Immunosenescence profiles are not associated with muscle strength, physical performance and sarcopenia risk in very old adults: The Newcastle 85+ Study

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\textbf{A B S T R A C T}

Decline in immune system function (immunosenescence) has been implicated in several age-related disorders. However, little is known about whether alteration in T-cell senescence, a process underlying immunological ageing, is related to muscle health in very old adults (aged ≥85 years). Using data from the Newcastle 85+ Study, we aimed to (a) derive and characterise immunosenescence profiles by clustering 13 baseline immunosenescence-related biomarkers of lymphocyte compartments in 657 participants; (b) explore the association between the profiles and 5-year change in muscle strength (grip strength) and physical performance (Timed Up-and-Go test), and (c) determine whether immunosenescence profiles predict 3-year incident sarcopenia. Two distinct clusters were identified; Cluster 1 (Senescent-like phenotype, \(n = 421\)), and Cluster 2 (Less senescent-like phenotype, \(n = 236\)) in individuals with complete biomarker data. Although Cluster 1 was characterised by T-cell senescence (e.g., higher frequency of CD4 and CD8 senescence-like effector memory cells), and elements of the immune risk profile (lower CD4/CD8 ratio, CMV+), it was not associated with change in muscle function over time, or with prevalent or incident sarcopenia. Future studies will determine whether more in-depth characterisation or change in T-cell phenotypes predict the decline in muscle health in late adulthood.

\textbf{1. Introduction}

A progressive loss of skeletal muscle strength and mass (sarcopenia) leads to impaired function, and increased risk of disability, frailty, and death in older adults (Cruz-Jentoft et al., 2019; Cruz-Jentoft and Sayer, 2019). Alterations in metabolic, hormonal and immune factors have been implicated in the pathogenesis of sarcopenia and age-related loss of muscle function (Cruz-Jentoft and Sayer, 2019; Wilson et al., 2017). Specifically, ageing of the immune system (immunosenescence) is an integral part of the intrinsic biology of ageing, characterised by accumulation of molecular and cellular damage, which results in the appearance of a diverse array of pathobiological hallmarks (López-Otín et al., 2013; Kirkwood, 2005). In this scenario, overlap among causal pathways might be revealed by links between sarcopenia and immunosenescence.

Although research about the role of immunosenescence in sarcopenia, independently or through inflammation, lags behind other mechanistic studies, the evidence linking immune system and skeletal muscle is growing (Nelke et al., 2016; Saini et al., 2017; Schiaffino et al., 2017; Wilson et al., 2017). With regard to immune factors, older adults experience profound change in immune system function due to systemic decline in both adaptive and innate immunity (Licastro et al., 2005; Le Page et al., 2018; Moro-García et al., 2013; Pawelec et al., 2016; Ventura et al., 2017), resulting in reduced ability to fight infections and tumours, and increased chronic low-grade inflammation (immflamaging) (Moro-García et al., 2013; Ventura et al., 2017). The main features of immunosenescence in adaptive immunity include reduced numbers of naïve T-cell lymphocytes due to thymic atrophy, and expansion of highly differentiated, antigen-specific T-cells, such as cytotoxic CD8⁺ and CD28⁻ T-cells (Le Page et al., 2018; Moro-
Garcia et al., 2013). These lymphocyte compartments, characterised by replicative senescence, accumulate with advancing age in healthy older adults, in those with autoimmune disorders, and in patients exposed to chronic viral infections, such as cytomegalovirus (CMV) (Pawelec et al., 2010). Seropositivity for CMV (CMV + ), an element of the ‘immune risk profile’ (Pawelec et al., 2010), has been recognised as one of the main drivers of highly differentiated (senescent) CD8 + T lymphocytes (Jergović et al., 2019). An inverted CD4:CD8 ratio of < 1, another element of the immune risk profile (Jergović et al., 2019), has been linked to a depleted naïve T cell pool and compromised immune response to new pathogens and vaccinations in older adults (Ventura et al., 2017; Xu and Larbi, 2017). Although less profound, another key feature of the ageing immune system is defects in the function of innate immune cells such as natural killer (NK) cells, neutrophils, macrophages, and dendritic cells (Ventura et al., 2017; Wilson et al., 2017).

The immunosenescence phenotype includes inflammingeaging, which is characterised by increased production of pro-inflammatory cytokines (e.g. interleukin 1β (IL-1β), IL-6, tumour necrosis factor α (TNF-α)) and other inflammatory markers by senescent cells and adipose tissue (Ventura et al., 2017; Wilson et al., 2017). This chronic low-grade inflammation is induced, in part, by constant antigen exposure (infections) and oxidative stress, and has been implicated in pathophysiology of several age-related diseases, including sarcopenia (Michaud et al., 2013; Ventura et al., 2017; Wilson et al., 2017). Recently, immunosenescence has been shown to play a role in age-related muscle atrophy, myofibre denervation and reduced regeneration upon injury (Saini et al., 2016; Schiaffino et al., 2017) in animal models. Also, skeletal muscle has been proposed as a regulator of the immune system, partly by secretion of myokines affecting the maintenance and regulation of immune cells and having pro-inflammatory and catabolic effects (reviewed in Nelke et al., 2019).

Studies that have investigated the link between markers of immunosenescence and physical functioning, sarcopenia and frailty in older adults are sparse, and yielded mixed results based on cross-sectional analyses of a few biomarkers. Lower lymphocyte count was independently associated with a higher risk of combined sarcopenia and frailty in Spanish older patients (aged 77.3 ± 8.4 years) recruited from hospitals and outpatient clinics with at least two chronic conditions at admission (Bernabé-Wittel et al., 2019). A negative correlation between the lymphocyte count and frailty, but a positive association between the lymphocyte count and hand grip strength was observed in Spanish older women (aged 84.2 ± 6.5 years) living in the community (Fernández-Garrido et al., 2014). In the Berlin Aging Study (BASE-II) study of older adults (aged 60-85 years), several immunosenescence-related biomarkers (CMV +, leukocyte telomere length, IL-6 levels) were not associated with grip strength (Goldbeck et al., 2016). In the Lothian Birth Cohort 1936 of older adults aged ≥70 years, CMV + was associated with smaller neck muscle cross-sectional area even after adjustment for IL-6 in men but not in women (Kilgour et al., 2013). A strong male-specific correlation was observed between the increased proportion of the CD27- IgD- B late memory cells and the physical capacity decline and frailty in a subcohort of The Vitality 90+ study of Finish nonagenarians (Nevalainen et al., 2019). In the BELFRAIL Study of community-dwelling older adults aged ≥80 years (the very old) from Belgium, a CD4:CD8 ratio > 5 was counterintuitively associated with impaired physical performance (Short Physical Performance Battery) in CMV + participants (Adriaensen et al., 2017).

Utilising longitudinal data from the Newcastle 85+ Study, we have described previously the trajectories of muscle strength and physical performance in very old adults (Granic et al., 2016; Granic et al., 2018), and investigated their relationship to potential determinants such as other biological markers, including inflammation and serum vitamin D (Granic et al., 2017a; Granic et al., 2017b). However to our knowledge, no prospective studies have investigated the association between immunosenescence profiles defined from a set of immunosenescence-related biomarkers and trajectories of muscle strength and physical performance, or risk of sarcopenia in very old adults. Therefore, employing data from the Newcastle 85+ Study, we aimed to: (a) derive immunosenescence profiles by clustering immunosenescence-related biomarkers of lymphocyte compartments and to characterise them in relation to socio-demographic, health, and lifestyle factors; (b) explore the association between the profiles, muscle strength (grip strength) and physical performance (Timed Up-and-Go test, TUG) decline over 5 years, and (c) determine whether immunosenescence profiles were associated with the risk of prevalent and 3-year incident sarcopenia in very old adults.

2. Materials and Methods

2.1. Study population

The Newcastle 85+ Study is a prospective, population-based study of very old adults living in Newcastle and Tyneside area, United Kingdom. The study aimed to investigate biological, psychological and social influences of ageing of individuals born in 1921 at baseline (2006/07) and their health trajectories over 5 years (1.5- (wave 2), 3- (wave 3), and 5-year (wave 4) follow-up). The study details have been described previously (Collerton et al., 2007; Collerton et al., 2009) and are available at http://research.ncl.ac.uk/85plus/. A complete multi-dimensional health assessment at baseline (wave 1), including the review of general practice records, was available for 845 participants. Of those, 657 (77.8%) had complete values for 13 immunosenescence-re related biomarkers of lymphocyte compartments to establish immunosenescence profiles comprising the analytic sample for the present study at baseline.

The study was approved by the Newcastle and North Tyneside 1 Research Ethics Committee, and conducted in accordance with the Declaration of Helsinki. A written informed consent was obtained from all participants; for those who lacked the capacity to consent, the consent was obtained from a relative or carer.

2.2. Blood-based biomarkers

Blood-based biomarkers from 749 participants were analysed in peripheral blood samples drawn between 7-10:30 am after an overnight fast (no drinks including coffee and tea, and food), and delivered to the laboratory (the Royal Victoria Infirmary, Newcastle upon Tyne, UK) for initial processing within 1 hour as described previously (Martin-Ruiz et al., 2011; Spyridopoulos et al., 2016). All blood samples were collected within 6 months post-baseline assessments, except for CMV serostatus (within 18 months) (Martin-Ruiz et al., 2011).

2.2.1. Lymphocyte compartments

Lymphocyte immunophenotyping and gating strategy has been described in detail previously (Martin-Ruiz et al., 2011; Spyridopoulos et al., 2016). Briefly, we used 4-colour flow-cytometry (Becton Dickson FACScan Flow Cytometer) and fluorescence-labelled antibodies (BD Bioscience, Oxford UK) to analyse blood samples. The marker combination to define lymphocyte compartments are described in Table A.1 (Appendix A). The senescence-like phenotype in T-cells was defined as the lack of CD27 and CD28 receptor expression in the CD4 subset (marker combination: CD4 + CD45RO CD27-CD28-) and the lack of CD45RD and CD27 expression in the CD8 T effector memory cells (TEMRA; marker combination: CD3 + CD8 + CD45RO CD27-), as previously described by us (Spyridopoulos et al., 2016). These marker combinations were regarded as appropriate markers of a senescence-like T-cells phenotype because of the presence of telomere dysfunction and reduced proliferation in the cells (discussed in Spyridopoulos et al., 2016). Lymphocyte compartments frequencies that were considered to establish immunosenescence profiles and the frequency of CD4 and CD8 and B cells ratios are reported in Table A.2.
2.2.2. Other biomarkers

For CMV seropositivity, CMV IgG concentration was determined using bioMerieux VIDAS (bioMerieux SA, France) fluorescent assay (sensitivity: 99.2%; specificity: 100%) and expressed in arbitrary units (AU < 4 for CMV-, and AU ≥ 6 for CMV+) (Spyridopoulos et al., 2016). C-reactive protein (CRP) was measured using Dade Behring CardioPhase high sensitivity CRP assay (Martin-Ruiz et al., 2011). Production of interleukin-6 (IL-6) was measured in supernatant of lipopolysaccharide-stimulated peripheral blood mononuclear cells (PBMC) by electrochemiluminescence as described (Martin-Ruiz et al., 2011). CMV seropositivity and a low CD4/CD8 ratio of < 1 have been regarded as elements of the immune risk profile, a simplified parameter of an ageing immune system.

2.3. Muscle strength and physical performance

2.3.1. Grip strength

Grip strength was measured using a hand-held dynamometer (Takei AS401 digital 0-100 kg x 0.1kgLCD) (Martin-Ruiz et al., 2011). In a standing position and with the elbows at approximately 180° angle, participants were instructed to squeeze the dynamometer as hard as possible alternating between the hands. Two measurements (in kg) for each hand were obtained and the maximum of four measurements for each participant (mean (M), standard deviation (SD)) was calculated (Roberts et al., 2011), and used in the analysis. In the analytic sample (n = 657), data to calculate the maximum grip strength was available for 646 (98.3%) participants at baseline (wave 1), 499 (76%) at wave 2, 380 (57.8%) at wave 3, and 254 (38.7%) at wave 4.

2.3.2. Timed Up-and-Go (TUG) test

Physical performance was assessed by the TUG test (Podsiadlo and Richardson1991). The time needed to get up from a chair (seat height 46 cm from the floor), walk in straight line for 3 m to and back from a marker placed on the floor, and sit back on the chair was recorded in seconds (s) with a stopwatch. Each participant performed the test only once and the use of walking aids (e.g. cane, walking frame, and wheeled walker) was documented at each wave. In the analytic sample, TUG data was available for 613 (93.3%) participants at baseline, 454 (69.1%) at wave 2, 339 (51.6%) at wave 3, and 231 (35.2%) at wave 4.

2.4. Sarcopenia

To establish the prevalence (wave 1 and wave 3) and incidence of sarcopenia (wave 3) in 657 participants, we used the revised European Working Group for Sarcopenia in Older People (EWGSOP 2) algorithm (Cruz-Jentoft et al., 2019; Cruz-Jentoft and Sayer, 2019). Sarcopenia was defined as the presence of both weak grip strength (< 27 kg in men, and < 16 kg in women) and low skeletal muscle index (SMI; skeletal muscle mass divided by height square, kg/m²) of < 8.87 kg/m² in men, and < 6.67 kg/m² in women (as described previously in Dodds et al., 2017 in this cohort). Complete data for prevalent sarcopenia in the analytic sample was available for 498 (91%) at baseline, and 332 (50.5%) at wave 3. The severity of sarcopenia was confirmed by the presence of low TUG (≥ 20 s). Complete data (grip strength, SMI, and TUG) to establish severe sarcopenia was available for 587 (89.3%) participants at baseline, and 320 (48.7%) at follow-up (wave 3). Body composition, including muscle mass was estimated with the Tanita-305 bioimpedance inbuilt algorithm (Tanita Corp., Tokyo, Japan).

2.5. Other measures and covariates

We used several socio-demographic, anthropometric, and health variables assessed at baseline to describe the immunosenescence profiles or to include them in multivariable analyses. The levels for all categorical variables are described in Table 1. Socio-demographic variable included sex, education, and social class coded to the National Statistics Socio-economic Classification (NS-SEC) system (Chandola and Jenkinson, 2000). Anthropometry included fat-free mass assessed by bioimpedance, height, and BMI. Other health-related variables were self-rated health compared with others of the same age, number of diseases reported from general practice records, cognitive impairment (Standardised Mini-mental State Examination, SMMSE), self-reported physical activity (Granic et al., 2019), and arthritis in hands (of any kind, including osteoarthritis, rheumatoid arthritis, other arthritis and non-specified arthritis in one or both hands). Overall attrition over 5-year follow-up (wave 2 to 4) was categorised as completing the study or not (due to mortality or withdrawal).

Self-reported physical activity was assessed with a validated physical activity questionnaire, and physical activity score (range 0-18) calculated as described previously (Granic et al., 2019). Briefly, from the frequency of each activity (i.e. ≥ 3 times/week (score 3); 1-2 times/week (score 2); 1-2 times/month (score 1), and hardly ever/never (score 0)) we created three outcome variables: very energetic, moderately energetic, and mildly energetic activities (score range of 0-3 for each). An overall physical activity score was derived using the following formula: (3 × very energetic activities score) + (2 × moderately energetic activities score) + (1 × mildly energetic activities score), and categorised into low (0-1), medium (2-6), and high (7-18).

3. Statistical analysis

3.1. Derivation and characteristics of immunosenescence profiles

Immunosenescence profiles were established in 657 participants with complete biomarker data at baseline describing lymphocyte compartments frequencies (analytic sample) using the SPSS Two Step clustering procedure (Table A.2). The best cluster solution was achieved with 13 immunosenescence-related biomarkers (i.e. Memory and Naïve B cells, Natural killer (NK) cells, NK T cells, total, Naïve and Memory CD4 and CD8 T cells, CD4 senescence-like effector memory cells (CD4TEMRA), Senescent Naïve CD4, and CD8 senescent-like effector memory (CD8TEMRA) cells) (Fig. 1, Supplementary Table 2). Based on the Two Step statistical parameter (importance factor ranging from 0 to 1), immunosenescence-related biomarkers contributing little to cluster separation were omitted (e.g. importance factor for Leukocytes, total B cells, CD4/CD8 ratio, CMV + was ≤ 0.001, indicating a negligible contribution to cluster separation). Non-normally distributed biomarkers (all except total and Naïve CD4 T, total CD8, and NK cells) were log10 transformed and CD4TEMRA was transformed as $\log_2$. We used automatic selection and the Bayesian Information Criterion (BIC) to determine the optimal cluster number and a Euclidean distance criterion for cluster separation. The robustness and stability of the final cluster solution was re-evaluated by random ordering of cases (4 times) and by comparing cluster characteristics.

To describe immunosenescence profiles’ characteristics (Table 1), we used a Student t-test to compare normally distributed continuous variables, a Mann-Whitney U test for non-normally distributed and ordered variables, and a Chi-square test for categorical variables at $\alpha < 0.05$.

3.2. Association between immunosenescence profiles and grip strength and Timed Up-and-Go test over 5 years

Using all available data in participants with established immunosenescence profiles (n = 657), we conducted multilevel linear modelling (West, 2009) to examine the association between the profiles (clusters) and grip strength and TUG initial level and rate of change over 5 years, and fitted the following linear growth curve models for grip strength and TUG separately. For grip strength, Model 1 was unadjusted and included ‘time’ (continuous (in years) to examine the linear trend of time in the study). Model 2 was adjusted for immunosenescence profiles (to test whether initial status (intercept)
Table 1
Characteristics of immunosenescence profiles in the Newcastle 85+ Study.

| Characteristics                      | All  | Cluster 1 'Senescent-like phenotype' | Cluster 2 'Less senescent-like phenotype' | P   |
|--------------------------------------|------|-------------------------------------|------------------------------------------|-----|
|                                      | % (n) | 657                                 | 64 (421)                                 | 36 (236) |
| **Sociodemographic**                 |      |                                     |                                          |     |
| Sex, % (n)                           |      |                                     |                                          |     |
| men                                  | 38.4 (252) | 67.9 (171)                          | 32.1 (81)                                | 0.11 |
| women                                | 61.6 (405) | 61.7 (250)                          | 38.3 (155)                               |     |
| Education, % (n)                     |      |                                     |                                          | 0.001 |
| 0-9 years                            | 63.0 (409) | 68.7 (281)                          | 31.3 (128)                               |     |
| 10-11 years                          | 24.3 (158) | 62.7 (99)                           | 37.3 (59)                                |     |
| ≥ 12 years                           | 12.6 (82)  | 47.6 (39)                           | 52.4 (43)                                |     |
| Occupational class, % (n)            |      |                                     |                                          | < 0.001 |
| routine/ manual                      | 50.8 (320) | 72.5 (232)                          | 27.5 (88)                                |     |
| intermediate                         | 14.9 (94)  | 59.6 (56)                           | 40.4 (38)                                |     |
| higher managerial/administrative     | 34.3 (216) | 53.7 (116)                          | 46.3 (100)                               |     |
| **Anthropometry**                    |      |                                     |                                          |     |
| fat-free mass (kg), M (SD)           | 45.2 (9) | 45.7 (9.4)                          | 44.3 (8.2)                               | 0.07 |
| height (cm), M (SD)                  | 161.4 (7.7) | 161.6 (7.7)                          | 161.2 (7.4)                              | 0.55 |
| BMI, % (n)                           |      |                                     |                                          | 0.32 |
| < 18.5 (underweight)                 | 6.9 (42) | 6.9 (27)                            | 6.8 (15)                                 |     |
| > 18.5 < 25 (normal)                 | 51.5 (316) | 48.9 (192)                          | 56.4 (124)                               |     |
| > 25 < 30 (overweight)               | 31.3 (192) | 33.1 (130)                          | 28.2 (62)                                |     |
| > 30 (obese)                         | 10.3 (63)  | 11.2 (44)                           | 8.6 (19)                                 |     |
| **Health variables**                 |      |                                     |                                          | 0.83 |
| Self-rated health, % (n)             |      |                                     |                                          |     |
| excellent/very good                  | 40 (259) | 63.6 (164)                          | 36.4 (94)                                |     |
| good                                 | 37.9 (245) | 63.7 (156)                          | 36.3 (89)                                |     |
| fair/poor                            | 22.1 (143) | 66.4 (95)                           | 33.6 (48)                                |     |
| Number of diseases, % (n)            |      |                                     |                                          | 0.4 |
| 0-1                                  | 28.9 (190) | 29.1 (123)                          | 28.4 (67)                                |     |
| 2                                    | 30.7 (202) | 29.0 (122)                          | 33.9 (80)                                |     |
| ≥ 3                                  | 40.3 (265) | 41.8 (176)                          | 37.7 (89)                                |     |
| Cognitive impairment, % (n)          |      |                                     |                                          | 0.91 |
| impaired (SMMSE 0-25)                | 27.3 (477) | 27.1 (114)                          | 27.5 (65)                                |     |
| normal (SMMSE 26-30)                 | 72.7 (477) | 72.9 (306)                          | 72.5 (171)                               |     |
| Self-reported physical activity, % (n) |      |                                     |                                          | 0.5 |
| low (score 0-1)                      | 21.6 (141) | 22.4 (94)                           | 20.1 (47)                                |     |
| medium (score 2-6)                   | 282 (43.1) | 41.4 (174)                          | 46.2 (108)                               |     |
| high (score 7-18)                    | 35.3 (231) | 36.2 (152)                          | 33.8 (79)                                |     |
| Arthritis in hands, % (n)            |      |                                     |                                          | 0.8 |
| no                                   | 92.9 (600) | 65 (390)                            | 35 (210)                                 |     |
| yes                                  | 7.1 (46)  | 52.2 (24)                           | 47.8 (22)                                |     |
| Use of walking aids (baseline), % (n)|      |                                     |                                          | 0.9 |
| no                                   | 82.5 (506) | 64.2 (325)                          | 35.8 (181)                               |     |
| yes                                  | 17.5 (107) | 36.8 (68)                           | 36.4 (39)                                |     |
| Overall attrition over 5 years, % (n)|      |                                     |                                          | 0.05 |
| completed the study                  | 43.7 (287) | 59.9 (172)                          | 40.1 (115)                               |     |
| dropped out                          | 56.3 (370) | 67.3 (249)                          | 32.7 (121)                               |     |
| **Sarcopenia components (baseline), M (SD)** |      |                                     |                                          |     |
| Grip strength (kg), men/women        | 27.5 (6.9)/ 15.5 (4.7) | 27.2 (6.6)/ 15.5 (4.4) | 28.1 (7.6)/ 15.4 (5.1) | 0.39/ 0.75 |
| SMI (kg/m²), men/women               | 9.9 (1.9)/ 7.7 (2.3) | 9.9 (2.1)/ 7.8 (2.3) | 9.92 (1.65)/ 7.6 (2.13) | 0.57/ 0.61 |
| TUG (s)                              | 18.3 (14)  | 19 (51)                             | 17.1 (10.8)                              | 0.26 |
| **Sarcopenia components (wave 3), M (SD)** |      |                                     |                                          |     |
| Grip strength (kg), men/women        | 25.8 (6.9)/ 14.8 (4.6) | 25.4 (7.5)/ 14.9 (4.7) | 26.6 (5.4)/ 14.7 (4.5) | 0.05/ 0.78 |
| SMI (kg/m²), men/women               | 10.4 (2.4)/ 7.9 (2.1) | 10.4 (2.6)/ 8.0 (2.2) | 10.5 (1.8)/ 7.8 (2.0) | 0.36/ 0.6 |
| TUG (s)                              | 21.4 (17.5) | 21.9 (20)                           | 20.5 (12)                                | 0.97 |
| **Sarcopenia, yes % (n)**            |      |                                     |                                          |     |
| prevalent (baseline)                 | 18.1 (108) | 66.7 (72)                           | 33.3 (36)                                | 0.48 |
| severe prevalent                     | 4.9 (29)  | 62.1 (18)                           | 37.9 (11)                                | 0.87 |
| prevalent (wave 3)                   | 15.4 (51) | 68.6 (35)                           | 31.4 (16)                                | 0.2 |
| severe prevalent (wave 3)            | 5 (16)  | 56.3 (9)                            | 43.8 (7)                                 | 0.69 |

(continued on next page)
used by clusters), and included an interaction of clusters and ‘time’ to test for varying rates of change in grip strength by immunosenescence profiles. Model 3 was further adjusted for a set of covariates previously shown to be significantly associated with muscle strength and physical performance in this cohort (Granic et al., 2016; Granic et al., 2017a; Granic et al., 2017b; Granic et al., 2019): sex, self-rated health, disease count, cognitive status, physical activity, sex-specific fat-free mass, sex-specific height, and overall attrition (Table 2). We used the same models for TUG, except Model 1 also tested for non-liner trend of time (time2). Model 3 was further adjusted for arthritis in hands for grip strength, and walking aids for TUG test. All covariates were time-invariant variables (assessed at baseline), except for use of walking aids (assessed at each wave). Random effects included random slopes and intercepts. Negative (declining) β estimates for grip strength, and positive (increasing) β estimates for TUG indicated poor performance. The SPSS MIXED procedure (SPSS, IBM Corporation, Armonk, NY, USA), with restricted maximum likelihood method and a scaled identity matrix (id) at Level 1 (within-group change), and unstructured (UN) covariance matrix at Level 2 (between-group change) were used to generate parameter estimates (β) for effects.

### 3.3. Association between immunosenescence profiles and sarcopenia

We used logistic regression (odds ratio (OR) 95% CI) to explore the association between prevalent sarcopenia (baseline and 3-year follow up) and 3-year incident sarcopenia. Model 1 was unadjusted, and Model 2 was adjusted for sociodemographic factors (social class, education), BMI, and cognitive status (Table 3). These covariates were significantly associated with sarcopenia (defined with EWGSOP1 definition) in this cohort as described previously (Dodds et al., 2017).

### 3.4. Sensitivity analyses

Participants with and without immunosenescent profiles were compared across key descriptive variables included in Table 1 (details not shown). In multivariable sensitivity analysis, we explored the association between individual immunosenescent-related biomarkers and grip strength and TUG test (linear mixed models), and sarcopenia (logistic regression models). We reported selected results in Table A.3 and Table A.4 (Appendix A).

### 4. Results

#### 4.1. Characteristics of immunosenescence profiles

Except for self-reported physical activity (p = 0.03) and attrition (p = 0.001), participants with complete data to establish immunosenescence profiles (n = 657) did not differ across a range of descriptive variables from those who missed baseline data for cluster derivation (n = 188, 22.2%) (details not shown).

We identified two distinct immunosenescence profiles: Cluster 1 (‘Senescent-like phenotype’, n = 421), and Cluster 2 (‘Less senescent-like phenotype’, n = 236) (Fig. 1) that differ by socio-demographic factors, immune risk ratios, and inflammatory biomarkers (Table 1, Table A.2). Eight immunosenescence-related biomarkers contributed the most to cluster separation, notably (in order of importance from bottom-up) Memory CD4, NKT, total CD8 T, CD8TEMRA, CD4TEMRA, Memory CD8 T, total CD4 T, and Naïve CD4 T-cells (Fig. 1).

Specifically, Memory CD4 cells contributed the most (Two Step importance factor of 1), and NK cells contributed the least to cluster separation (Two Step importance factors of 0.01) (range 0-1; Table A.2).

Participants in Cluster 1 (‘Senescent-like phenotype’) had higher frequency of Memory CD4, NKT, total CD8 T, CD8TEMRA, CD4TEMRA, Memory CD8 T-cells (all p < 0.001), and lower frequency of total CD4 and Naïve CD4 T-cells (both p < 0.001) compared to participants in Cluster 2 (‘Less senescent-like phenotype’) (Table A.2). They also had lower CD4/CD8 ratio, but higher Memory CD4/Naïve CD4 and Memory CD8 T/Naïve CD8 T ratios, were more likely to be CMV+ (all p < 0.001), had higher CRP (p = 0.04) and stimulated IL-6 (p = 0.01) concentrations (Table A.2). They were less educated, and belonged to a lower social class (both p < 0.001), but did not differ on any other variables.

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**Table 1 (continued)**

| Characteristics | All | Cluster 1 | Cluster 2 | p |
|-----------------|-----|-----------|-----------|---|
| % (n)           | 657 | 64 (421)  | 36 (236)  | .51|
| incident cases (wave 3) | 7.4 (24) | 66.7 (16) | 33.3 (8) | .51 |

BMI, body mass index; M, mean; SMI, skeletal muscle index; SMMSE, Standardised Mini-mental State Examination; SD, standard deviation; TUG, Timed Up-and-Go Test.

* Sarcopenia was determined based on grip strength and skeletal muscle index (SMI) sex-specific cut-offs (Dodds et al., 2017), and sarcopenia severity confirmed by the presence of low Timed Up-and-Go test (TUG) (Cruz-Jentoft et al., 2019).

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36%; grey pie chart and bars) had higher frequency of total CD4 T and Naïve CD4 T-cells (both p < 0.001). Error bars represent standard deviation of the mean (SEM). *** p < 0.001; ** p < 0.005.
Table 2

β estimates of mixed models for muscle strength (grip strength) and physical performance (Timed Up-and-Go test) decline by immunosenesence profiles* over 5 years in the Newcastle 85+ Study.

| Aspect | Effect | Model 1 | Model 2 | Model 3 |
|--------|--------|---------|---------|---------|
|        |        | β (SE)  | p      | β (SE)  | p     | β (SE)  | p     |
|        |        |         |        |         |        |         |        |
| Grip strength | Time | −0.20 (0.4) | 0.001 | −0.17 (0.09) | 0.08 | −0.18 (0.1) | 0.06 |
|         | Cluster 1 | 0.45 (0.83) | 0.59 | 0.35 (0.54) | 0.51 |
|         | Cluster 2 (ref) | 0 | 0 | 0 | 0 |
|         | Slope | −0.06 (0.12) | 0.61 | −0.07 (0.12) | 0.55 |
|         | Cluster 1 × Time | 0 | 0 | 0 | 0 |
|         | Cluster 2 × Time (ref) | −0.06 (0.12) | 0.61 | −0.07 (0.12) | 0.55 |
| TUG | Time | 0.06 (0.01) | < 0.001 | 0.06 (0.001) | < 0.001 | 0.07 (0.01) | < 0.001 |
|        | Time² | −0.01 (0.001) | < 0.001 | −0.01 (0.001) | < 0.001 | −0.01 (0.001) | < 0.001 |
|         | Cluster 1 | 0.03 (0.02) | 0.14 | 0.02 (0.01) | 0.12 |
|         | Cluster 2 (ref) | 0 | 0 | 0 | 0 |
|         | Slope | −0.003 (0.01) | 0.51 | −0.004 (0.004) | 0.28 |
|         | Cluster 1 × Time | 0 | 0 | 0 | 0 |
|         | Cluster 2 × Time (ref) | −0.003 (0.01) | 0.51 | −0.004 (0.004) | 0.28 |

TUG, Timed Up-and-Go test; ref, reference. Model 1 was unadjusted and included linear (Time) and quadratic (Time²) trend of time (for TUG), random slopes and intercept. Model 2 was further adjusted for immunosenesence profiles and interaction terms (Cluster × Time). Model 3 was further adjusted for sex, self-rated health, disease count, cognitive status, physical activity, sex-specific fat-free mass, sex-specific height, and overall attrition. Grip strength model also included the presence of arthritis in hands, and TUG test model use of walking aids.

* Immunosenesence profiles: Cluster 1 (‘Senescent-like phenotype’), Cluster 2 (‘Less senescent-like phenotype’), Cluster 4 (‘Less senescence-like phenotype’), Cluster 5 (‘Senescence-like phenotype’).

Table 3

Association between immunosenesence profiles and prevalent and incident sarcopenia in the Newcastle 85+ Study.

| Event | Model 1 | p | Model 2 | p |
|-------|---------|---|---------|---|
| Prevalent sarcopenia (wave 1) | OR (95% CI) | 0.48 | OR (95% CI) | 0.28 |
| Cluster 1 | 1.17 (0.75-1.82) | 1.31 (0.80-2.13) | |
| Cluster 2 (ref) | 1 | 1 | |
| Prevalent sarcopenia (wave 3) | OR (95% CI) | 0.2 | OR (95% CI) | 0.09 |
| Cluster 1 | 1.52 (0.80-2.87) | 1.80 (0.91-3.59) | |
| Cluster 2 (ref) | 1 | 1 | |
| Incident sarcopenia (wave 3) | OR (95% CI) | 0.51 | OR (95% CI) | 0.41 |
| Cluster 1 | 1.34 (0.56-3.24) | 1.45 (0.59-3.65) | |
| Cluster 2 (ref) | 1 | 1 | |

ref, reference; Cluster 1 (‘Senescent-like phenotype’), Cluster 2 (‘Less senescence-like phenotype’). Model 1 is unadjusted. Model 2 is adjusted for education, socio-economic status, BMI, and cognitive impairment.

4.2. Change in muscle strength (grip strength) and physical performance (Timed Up-and-Go test) over 5 years across immunosenesence profiles

Table 2 presents β estimates of mixed effect models for grip strength and TUG change over time in 657 participants with established immunosenesence profiles. In the unadjusted model (Model 1), there was a significant fixed effect of time on grip strength (−0.2 kg decline / year; p = 0.01), and TUG (a loss of 0.06 log₁₀ s/ year; p < 0.001) with a small deceleration over time (Time²: −0.01 log₁₀ s/ year; p < 0.001). In all adjusted models, Cluster 1 (‘Senescent-like phenotype’) was not associated with baseline grip strength or TUG and their change over time compared with Cluster 2 (‘Less senescent-like phenotype’) (e.g. Model 3: grip strength in all participants β (SE) = −0.35 (0.54), p = 0.51, and TUG β (SE) = 0.02 (0.01), p = 0.12). Trajectories of muscle function (grip strength and TUG) by immunosenesence profiles (Model 3) over 5 years are presented in Fig. 2, showing an overall decline over time, but no differences in change in either grip strength or TUG across the profiles. Also, in supplementary analyses, we found no association between individual immunosenesence-related biomarkers, CD4/CD8 ratio (continuous and categorised), and CMV seropositivity and muscle strength and physical performance in the very old (Table A.3).

4.3. Baseline and 3-year prevalent and incident sarcopenia across immunosenesence profiles

A flow diagram of participants in the analytic sample with data available to establish prevalent and incident sarcopenia and those lost to follow-up over 3 years is presented in Fig. 3. Briefly, of 598 participants with an immunosenesence profile and complete data (grip strength, skeletal muscle index) to establish prevalent sarcopenia, 108 (18.1%; approximately 1 in 5) had sarcopenia at baseline. Of those, 29 had severe sarcopenia confirmed by low TUG test. Two hundred and fifty-five participants were lost to 3-year follow-up (67.1% due to mortality), and 332 had data to establish prevalent sarcopenia at wave 3. Of those, 51 (15.4%) had sarcopenia, and 24 (47.1%) were incident cases. Interestingly, 252 (38.4% of the analytic sample) participants stayed sarcopenia free over 3 years.

Although there were twice as many prevalent cases of sarcopenia (both at baseline and 3-year follow-up) and incident cases of sarcopenia in Cluster 1 (‘Senescent-like phenotype’) compared with Cluster 2 (‘Less senescent phenotype’), these differences were not statistically significant (Table 1). In addition, although the OR were raised in unadjusted and adjusted logistic regression models, we found no statistically significant associations between immunosenesence profiles and sarcopenia. Specifically, being in Cluster 1 was not associated with the risk of prevalent sarcopenia at baseline (OR [95% CI]: 1.31 [0.80-2.13], p = 0.28) and 3-year follow-up (1.80 [0.91-3.59], p = 0.09), and did not predict incident sarcopenia (1.45 [0.95-3.65], p = 0.41) in Model 2 adjusted for education, socio-economic status, BMI, and cognitive impairment (Table 3). Individual immunosenesence-related biomarkers were not associated with either prevalent or incident sarcopenia (Table A.4).
5. Discussion

We analysed 5-year change in muscle strength (grip strength) and physical performance (TUG) and 3-year incident sarcopenia in 657 very old adults from the Newcastle 85+ study in relation to immunosenescence profiles derived from 13 immunosenescence-related biomarkers of lymphocyte compartments. Participants with the ‘Senescent-like phenotype’ characterised by several indicators of immunosenescence (e.g. higher frequency of CD4 and CD8 effector memory cells with a senescence-like phenotype, CMV+, and depleted Naive CD4 T-cells pool) and with the ‘Less senescent-like phenotype’ experienced similar decline in grip strength and TUG over time. Furthermore, the ‘Senescent-like phenotype’ was not associated with prevalent and incident sarcopenia. To the best of our knowledge, this is the first prospective study to investigate the role of immunosenescence, defined a posteriori from multiple biomarkers in human peripheral blood, on muscle health and function in very old adults.

Current understanding about how immunosenescence influences skeletal muscle function with ageing is inconclusive and limited to cross-sectional associations between a selected number of individual immunosenescence biomarkers and measures of muscle mass, strength, and function (Adriaensen et al., 2017; Bernabeu-Wittel et al., 2019; Fernández-Garrido et al., 2014; Goldeck et al., 2016; Kilgour et al., 2013; Nevalainen et al., 2019). These studies have based their rationale for the biomarker inclusion on previous research showing a positive association between immunosenescence and poor health (e.g. coronary heart disease, cognitive decline, and mortality) (Spyridopoulos et al., 2009; Spyridopoulos et al., 2016), and mechanistic hypotheses linking immunosenescence, inflammation and oxidative stress (Jergović et al., 2019; Ventura et al., 2017; Wilson et al., 2017) through either mediating effects of chronic antigen exposure (e.g. CMV+) (Adriaensen et al., 2017; Goldeck et al., 2016; Kilgour et al., 2013) or independently (Bernabeu-Wittel et al., 2019; Fernández-Garrido et al., 2014). In addition, a few studies have reported the association between an inverted CD4:CD8 ratio of < 1, an element of the immune risk profile explained by a higher proportion of late-differentiated memory CD8+ T-cells and CMV+, and poor physical functioning in older adults (Moro-García et al., 2012), but not in the very old (Adriaensen et al., 2017; Formiga et al., 2011). Instead of using a single biomarker approach, we derived a composite measure of immunosenescence by clustering individuals into two distinct, non-overlapping groups based on their unique immunosenescence profiles derived from a set of blood biomarkers of lymphocyte compartments. A ‘Senescence-like phenotype’ (Cluster 1) did not differ from a ‘Less senescent phenotype’ (Cluster 2) across a range of health and lifestyle factors, and was not associated with sarcopenia and muscle function. Interestingly, those in Cluster 1 had lower socioeconomic status compared to those in Cluster 2, suggesting a long-term negative effect of social class on physiology and health (discussed in Cavigelli and Chaudhry, 2012). In sensitivity analyses, we also found no association between individual immunosenescence biomarkers and muscle health, including those contributing to the immune risk profile (CMV + and CD4:CD8 ratio of < 1).

There may be several reasons for these negative findings. First,
Biomarkers associated with an increased risk of adverse health in young old (aged 65-74 years) may become less detrimental, neutral or protective in very old adults (aged ≥85 years). For example, having elements of immunosenescence such as a lower proportion of naïve CD8+ T-cells and a higher proportion of memory cells was associated with longer survival over 8 years in the Leiden 85+ study (Derhovanessian et al., 2013), which the authors proposed contributed to better immunesurveillance by non-senescence effector memory cells and control of antigens reactivation such CMV.

Second, the ubiquity of CMV infection in the very old and associated immunomodelling in the lymphocyte subsets (e.g. expansion of late-differentiated CD4+ and CD8+ T-cells, CD4:CD8 ratio inversion) may provide a survival and functioning advantage in later life. As in the Leiden 85+ population (Derhovanessian et al., 2013) and other cohorts of the very old (Matheï et al., 2015), CMV seropositivity was high in the Newcastle 85+ Study (641 individuals were CMV+ or 85.6% of those tested). Equally, the proportion of the octogenarians with an inverted CD4:CD8 ratio (< 1) was comparable between our and other

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![Flow diagram of participants with immunosenescence profile and available data to establish prevalent and incident sarcopenia over 3-year follow-up in the Newcastle 85+ Study. At baseline, 598 (91%) participants with immunosenescence profile had complete data to establish prevalent sarcopenia (grip strength and muscle mass) based on the European Working Group for Sarcopenia in Older People (EWGSOP 2) algorithm (Cruz-Jentoft et al., 2019). Of those, 108 (18.1%) had sarcopenia (light-grey box). At wave 3 (3-year follow-up), 332 participants had data to establish sarcopenia. Of those, 51 (15.4%) had sarcopenia (light-grey box), and 24 were incident cases (dark grey box). Loss to follow-up was mostly due to mortality.](image-url)
populations (i.e. 15% in OCTO longitudinal Swedish study of 85-year olds (Wikby et al., 1998), and 17% in the Newcastle 85+ ). The inverted ratio was not associated with increased mortality in octogenarians from the OCTABAIX immune study (Formiga et al., 2014), and was no longer present in 90 and 100-year olds in the Swedish NONA immune longitudinal study (Strindhall et al., 2007), suggesting selection against those with the risk factor at younger ages. Previously, we have shown in the Newcastle 85+ Study that CMV seropositivity and a low CD4:CD8 ratio were not significant predictors of all-cause mortality (Spyridopoulos et al., 2016) and frailty (Collerton et al., 2012), but CMV (although not the CD4:CD8 ratio) and senescence in the CD4 and CD8 T-cells compartments were predictors of cardiovascular mortality and deaths from myocardial infarctions and stroke (Spyridopoulos et al., 2016).

However, there is a possibility that the expansion of non-senescent effector T-cells and the immunosurveillance mediated by other lymphocyte compartments may prevent antigen reactivation and subsequent inflammatory response, which has been shown to negatively affect muscle function in the very old (Granic et al., 2017b). Indeed, a recent in-depth phenotyping of T-cell compartments using a 8-colour flow-cytometry assay in a subcohort of the Newcastle 85+ Study participants has revealed that a high proportion of CD27- CD28+ CD8 effector memory cells (CD8TEMRA) was associated with a reduced risk of all-cause mortality and cardiovascular and non-cardiovascular diseases independently of CMV serostatus and other risk factors (Martín-Ruiz et al., 2020). Future studies in this cohort will determine whether immunosenescence profiles derived from a more in-depth description of circulating T-cell compartments will corroborate the null findings for sarcopenia.

Third, all biomarkers were evaluated at baseline and were based on the 4-colour flow cytometric protocol. To model the effect of immunosenescence on change in muscle strength (grip strength) and physical performance (TUG) over time, the change in immunosenescence profiles and individual immunosenescence biomarkers may be a more suitable measure of exposure. Also, the use of a more comprehensive assay to characterise T-cell phenotypes may minimise any misclassification of the exposure, and provide better insights into the relationship between immunosenescence and muscle health in later life.

Lastly, as reported in other cohorts of the very old, about 40% of the participants were lost to follow-up by wave 3 mostly due to mortality, thus the analyses may have been underpowered to detect significant associations. Although those who were lost to follow-up were not more likely to belong to a ‘Senescence-like phenotype’, they were more likely to have severe sarcopenia at baseline (details not shown) compared to participants with a ‘Less senescence-like phenotype’, suggesting survival bias and more robust individuals completing the study. In addition, 252 (99 men and 153 women; 38% of the analytic sample) stayed sarcopenia-free over 3 years, indicating the presence of a subcohort of very robust individuals.

5.1. Strengths and limitations

The strengths of our study include its longitudinal design, size, a single year birth cohort controlling for the effect of age, inclusion of participants living in institutions and those with cognitive impairments, and a range of immunosenescence biomarkers, muscle health measures, and covariates included in the prospective analyses. The study has several limitations. The lack of associations could be due to the limitations in our outcome measures (e.g. grip strength as a measure of muscle strength; use of bioimpedance to estimate muscle mass) or in the biomarker selection. Other sarcopenia-associated biomarkers (e.g., myostatin), not available in this cohort, may have been more relevant. Although we evaluated the robustness of the cluster solution, the associations may have been affected by the choice of available immunosenescence biomarkers. The Two Step procedure is an a posteriori, exploratory technique dependent on data at hand and statistical parameters (such as importance factor) for the inclusion of biomarkers, which may exclude biologically relevant biomarkers. We used a 4-colour flow-cytometry assay to describe T-cell compartments, which may be limited in adequately distinguishing between senescent and non-senescent memory cells (Prlic et al., 2012). There is limited comparison with other studies, and the results need to be repeated in other cohorts involving very old adults. We used a composite measure of immunosenescence, which should not undermine the utility of the individual biomarker approach. The results have limited generalisability to non-White very old populations.

5.2. Conclusions

We used prospective data to investigate the association between immunosenescence profiles derived from 13 biomarkers of lymphocyte compartments, grip strength, physical performance and incident sarcopenia in the Newcastle 85+ Study. We found no association between immunosenescence and muscle health in very old adults. Cluster 1 (‘Senescence-like phenotype’) characterised by T-cell senescence and elements of the immune risk profile (e.g., CMV+) was not significantly associated with muscle strength (grip strength), physical performance (TUG) and incident sarcopenia compared with Cluster 2 (‘Less senescence-like phenotype’) in very old adults. Further studies from this and other larger cohorts are needed to determine whether more in-depth characterisation of T-cell phenotypes and change in immunosenescence biomarkers predicts the decline in muscle health and function in late adulthood.

6. Authors’ contributions

AG contributed to: conceptualisation of the manuscript design, research goals and aims; formal analysis and conduction of the research; methodology; validation of the results; writing and original draft preparation; writing review and editing; visualisation. CM-R contributed to: The Newcastle 85+ Study biomarker research aims and goals; design of flow cytometric experiments; performed and supervised flow cytometric experiments and analysed flow cytometric data; critical review of the manuscript for intellectual/scientific content; validation of the results. RMD contributed to: critical review of the manuscript for intellectual/scientific content; validation and interpretation of the results. TBLK and TvZ contributed to: The Newcastle 85+ Study design, research goal and aims, funding, resources acquisition and provision; conceptualisation of the manuscript design, aims and goals; critical review of the manuscript for intellectual/scientific content. LR (The Newcastle 85+ Study PI) and KS contributed to critical review of the manuscript for scientific content and the results interpretation. AAS contributed to: the manuscript research goals and aims; critical review of the manuscript for intellectual/scientific content. All authors read and approved to the final manuscript.

7. Ethics approval and consent to participate

The study was approved by the Newcastle & North Tyneside Local Research Ethics Committee 1 (Ref: 06/Q0905/2). Signed informed consent was obtained from each participant. For those lacking capacity, the consent was sought from their consultee or carer.

8. Availability of data and materials

The datasets used and/or analysed for the current study are available from the Newcastle 85+ Study Data Guardians led and chaired by Professor Carol Jagger (carol.jagger@newcastle.ac.uk) on reasonable request through the study website (http://research.ncl.ac.uk/85plus). The data analysis code underpinning this publication has been archived in the Newcastle University Data Repository (https://data.ncl.ac.uk/) at
https://doi.org/10.25405/data.ncl.12251231.

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Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:http://dx.doi.org/10.1016/j.jad.2020.111321.

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