Mechanisms of Ageing and Development 132 (2011) 496–502

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

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

Assessment of a large panel of candidate biomarkers of ageing in the Newcastle 85+ study

Carmen Martin-Ruiz [a], Carol Jagger [a], Andrew Kingston [a], Joanna Collerton [a], Michael Catt [a], Karen Davies [a], Mick Dunn [b], Catharien Hilkens [b], Bernard Keavney [c], Simon H.S. Pearce [c], Wendy P.J. den Elzen [d], Duncan Talbot [e], Laura Wiley [a], John Bond [a,f], John C. Mathers [a], Martin P. Eccles [a,f], Louise Robinson [a,f], Oliver James [b], Thomas B.L. Kirkwood [a,*], Thomas von Zglinicki [a]

[a] Institute for Ageing and Health, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne NE4 5PL, UK
[b] Institute of Cellular Medicine, Newcastle University, Framlington Place, Newcastle upon Tyne NE2 4HH, UK
[c] Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne NE1 3BZ, UK
[d] Department of Public Health and Primary Care, Leiden University Medical Center, Post zone V-0-P, P.O. Box 9600, 2300 RC Leiden, The Netherlands
[e] Unilever Discover, Colworth Science Park, Sharnbrook, Bedfordshire MK44 1LQ, UK
[f] Institute of Health and Society, Newcastle University, Baddiley Clark Building, Richardson Road, Newcastle upon Tyne NE2 4AX, UK

ARTICLE INFO

Article history:
Received 28 June 2011
Received in revised form 5 August 2011
Accepted 8 August 2011
Available online 16 August 2011

Keywords:
Biomarkers
Ageing
Health status
Cohort study

ABSTRACT

Sensitive and specific biomarkers of ageing are needed to evaluate interventions to extend health span. However, there is growing evidence that information provided by candidate biomarkers may change with age itself. Little is yet known about the value of candidate biomarkers in those over 85 years, currently the fastest growing population sub-group in many countries. This study assessed a large panel of candidate biomarkers in a cohort of 85 years old by studying comparative associations with health status. Using a cross-sectional sample of 852 individuals aged 85, we performed uni- and multi-variable analyses of associations between 74 candidate biomarkers and 4 health-status measures: viz. multi-morbidity, cognitive impairment, disability and proximity to death as measured by mortality within 1.5 years. We defined as most informative any measures that were significantly associated with at least two of the health-status measures in multivariable analyses in this age group. 10 out of 74 tested candidates fulfilled this criterion, while several proposed biomarkers of ageing, notably inflammation and immune risk markers and telomere length, did not. As future data accrues on health outcomes within the cohort, it will become possible also to evaluate the predictive value of these and others of the candidate biomarkers.

1. Introduction

In biological terms, human ageing is a complex, heterogeneous and very gradual process. This makes the development of biomarkers of human ageing both important and difficult (Kirkwood, 1998) and, despite numerous efforts, success to date has been limited (Sprott, 2010). Furthermore, biomarkers of ageing have been considered for a variety of purposes, which are not always distinguished sufficiently (Kirkwood, 1998). Most investigations treated biomarkers as tools for *comparing* rates of ageing in different populations, or in different sub-groups within a single population. Others however sought biomarkers for person-specific *prediction*. This is much more challenging, mostly because ageing as a biological process is not well defined at the individual level. We here describe an evaluation of candidate biomarkers in the baseline data of a prospective, cohort study. The present analysis is thus comparative in nature but in time there will be opportunity to relate our comparative findings to future predictive utility, and we look forward also to opportunities to compare our findings with other initiatives such as the ongoing EU Mark-Age project (http://edukon.biologie.uni-konstanz.de/mark-age).

Whether seeking comparative or predictive biomarkers no single measure has yet qualified as a sensitive and specific biomarker of ageing. This has resulted in the attempted use of panels of measures that associate with survival, health at old age, frailty, age-related (multi-) morbidity or disability. 'Classical' measures used for this purpose include indicators of physical function, body mass and composition, cardiovascular function, metabolic activity, haematological function, inflammation, endocrine function and micronutrient status. Few of these measures

* Corresponding author. Tel.: +44 191 248 1103; fax: +44 191 248 1101.
E-mail address: tom.kirkwood@ncl.ac.uk (T.B.L. Kirkwood).

0047-6374/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mad.2011.08.001

conform to published criteria for biomarkers of ageing (Baker and Sprott, 1988).

Recent years have seen candidate biomarkers beginning to emerge from new insights into the mechanisms of biological ageing. These include telomere length and other markers of peripheral blood (immune) cell senescence, as well as measures of accumulated oxidative damage and of DNA repair capacity. Peripheral blood cell telomere length (Cawthon et al., 2003; Houben et al., 2010; Martin-Ruiz et al., 2005, 2006) and indicators of immunosenescence (Derhovanessian et al., 2010; Strindhall et al., 2007; Wikby et al., 2008) have been shown frequently, but not always, to be associated with age-related morbidity and mortality, while oxidative damage or DNA repair markers were seldom validated in human populations.

Several disease-specific biomarkers have been developed and tested for conditions in which biological age is the single biggest risk factor. However, despite this evidence for an intimate link with the ageing process, very few of these candidates have been tested in parallel with generic biomarkers of ageing. For instance N-terminal proB-type natriuretic peptide (BNP), a well-established marker for cardiovascular disease, has been shown to predict mortality in older adults even without specific cardiac diagnoses (Vaes et al., 2009). In subclinical hypothyroidism, serum levels of thyroid-stimulating hormone (TSH) were reported to increase with age, although these data remain controversial (Fatourechi, 2007; Ochs et al., 2008).

Even where biomarkers of age-related disease or ageing have been documented in younger-old populations (typically aged 60–85), these findings are inconsistent when studied in the oldest-old (aged 85 and above). For example, blood pressure (Euser et al., 2009; van Bemmel et al., 2006), indicators of metabolic syndrome (van den Berg et al., 2007) and telomere length (Martin-Ruiz et al., 2006) did not associate significantly with age-related morbidity or mortality in at least one previous population-based study of the oldest-old. Thus, in general, the utility of biomarkers of ageing and age-related diseases in understanding the health trajectories of the oldest-old is unexplored territory. It is important that this lacuna is filled given the rapid growth in the number of very old people in many contemporary populations. Identification of biomarkers of ageing and health status will not only lead to more robust evidence to facilitate the development and targeting of interventions to improve health and avert high-cost dependency, but will also aid investigations of the links between ageing and disease.

The current paper reports analyses of data from baseline assessments made within the Newcastle 85+ study (Collerton et al., 2007, 2009). We examine the extent to which a total of 74 candidate biomarkers, including novel (ageing-specific), classical and disease-specific candidates, are associated with the presence of adverse health states: multi-morbidity, cognitive impairment, disability and short term (1.5 years) mortality. As ageing is a multidimensional process, we define a candidate biomarker as informative in this population about ageing itself (as opposed to a single dimension of age-related change), if the candidate marker shows significant associations in the same direction with at least two of the health-status measures in a multivariable analysis.

2. Methods

2.1. Study population

The Newcastle 85+ study recruited a cohort of 1042 individuals aged around 85 years (Supplementary Fig. S1) all born in 1921 and registered with a participating National Health Service (NHS) general practice in the areas of Newcastle upon Tyne or North Tyneside in the north-east of England (Collerton et al., 2007, 2009). Given the near universal coverage of the NHS among people of this age and in this region, effectively all those in the age group were approached to participate, including those in care homes and with disabilities and/or cognitive impairment. Following extensive preparative work and refinement of recruitment procedures for this age group (Davies et al., 2010), a good level of recruitment was achieved and previous analyses have confirmed that there were no important differences between those who agreed to take part and the background characteristics of the population, indicating a high degree of representativeness in the study sample (Collerton et al., 2009). At baseline, participants underwent a detailed multidimensional health assessment by trained research nurses in their usual residence (own home or institution), comprising questionnaires, measurements, function tests, a fasting blood sample, and a review of medical records held by the general practice. Participants could decline elements of the protocol.

2.2. Anthropometry, blood pressure and physical function

Assessment procedures are described in Supplementary methods.

2.3. Blood-based candidate biomarkers

After an overnight fast, 40 ml blood was drawn from the antecubital vein between 7:00 and 10:30 am. Great attention was paid to getting blood samples to the laboratory as quickly as possible and 95% of samples were received for processing within 1 h of venepuncture. Aliquots of serum, EDTA plasma, LiHep plasma and peripheral blood mononuclear cells (PBMC) were obtained and analysed as described in Supplementary methods.

2.4. Health-status measures

Cognitive impairment was assessed by the Standardised Mini-Mental State Examination (SMMSE) with scores ranging from 0 (impaired) to 30. A disability score (maximum score 17) was calculated from 17 self-reported Activities of Daily Living (ADLs) with participants scoring 1 for each activity in which they required help or were only independent with difficulty and 0 otherwise. A disease count (maximum score 18) was calculated from selected chronic diseases (Collerton et al., 2009) with participants scoring 1 if the disease was present and 0 if absent. For the disease and disability scores, participants were assigned a value only if all variables were present. To score death-within-18-months as a measure of health status, data on deaths among the study participants were obtained from the Medical Research Information Service. Survival time was calculated from date of first interview to death or censored at 18 months.

2.5. Statistical analysis

Albumin, cortisol, glucose, low-density lipoprotein (LDL) cholesterol, triglycerides, body composition measures and vitamin B6 are affected by fasting status and non-fasting blood samples (less than 5% of the samples) were re-coded as missing for these measures. Cortisol, thyroid hormones and homocysteine are affected by time of day of collection and samples collected after midday were re-coded as missing ($n = 1$).

Candidate biomarker distributions by gender are presented as means, standard deviations and percentage under, within and above normative ranges (where available). Normative ranges from the Department of Clinical Biochemistry at Newcastle Royal Victoria Infirmary were used for: cortisol, C-reactive protein (CRP), ferritin, glucose, glycated haemoglobin (HbA1c), high-density lipoprotein (HDL) cholesterol, triglycerides, urate, sodium, potassium, urea, creatinine, phosphate, alkaline phosphatase, total protein, albumin, bilirubin, alanine transaminase (ALT), thyroid-stimulating hormone (TSH), thyroxin (FT4) and triiodothyronine (FT3); for the remainder normative ranges were taken from The Medical Council of Canada 2010 (http://www.mcc.ca/objectives_online/objectives.pl?lang=english&loc=values).

Gender differences in candidate biomarkers were assessed by *t*-tests with log-transformations applied where distributions showed marked positive skewness.

We used ordinal polytomous regression to test associations between candidate biomarkers and the four health-status measures categorised as: SMMSE 0–17/18–21/22–25/26–30; disability score 0/1–6/7–12/13–17; disease count 1–2/3–6/7–18 (no participants had a disease count of zero). For each candidate biomarker gender-specific deciles were calculated and participants allocated to one of three categories: <10th percentile, 10–90th percentile, >90th percentile. In regression models each candidate biomarker was first entered singly and then multivariable models were constructed using backwards elimination, in both cases with adjustment for gender. The middle (10–90th percentile) category was used as the reference category for the candidate biomarkers and missing values were imputed to the middle category, to maximise the potential sample size. A significance level of 5% was used throughout. Cox proportional hazards regression modelling was used for associations between candidate biomarkers and short-term (18 month) survival with proportional hazards verified via scaled Schoenfeld residuals. Prior to fitting the multivariable models potential multicollinearity was assessed by Variance Inflation Factors (VIFs) and biomarkers with VIFs > 4 were removed (Total body water and Fat free mass). Sensitivity of the multivariable models to the choice of 10/90th percentile was assessed by repeating model fitting with 20/80th and 25/75th percentiles. Goodness of fit of models was compared by the *C* statistic (or area under the receiver operating characteristic curve) from fitting logistic regression models with dichotomized health status (18-month survival, SMMSE 0–17/18+ and 0–25/26–30, disability score 0/1+ and 0–12/13–17, and

disease count 1–2/3+ and 1–6/7–18) as the response variable, the independent variables being the combination of biomarkers from the final multivariable models. All statistical analyses were performed using STATA version 10.1.

3. Results

Of the 852 participants who underwent multidimensional health assessment, blood-based data are available for 719–778 (depending on assay, Supplementary Table S1). Summary statistics for each candidate biomarker for men and women separately and the significance of the gender difference are given in Supplementary Table S1. There were significant between-gender differences for the majority of measures. For blood-based candidate biomarkers where normative ranges were available, Fig. 1 shows the percentage of participants falling into each of the three categories: below, within and above normal range. Large proportions of participants had levels above the normative range for creatinine (47.4%), urea (33.9%), urate (30.5%), vitamin D (26.9%), LDL cholesterol (38.8%), HDL cholesterol (30.3%), total cholesterol (38.7%), HbA1c (25.8%), and CRP (30.5%), or levels below the normative range for lymphocytes (28.4%), red blood cells (39.4%) and haematocrit (41.6%).

Supplementary Table S2 shows the strength of association between each candidate biomarker singly (adjusted for gender) and disease count, disability score and SMMSE score by odds ratios (OR) and for mortality by hazard ratios (HR), candidate biomarkers being included where there was a significant association with at least one of the health status measures. For 11 candidate biomarkers (total protein, apolipoprotein A1 (ApoA1), HDL cholesterol, free T3, haematocrit, haemoglobin, red blood cell

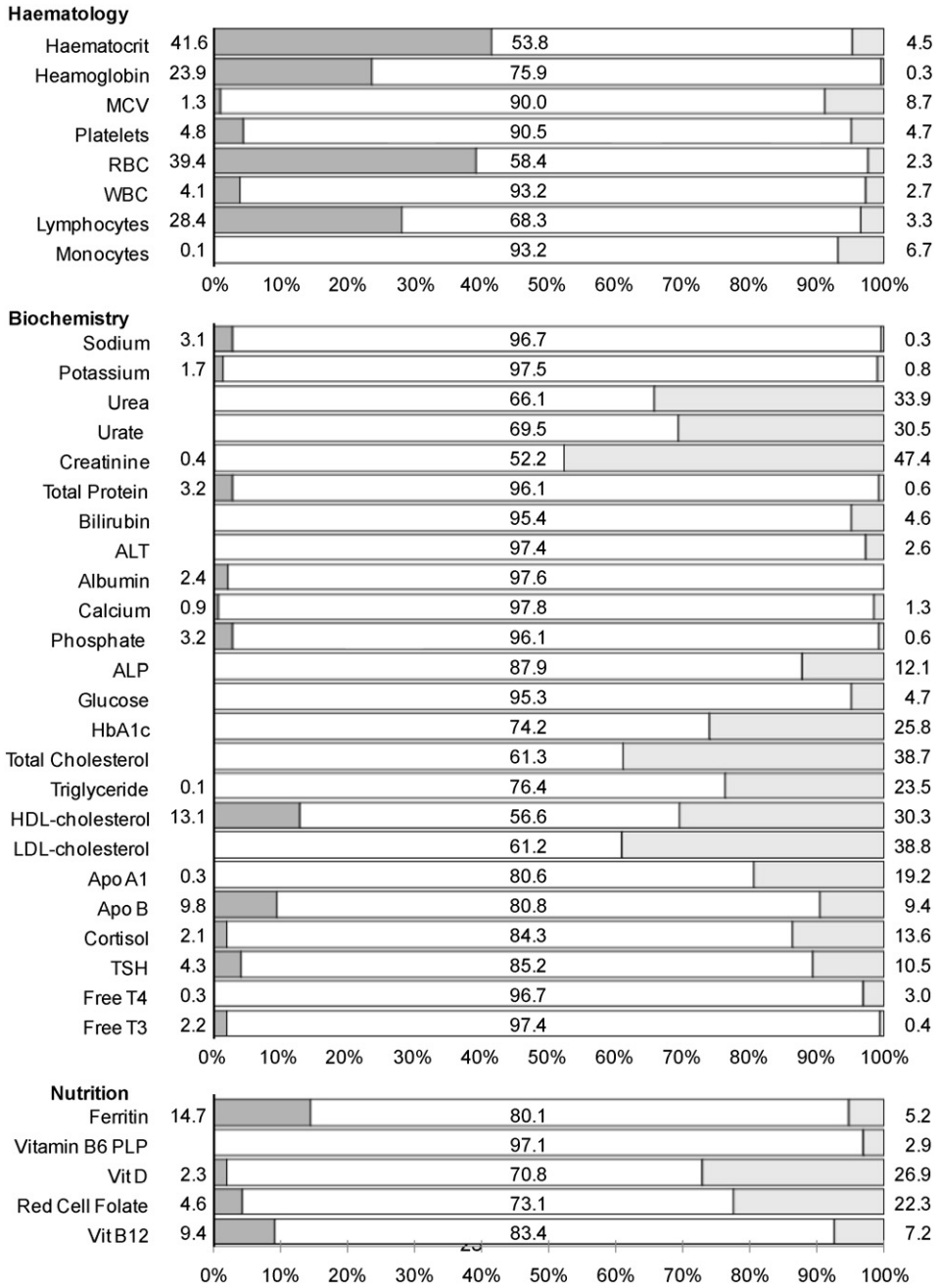

Fig. 1. Comparison of haematological, biochemical and nutritional data from the study population with normal ranges. Percentages of the study population that fall into the normal range (white), are below (dark grey, left) or above (light grey, right) the normal range limits are shown.

count (RBC), systolic blood pressure, handgrip strength, forced expiratory volume in 1 s (FEV), peak expiratory flow rate (PEFR)) low levels were associated with significantly higher chances of all 4 adverse health-status measures, and for 3 candidate biomarkers (PEFR, BNP and timed-up-and go (TUG)) high levels were significantly associated with the poorer health-status measures. For 9 further candidate biomarkers (forced vital capacity (FVC), calcium, ALT, HbA1c, albumin, creatinine, bilirubin, vitamin D, vitamin B6) low levels were associated with significantly higher chances of 3 adverse health-status measures. For a number of candidate biomarkers there was consistency across the distribution with low (or high) values being significantly 'protective' and high (or low) values being significantly 'harmful', though only PEFR showed this across all 4 health-status measures while handgrip strength, TUG and HDL cholesterol were significant for 3 health-status measures.

In the multivariable models, 49 of the 74 candidate biomarkers were significantly associated with at least one of the 4 health-status measures, as either 'harmful', 'protective' or both (Table 1). TUG and BNP were the only candidate biomarkers that were associated with all 4 health-status measures. Handgrip strength (both high and low percentiles) was consistently associated with 3 of the 4 health-status measures (except mortality). 10 of the tested 74 candidate markers (three of them measuring anaemia-associated parameters) were concordantly associated with at least 2 age-related health-status measures (Table 2). The models fitted well with *C* statistics for the four health-status measures ranging from 0.77 to 0.89. The selection of biomarkers included in the final multivariable models was somewhat sensitive to the choice of 10/90th percentile although around 30% of the biomarkers were stable across all three cut-points and models using other cut-points did not fit significantly better. Importantly, 67% of the associations included in Table 2 were also retained in models using at least one additional cut-point.

4. Discussion

A large number of the reference values for our candidate biomarkers are one-sided in the sense that only values above (or below) a reference level are considered 'abnormal'. As others (Seplaki et al., 2004) have done, we tested the top and bottom deciles against our 4 health-status measures, since we sought to identify potentially 'protective' and 'harmful' effects as well as 'U' shaped associations. Moreover the 'normal' ranges were generally produced for younger age groups and their validity for the oldest-old is uncertain, as reflected in the large proportions of participants falling outside accepted normal ranges (Fig. 1). We recognize, of course, that the use of top and bottom deciles is to some extent arbitrary and other cut-offs were considered. While these resulted in some differences in the associations detected, the main results were largely unaffected and we therefore believe that the use of top and bottom deciles is reasonable for the present purpose.

We found 49 of the candidate biomarkers to be associated with at least one health-status measure in a multivariable analysis (Table 1). Some candidate biomarkers showed opposing associations for different health-status measures. For instance, low body fat was associated with low disability, but increased mortality. While red blood cell (RBC)-associated data showed a coherent picture, white blood-cell (WBC) associated data (WBC count, lymphocytes, monocytes and neutrophils) did not. High total protein content was associated with low morbidity, but high mortality, and the opposite was true for low bilirubin. ALT associated coherently with disability, but not with morbidity.

A smaller number of candidates fulfilled the above criterion for an informative biomarker of ageing. These are summarized in Table 2.

High blood pressure (BP) is a risk factor for mortality in younger populations but has not been found to be so in 85 years old (van Bemmel et al., 2006), in whom it has instead been associated with improved cognitive function (Euser et al., 2009). Our data confirmed these findings. Previous studies also show low BP is associated with cognitive impairment and disability, as did ours, which however was not adjusted for anti-hypertensive medication.

Measures of physical function (hand grip strength, TUG and FEV) are well established biomarkers of ageing, especially in younger populations (Bohannon, 2008; Cooper et al., 2010; Rolland et al., 2006; Sabia et al., 2010). Our data confirmed these measures as informative biomarkers of ageing in the oldest-old.

As in other studies (Guralnik et al., 2004), anaemia was highly prevalent in the Newcastle 85+ study (Collerton et al., 2009). In 85-year-olds, anaemia has been proposed as an independent predictor of mortality (den Elzen et al., 2009). The associations between anaemia-related measures and multiple health-status measures in our population support a role for anaemia as a biomarker of functional decline in ageing.

BNP is elevated in the presence of heart failure, and BNP measurement is included in diagnostic algorithms for heart failure in patients presenting with symptoms. This might explain the association between BNP and both multimorbidity and mortality in our study, although high BNP has been shown to predict mortality in octa- and nonagenarians independent of specific cardiac diagnoses (Kistorp et al., 2005; Vaes et al., 2009). In addition, high BNP concentrations associated with low cognitive function and low BNP with low levels of disability in our population. It remains to be established whether this is mediated via the impact of cardiovascular disease on cognition but, irrespective of mechanisms, our data suggest BNP to be an informative general marker of age-related dysfunction.

Low free T3 levels were associated with increased risk for morbidity and mortality in our study. These findings are consistent with other studies of aged populations, showing both an association of low serum T3 with reduced parameters of physical performance and muscle strength (van den Beld et al., 2005), and with increasing disease-burden and mortality (Forestier et al., 2009; van den Beld et al., 2005). Low serum free T3 is classically associated with caloric restriction/starvation, as well as with non-thyroidal illness as a component of the 'sick-euthyroid' syndrome. Reduced hepatic type 1 deiodinase activity (Donda and Lemarchand-Beraud, 1989), leading to low serum T3 has been suggested as a feature of advancing age. However our findings contrast to those of a study of healthy centenarians, which suggested that low serum FT3 was a feature of age alone (Mariotti et al., 1993).

We found low concentrations of vitamin D to be associated with increased multimorbidity and cognitive impairment, as previously shown (Anderson et al., 2010; Llewellyn et al., 2010). The large proportion of our participants with supra-normal vitamin D concentrations (see Fig. 1), and its association with cognitive impairment, were probably due to the vitamin supplementation routinely performed in most nursing and EMI homes.

We did not find convincing evidence that several candidate biomarkers including inflammatory markers, telomere length and CD4/CD8 T lymphocyte ratio, were associated with adverse health-status measures. Persistent low grade inflammation is a driver of the ageing process (Franceschi et al., 2000) and high concentrations of CRP and serum interleukins are associated with various age-related health-status measures (Schaap et al., 2009; Tiainen et al., 2010). In our univariate analyses (Table S2), high CRP concentrations were associated with multimorbidity, disability and mortality but only the association with disability remained in the multivariable models. Similarly, low levels of stimulated cytokine release were associated with a single health-status

Table 1
Associations of candidate biomarker values above 90th percentile and below 10th percentile (relative to 10–90th percentile) with disease count, cognitive impairment, disability and mortality. Health-status measures are from multivariable models after backwards elimination adjusted for gender. Only significant associations are shown.

	Disease count		Cognitive Impairment		Disability count		Mortality	
	OR (95%CI)	*p* Value	OR (95%CI)	*p* Value	OR (95%CI)	*p* Value	HR (95%CI)	*p* Value
Anthropometry								
Fat % <10					0.49 (0.29–0.81)	0.006	2.19 (1.22–3.94)	0.009
BMI <10	0.40 (0.21–0.73)	0.003						
Blood pressure								
BP systolic <10			2.02 (1.17–3.48)	0.011	1.79 (1.09–2.95)	0.021		
BP diastolic <10	1.90 (1.03–3.53)	0.04						
Physical function								
Hand grip strength <10	2.78 (1.48–5.23)	0.002	3.64 (2.17–6.09)	<0.001	4.67 (2.74–7.97)	<0.001		
Hand grip strength >90	0.42 (0.23–0.77)	0.004	0.44 (0.20–0.95)	0.037	0.51 (0.30–0.85)	0.011		
TUG <10	0.48 (0.27–0.88)	0.018			0.26 (0.15–0.44)	<0.001		
TUG >90			1.85 (1.10–3.12)	0.021	9.02 (5.39–15.10)	<0.001	1.97 (1.14–3.43)	0.016
PEFR <10	1.92 (1.04–3.58)	0.038						
FEV <10			2.26 (1.32–3.87)	0.003	3.17 (1.87–5.38)	<0.001		
FEV >90					0.58 (0.35–0.99)	0.045		
FVC >90			0.43 (0.21–0.87)	0.018				
Haematology								
Haematocrit <10			1.99 (1.17–3.40)	0.011			2.23 (1.28–3.90)	0.005
Haemoglobin >90			0.48 (0.25–0.97)	0.041			0.27 (0.10–0.76)	0.013
MCV >90					2.02 (1.19–3.44)	0.009		
RBC <10	2.31 (1.24–4.29)	0.008			2.14 (1.303.54)	0.003		
WBC <10	0.52 (0.28–0.98)	0.042						
WBC >90			0.37 (0.17–0.80)	0.012	0.52 (0.31–0.88)	0.016		
Lymphocytes <10							0.30 (0.14–0.67)	0.003
Monocytes >90			0.41 (0.20–0.87)	0.021			2.06 (1.19–3.60)	0.01
Neutrophils >90							0.48 (0.25–0.92)	0.026
Biochemistry								
Sodium <10			0.48 (0.24–0.98)	0.043				
Sodium >90					1.83 (1.09–3.08)	0.023		
Phosphate <10			1.79 (1.04–3.08)	0.035				
Urate <10							2.26 (1.19–4.28)	0.013
Urate >90			0.37 (0.19–0.71)	0.003				
Creatinine <10					1.92 (1.15–3.19)	0.011		
Glucose >90	2.50 (1.32–4.73)	0.005						
Total protein <10	2.08 (1.07–4.02)	0.031					3.12 (1.74–5.60)	<0.001
Total protein >90	0.46 (0.24–0.88)	0.02					2.00 (1.06–3.79)	0.033
Bilirubin <10	1.80 (1.01–3.22)	0.046					0.50 (0.26–0.96)	0.037
ALT <10	0.40 (0.21–0.76)	0.005			3.21 (1.91–5.39)	<0.001		
ALT >90					0.55 (0.32–0.94)	0.029		
Albumin <10			2.86 (1.64–4.98)	<0.001				
Calcium <10							2.89 (1.47–5.67)	0.002
Adjusted calcium <10							0.24 (0.09–0.61)	0.003
Adjusted calcium >90							2.82 (1.55–5.14)	0.001
HbA1c <10					1.96 (1.16–3.33)	0.013		
Triglycerides >90					2.38 (1.37–4.15)	0.002		
HDL <10							2.07 (1.17–3.69)	0.013
HDL >90							0.20 (0.06–0.64)	0.007
LDL >90					0.30 (0.18–0.51)	<0.001		
Total cholesterol <10	2.64 (1.44–4.82)	0.002						
Total cholesterol >90	1.98 (1.05–3.72)	0.034						
Total cholesterol/HDL cholesterol >90			2.30 (1.33–3.98)	0.003				
Apo A1 <10					2.29 (1.26–4.14)	0.006		
Apo A1 >90							3.34 (1.45–7.68)	0.005
Apo B <10							2.51 (1.21–5.18)	0.012
Cortisol >90							2.38 (1.36–4.17)	0.002
Free T3 <10	2.40 (1.27–4.50)	0.006					2.23 (1.30–3.81)	0.004
Free T3 >90	0.44 (0.24–0.83)	0.012						
Free T4 >90	2.07 (1.12–3.83)	0.02						
hsCRP <10					0.49 (0.29–0.82)	0.007		
hsCRP >90					1.91 (1.18–3.09)	0.008		
NT-pro BNP <10	0.51 (0.28–0.92)	0.027			0.60 (0.36–1.00)	0.048		
NT-pro BNP >90	5.97 (3.23–11.04)	<0.001	1.73 (1.03–2.91)	0.038			4.31 (2.61–7.13)	<0.001
Nutritional markers								
Ferritin <10	2.61 (1.39–4.90)	0.003						
Red cell folate <10	0.53 (0.29–0.97)	0.039						
Homocysteine <10					0.43 (0.25–0.73)	0.002		
Vitamin B2 <10							0.29 (0.13–0.68)	0.004
Vitamin B2 >90							0.39 (0.17–0.94)	0.035
Vitamin B6 PA < 10							3.05 (1.68–5.53)	<0.001
Vitamin B12 >90					1.76 (1.06–2.93)	0.03		
Vitamin D <10	1.89 (1.06–3.39)	0.031	1.86 (1.12–3.09)	0.017				
Vitamin D >90			1.86 (1.07–3.24)	0.028				
Inflammatory response								
IL-6 <10			0.43 (0.22–0.84)	0.013				

Table 1 (*Continued*)

	Disease count		Cognitive Impairment		Disability count		Mortality	
	OR (95%CI)	*p* Value	OR (95%CI)	*p* Value	OR (95%CI)	*p* Value	HR (95%CI)	*p* Value
TNF-alpha <10	0.43 (0.24–0.78)	0.006						
F2 alpha <10			0.50 (0.26–0.96)	0.037				
Immunosenescence								
B cells memory/naïve <10					2.17 (1.27–3.69)	0.004	0.32 (0.13–0.80)	0.015
CD4 memory/naive							0.40 (0.17–0.91)	0.029
CD8 memory/naïve >90			0.40 (0.19–0.84)	0.014				
Cell ageing and ROS								
DNA damage <10							0.33 (0.14–0.80)	0.014

Table 2
Informative biomarkers of ageing in the Newcastle 85+ study. Markers associating coherently with more than one age-related health-status measure in a multivariable analysis fitted with the 10/90th percentile. Direction of association ('harmful' or 'protective') is indicated. Markers that are retained in analyses with the 20/80th percentiles are shown in bold, and those retained with the 25/75th percentiles in italics.

Biomarker	Multi-morbidity	Cognitive impairment	Disability	Mortality
Systolic BP		*Low: harmful*	**Low: harmful**	
Hand grip strength	**Low: harmful** **High: protective**	**Low: harmful** High: protective	**Low: harmful** **High: protective**	
Timed up and go	**Low: protective**	**High: harmful**	**Low: protective** **High: harmful**	High: harmful
FEV		Low: harmful	Low: harmful **High: protective**	
Haematocrit		**Low: harmful**		Low: harmful
Haemoglobin		**High: protective**		**High: protective**
RBC count	Low: harmful		Low: harmful	
Free T3	**Low: harmful** *High: protective*			Low: harmful
BNP	Low: protective **High: harmful**	**High: harmful**	Low: protective	**High: harmful**
Vitamin D	Low: harmful	**Low: harmful** **High: harmful**		

measure only. Therefore, serum CRP or stimulated cytokine release were not classified as informative biomarkers of ageing in our population.

Low peripheral blood cell telomere length is associated with, and predictive for, cognitive dysfunction, various age-associated diseases and mortality (Cawthon et al., 2003; Houben et al., 2010; Martin-Ruiz et al., 2005, 2006), though mostly in younger populations (up to age 75). Like others, we previously observed an absence of associations between telomere length and age-related morbidity and mortality in oldest-old groups (Houben et al., 2010; Martin-Ruiz et al., 2005), and this has now been confirmed in our new population.

Low CD4/CD8T lymphocyte ratio was associated with low survival in Swedish longitudinal studies of octogenarians and nonagenarians (Strindhall et al., 2007) and suggested as the central feature of an 'immune risk profile'. We did not find any significant association of the extreme percentiles of the CD4/CD8 ratios with survival or any other health-status measure. This might reflect the short time interval for survival and will be re-examined in future work with longer follow-up times and more comprehensive characterization of the immune risk phenotype.

In summary, we tested 74 candidate biomarkers simultaneously against age-related functional decline in a cohort of 85-year-old. We found 10 to meet our criteria for an informative biomarker of ageing. Full validation of a biomarker of ageing in this age group will require demonstration of predictive capability. We will be able to assess this in the coming years as longer-term follow-up becomes available.

Funding

This work was supported by the Medical Research Council and Unilever Discover Colworth (G0601333); the Medical Research Council and the Biotechnology and Biological Sciences Research Council (G0500997), the British Heart Foundation (PG/08/026/24712) and the UK NIHR Biomedical Research Centre for Ageing and Age-related Disease award to the Newcastle upon Tyne Foundation Hospitals NHS Trust.

Acknowledgements

Our special thanks go to the study participants for their time and personal information. We thank the research nurses and the biomarker technicians Claire Kolenda, Craig Parker, and Anna Tang. We further thank NHS North of Tyne and participating local general practices.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mad.2011.08.001.

References

Anderson, J.L., May, H.T., Horne, B.D., Bair, T.L., Hall, N.L., Carlquist, J.F., Lappe, D.L., Muhlestein, J.B., 2010. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. Am. J. Cardiol. 106, 963–968.
Baker 3rd, G.T., Sprott, R.L., 1988. Biomarkers of aging. Exp. Gerontol. 23, 223–239.
Bohannon, R.W., 2008. Hand-grip dynamometry predicts future outcomes in aging adults. J. Geriatr. Phys. Ther. 31, 3–10.
Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenko, A., Kerber, R.A., 2003. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 361, 393–395.
Collerton, J., Barrass, K., Bond, J., Eccles, M., Jagger, C., James, O., Martin-Ruiz, C., Robinson, A.L., Von Zglinicki, T., Kirkwood, T., 2007. The Newcastle 85+ study: biological, clinical and psychosocial factors associated with health ageing: study protocol. BMC Geriatr. 7, 14.
Collerton, J., Davies, K., Jagger, C., Kingston, A., Bond, J., Eccles, M.P., Robinson, A.L., Martin-Ruiz, C., Von Zglinicki, T., James, O.F.W., Kirkwood, T., 2009. Health and

disease in 85 years olds: baseline findings from the Newcastle 85+ cohort study. BMJ 399 (Part b4904), 1–11.
Cooper, R., Kuh, D., Hardy, R., 2010. Objectively measured physical capability levels and mortality: systematic review and meta-analysis. BMJ 341, c4467.
Davies, K., Collerton, J.C., Jagger, C., Bond, J., Barker, S.A.H., Edwards, J., Hughes, J., Hunt, J.M., Robinson, A.L., 2010. Engaging the oldest old in research: lessons from the Newcastle 85+ study. BMC Geriatr. 10 .
den Elzen, W.P., Willems, J.M., Westendorp, R.G., de Craen, A.J., Assendelft, W.J., Gussekloo, J., 2009. Effect of anemia and comorbidity on functional status and mortality in old age: results from the Leiden 85-plus Study. CMAJ 3–4.
Derhovanessian, E., Maier, A.B., Beck, R., Jahn, G., Hahnel, K., Slagboom, P.E., de Craen, A.J., Westendorp, R.G., Pawelec, G., 2010. Hallmark features of immunosenescence are absent in familial longevity. J. Immunol. 185, 4618–4624.
Donda, A., Lemarchand-Beraud, T., 1989. Aging alters the activity of 5′-deiodinase in the adenohypophysis, thyroid gland, and liver of the male rat. Endocrinology 124, 1305–1309.
Euser, S.M., van Bemmel, T., Schram, M.T., Gussekloo, J., Hofman, A., Westendorp, R.G., Breteler, M.M., 2009. The effect of age on the association between blood pressure and cognitive function later in life. J. Am. Geriatr. Soc. 57, 1232–1237.
Fatourechi, V., 2007. Upper limit of normal serum thyroid-stimulating hormone: a moving and now an aging target? J. Clin. Endocrinol. Metab. 92, 4560–4562.
Forestier, E., Vinzio, S., Sapin, R., Schlienger, J.L., Goichot, B., 2009. Increased reverse triiodothyronine is associated with shorter survival in independently-living elderly: the Alsanut study. Eur. J. Endocrinol. 160, 207–214.
Franceschi, C., Bonafe, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann. N. Y. Acad. Sci. 908, 244–254.
Guralnik, J.M., Eisenstaedt, R.S., Ferrucci, L., Klein, H.G., Woodman, R.C., 2004. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. Blood 104, 2263–2268.
Houben, J.M., Giltay, E.J., Rius-Ottenheim, N., Hageman, G.J., Kromhout, D., 2010. Telomere length and mortality in elderly men: the Zutphen elderly study. J. Gerontol. A. Biol. Sci. Med. Sci..
Kirkwood, T.B., 1998. Alex Comfort and the measure of aging. Exp. Gerontol. 33, 135–140.
Kistorp, C., Raymond, I., Pedersen, F., Gustafsson, F., Faber, J., Hildebrandt, P., 2005. N-terminal pro-brain natriuretic peptide. C-reactive protein, and urinary albumin levels as predictors of mortality and cardiovascular events in older adults. JAMA 293, 1609–1616.
Llewellyn, D.J., Lang, I.A., Langa, K.M., Muniz-Terrera, G., Phillips, C.L., Cherubini, A., Ferrucci, L., Melzer, D., 2010. Vitamin D and risk of cognitive decline in elderly persons. Arch. Intern. Med. 170, 1135–1141.
Mariotti, S., Barbesino, G., Caturegli, P., Bartalena, L., Sansoni, P., Fagnoni, F., Monti, D., Fagiolo, U., Franceschi, C., Pinchera, A., 1993. Complex alteration of thyroid function in healthy centenarians. J. Clin. Endocrinol. Metab. 77, 1130–1134.
Martin-Ruiz, C., Dickinson, H.O., Keys, B., Rowan, E., Kenny, R.A., Von Zglinicki, T., 2006. Telomere length predicts poststroke mortality, dementia, and cognitive decline. Ann. Neurol. 60, 174–180.
Martin-Ruiz, C.M., Gussekloo, J., van Heemst, D., von Zglinicki, T., Westendorp, R.G., 2005. Telomere length in white blood cells is not associated with morbidity or mortality in the oldest old: a population-based study. Aging Cell 4, 287–290.
Ochs, N., Auer, R., Bauer, D.C., Nanchen, D., Gussekloo, J., Cornuz, J., Rodondi, N., 2008. Meta-analysis: subclinical thyroid dysfunction and the risk for coronary heart disease and mortality. Ann. Intern. Med. 148, 832–845.
Rolland, Y., Lauwers-Cances, V., Cesari, M., Vellas, B., Pahor, M., Grandjean, H., 2006. Physical performance measures as predictors of mortality in a cohort of community-dwelling older French women. Eur. J. Epidemiol. 21, 113–122.
Sabia, S., Shipley, M., Elbaz, A., Marmot, M., Kivimaki, M., Kauffmann, F., Singh-Manoux, A., 2010. Why does lung function predict mortality? Results from the Whitehall II Cohort Study. Am. J. Epidemiol. 172, 1415–1423.
Schaap, L.A., Pluijm, S.M., Deeg, D.J., Harris, T.B., Kritchevsky, S.B., Newman, A.B., Colbert, L.H., Pahor, M., Rubin, S.M., Tylavsky, F.A., Visser, M., 2009. Higher inflammatory marker levels in older persons: associations with 5-year change in muscle mass and muscle strength. J. Gerontol. A. Biol. Sci. Med. Sci. 64, 1183–1189.
Seplaki, C.L., Goldman, N., Weinstein, M., Lin, Y., 2004. How Are Biomarkers Related to Physical and Mental Well-Being? J. Gerontol. Biol. Sci. 59A, 201–217.
Sprott, R.L., 2010. Biomarkers of aging and disease: introduction and definitions. Exp. Gerontol. 45, 2–4.
Strindhall, J., Nilsson, B.O., Lofgren, S., Ernerudh, J., Pawelec, G., Johansson, B., Wikby, A., 2007. No immune risk profile among individuals who reach 100 years of age: findings from the Swedish NONA immune longitudinal study. Exp. Gerontol. 42, 753–761.
Tiainen, K., Hurme, M., Hervonen, A., Luukkaala, T., Jylha, M., 2010. Inflammatory markers and physical performance among nonagenarians. J. Gerontol. A. Biol. Sci. Med. Sci. 65, 658–663.
Vaes, B., de Ruijter, W., Degryse, J., Westendorp, R.G., Gussekloo, J., 2009. Clinical relevance of a raised plasma N-terminal pro-brain natriuretic peptide level in a population-based cohort of nonagenarians. J. Am. Geriatr. Soc. 57, 823–829.
van Bemmel, T., Vinkers, D.J., Macfarlane, P.W., Gussekloo, J., Westendorp, R.G., 2006. Markers of autonomic tone on a standard ECG are predictive of mortality in old age. Int. J. Cardiol. 107, 36–41.
van den Beld, A.W., Visser, T.J., Feelders, R.A., Grobbee, D.E., Lamberts, S.W., 2005. Thyroid hormone concentrations, disease, physical function, and mortality in elderly men. J. Clin. Endocrinol. Metab. 90, 6403–6409.
van den Berg, E., Biessels, G.J., de Craen, A.J., Gussekloo, J., Westendorp, R.G., 2007. The metabolic syndrome is associated with decelerated cognitive decline in the oldest old. Neurology 69, 979–985.
Wikby, A., Månsson, I., Johansson, B., Strindhall, J., Nilsson, S., 2008. The immune risk profile is associated with age and gender: findings from three Swedish population studies of individuals 20–100 years of age. Biogerontology 9, 299–308.