in with the outcome variables aging, mortality, or frailty/sarcopenia. Abstracts were then reviewed. Criteria for selection was that participants were human, healthy and that data should be longitudinal, cross-sectional studies and decade splits were noted, but not included in final determinations. Thirty separate searchers were run producing a total number of 279,988 articles; 83 of these articles were related. If a recent meta-analysis or systematic review was found in the area this article was used.
Immune function	The review of journal articles was carried out by searching the database Medline with the following search terms: ((((Ageing) OR Aging) OR Age differences) AND _____ AND _____. The first blank was filled with one of the biomarkers: B Cells, CMV, C-reactive protein, Dendritic, IL-6, IL1β, TNFα, IL10, Natural Killer Cells, Neutrophils, Telomeres, and T-Cells. The second blank was filled with the outcome variable mortality or infection. This approach resulted in 27 separate searches producing a total number of 3862 articles, 49 of these articles were related. This search approach did not pull up articles such as the NONA and OCTA studies, in this instance reference sections from articles that did relate informed other articles that were related.
Physiological function	Pubmed was searched using the following terms related to ageing and ageing-related phenotypes (i.e. mortality, longevity, lifespan) and:

	Lung function, Forced expiratory volume (FEV1), Spirometry. Bone health, Bone density, bone mass hip, Ultrasound attenuation at heel, Dual X-ray Absorptiometry (DEXA, DXA), Muscle Mass, Estimated leg muscle mass, Dual X ray Absorptiometry (DEXA, DXA), Body impedance, body mass index (BMI), waist circumference Cardiovascular disease risk factors, Systolic blood pressure, Glucose metabolism, Glycated haemoglobin, fasting and non-fasting plasma glucose, postprandial plasma glucose Lipid profile, total cholesterol, Low density lipoprotein cholesterol and high density lipoprotein cholesterol, triglycerides, triacylglycerol Systematic reviews, meta-analysis, cohort (studies)

Appendix 2. Descriptions of the Recommended Physiologic function domains
Lung function The lungs' structure and function undergoes significant changes with age, with enlargement of alveolar ducts and respiratory bronchioles, and loss of septal tissue between alveoli.[245] Elastic tissue decreases and fibrous tissue increases, and structure and contractile function of respiratory muscles changes with decline in muscle strength. Lung function can be assessed using spirometry which includes estimates of vital capacity, and forced expiratory volume in a second (FEV1), the most common measure documented in epidemiologic studies. Spirometry measures are highly dependent on the subject's cooperation and effort, and is normally repeated at least three times to ensure a satisfactory measurement.
Bone health Functions of the skeleton include support of the body, body movement, mineral storage and homeostasis, maintenance of acid base balance and storage for bone marrow.[89] Assessment of bone health in relation to ageing has largely focussed on changes in bone mass and structure and associated increased fracture risk though the other functions are also highly relevant for ageing. With ageing, the balance between rates of bone formation and bone resorption is disturbed, leading to a decrease in bone mass and remodelling and particularly thinning of cortical bone. Together with changes in calcium metabolism, hormonal regulation and matrix composition, ageing is associated also with loss of tensile, compression, torsional and bending strength. Techniques for measuring bone mass include dual x-yay absorptiometry (DXA) at the hip, spine or whole body, broadband ultrasound attenuation

(BUA), usually at the heel, and quantitative computed tomography (CT).[98 246] The most common and feasible methods are DXA (usually at hip and spine) and BUA of heel.

Body mass and body composition

Ageing is associated with body composition changes such as increases in body fat, reduction in muscle mass (together with reductions in organs mass) in humans. There is loss of muscle fibre, particularly fast contracting type-II fibres, decrease in synthesis of contractile protein and reduction of mitochondrial mass. There is also reduction in spinal cells of the anterior horn and changes in the myoneural junction.[89 247-253] The European Working Group on Sarcopenia in older people listed a range of techniques to assess sarcopenia including muscle mass, muscle strength, and physical performance.[73] Functional measures are discussed in the physical capability section of this paper. Muscle mass can be assessed using CT, magnetic resonance imaging (MRI), Dual energy X ray absorptiometry (DXA), bioimpedance analysis (BIA), and indirect measures such as body potassium per fat free soft tissue. While CT and MRI are considered very precise imaging systems, high cost, limited access to equipment and concerns about radiation exposure limit the use of these methods. Bioimpedance analysis, the most feasible for general use, estimates the volume of fat and lean body mass and prediction equations have been developed. DXA is a method used to estimate fat free mass and leg muscle mass.[254 255]

Cardiovascular function

Ageing of the cardiovascular system is associated with ageing of cardiac muscle and function as well as with the vascular wall. Arteriosclerosis is vascular damage leading to progressive thickening and loss of resiliency of the arterial wall. Atherosclerosis refers specifically to thickening of the

arterial wall due to plaques characterised by intimal fatty accumulation and subintimal increase in fatty tissues.[256 257] While atherosclerosis is not necessarily a concomitant of ageing, in most societies, ageing is associated with changes in the vascular endothelium and increase in atherosclerosis and consequent risk of compromise to the vascular supply of vital organs including the heart resulting in coronary heart disease, the brain (cerebrovascular disease) and peripheral organs such as the kidney and lower limbs.

While there are standard clinical procedures for imaging of the vasculature such as CT and ultrasound of the carotid arteries, these are not feasible for general use. CVD epidemiology has very well documented physiological factors which increased risk of CVD. While there are now dozens of such factors encompassing inflammation and haemostasis,[120-124] the classical widely measured and well documented physiological factors related to risk of atherosclerosis are blood pressure and lipid profile: total cholesterol, low density lipoprotein cholesterol and high density lipoprotein cholesterol and triglycerides.

Glucose metabolism

Ageing is associated with alterations in insulin receptors, decreased number of glucose transporter units in target cells, alterations in carbohydrate metabolism including decrease in body muscle mass, increase in adiposity, decreased cellular glucose oxidation and increased liver gluconeogenesis. This results in lower glucose tolerance as measured by impaired ability to lower blood glucose after a standard glucose load. There are several measures of glucose tolerance with the clinically accepted measures for diagnosis of diabetes mellitus being the fasting and postprandial blood glucose concentration. More recently glycated haemoglobin, a measure of usual glucose concentrations over the preceding few months which does not require fasting or a glucose challenge, has also been suggested as a feasible indicator of glucose metabolism.[135]

Appendix 3. Problems and considerations in establishing cognition-related biomarkers of healthy ageing
1. Measures are not just markers of function (unlike biochemical measures), they are the function; they are outcomes as well as markers.
2. The taxonomy of cognitive functions is not given, so one must address which domains are recognised and thought to be important, and how they co-vary; and recognise the discussions and debates that surround this.
3. Domains of cognition have many putative markers; there are thousands of cognitive tests, including many alternatives for even the best-agreed cognitive domains.
4. There are few studies of older people that use the same battery of cognitive tests, and not all studies cover all domains; as a result, there is considerable difficulty in harmonisation.
5. All cognitive tests tend to covary—people who score well one any one test of any one cognitive domain tend to score well on all other tests of all other cognitive domains—and, therefore, the sources of variance in cognitive tests do not just lie at the level of the test; there are higher-order sources of variance.
6. There is some tension between using standard psychometric tests to assess domains of cognitive function in a quite broad way, and more experimental tests that are used to assess specific cognitive processes.
7. We need to recognise that there are some attempts to establish standard cognitive batteries, the most relevant to ageing being the recently-devised NIH Toolbox.
8. There is time: cognitive tests take time to assess, and there is almost always a time limit for what can be assessed, and so comprehensiveness of coverage has to be considered in the light of the time available and the likelihood of fatiguing the participant.
9. Some cognitive tests are designed for the normal range of function, and some for people who have cognitive pathology. Here, we concentrate on the normal range of function.
10. Some cognitive tests are free and some are proprietary, which affects researchers' choices.
11. Cognitive tests take time and the length of the battery of tests is an important consideration.
12. Cognitive tests often have familiarity and practice effects, which is often a concern in longitudinal studies where the same participant is retested at different points in time.
13. Some tests can be completed without a tester present and some need one-to-one interviews.
14. Some are computerised, and some are 'paper-and-pencil'. Some have alternative forms and some do not.
15. There are often many small variants of the same type of test.
16. Many cognitive tests have verbal or other language and culturally-specific content, making them unsuitable for certain populations.

Appendix 4. Descriptions of the Cognitive subdomains and tests relevant to ageing selected from longitudinal studies of ageing	
Domains	**Tests**
Executive Function Executive functions are involved in goal-directed behaviour. They allow us to plan and organise future behaviour, to initiate and inhibit actions and to monitor and change behaviour as needed. They are associated with the frontal lobes and have considerably overlapped with aspects of attention and working memory.[258 259] There are many tests which attempt to focus on different aspects of executive function, including word generation (verbal fluency), inhibition, decision-making and attention. Many of these, including the recommended test of Verbal Fluency, have been shown to deteriorate with age.[260 261]	**Verbal Fluency[175]** This test is used as a measure of Executive Function. It involves participants producing as many words as possible in a given time-frame and defined by certain rules. The two most common types of Verbal Fluency test are: 1. phonemic fluency, in which the participant names beginning with certain letters (e.g. C, A, F), or 2. category fluency, in which the participant names words within a certain category (e.g. animals). Both are quick and widely used measures.
Processing Speed This refers to the speed that the brain can process information. Processing speed seems to be a factor that underlies many other, higher-level processes. It slows considerably with age[261-265] and this slowing is often thought to be a factor in the deterioration of other cognitive functions, such as problem solving, planning and remembering. It is often tested using simple, timed tasks that place	**Digit Symbol Coding[266]** This test is measure of Processing Speed. It is a simple pencil and paper task which involves the participant in copying various symbols as fast as possible. Each symbol has a corresponding number and the participant has to copy down the appropriate symbol for each number. The goal is to copy as many items as possible in a short time frame (usually 90 or 120 seconds)

Appendix 4. Descriptions of the Cognitive subdomains and tests relevant to ageing selected from longitudinal studies of ageing	
Domains	**Tests**
few demands on our cognitive resources.	
Verbal Memory and Learning These refer to our ability to remember and learn verbal material. There are many types of memory and various distinctions including that between verbal and visual memory.[264 267] Verbal memory could refer to our ability to remember a story that was read to us, or a list of words or numbers, and it has been shown to deteriorate significantly with age.[268-272]	**Rey Auditory Verbal Learning Test[175]** This test is used as a measure of Verbal memory and learning. A list of words (list A) is presented to the participant who recalls as many of them as possible. This same procedure is repeated 4 times with the same list. A new list (List B) is then presented and recalled once (this list is designed to act as a distractor). The participant then has to recall as many of the words from List A as possible, this time without being shown the list first.
Attention Attention refers to our capacity to attend to information and stimuli. It is a broad area and we use our attention in a number of different ways. Sometimes we need to switch between tasks and divide our attention, sometimes we need to sustain our attention on specific tasks, and sometimes we need to inhibit irrelevant information in order to pay attention to the right things. Sometimes our attention needs to be highly selective about what it focuses on, and sometimes less so. Many tests exist to look at all of these areas and more, and while there is some fluctuation in how ageing affects aspects of	**Stroop Test[175]** This test is recommended as a test of attention, though has considerable overlap with executive function as well.[259 260] Participants are shown a card with the names of various colours spelled out on it (colour words). These colour words are either printed in black, or in different colours, and there are different conditions to the test. In the congruous condition, the colour words are printed in their corresponding colours; in the incongruous condition, the colour words are printed in different colours than that which they spell. The participant is tasked either with reading the colour word, or naming

Appendix 4. Descriptions of the Cognitive subdomains and tests relevant to ageing selected from longitudinal studies of ageing	
Domains	**Tests**
attention, it has generally been shown to decline with age.[273-275]	the colour it is printed in. There is another baseline condition where the participant is just shown a series of colour dots or shapes and has to name the colours. Several alternate versions of this test exist.
Working Memory Working memory refers to a temporary store of information that disappears after a short period of time, and deteriorates as we get older.[261 264] It is the type of memory that allows us hold on to information long enough to build or work on; it is the combination of retention and manipulation of new information that characterises working memory. For example, it enables us to hold on to a telephone number long enough to dial it, or to remember what someone has just said long enough for us to reply, or in mental arithmetic to remember a number long enough to add something to it or manipulate it in some way.	**Digit Span Backwards[266]** This test provides a measure of working memory. A string of digits are read out to participants at a rate of approximately 1 digit per second, and the participant has to recall them in reverse order. The test starts with strings of 2 digits and increases to strings of 8 digits. When a participant fails to get 2 strings of the same length correct, the test is stopped.

Appendix 4. Descriptions of the Cognitive subdomains and tests relevant to ageing selected from longitudinal studies of ageing	
Domains	**Tests**
Crystallised Ability Crystallised ability refers to the knowledge that we have acquired over a lifetime, such as our general knowledge about the world, or our understanding and use of language. It is often contrasted with "fluid abilities", which relate to our ability to reason about things and solve problems. Contrary to most other aspects of cognition, it tends to improve or remain stable with age and often stays intact despite other aspects of thinking deteriorating.[261 262 264] Common tests for crystallised ability include tests of language, vocabulary and comprehension.	**Boston Naming Test[175]** This is a test of crystallised ability. Participants are shown a series of line drawings of objects and have to name them. The objects depicted are simple to start and increase in difficulty..
Reasoning This refers to our ability to reason logically about things, see patterns and solve problems. It is seen as a "fluid ability" and frequently contrasted with "crystallised ability", described above. Unlike crystallised ability, reasoning and fluid abilities decline significantly with age.[262 263 265] Reasoning is sometimes further divided into verbal	**Raven's Progressive Matrices[175]** This is a test of non-verbal reasoning. The participant is shown a series of diagrams, each of which has a piece missing. The participant has to figure out the pattern or sequence in the diagram and choose the missing piece from a series of options below.

Appendix 4. Descriptions of the Cognitive subdomains and tests relevant to ageing selected from longitudinal studies of ageing	
Domains	**Tests**
reasoning and non-verbal reasoning, with a key feature of the latter being that it has good cross-cultural validity due to its lack of dependence on reading and languages. While reasoning would be a highly recommended domain for studying cognitive aging, a slight disadvantage is that most reliable tests of reasoning ability take a relatively long time to administer.	
Visual Memory This refers to our ability to remember visual information. As mentioned above, memory is often subdivided into different processes. Visual and verbal memory are frequently contrasted in the literature on cognition, and visual and spatial memory are sometimes described as distinct processes too, with spatial memory referring more to the spatial organisation of scenes or events, and visual memory relating more to memory of visual features. Various tests exist to test visual memory, generally focused around remembering pictures, visual scenes and images, and our ability to do so has been	**Benton Visual Retention Test**[175] This is a test of visual memory. There are several different ways of administering the test,[175] but the essence is that a participant is shown a series of designs of increasing difficulty and has to draw them from memory on a blank piece of paper. The length of time that each design is displayed for and the gap between viewing the design and reproducing it vary depending on the type of administration.

Appendix 4. Descriptions of the Cognitive subdomains and tests relevant to ageing selected from longitudinal studies of ageing	
Domains	**Tests**
shown to deteriorate with age.[264]	
Visuo-Spatial Ability Visuo-spatial ability refers to our ability to interpret and manipulate visual and spatial information, than to memorise it, although it could be argued that memory for this type of information is a process within the visuo-spatial domain. It is often tested by asking participants to copy or construct replicas of figures or patterns and again it has been found to deteriorate with age. [261-263 265]	**Block Design[266]** This is a test of visuo-spatial ability. The participant is given a pattern and must replicate it using a number of blocks. Each of the blocks has six sides, two of which are coloured red, two of which are coloured white, and two of which are half red and half white. Rather like a jigsaw, the participant has to piece the blocks together to replicate the pattern as fast as possible. The test starts with straight forward items and increases in difficulty as it progresses.

Appendix 5. Practicalities considered when selecting tests
1) Used widely by other studies: harmonisation
2) Ease of administration
3) Training needed for tester consistency
4) Cost
5) Norms available for a wide age range or not
6) Time taken to administer
7) Affected by sensory or motor limitations
8) Equipment required
9) Language or other cultural limitations
10) Practice effects
11) Likelihood of fatigue
12) Reliability
13) Validity
14) The aim of the study: follow-up, intervention, diagnosis, etc.
15) Setting: e.g., healthy (dealt with here) or pathological cognitive ageing
16) Possibility of adaptive testing

Appendix 6. Most widely used cognitive tests and related information.								
Cognitive Domain	**Test**	**No of Cohorts**	**Approx. time to administer**	**Age range (norms)**	**Cost (often refers to full battery rather than individual test)**	**Ease of Use**	**Use with other countries**	**References**
Executive Function	Verbal Fluency	41	~5 mins	6-95	Free	+++	Spanish (Spain, Mexico, Latino populations), Cantonese, Hebrew, Greek	Strauss *et al*. (2006)[175]
	Trail Making Test -B	12	~5 mins	9-89	$50	+++	Arabic, Chinese, Hebrew versions available	Strauss *et al*. (2006)[175]
	Stroop	11	~5 mins	8-89	Free - $150	+++	Spanish & Cantonese versions	Strauss *et al*. (2006)[175]

Appendix 6. Most widely used cognitive tests and related information.								
Cognitive Domain	**Test**	**No of Cohorts**	**Approx. time to administer**	**Age range (norms)**	**Cost (often refers to full battery rather than individual test)**	**Ease of Use**	**Use with other countries**	**References**
Processing Speed	Digit-Symbol Coding	18	~5 mins	16-89	WAIS III = $450, WAIS IV = $1,120	+++	Canadian version	Wechsler (1997b)[266]
	Reaction Time	10	~5 mins	–	Deary-Liewald Task = Free	+++	–	Deary *et al*., 2011[276]
	Trail Making Test –A	10	~5 mins	9-89	$50	+++	Arabic, Chinese, Hebrew versions available	Strauss *et al*. (2006)[175]

Appendix 6. Most widely used cognitive tests and related information.								
Cognitive Domain	**Test**	**No of Cohorts**	**Approx. time to administer**	**Age range (norms)**	**Cost (often refers to full battery rather than individual test)**	**Ease of Use**	**Use with other countries**	**References**
Attention	Stroop	11	~5 mins	8-89	Free - $150	+++	Spanish & cantonese versions	Strauss *et al*. (2006)[175]
	Reaction Time	10	~5 mins	–	Deary-Liewald Task = Free	+++	–	Deary *et al*., 2011[276]
	letter cancellation	3	~5 mins	–	Free	+++	–	Lezak *et al*. (2012)[244]
Verbal Memory & Learning	Word List Recall & Verbal Learning tests	34	Many versions: ~2-45 mins with delay	–	Free	+++	–	Lezak *et al*. (2012)[244]
	Rey Auditory Verbal Learning Test (RAVLT)	7	~10-15 mins	6-89	Free (english version)	+++	Chinese, Spanish, Hebrew, German, Flemish, Dutch	Strauss *et al*. (2006)[175]
	Logical memory	12	~10-40 mins with delay	16-89	WMS III = $450, WMS IV = $720	++	–	Wechsler, 1997[266]

Appendix 6. Most widely used cognitive tests and related information.								
Cognitive Domain	**Test**	**No of Cohorts**	**Approx. time to administer**	**Age range (norms)**	**Cost (often refers to full battery rather than individual test)**	**Ease of Use**	**Use with other countries**	**References**
	Digit span forward	12	~5 mins	16-89	WAIS III = $450, WAIS IV = $1,120	+++	Canadian version	Wechsler, 1997[266]
	California Verbal Learning Test	3	~20-50 mins with delay	16-89	$675	+++	–	Strauss *et al*. (2006)[175]
	Buschke Selective Reminder Test	3	~30 mins	5-91	Free	+++	Spanish, Hebrew	Strauss *et al*. (2006)[175]
Visual memory	Benton Visual Retention Test (BVRT)	6	~5-20 mins	8-80+	5th Ed = $239	+++	Used in China, Egypt, India, Venezuela	Strauss *et al*. (2006)[175]
	Spatial span	5	~5-10 mins	16-89	WMS III = $450, WMS IV = $720	++	–	Wechsler (1997)[266]
	Visual reproduction memory	4	5-45 mins with delay	16-89	WMS III = $450, WMS IV = $720	+++	–	Wechsler (1997)[266]
	Digit Span backward	15	~5 mins	16-89	WAIS III = $450, WAIS IV = $1,120	+++	Canadian version	Wechsler (1997)[266]

Appendix 6. Most widely used cognitive tests and related information.								
Cognitive Domain	**Test**	**No of Cohorts**	**Approx. time to administer**	**Age range (norms)**	**Cost (often refers to full battery rather than individual test)**	**Ease of Use**	**Use with other countries**	**References**
Working Memory	Serial 7s subtraction test	5	~2 mins	_	MMSE= $140	+++	_	Strauss *et al*. (2006)[175]
	Spatial span backwards	5	~5 mins	16-89	WMS III = $450, WMS IV = $720	++	_	Wechsler (1997)[266]
Crystallised Ability	Boston Naming Test	12	~10-20 mins	20-85	$121	+++	Spanish version. Also used with following languages: Chinese, Italian, Jamaican, Dutch, Korean & French-Canadian	Strauss *et al*. (2006)[175]; www.pearsonassessments.com
	National Adult Reading Test (NART)	8	~5 mins	18-97	Free	++	United States, Canada	Strauss *et al*. (2006)[175]

Appendix 6. Most widely used cognitive tests and related information.								
Cognitive Domain	**Test**	**No of Cohorts**	**Approx. time to administer**	**Age range (norms)**	**Cost (often refers to full battery rather than individual test)**	**Ease of Use**	**Use with other countries**	**References**
	Mill Hill Vocabulary Test (MHV)	7	~10 mins	13-80	Raven's SPM + MHV = £260,	+++	–	Lezak *et al*. (2012)[244]
Reasoning	Raven's progressive matrices	9	~40-60 mins	6.5-70+	SPM = $209, APM = $245, Comprehensive kit = $887	+++	Tested with African American, Caucasian, Hispanic, Asian, African, East Indian groups.	Strauss *et al*. (2006)[175]
	Number Series	4	~ 5 min	–	–	+++	–	–
	Alice Heim 4 (AH4)	4	~40 mins	–	–	+++	–	–
	Matrix Reasoning	2	~10-15 mins	16-89	WAIS III = $450, WAIS IV = $1,120	+++	Canadian version	Wechsler (1997)[266]

Appendix 6. Most widely used cognitive tests and related information.								
Cognitive Domain	**Test**	**No of Cohorts**	**Approx. time to administer**	**Age range (norms)**	**Cost (often refers to full battery rather than individual test)**	**Ease of Use**	**Use with other countries**	**References**
	Cattell Culture Fair	2	–	–	–	+++	–	–
Visuo-Spatial Ability	Block design	10	~10-15 mins	16-89	WAIS III = $450, WAIS IV = $1,120	++	Canadian version	Wechsler (1997)[266]
	Mental Rotation	5	~5-15 mins	–	–	++	–	Lezak *et al*. (2012)[244]
	Figure copy	4	Many versions: ~2-45 mins with delay	–	MMSE= $140 Rey Complex Fig = Free - $200	+++	–	Strauss *et al*. (2006)[175]

Appendix 7. Findings on biomarkers of endocrine function
Sex hormones The relationship between sex hormones and longitudinal decline with ageing is well established. There is a linear association between age and likelihood of having low testosterone levels,[277] most notably in men over the age of 60.[278] Studies show a yearly decline ranging between .121 n/mol and .382 n/mol in men[189 279 280] and another study showing a -1.6% decrease yearly.[188] Estrogen also declines with age and production begins to decline 1 or 2 years before menopause (Henderson, 2009; Ralston *et al*., 1990).[190] Both low levels of estrogen and testosterone have been related to mortality, with a strong association reported between low levels of testosterone and cardiovascular disease (CVD) mortality.[281-285] However, a recent meta-analysis found no relationship between testosterone and cardiovascular disease and proposed testosterone may be a marker of poor health in general.[286] Estrogen replacement therapy has been linked to reduced levels of mortality;[283 284] however, this is only evident in early studies. Frailty and bone mass loss has been associated with low levels of testosterone and estrogen.[287 288] Testosterone treatments have been successful in increasing bone density in men.[202] Estrogen replacement therapy increases bone density and then prevents decreases over time.[203-205 287 289-291]
DHEAS and DHEAS:Cortisol DHEAS declines longitudinally with age,[195 292] with one study reporting a 5.2% decrease annually.[188] The relationship between mortality and DHEAS has produced mixed results, roughly 60% of the studies report a linear relationship[192-196] and 40% report no relationship.[199-201] There is little evidence relating DHEAS:Cortisol with ageing and mortality. Both DHEAS and DHEAS:Cortisol have been related to frailty,[182 196 293] however more research is needed in this area.

Growth Hormone/IGF-1

Growth Hormone and IGF-1 decline with age, after 30 or 40 years in men,[294] in healthy adults and is well documented.[191 295-298] Both high and low levels of IGF-1 have been associated with mortality.[197 198] Low levels of IGF-1 has been related to reduced strength[299] and treatment of growth hormone in men helped a number of ageing symptoms, but did not change bone mass.[296]

Melatonin

Plasma melatonin declines with age[206] in the early 60s and bottoms out in those 70 and older.[300] However, this may not be characteristic of healthy ageing.[301]

Adiponectin

It is well recognized that adiponectin increases with ageing cross-sectionally and that the relationship is more pronounced in men.[143 302] One study has shown this longitudinally.[185] There is a strong link between adiponectin and mortality, independent of baseline body mass index or change in body mass index.[186 187 303-305] Little research has related adiponectin to frailty; however, one study reported changes in adiponectin to be related to increases in physical disability.[185]

Leptin

There is a development of central resistance to leptin with aging.[306] In a study of people over the age of 100 those with the lowest teritle of leptin had a higher mortality risk[303 304] and in a study of an older population leptin was not related to mortality.[307]

Ghrelin

The effect of healthy ageing on ghrelin has not yet been clarified.

Appendix 8. Findings on biomarkers of immune function
Immune Cells
B-Cells One mark of the ageing immune system is a skewing towards myeloid lineage cells (monocytes, neutrophils) and away from lymphoid cells. Cross-sectional evidence comparing young and old adults has shown decreases in the overall percentage of B-cells,[308] overall B-cell numbers,[309] and B-cell diversity.[310] B-cells become more differentiated, losing CD27 and the percentage of CD27 negative B-cells is higher in older adults. Longitudinal research is needed to directly link these changes with outcomes such as infection and mortality.
T-cells There is a significant literature concerning shortening of telomere length with age, but this also represents an indicator of pathogen exposure across the lifetime rather than solely physiological ageing.[311] Changes in T-cell phenotype with age have been well studied. A large cross sectional study found that the CD4:CD8 ratio increased only very slightly with age.[214] Two longitudinal studies found that those over 85 year olds with a CD4:CD8 ratio of less than 1 had increased mortality and this measure is thus part of the IRP.[312-314] Those reaching 100 years did not have this profile.[221] In a third larger population based study in 20-94 year olds the same group reported that the percentage of individuals with the inverted CD4:CD8 ratio rose from 8% in 20-59 year olds to 16% in 60-94 year olds.[216] Individuals aged 60 and over with the inverted CD4:CD8 ratio had a higher mortality rate. This study also found that the inverted ratio was more common in males. The inverted CD4:CD8 ratio could reflect the high percentage of CD8 positive cells arising in CMV seropositive adults, another element of the IRP. An increase in the memory:naive T-cell ratio and increased levels of CD28 negative cells with ageing is also well documented, though its association with mortality is not established. It may well however better reflect the ageing process as it is influenced by both thymic atrophy and lifetime pathogen exposure. Thymic atrophy is well documented and occurs at a rate of 3% per annum in humans and thus it meets many requirements of a potential biomarker. Further research is needed to determine its utility as a biomarker of ageing.

Dendritic cells

Recently impaired production of dendritic cells has been related to an impaired CD8+ T cell response to the influenza vaccine,[315] but there are no data on the association between Dendritic cell numbers or functions and age-related mortality.

Natural Killer (NK) Cells

Many cross-sectional studies report increases in total NK-cell numbers with age; but this is accompanied by a reduction in the numbers of this cell type that have cytotoxic capacity, resulting in reduced killing ability overall. Two longitudinal studies from the same group have reported a relationship between a decrease in natural killer cell function (cytotoxicity) and increased rate of infection and mortality among care home Japanese residents.[217] Further research is required to confirm NK-cell functional decline as associated with mortality

Neutrophils

Higher baseline numbers of circulating neutrophils have been reported and reflect the skewing of haemopoiesis towards the myeloid lineage. Increased neutrophil numbers was associated with increased mortality in women 170 and higher levels of neutrophils predicted mortality among those over 100 years of age.167 More research is needed examining the association between neutrophil functional decline with ageing, which is well documented, and mortality.

Telomere length in immune cells

Research examining the relationship between telomere length and mortality has produced mixed results. Some studies have reported a modest relationship between shorter telomere length or change in length and mortality.[316-319] However, the majority of studies show no relationship between telomere length and mortality.[224 225 316 320-324] As stated above, if peripheral blood mononuclear cells (PBMC) are used to measure telomere attrition, there is risk for confounding due to antigen exposure across the lifespan as immune cells proliferate upon antigen exposure and thus shorten their telomeres. The majority of studies looking at PBMC telomere length and mortality have been in diseased populations, predominantly cancer. Using non-immune cells such as skin fibroblasts, would remove this confounding. Some studies have assessed proliferation capacity (population doublings) in skin fibroblasts, an indirect measure of telomere length, and found a shortening with

age, but its association with mortality remains to be established.

Cytomegalovirus (CMV)

The risk of being infected with Cytomegalovirus increases with age.[325 326] CMV has been related to functional impairment in the elderly.[327 328] Two recent studies have directly linked CMV with mortality showing a positive association between CMV seropositivity and mortality.[329 330] In addition, CMV seropositivity is part of the IRP. Participants over the age of 80 with IRP had a significantly increased mortality rate compared to those who were not over a 4 year period.[325 331 332] The limitation of the IRP is that it only considered adaptive immunity, though it did also include inflammatory cytokines. In addition the study only considered the oldest old, i.e. over 80 year olds, thus whether the IRP holds at younger ages is not known.

Inflammation status

C-reactive protein (CRP)

Increased levels of the inflammatory marker CRP have been related to all-cause and specific causes of mortality,[123 186 333-337] although one study only found this association in men.[338] Data from the Cardiovascular Health Study All Stars reported that a doubling in CRP over a 9 year period was a predictor of all-cause mortality and increases in cognitive and physical impairment, However, CRP level at 9 years was a stronger predictor than change over time.[339]

Pro-inflammatory cytokines

Interleukin 6 (IL-6)

Interleukin 6 (IL-6) is a strong predictor of mortality and is an element within the IRP,[186 335 338 340-342] however, another recent study found that IL-6 did not independently predict mortality.[307] Other pro-inflammatory cytokines, namely IL1b and TNF, are also now appearing as elements in immune ageing studies. They give very similar results to IL6 and for example were associated with physical frailty in the Hertfordshire Ageing Study.[196]

Interleukin 10 (IL-10)

Inflammageing is also associated with reduced levels of pro-infllammatory cytokines, notably IL-10, but has been less well studied to date as knowledge of these cytokines and reagents for measuring them is more recent. More research is required before the decline in this cytokine could be confirmed as a marker of ageing.

Appendix 9. Area under the curve (C-statistic) for the association between biomarkers of ageing and mortality					
Domain	**Biomarker**	**Mortality discrimination C-statistic**	**Other variables in model**	**Length Follow up**	**Reference**
Age		0.521 to 0.524	Crude risk	10 years	[343]
Lung function	Spirometry: Forced Expiratory Volume in 1 sec (FEV1)	0.65	Crude risk	median 28 months (range, 4 to 68)	[344]
Body composition	Body mass, Body Mass Index	0.524 to 0.572	Crude risk	10 years	[343]
Cardiovascular function	Systolic blood pressure Sphygmomanometry	0.543 to 0.587 0.74	Crude risk Age-adjusted	10 years	[241 343]
	Lipid profile: total cholesterol	0.515 0.72	Crude risk Age-adjusted	10 years	[343]
	LDL- cholesterol	0.60 0.71 to 0.73 0.81	Crude risk Age-adjusted Adjusted for age, smoking status, the presence or absence of diabetes mellitus, blood pressure, and use or nonuse of hormone-replacement therapy.	8 years	[241 343 345]
	HDL- cholesterol	0.554 to 0.591 0.73	Crude risk Age-adjusted	10 years	[343]

Appendix 9. Area under the curve (C-statistic) for the association between biomarkers of ageing and mortality					
Domain	**Biomarker**	**Mortality discrimination C-statistic**	**Other variables in model**	**Length Follow up**	**Reference**
Immune function	CRP	0.61 to 0.64 0.74 0.81	Crude risk Age-adjusted Adjusted for age, smoking status, the presence or absence of diabetes mellitus, blood pressure, and use or nonuse of hormone-replacement therapy.	8 years	[241 343 345]
Locomotor function	Gait (Walking) speed	0.68 (95% CI 0.63-0.73)	Sex	13 years	[65]
	Chair rising	0.66 (95% CI 0.60-0.72)	Sex	13 years	[65]
Strength	Grip strength	0.63 (95% CI 0.56-0.69)	Sex	13 years	[65]
Balance	One leg stand	0.70 (95% CI 0.64-0.76)	Sex	13 years	[65]

Appendix 10. List of participants at the Workshop held in Newcastle, UK October 2012	
Name	**Affiliation**
Prof John C. Mathers	Newcastle University, UK
Prof Tim Cawston	Newcastle University, UK
Prof Richard Gershon	Northwestern University, USA
Prof Joe Verghese	Albert Einstein College of Medicine, USA
Prof Christian Drevon	University of Oslo, Norway
Prof Daan Kromhout	Wageningen University, Netherlands
Prof Oscar Franco	Erasmus University, Netherlands
Prof Rudi Westendorp	University of Leiden, Netherlands
Dr Sabita Soedamah-Muthu	Wageningen University, Netherlands
Dr Janine Felix	Erasmus University, Netherlands
Prof Ian Deary	Edinburgh University, UK
Prof Janet M. Lord	Birmingham University, UK
Prof Diana Kuh	MRC Unit for Lifelong Health and Ageing, UK
Prof Kay Tee Khaw	Cambridge University, UK
Dr Victoria Keevil	Cambridge University
Dr Janet Valentine	Medical Research Council
Dr Louisa Jenkin	BBSRC
Dr Rachel Cooper	MRC Unit for Lifelong Health and Ageing, UK
Dr Jack Nissan	Edinburgh University, UK
Dr Annie Ginty	Birmingham University, UK
Prof Gail Mountain	Sheffield University, UK
Prof Naveed Sattar	Glasgow University, UK
Prof Christina Victor	Brunel University, UK
Prof Marion McMurdo	Dundee University, UK
Prof Patricia Schofield	Greenwich University
Prof Philip Rowe	Strathclyde University
Prof Elaine Dennison	Southampton University
Prof Eugene Milne	NHS North East
Dr Riccardo Marioni	Cambridge University
Mr Theodore Cosco	Cambridge University
Dr Lynn McInnes	Northumbria University
Prof Carol Jagger	Newcastle University
Mrs Karen Davies	Newcastle University
Dr Mario Siervo	Newcastle University
Dr Blossom Stephan	Newcastle University
Prof Jim Edwardson	Newcastle University
Prof Louise Robinson	Newcastle University
Prof Mark Pearce	Newcastle University
Dr Carmen Martin-Ruiz	Newcastle University
Dr Antoneta Granic	Newcastle University
Dr Alex Munro	Newcastle University
Prof Martin White	Newcastle University
Prof Lynn Rochester	Newcastle University

Prof Falko Sniehotta	Newcastle University
Prof Paula Moynihan	Newcastle University
Prof Thomas D. Meyer	Newcastle University
Prof Suzanne Moffatt	Newcastle University
Prof Ashley J. Adamson	Newcastle University
Prof Mike Catt	Newcastle University
Prof Thomas von Zglinicki	Newcastle University
Dr Jose Lara	Newcastle University
Dr Ben Heaven	Newcastle University
Dr Nicki Hobbs	Newcastle University
Dr Alan Godfrey	Newcastle University
Dr Elizabeth Evans	Newcastle University
Dr Claire Cleland	Newcastle University
Dr Laura Brown	Manchester Metropolitan University
Mrs Sanchia Coatsworth	Newcastle University
Miss Evelyn Barron	Newcastle University
Miss Caroline Shaw	Newcastle University
Miss Suzanne MacDonald	Newcastle University