**Appendix 2:** **Methods-Supplementary information on the categorization of aging phenotypes**

* Procedures for assessment of non-fatal and fatal cardiovascular events and non-cardiovascular death [1]. Briefly, coronary heart disease status was based on clinically verified events including myocardial infarction [2] and definite angina [3]. Stroke was assessed using a self-reported measure of physician diagnosis. Follow up for mortality was performed through the national mortality register kept by the National Health Services Central Registry, using the NHS identification number assigned to each British citizen. The International Classification of Diseases, Ninth Revision (ICD-9), and 10th Revision (ICD-10) codes were used to define cardiovascular disease mortality (ICD-9 390.0-458.9, ICD-10 I00-I99). Non-cardiovascular disease mortality included all remaining deaths not classified as cardiovascular disease.
* Criteria for successful aging [1]:

(1) No history of the following chronic diseases: cancer (assessed from National Cancer Registry), coronary heart disease, stroke, and diabetes determined by self-report of doctor diagnosis, use of anti-diabetic medication or oral glucose tolerance test (a fasting glucose>=7.0mmol/l, a 2-h post-load glucose >=11.1mmol/l)[4] ;

(2) Good cognitive, physical, respiratory and cardiovascular functioning and absence of disability. All functional measures were assessed by a trained nurse using standard protocols and have been detailed previously[1]. Cognitive functioning was assessed using a score of global cognition calculated from 5 cognitive tests; physical functioning using walking speed over a 8-foot walking course [5]; respiratory function using forced expiratory volume in one second/height2 (L/m2) [6]; and cardiovascular function using systolic blood pressure (average of 2 measurements in the sitting position after a 5-minute rest using the sphygmomanometer OMRON HEM 907, Omron, Milton Keynes, UK). Poor functioning was defined as scores in the worst sex- and age-standardised quintile. Participants having difficulties with one or more activities assessed using instrumental and basic activities of daily living questionnaire [7] [8]were categorized as disabled;

(3) Good mental health defined as scoring >42 on the mental health component summary scale of the Short Form General Health Survey [9].

**References**

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**Appendix 3: Details of the sensitivity analyses**

To examine potential competing risk bias, the association between inflammation and aging phenotypes was assessed using multinomial regression. We estimated odds of successful aging and the two unhealthy aging outcomes within a single analysis with normal ageing as the common reference point for all three outcomesthus avoiding the substantial overlap in the different health components of aging (Appendix 5).

Three sets of sensitivity analyses were also carried out to assess the extent to which the association between inflammation and aging phenotypes were driven by obesity, use of anti-inflammatory drugs and acute inflammation, by excluding successively (1) obese participants (defined by a body mass index ≥30kg/m2); (2) users of anti-inflammatory medications and (3) participants with acute inflammation (C-reactive protein >10 mg/L) (Appendix 6).

To assess whether the sample selection may affect the associations, two supplementary analyses were performed. First we compared the odds ratios of non-cardiovascular mortality for all 5353 participants with complete data on inflammation and cause of death to the corresponding odds ratios for the 3044 participants included in the main analysis. Second, from the sample of 4165 participants with complete data on aging phenotypes we applied a multiple imputation method to deal with missing data on inflammation and covariates.

Finally, we used net reclassification improvement statistics [1, 2] to examine the extent by which assessing inflammation level by using two repeated measurements – as compared to using a single measurement - provide a stronger predictor for future aging phenotypes (Appendix 7).

**References**

1. Pencina, M.J., et al., *Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond.* Stat Med, 2008. **27**(2): p. 157-72.

2. Pencina, M.J., R.B. D'Agostino, Sr., and E.W. Steyerberg, *Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers.* Stat Med, 2011. **30**(1): p. 11-21.

**Appendix 5: Full adjusted association between interleukin-6 levels at baseline (1997-99) and over the 5-y exposure period (5-y before baseline and at baseline) and subsequent aging phenotype at 10-y of follow-up within a single analytic setting with normal ageing as the common reference point for all three outcomes (n=3044)\***

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Successful aging | | | | Fatal or Non Fatal  cardiovascular events | | | | Non CVD Death | | | | |
|  |  | |  |  |  | |  |  |  | | |  |  |
|  |  | OR | 95 %CI |  |  | OR | 95 %CI |  |  | | OR | 95 %CI |  |
| Interleukin-6 levels at baseline |  | |  |  |  | |  |  |  | | |  |  |
| Low (756) |  | 1 | ref |  |  | 1 | ref |  |  | | 1 | ref |  |
| Int. (1456) |  | 0.73 | 0.59;0.89 |  |  | 1.27 | 0.90;1.80 |  |  | | 1.20 | 0.70;2.05 |  |
| High (832) |  | 0.50 | 0.38;0.65 |  |  | 1.46 | 1.00;2.14 |  |  | | 2.20 | 1.27;3.80 |  |
|  | | | | | | | | | |  | | | |
| No. of times interleukin-6 was high over the 5-y exposure period\*\* | | | | | | | | | |  | | | |
| 0 (1867) |  | 1 | ref |  |  | 1 | ref |  |  | | 1 | ref |  |
| 1 (791) |  | 0.69 | 0.55;0.86 |  |  | 1.27 | 0.96;1.68 |  |  | | 1.23 | 0.82;1.86 |  |
| 2 (386) |  | 0.60 | 0.43;0.83 |  |  | 1.48 | 1.04;2.11 |  |  | | 2.13 | 1.36;3.35 |  |

To examine potential competing risk bias, the association between inflammation and aging phenotypes was assessed using multinomial regression. We estimated odds of successful aging and the two unhealthy aging outcomes within a single analysis with normal ageing as the common reference point for all three outcomesthus avoiding the substantial overlap in the different health components of aging

\*Multinomial logistic regression to analyze associations between inflammation and the 4-category aging outcome: (1) successful aging, (2) cardiovascular events at follow-up, (3) non-cardiovascular death and (4) normal aging (the non-case category for each of the other categories). Normal Aging was the “non-case” category for all aging phenotype outcomes presented. Models were adjusted for sex, age, socio-economic status, smoking habits, physical activity, acute inflammation and use of anti-inflammatory drugs

\*\*Interleukin-6 was measured twice (5 years before baseline and at baseline). A value of “2” indicates both measurements were high, a value of “1” that either measurement was high and a value of “0” indicates that none of these measurements was high.

**Appendix 6: Full adjusted\* association between interleukin-6 levels over the 5-y exposure period (5-y before baseline and at baseline) and subsequent aging phenotypes at 10-year follow-up- Sensitivity analyses-**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. of times interleukin-6 was high over the 5-y exposure period\*\* | Successful aging\* | | | | Fatal or Non Fatal  cardiovascular events\* | | | | Non-cardiovascular Death\* | | | |
|  | |  |  |  | |  |  |  | |  |  |
| *N cases* | OR | 95 %CI |  | *N cases* | OR | 95 %CI |  | *N cases* | OR | 95 %CI |  |
| After excluding the 361 obese participants † | | | | | | | | | | | | |
| 0 | *505* | 1 | Ref |  | *157* |  | Ref |  | *63* |  | Ref |  |
| 1 | *127* | 0.68 | 0.54;0.86 |  | *84* | 1.38 | 1.03;1.84 |  | *31* | 1.19 | 0.75;1.87 |  |
| 2 | *43* | 0.62 | 0.44;0.89 |  | *40* | 1.34 | 0.89;2.01 |  | *27* | 2.09 | 1.26;3.49 |  |
| After excluding the 295 anti-inflammatory drugs users | | | | | | | | | | | | |
| 0 | *501* |  | Ref |  | *143* |  | Ref |  | *61* |  | Ref |  |
| 1 | *137* | 0.67 | 0.54;0.84 |  | *85* | 1.37 | 1.04;1.81 |  | *33* | 1.34 | 0.88;2.02 |  |
| 2 | *46* | 0.54 | 0.38;0.76 |  | *52* | 1.64 | 1.14;2.35 |  | *29* | 2.44 | 1.55;3.82 |  |
| After excluding the 86 participants with acute inflammation | | | | | |  |  |  |  |  |  |  |
| 0 | *525* |  | Ref |  | *164* |  | Ref |  | *67* |  | Ref |  |
| 1 | *141* | 0.66 | 0.53;0.81 |  | *93* | 1.44 | 1.07;1.94 |  | *40* | 1.24 | 0.80;1.94 |  |
| 2 | *46* | 0.53 | 0.38;0.75 |  | *51* | 1.77 | 1.22;2.58 |  | *36* | 2.13 | 1.30;3.49 |  |

Three sets of sensitivity analyses were carried out to assess the extent to which the association between inflammation and aging phenotypes were driven by obesity, use of anti-inflammatory drugs and acute inflammation, by excluding successively (1) obese participants (defined by a body mass index ≥30kg/m2); (2) users of anti-inflammatory medications and (3) participants with acute inflammation (C-reactive protein >10 mg/L)

\*Results from logistic regression adjusted for sex, age, socio-economic status, smoking habits, physical activity, acute inflammation and use of anti-inflammatory drugs.

\*\*Interleukin-6 was measured twice (5 years before baseline and at baseline). A value of “2” indicates both measurements were high, a value of “1” that either measurement was high and a value of “0” indicates that none of these measurements was high.

**Appendix 7**: Computation of net reclassification improvement statistics characterizing the difference in predictive ability when inflammation was assessed using 2 measures (Model2) versus one measure (Model1)

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Successful aging, n= 721 | | | | Fatal or non-fatal  cardiovascular disease events, n=321 | | | | Non-cardiovascular death, n= 147 | | | |
|  |  | OR | 95 %CI |  |  | OR | 95 %CI |  |  | OR | 95 %CI |  |
|  | | | | | | | |  |  | |  |  |
| **Model 1: Interleukin-6 assessed at baseline** | | | | | | | | |  | | | |
| Low |  | 1 | Ref |  |  | 1 | Ref |  |  | 1 | Ref |  |
| Int. |  | 0.70 | 0.58-0.86 |  |  | 1.41 | 1.00-1.99 |  |  | 1.33 | 0.78-2.28 |  |
| High |  | 045 | 0.35-0.59 |  |  | 1.76 | 1.21-2.55 |  |  | 2.64 | 1.53-4.55 |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Model 2 :Interleukin-6 assessed 5-y before baseline and at baseline** | | | | | | | | | | | | |
| Interleukin-6 assessed 5-y before baseline | | | | |  |  |  |  |  |  |  |  |
| Low |  | 1 | Ref |  |  | 1 | Ref |  |  | 1 | Ref |  |
| Intermediate. |  | 0.67 | 0.55-0.83 |  |  | 1.39 | 0.98-1.97 |  |  | 1.62 | 0.91-3.06 |  |
| High |  | 0.65 | 0.49-0.85 |  |  | 1.73 | 1.16-2.58 |  |  | 1.67 | 0.95-2.79 |  |
| Interleukin-6 assessed at baseline |  |  |  |  |  |  |  |  |  |  |  |  |
| Low |  | 1 | Ref |  |  | 1 | Ref |  |  | 1 | Ref |  |
| Intermediate |  | 0.79 | 0.64-0.97 |  |  | *1.28* | 0.90-1.81 |  |  | 1.18 | 0.67-2.05 |  |
| High |  | 0.53 | 0.41-0.71 |  |  | 1.46 | 0.98-2.17 |  |  | 2.24 | 1.26-3.98 |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Net Reclassification Improvement (NRI) between Model 1 and Model 2\*** |  | NRI | Standard Error | p |  | NRI† | Standard Error | p |  | NRI† | Standard Error | p |
|  | 0.236 | 0.040 | <.001 |  | 0.154 | 0.058 | 0.009 |  | 0.221 | 0.062 | 0.009 |

Analyses assessed the associations of inflammation with:

(1) successful aging (non-cases: normal aging phenotype, cardiovascular disease events and non-cardiovascular deaths combined), total n=3044

(2) cardiovascular disease event (non-cases: successful and normal aging phenotypes combined), total n=2897. The 147 participants with non-cardiovascular death were excluded.

(3) Non-cardiovascular death (non-cases: successful and normal aging phenotypes combined), total n=2723. The 321 participants with a cardiovascular disease event were excluded.

Results from logistic regression adjusted for sex, age, socio-economic status, smoking status, physical activity, acute inflammation and use of anti-inflammatory drugs

\* NRI, Net reclassification improvement index. Calculation of the NRI statistics [1, 2] provides with the difference in predictive ability when inflammation was assessed using 2 **Interleukin-6** measurements (both 5-y before baseline and at baseline) versus one **Interleukin-6** measurement (at baseline) by comparing the predicted risk levels among participants who developed a specific aging phenotype and those who did not.

**References**

1. Pencina, M.J., et al., *Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond.* Stat Med, 2008. **27**(2): p. 157-72.

2. Pencina, M.J., R.B. D'Agostino, Sr., and E.W. Steyerberg, *Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers.* Stat Med, 2011. **30**(1): p. 11-21.