Immunosenescence profiles of lymphocyte compartments and multiple long-term conditions (multimorbidity) in very old adults: The Newcastle 85+ Study

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ABSTRACT

Immunosenescence, a decline in immune system function, has been linked to several age-related diseases and ageing syndromes. Very old adults (aged ≥ 85 years) live with multiple long-term conditions (MLTC, also known as multimorbidity)—a complex phenomenon of poor health defined by either counts, indices, or patterns, but little is known about the relationship between an ageing immune system and MLTC in this age group. We utilised baseline data from the Newcastle 85+ Study to investigate the associations between previously defined immunosenescence profiles of lymphocyte compartments and MLTC counts and patterns (from 16 chronic diseases/ageing syndromes). Seven hundred and three participants had MLTC and complete data for all 16 conditions, a median and mean of 5 (range 2–11) and 62.2% had ≥ 5 conditions. Three distinct MLTC patterns emerged by clustering: Cluster 1 (‘Low frequency cardiometabolic-cerebrovascular diseases’, n = 209), Cluster 2 (‘High ageing syndromes-arthritis’, n = 240), and Cluster 3 (‘Hypertensive-renal impairment’, n = 254). Although having a more senescent phenotype, characterised by higher frequency of CD4 and CD8 senescence-like effector memory cells and lower CD4/CD8 ratio, was not associated with MLTC compared with less senescent phenotype, the results warrant further investigation, including whether immunosenescence drives change in MLTC and influences MLTC severity in late adulthood.

1. Introduction

Ageing is associated with morphological and physiological changes at the cellular, organ, and system level in the human body, including the immune system, which lead to functional decline, disease, and death. Age-related alterations in the immune system, also known as immunosenescence, are multifaceted and involve both innate and adaptive immunity (reviewed in Sadighi Akha, 2018; Castelo-Branco and Soveral, 2014; Nikolich-Zugich, 2018, Pawelec, 2018, Weiskopf et al., 2009), with changes in the latter being most profound, particularly in T lymphocytes (T cells) (Akbar et al., 2016; Le Page et al., 2018; Maue et al., 2009; Xu and Larbi, 2017). Several characteristics of age-associated decline in adaptive immunity have been described in the literature, including marked reduction in naïve T- and B cells in the circulation, increase in memory cells (Sadigh Akha, 2018; Nikolich-Zugich, 2018; Pawelec, 2018), and inflammaging—a low-grade chronic inflammation—recognised as a hallmark of ageing and underlying several age-related diseases (reviewed in Fülöp et al., 2021, Santoro et al., 2022).

Abbreviations: CMV, cytomegalovirus; IF, importance factor; MLTC, multiple long-term conditions; PCA, principal component analysis.

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Briefly, there is a decrease in peripheral naïve T and B cells with ageing partially due to thymic involution and the shrinkage of their diversity and clonal distribution after the age of 50 years (reviewed in Thomas et al., 2020). Life-long antigen exposure results in increase of a sub-population of T cells with a late-differentiated phenotype, such as late-differentiated CD4⁺ and CD8⁺ effector memory cells that harbour replicative senescence and production of pro-inflammatory cytokines (Akbar et al., 2016; Le Page et al., 2018; Nikolich-Zugich, 2018). Specifically, T cells reactive to cytomegalovirus (CMV) dominate the repertoire of memory T cells in older adults contributing to decreased memory compartments diversity, and accumulation of differently differentiated CD8⁺ effector memory cells with low expression of CD28 co-stimulatory molecule (with or without CD27 expression) and shorter telomeres (reviewed in Aiello et al., 2019; Pawelec, 2018; Weiskopf et al., 2009). There is also a decrease in the number and diversity of circulating B cells, and an impaired ability to produce high affinity protective antibodies (Sadighi Akha, 2018; Nikolich-Zugich, 2018; Pawelec, 2018). These changes in quality and quantity of T and B cells result in an inadequate immune response to novel pathogens, poor response to vaccination, increased risk of infections and age-related diseases such as neurodegenerative, cardiovascular (atherosclerosis), renal, and metabolic diseases, and cancers (Barbè-Tuana et al., 2020; Fülöp et al., 2016). Additionally, studies in Swedish octo- and nonagenarians have defined an immune risk profile characterised by an inverted CD4/CD8 ratio, CMV seropositivity, and severe reduction of B cells to be associated with higher mortality (reviewed in Pawelec, 2018).

Advancing age is a major risk factor for higher prevalence and incidence of multiple long-term conditions (MLTC, also known as multimorbidity) in the population (hereafter MLTC) (Kudesia et al., 2021; Marengoni et al., 2011; Xu et al., 2017). MLTC is a complex phenomenon of compromised state of health with ageing defined commonly as a presence of ≥2 coexisting chronic diseases, conditions, and somatic risk factors (by counts) in one person (WHO, 2015; Calderón-Larrañaga et al., 2017; Sasseville et al., 2019) from a list of at least 12 frequent diagnoses encountered in primary care (Fortin et al., 2012). The WHO definition is the commonest and simplest approach in defining MLTC in epidemiological studies (Xu et al., 2017). Additionally, MLTC indices (or weighted counts) are used to account for disease severity (Diederichs et al., 2011; Stirland et al., 2020), whilst MLTC patterns are generated via data reduction statistical methods to understand diseases groupings (Foguet-Boreu et al., 2015; Prados-Torres et al., 2014; Rajoo et al., 2021; Vetranio et al., 2020).

Very old adults (defined as either aged 80 years and over (≥80) or 85 years and over (≥85)) are the fastest growing group in many societies (United Nations, 2019). Living with MLTC is a norm for this age group ( Barnett et al., 2012; Salive, 2013), and despite the differences in operationalisation of MLTC (e.g., the number and type of diseases/conditions included) across studies, the overall prevalence of MLTC in the very old is high, ranging from 55% to 98% (Marengoni et al., 2011), with significant complexity in patterns (Collerton et al., 2016; Dong et al., 2015; Foguet-Boreu et al., 2015). Additionally, MLTC often overlaps with frailty, polypharmacy, and functional impairment in an older person due to the widespread deficit accumulations raising the need for a tailored approach to healthcare (Yarnall et al., 2017).

Although a number of studies have investigated the role of T cell senescence in several age-related diseases that share similar patholog- ical mechanisms such as inflamming (Barbè-Tuana et al., 2020; Chen et al., 2020; Fülöp et al., 2016; Santoro et al., 2021), studies that link MLTC defined in terms of counts, indices, and patterns with multiple immune biomarkers are lacking. Understanding the role of age-associated decline in immune function in MLTC could help find solutions for their prevention and treatment (Aiello et al., 2019; Duggal et al., 2019; Weyand and Goronzy, 2016).

To our knowledge, no studies have investigated the association between immunosenescence profiles determined from a set of immune biomarkers and MLTC in the very old. Utilisation of data reduction techniques (e.g., clustering) allows for the detection of natural groupings (clusters or patterns) in the data that would otherwise not be obvious and permits the derivation of summary variables underlying a common construct (e.g., immunosenescence profiles) (Appendix A).

Therefore, the aim of this study was to investigate the association between the summary variables characterising senescence in lymphocyte compartments (i.e., previously defined immunosenescence profiles) (Granic et al., 2020) and MLTC defined using counts (≥2 chronic diseases and ageing syndromes) and patterns utilising baseline data from the Newcastle 85+ Study.

2. Materials and Methods

2.1. Study population and MLTC sample

The Newcastle 85+ Study is a longitudinal, population-based study of very old adults living in Newcastle and the Tyneside area, United Kingdom. The study aimed to explore biological, psychological and social influences of ageing of individuals born in 1921 at baseline (2006/07) and their health trajectories over 5 years. The study has been described in detail previously (Collerton et al., 2007, 2009) and further information is available at http://research.ncl.ac.uk/85plus/. A complete multidimensional health assessment, including the review of general practice records (i.e., diseases, medication, use of healthcare services), was available for 845 participants at baseline. Of those, 819 (96.9%) had multimorbidity (≥2 out of 16 chronic diseases and ageing syndromes) based on available data. We excluded 116 (14.2%) participants who did not have complete information for all 16 conditions leading to a MLTC sample of 703 participants (Fig. 1). Of those, 85.1% (n = 598) also had complete data for 13 immune biomarkers of lymphocyte compartments used previously to establish two immunosenescence profiles (Granic et al., 2020) (Appendix A).

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

2.2. Immunosenescence profiles of lymphocyte compartments

Details about blood-based biomarker collection, lymphocyte immunophenotyping, and the marker combination used to define lymphocyte compartments have been described previously (Martin-Ruiz et al., 2011; Spyridopoulos et al., 2016), including the strategy used to derive and characterise immunosenescence profiles (Granic et al., 2020). Additional information about 13 immune markers of lymphocyte compartments and immunosenescence profile derivation are presented in Appendix A (Table A.1, Table A.2, Supplementary Methods). Briefly, blood-based biomarkers from 749 participants after an overnight fast were initially analysed in peripheral blood samples at the Royal Victoria Infirmary, Newcastle upon Tyne, UK. For lymphocyte immunophenotyping, we used 4-colour flow-cytometry (Becton Dickson FACScan Flow Cytometer) and fluorescence-labelled antibodies (BD Bioscience, Oxford UK) and the marker combination as described (Martin-Ruiz et al., 2011; Spyridopoulos et al., 2016) (see Supplementary material, Fig. A.1 for an example of gating strategy). Specifically, the senescence phenotype in T-cells was defined as the lack of CD27 and CD28 receptor expression in the CD4 subset (marker combination: CD4⁺CD27⁻CD28⁻) and the lack of CD45RA and CD27 expression in the CD8 T effector memory cells (TEMRA; marker combination: CD3⁺CD8⁺CD45RA⁻CD27⁻) (Table A.1). The rationale for using these markers has been previously discussed (Spyridopoulos et al., 2016). Namely, the marker combinations were regarded as adequate markers of senescence in T-cells because of the presence of telomere dysfunction and replicative
3. Multiple long-term conditions

2.3.1. Chronic diseases and ageing syndromes

We selected 10 chronic diseases/disease groups and 6 ageing syndromes/impairments with more than 3% prevalence and less than 10% missing data in 845 participants with both multidimensional health assessment and medical records review at baseline (Fig. 1). Other criteria included their known impact on morbidity (Diederichs et al., 2011), disease co-occurrence and patterning (Xu et al., 2017), health care use, disability, and mortality (Collerton et al., 2016; Kingston et al., 2014). Table B.1 (Appendix B) lists chronic diseases/disease groups and ageing syndromes, their ascertainment criteria, prevalence, and the frequency of missing data in 845 participants. We excluded depression, sarcopenia, and frailty because of missing data > 10%. For example, only 552 participants out of 845 had their physical frailty status assigned (robust, pre-frail, frail) based on the Fried’s frailty criteria (Collerton et al., 2012). Details about operationalisation of frailty in the Newcastle 85+ Study have been described elsewhere (Collerton et al., 2012). Additionally, depression was assessed using the 15-item Geriatric Depression Scale, a measure not suitable for individuals with cognitive impairment.

2.3.2. Definition of MLTC

MLTC was defined as having ≥ 2 chronic diseases and ageing syndromes (WHO, 2015; Calderón-Larrañaga et al., 2017; Salive, 2013; Sasseville et al., 2019). Presence of diseases/ageing syndromes (coded ‘1’ for each) was expressed as a summary score (continuous variable; possible range 2–16) (Table B.1). Because the very old have high prevalence of MLTC (Collerton et al., 2016; Formiga et al., 2013; Salive, 2013; Steinhagen-Thiessen and Borchelt, 2010) and to separate those with a substantial disease burden by counts, the scores were also categorised into two groups using a median/mean of conditions in this cohort as a cut-off (< 5 and ≥ 5 MLTC). Furthermore, MLTC was defined using patterns from all 16 chronic diseases/ageing syndromes by an aposteriori technique in 703 participants with complete data as described below.

2.4. Other measures and covariates

We looked at the relationship between several sociodemographic, health, and lifestyle variables assessed at baseline and MLTC patterns and included them in multivariable analyses. The levels for all categorical variables are described in Table 1. Socio-demographic variables included sex, education, and occupational class (coded according to the National Statistics Socio-economic Classification (NS-SEC) system (Chandola and Jenkinson, 2000)). Other health-related variable was self-rated health compared with others of the same age. Lifestyle variables included BMI (kg of body weight/m² of height), current alcohol intake, smoking status, and self-reported physical activity (Granic et al., 2019). 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) 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)). The scores were used to create three outcome variables (i.e., very energetic, moderately energetic, and mildly energetic activities), and an overall physical activity score calculated with the formula ((3 × very energetic score) + (2 × moderately energetic score) + (1 × mildly energetic score)).
## Table 1
Characteristics of MLTC patterns in the sample with complete data for 16 chronic diseases and ageing syndromes in the Newcastle 85+ Study.

| Characteristics | All | Cluster 1 | Cluster 2 | Cluster 3 | p |
|-----------------|-----|-----------|-----------|-----------|---|
| % (n)           | 703 | 209 (29.7)| 240 (34.1)| 254 (36.1)| 0.13 |
| **Sociodemographic** |     |           |           |           |     |
| Sex, % (n)      |     |           |           |           |     |
| male            | 276 | 89 (42.6) | 82 (34.2) | 105 (41.3)| 0.07 |
| female          | 427 | 120 (57.4)| 158 (65.8)| 149 (58.7)|     |
| Education, % (n) |     |           |           |           | 0.07 |
| 0-9 years       | 452 | 121 (58.5)| 159 (66.5)| 172 (67.7)|     |
| 10-11 years     | 162 | 53 (26.1) | 53 (22.2) | 55 (21.7) |     |
| ≥ 12 years      | 86  | 32 (15.5) | 27 (11.3) | 27 (10.6) |     |
| Occupational class, % (n) |     |           |           |           | 0.37 |
| routine/manual  | 345 | 95 (47.0) | 124 (54.1)| 126 (51.9)|     |
| intermediate     | 94  | 32 (15.8) | 34 (14.8) | 28 (11.5) |     |
| higher managerial/ administrative | 235 | 75 (37.1) | 71 (31.0) | 89 (36.6) |     |
| **Health variables** |     |           |           |           |     |
| Number of MLTC, M (SD) | 5.29 | 3.60 (1.20)| 7.13 (1.51)| 4.95 (1.47)| < 0.001 |
| median (range)  | 5   | 3 (2-7)   | 7 (4-11)  | 5 (2-10)  |     |
| MLTC groups, n (%) |     |           |           |           | < 0.001 |
| < 5 diseases/ageing syndromes | 266 | 163 (78.0)| 6 (2.5)   | 97 (38.2) |     |
| ≥ 5 diseases/ageing syndromes | 437 | 46 (22.0) | 234 (97.5)| 157 (61.8)|     |
| Immunosenescence profile, n (%) |     |           |           |           | 0.72 |
| 'Senescent-like phenotype' | 384 | 111 (18.6)| 136 (22.7)| 137 (22.9)|     |
| 'Less senescent-like phenotype' | 214 | 68 (31.8) | 70 (32.7) | 76 (35.5) |     |
| CD4/CD8 ratio   |     |           |           |           | 0.36 |
| < 1             | 110 | 32 (17.2) | 44 (20.1) | 34 (15.0) |     |
| ≥ 1             | 522 | 154 (82.8)| 175 (79.9)| 193 (85.0)|     |
| **CMV seropositivity, % (n)** |     |           |           |           | 0.85 |
| positive        | 573 | 172 (84.7)| 197 (86.4)| 204 (86.4)|     |
| negative        | 94  | 31 (15.3) | 31 (13.6) | 32 (13.6) |     |
| Self-rated health, % (n) |     |           |           |           | < 0.001 |
| excellent/very good | 287 | 95 (46.1) | 66 (27.6) | 126 (49.6) |     |
| good            | 262 | 73 (35.4) | 99 (41.4) | 90 (35.4) |     |
| fair/poor       | 150 | 38 (18.4) | 74 (31.0) | 38 (15.0) |     |
| **Lifestyle variables** |     |           |           |           |     |
| BMI, % (n)      |     |           |           |           | 0.1 |
| < 18.5 (underweight) | 43   | 20 (10.0) | 11 (4.9)  | 12 (5.1)  |     |
| > 18.5 < 25 (normal) | 329  | 108 (53.7)| 102 (45.5)| 119 (50.4)|     |
| > 25 < 30 (overweight) | 225  | 58 (28.9)| 86 (38.4) | 81 (34.3) |     |
| > 30 (obese)    | 64  | 15 (7.5)  | 25 (11.2) | 24 (10.2) |     |
| Current alcohol intake, n (%) |     |           |           |           | 0.03 |
| yes             | 440 | 146 (69.9)| 144 (60.0)| 150 (59.1)|     |
| no              | 263 | 63 (30.1) | 96 (40.0) | 104 (40.9)|     |
| Smoking status  |     |           |           |           | 0.1 |
| never           | 250 | 64 (30.6)| 91 (37.9) | 95 (37.5) |     |
| former          | 414 | 127 (60.8)| 139 (57.9)| 148 (58.5) |     |
| current         | 38  | 18 (8.6)  | 10 (4.2)  | 10 (4.0)  | < 0.001 |
| Self-reported physical activity, % (n) |     |           |           |           |     |
| low (score 0-1) | 146 | 28 (13.4)| 67 (28.0) | 51 (20.2) |     |

(continued on next page)
activities score + \[2 \times \text{moderately energetic activities score} + 1 \times \text{mildly energetic activities score}\], and categorised into low (0–1), medium (2–6), and high (7–18).

Table 1 (continued)

| Characteristics | All | Cluster 1 | Cluster 2 | Cluster 3 | p |
|-----------------|-----|-----------|-----------|-----------|---|
| BMI, body mass index; M, mean; MLTC, multiple long-term conditions; SD, standard deviation. | | | | | |

| Chronic diseases/syndromes | All | Cluster 1 | Cluster 2 | Cluster 3 | p1 |
|----------------------------|-----|-----------|-----------|-----------|---|
| Hypertension yes | 419 (59.5) | 37 (17.7) | 138 (57.5) | | <0.001 |
| no | 285 (40.5) | 172 (82.3) | 102 (42.5) | 11 (4.3) | <0.001 |
| Cardiac diseases yes | 273 (38.8) | 27 (12.9) | 127 (52.9) | 119 (46.9) | <0.001 |
| no | 430 (61.2) | 182 (87.1) | 113 (47.1) | 135 (53.1) | <0.001 |
| Diabetes yes | 100 (14.2) | 4 (1.9) | 37 (15.4) | 59 (23.2) | <0.001 |
| no | 603 (85.8) | 205 (98.1) | 203 (84.6) | 195 (76.8) | <0.001 |
| Cerebrovascular diseases yes | 149 (21.2) | 18 (8.6) | 75 (31.3) | 56 (22.0) | <0.001 |
| no | 554 (78.8) | 191 (91.4) | 165 (68.8) | 196 (78.0) | <0.001 |
| Renal impairment yes | 173 (24.6) | 15 (7.2) | 69 (28.7) | 89 (35.0) | <0.001 |
| no | 530 (75.4) | 194 (92.8) | 117 (71.3) | 165 (65.0) | <0.001 |
| Respiratory diseases yes | 156 (22.2) | 55 (35.3) | 78 (50.0) | | <0.001 |
| no | 547 (77.8) | 154 (73.7) | 162 (67.5) | | <0.001 |
| Thyroid disease yes | 109 (15.5) | 11 (5.3) | 64 (26.7) | 34 (13.4) | <0.001 |
| no | 594 (84.5) | 205 (98.1) | 176 (73.3) | 220 (86.6) | <0.001 |
| Arthritis yes | 489 (69.6) | 136 (65.1) | 200 (83.3) | 153 (60.2) | <0.001 |
| no | 214 (30.4) | 73 (34.9) | 40 (16.7) | 101 (39.8) | <0.001 |
| Cancers yes | 105 (14.9) | 19 (9.1) | 56 (23.3) | 30 (11.8) | <0.001 |
| no | 598 (85.1) | 190 (90.9) | 184 (76.7) | 224 (88.2) | <0.001 |
| Osteoporosis yes | 87 (12.4) | 21 (10.0) | 46 (19.2) | 20 (7.9) | <0.001 |
| no | 616 (87.6) | 188 (90.0) | 194 (80.8) | 234 (92.1) | <0.001 |
| Ageing syndromes, n (%) | | | | | |
| Falls yes | 273 (38.8) | 35 (16.7) | 195 (81.3) | 43 (16.9) | <0.001 |
| no | 430 (61.2) | 174 (83.3) | 45 (18.8) | 211 (83.1) | <0.001 |
| Pain yes | 366 (52.1) | 126 (60.3) | 164 (68.3) | 76 (29.9) | <0.001 |
| no | 337 (47.9) | 83 (39.7) | 76 (31.7) | | <0.001 |
| Urinary incontinence yes | 224 (31.9) | 47 (22.5) | 124 (51.7) | 53 (20.9) | <0.001 |
| no | 479 (68.1) | 162 (77.5) | 116 (48.3) | 201 (79.1) | <0.001 |
| Visual impairment yes | 260 (37.0) | 55 (26.3) | 116 (48.3) | 89 (35.0) | <0.001 |
| no | 443 (63.0) | 154 (73.7) | 124 (51.7) | 165 (65.0) | <0.001 |
| Hearing impairment yes | 468 (66.6) | 125 (59.8) | 187 (77.9) | 156 (61.4) | <0.001 |
| no | 235 (33.4) | 84 (40.2) | 53 (22.1) | 98 (38.6) | 0.005 |
| Cognitive impairment yes | 69 (9.8) | 21 (10.0) | 34 (14.2) | 14 (5.5) | <0.001 |
| no | 634 (90.2) | 188 (90.0) | 206 (85.8) | 240 (94.5) | <0.001 |

1Chi-square analysis; numbers in bold indicate the cells contributing the most to MLTC patterns’ differences based on adjusted standardised residuals. MLTC, multiple long-term conditions.
3. Statistical analysis

3.1. Derivation and characteristics of MLTC patterns

MLTC patterns were established in 703 participants with complete data at baseline for 16 chronic diseases and ageing syndromes (Table B.1) using the SPSS Two-Step clustering. Details of this procedure are outlined in Appendix A (Supplementary Methods). Briefly, we used automatic selection and the Bayesian Information Criterion (BIC) to determine the optimal number of clusters and a log-likelihood distance criterion for cluster separation. The robustness and stability of the final cluster solution was re-evaluated by random ordering of cases (three times) and by comparing cluster characteristics. The Two-Step clustering statistical parameters such as importance factor (IF) ranging from 0 (low importance) to 1 (high importance) were inspected for each disease/ageing syndrome variable to determine which variables contributed the most to cluster separation (i.e., the IF of 0 defines low contribution, and IF of 1 high contribution in cluster separation).

To describe MLTC pattern characteristics (Table 1), we used a One-Way ANOVA to compare normally distributed continuous variables, a Kruskal-Wallis H test for non-normally distributed and ordered variables, and a Chi-square test for categorical variables at α < 0.05. The Chi-square test was also used to examine the prevalence of 16 conditions across the MLTC patterns (Table 2). The frequencies of 13 lymphocyte compartments used to derive immunosenescence profiles across MLTC groups (< 5 MLTC, ≥ 5 MLTC) and patterns are described in Appendix B (Table B.2 and Table B.3, respectively). For normally distributed variables, we used a Student t-test (Table B.2) and a One-Way ANOVA (Table B.3), and a Mann-Whitney U test (Table B.2) and a Kruskal-Wallis H test (Table B.3) for non-normally distributed variables.

3.2. Association between immunosenescence profiles and MLTC scores and patterns

Immunosenescence profiles were investigated in relation to MLTC (scores, groups, and patterns) using several multivariable analyses. We first examined the association between immunosenescence profiles and having ≥ 5 MLTC using logistic regression (odds ratio (OR) 95% CI) (Table 2). Model 1 was unadjusted, and Model 2 was adjusted for sociodemographic factors (sex, education, occupational class). Model 3 was additionally adjusted for lifestyle covariates (BMI, current alcohol intake, smoking status, and physical activity). Increased odds (> 1) would indicate higher risk of being in ≥ 5 MLTC group in participants having more senescent profile compared with those with less senescent profile after adjusting for relevant covariates (Table 3). These covariates were reported in the literature to be significantly associated with MLTC (Aminisani et al., 2020; Chudasama et al., 2021; Dhalvani et al., 2017; Fortin et al., 2014; Lefèvre et al., 2014). The models were repeated with MLTC counts (continuous) as an outcome (Fig. B.1) and key covariates using linear regression (Table B.4). The association between immunosenescence profiles and MLTC patterns were evaluated using multinomial logistic regression with the same set of covariates (Table 4). Increased odds (> 1) would indicate higher odds of belonging to a specific MLTC pattern in participants having more senescent profile compared with those with less senescent profile after adjustments for a set of covariates.

3.3. Supplementary analyses

We further examined the association between CD4/CD8 ratio < 1 (an element of immune risk profile) and MLTC counts and patterns in the MLTC sample, and separately in those with CMV seropositivity (a subsample of 573 participants with MLTC and assigned MLTC pattern). We reported selected results in Table B.5 and Table B.6. Additionally, all models from the main analyses with MLTC patterns, MLTC groups (< 5 and ≥ 5 conditions), and scores as outcomes were repeated in CMV seropositive participants (details not shown).

3.3.1. Principal component analysis

To examine the robustness of the findings with immunosenescence profiles of lymphocyte compartments, we used PCA with a Varimax rotation to reduce 13 immune biomarkers into a smaller set of immunosenescence components. The number of components was determined based on eigenvalues > 1, a scree plot, and the interpretability of the components. Variable loadings ≥ 0.3 were determined as significantly contributing to the component structure and lower loadings were omitted (Table B.7). To determine the robustness of the components solution we inspected the correlation matrix, Bartlett’s test of sphericity, and Kaiser-Meyer-Olkin measure of sample adequacy (details not shown). A Varimax rotation with Kaiser Normalization (orthogonal transformation) was used to improve the interpretability of immunosenescence components (Table B.7). The nomenclature for the components was given based on immune markers with the highest loading in each component. The principal components scores (continuous) were calculated using the Anderson-Rubin method, which were then standardised to yield a sample mean of 0 and standard deviation (SD) of 1. The components were used in the subsequent multivariate analyses as the independent variables with MLTC counts, groups, and patterns as the dependent variables (Table B.8 to Table B.10). We used the same statistical analyses and covariates as described in the Section 3.2.

4. Results

4.1. Prevalence of MLTC and characteristics of MLTC patterns

In the MLTC sample (n = 703), only 48 (6.8%) participants had 2 chronic diseases/ageing syndromes, whilst 79.1% had 3–7, and 14.1% had ≥ 8 diseases, the mean of 5.29 (SD = 2.01), and median of 5 diseases (Fig. B.1). Four hundred and thirty-seven participants (62.2%) were in ≥ 5 MLTC group (Table 1).

We identified three distinct MLTC patterns: Cluster 1 (‘Low frequency cardiometabolic-cerebrovascular diseases’ pattern, n = 209), Cluster 2 (‘High ageing syndromes-arthritis’ pattern, n = 240), and Cluster 3 (‘Hypertensive-renal impairment’ pattern, n = 254) (Fig. 2). Eleven chronic diseases and ageing syndromes contributed the most to cluster separation, particularly (in order of importance on the Y-axes

### Table 3

| Event | Model 1 | p   | Model 2 | p   | Model 3 | p   |
|-------|---------|-----|---------|-----|---------|-----|
| ≥ 5 MLTC group* | OR (95% CI) | 1.14 (0.81–1.61) | 0.45 | OR (95% CI) | 1.05 (0.73–1.50) | 0.79 | OR (95% CI) | 1.0 (0.68–1.47) | 0.99 |
| ‘Senescent-like phenotype’ | | | | | | |
| ‘Less senescent-like phenotype’ (ref) | | | | | | |

* ≥ 5 chronic diseases/ageing syndromes from counts compared with < 5 MLTC in logistic regression models.
MLTC, multiple long-term conditions; ref, reference.
Model 1 is unadjusted.
Model 2 is adjusted for sociodemographic variables (sex, education, occupational class).
Model 3 is additionally adjusted for lifestyle variables (BMI, alcohol intake, smoking status, and self-reported physical activity).
from bottom-up from the most to least important) hypertension, falls, cardiac diseases, pain, urinary incontinence, renal impairment, diabetes, respiratory diseases, thyroid disease, cerebrovascular diseases, and arthritis (IF scores from 1 (the most important) to 0.12 (the least important)). Osteoporosis and cognitive impairment contributed the least to cluster separation (Fig. 2).

The patterns differed by the mean and median number of 16 chronic diseases and ageing syndromes. They also varied by several health and lifestyle variables (self-reported health, physical activity, and current alcohol intake) (Table 1). Participants in Cluster 2 had more diseases, were more likely to report fair/poor health, and to be physically inactive compared with others. The prevalence of each chronic disease and ageing syndrome also differed across the patterns (Table 2). Compared with other clusters, Cluster 1 had the lowest prevalence of cardiac diseases, diabetes, cerebrovascular diseases, and renal impairment compared with other clusters (p < 0.001). Cluster 2 had the highest prevalence of thyroid disease, arthritis, cancer, osteoporosis, urinary incontinence, hearing, and visual impairment (p < 0.001). Cluster 3 had the highest prevalence of hypertension and renal impairment compared with other clusters.

Because of NK cells across MLTC groups (< 5 and ≥ 5 conditions), there were no statistically significant differences in immune biomarkers (lymphocyte compartments) by MLTC groups and patterns (Table B.2 and Table B.3).

4.2. Association between immunosenescence profiles and MLTC

Table 3 presents the OR (95% CI) of logistic regression models for being in ≥ 5 MLTC group by immunosenescence profiles of lymphocyte compartments. Belonging to ‘Senescent-like phenotype’ was not
associated with raised odds of having ≥5 MLTC compared to the membership in ‘Less senescent phenotype’ in both unadjusted and adjusted models. These results were corroborated in the linear regression models with MLTC counts as an outcome (Table B.4).

Table 4 presents the OR (95% CI) of multinomial logistic regression models for the risk of being in Cluster 2 (‘High ageing syndromes-arthritis’ pattern) and Cluster 3 (‘Hypertensive-renal impairment’ pattern) compared with Cluster 1 (‘Low frequency cardiometabolic- cerebrovascular’ pattern; reference group) by immunosenescence profiles. Although slightly raised for ‘Senescence-like phenotype’ in the Cluster 2 (‘High ageing syndromes-arthritis’ pattern), the OR were not significant.

4.3. Supplementary analyses: association between CD4/CD8 ratio < 1 and MLTC in the MLTC sample and sub-sample of CMV seropositive participants

Low CD4/CD8 ratio, an element of immune risk profile was not associated with the higher odds of being in the ≥5 MLTC group (e.g., OR [95% CI] = 1.14 [0.7–1.85], p = 0.6 in the fully adjusted model) (Table B.5). Similar non-significant results were obtained using linear regression models with MLTC counts as an outcome (details not shown).

Also, we observed no association between low CD4/CD8 ratio, MLTC counts (by ≥5 cut-off and continuous), and MLTC patterns in a sub-sample of participants who were positive for CMV (i.e., another element of immune risk profile) (Table B.6). No associations between MLTC (≥5 MLTC group, counts, and patterns) and immunosenescence profiles were found in 573 (61.1% female) CMV seropositive participants (e.g., in fully adjusted model (M3): OR [95% CI] = 0.95 [0.60–1.49], p = 0.81 for being in ≥5 MLTC if having ‘Senescent-like phenotype’; β [95% CI] = 0.02 [−0.31–0.51], p = 0.63; further details not shown).

4.3.1. Principal component analysis: association between immunosenescence components and MLTC in the MLTC sample

PCA with 13 immune biomarkers derived three components which explained 57.1% of the variance in the biomarkers: 34.3% (Component 1: ‘CD4TEMRA and CD8TEMRA-related’ component), 12.5% (Component 2: ‘CD4 cells-related’ component), and 10.25% (Component 3: ‘Memory B and NK cells-related’ component) (Table B.7). Communality coefficients were high for Memory CD4 (0.66) and NK cells (−0.79) which loaded the highest on Component 1 and Component 3, respectively. Naïve CD4 cells (0.71) and CD4 TEMRA (0.70) loaded the highest to Component 2 and Component 1, respectively. These immune biomarkers also contributed the most to the immunosenescence profiles separation in the Two-Step procedure.

In multivariate analyses with each immunosenescent component, no significant associations with MLTC (counts, groups, and patterns) (Table B.8 to Table B.10) were observed, except for Component 3 (‘Memory B and NK cell-related’ component) being significantly associated with 21% higher odds of belonging to the ≥5 MLTC group in Model 3, which was attenuated to non-significant by CMV+ in the fully adjusted model (Table B.8).

5. Discussion

5.1. Summary of findings

To our knowledge, this is the first study to investigate the cross-sectional associations between immunosenescence profiles, defined previously from multiple biomarkers of lymphocyte compartments in human peripheral blood (Granic et al., 2020), and MLTC (multimorbidity) in very old adults. MLTC were defined by counts and patterns from 16 common chronic diseases/groups and ageing syndromes each with the prevalence ≥3% in 703 participants. As described in other cohorts of the very old (Formiga et al., 2013; Salive, 2013; Steinhausen-Thiessen and Borchelt, 2010), over half lived with 5 and more age-related conditions. Having a more senescent phenotype 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 seropositivity), were not associated with higher odds of having ≥5 MLTC (sample mean and median compared with a less senescent phenotype. Also, no associations were found between a more senescent phenotype and MLTC patterns with the highest frequency of ageing syndromes and arthritis (Cluster 2) characterised by median of 7 conditions and 97.5% having ≥5 conditions. Equally, having elements of the immune risk profile was not associated with MLTC in very old adults. However, because we used a limited number of immune markers (T cells, NK cells, B cells, NK cells) and exploratory data reduction techniques that are greatly dependent on data at hand, the findings need to be interpreted with caution.

5.2. Interpretation of findings

Current understanding of how immunosenescence in lymphocyte compartments may influence the development of MLTC is based on epidemiological and mechanistic studies investigating the role of immunosenescence (lymphocyte subsets and the elements of immune risk profile) (Barbé-Tuana et al., 2020; Chen et al., 2020; Desdin-Micó et al., 2020; Fülöp et al., 2016; Santoro et al., 2021).

5.2.1. Age-associated decline in immune function (immunosenescence) and age-related diseases

Age-related diseases share common features of immunosenescence, and several important insights about the overlap between their mechanisms have been summarised in the recent reviews (Barbé-Tuana et al., 2020; Fülöp et al., 2016; Santoro et al., 2021). For example, higher number of CD4+CD28+ T cells have been reported in patients with heart diseases (e.g., angina, myocardial infarction, chronic heart failure). Atherosclerosis, an inflammatory disease and the pathological precursor of cardiovascular diseases, may be triggered by autoantigens and infectious agents stimulating infiltration of CD4+ T cells into the arterial walls. Senescent T cells such as cytotoxic CD8+ cells, CD8+CD28+ expansion and CD4+ memory cells were associated with atherosclerosis and coronary artery disease, and late-differentiated CD4+CD28- T cells with acute coronary diseases (reviewed in Fülöp et al., 2016).

Rheumatoid arthritis (RA) is described as a model of premature (accelerated) ageing of the immune system and coincides more frequently with cardiovascular diseases, cancers, lung diseases, osteoporosis, and neurological diseases (van Onna and Boonen, 2016), a trend that was also observed in this cohort. RA is characterised by a significant elevation of late-differentiated T cells (CD28+ CD4 and CD8 cells, with CD28+ including a subset of terminally differentiated CD45RA (TEMRA) memory cells) able to produce pro-inflammatory cytokines under stimulation (reviewed in Barbé-Tuana et al., 2020). Diabetes mellitus type 2 has been also regarded as a model of premature ageing of the immune system characterised by a decrease in CD4+ naïve cells alongside with an increased pool of memory CD4+ and effector CD4+ and CD8+ T cells and increased production of pro-inflammatory cytokines and oxidative stress (reviewed in Barbé-Tuana et al., 2020; Fülöp et al., 2016).

Cancer patients accumulate highly differentiated senescent cells (CD27+CD28-), which may affect the ability to use effective immunotherapies requiring the presence of co-stimulatory molecules. Additionally, impaired cancer immunity may also be driven by persistent chronic infection such as CMV seropositivity (reviewed in Barbé-Tuana et al., 2020; Fülöp et al., 2016), which is highly prevalent in the very old and a major driver of T cells senescence (Koch et al., 2007).

Our previous investigations of the associations between immunosenescence (lymphocyte subsets and the elements of immune risk profile) and chronic diseases, mortality, and frailty in the Newcastle 85+ Study have yielded mixed results (Collerton et al., 2012; Spyridopoulos et al., 2020; Fülöp et al., 2016; Santoro et al., 2021).
of the Newcastle 85 T-cell compartments using a 8-colour flow-cytometry assay in a sub-set of individuals with CMV seropositivity and a low CD4:CD8 ratio were not significant pre-existing conditions. However, a significant increase in effector memory cells and CD4 T cell frequencies between healthy and diseased older adults. Similarly, CD4 T effector memory cells and CD4 ‘CD8’ ‘CD57’ subsets were increased in all old diseased groups compared with YH, but no increase in CD4 ‘CD8’ ‘CD57’ was observed in all old diseased groups compared with young. Thus, along with the progressive decline in naïve T cell function, decreased T cell response to new antigens, increased frequency of terminally differentiated T cells, and decreased T cell receptor repertoire, a significant increase in senescent T cells (e.g., TEMRA, CD28 ‘CD57’ subsets) may characterise ageing with major age-related diseases, contributing to the increased levels of circulating cytokines observed in both metabolic and cardiovascular diseases, and inflamming.

When we compared the frequencies of 13 lymphocyte compartments included to derive immunosenesence profiles in the very old (Granic et al., 2020) across MLTC patterns and MLTC groups (< 5 versus ≥ 5 MLTC), we observed no statistically significant differences (p for trend range 0.07–0.97), except for NK cells being significantly higher in < 5 versus ≥ 5 MLTC group (18.02% (9.25) versus 15.65% (37.5%), p = 0.001; Table B.2). Cytoxic NK cells, as a part of the innate lymphocyte group and a key player in the early immune response against virus-infected and cancer cells, have been shown to be well preserved in healthy nonagenarians and centenarians and to extend their function to immune regulation, senescence cells clearance, and the initiation of adaptive immunity (reviewed in Santoro et al., 2021). Along with cancers, alterations in NK cell activity have been reported in other chronic inflammatory diseases, including diabetes, RA, atherosclerosis, and pulmonary diseases (Paris et al., 2017).”

5.2.2. MLTC and immunosenescence

To our knowledge, only a few studies have investigated the association between multiple chronic diseases and immunosenescence of lymphocyte compartments (Chen et al., 2020; Nilsson et al., 2002), and these have reported mixed results. None used dimension reduction techniques to define immunosenesence profiles from multiple immune system markers to create summary variables of ageing immune system.

In an immunological study of Swedish nonagenarians (the Swedish NONA Immune Study), morbidity and a health status determined using different immune study exclusion protocols (very healthy, moderately healthy, frail) were not associated with the T-cell immune risk phenotype related to CMV seropositivity (Nilsson et al., 2002). Namely, flow cytometry analysis of lymphocyte compartments detected no significant difference in CD4/CD8 ratio, CD3 ‘CD4’ ‘CD8’, CD3 ‘CD4’ ‘CD8’, CD8 ‘CD57’ ‘CD28’, CD8 ‘CD56’ ‘CD57’, or CD8 ‘CD56’ ‘CD57’ between the health-status groups in the oldest-old. A more recent clinical study compared alterations in T cell compartment (especially T cells replicative senescence) between young healthy adults (YH; age range 21–26 years), older healthy adults (OH; age range 65–82 years), and older adults with metabolic diseases (OM; age range 65–87 years), cardiovascular diseases (O-CVD; age range 65–99 years), and those with both disease groups (O-MD-CVD; age range 65–90 years) (Chen et al., 2020). Despite its small sample size per group (12–24 participants), the study observed significant changes in CD8 and CD4 T cell compartments with ageing and disease. Metabolic and cardiovascular diseases influenced the frequency of CD8 T cells subsets, including naïve T cells, effector memory T cells (TEM), and effector memory cells re-expressing CD45RA (TEMRA), along with the loss of CD28 and the gain of CD57 (i.e., recognised as the most senescent marker in both TEM and TEMRA subsets). Whilst the frequency of CD8 T cells and CD8 naïve cells were reduced in all older adult groups compared to YH, there was a significant decrease in CD8 naïve cells in CVD and MD-CVD compared with OH controls, and a significant increase in CD8 ‘TEMRA’ subset between healthy young and old groups, but a decrease of CD8 ‘TEMRA’ in older adults with MD and CVD compared with OH. Furthermore, there were no differences in CD28 ‘CD57’ ‘CD8’ ‘TEMRA’ frequencies between healthy and diseased older adults. Similarly, CD4 T effector memory cells and CD4 ‘CD28’ ‘CD57’ subsets were increased in all old diseased groups compared with YH, but no increase in CD4 ‘CD8’ ‘CD57’ TEMRA was observed in all old diseased groups compared with young. Thus, along with the progressive decline in naïve T cell function, decreased T cell response to new antigens, increased frequency of terminally differentiated T cells, and decreased T cell receptor repertoire, a significant increase in senescent T cells (e.g., TEMRA, CD28 ‘CD57’ subsets) may characterise ageing with major age-related diseases, contributing to the increased levels of circulating cytokines observed in both metabolic and cardiovascular diseases, and inflamming.

Because several commonly observed age-related diseases share similar features of immunosenescence (reviewed in Barbé-Tuana et al., 2020; Fülöp et al., 2016; Santoro et al., 2021), and supported by a strong genetic evidence for the role of innate and adaptive immunity in diverse chronic conditions from the text mining exercise of 1.85 million scientific abstract on human ageing (Fraser et al., 2022), it would have been expected that higher number of age-related conditions frequently observed in the very old might increase the likelihood of having a more senescent T cell-related phenotype. For example, Cluster 2 (‘High ageing syndromes-arthritis’ pattern) had the highest frequency of most conditions described above (Section 5.2.1) compared with other MLTC patterns. However, although present in 34% of participants, this MLTC pattern was not associated with a higher likelihood of belonging to a more senescent phenotype or having immune risk profile.

Several excellent recent reviews have highlighted a strong association between age-related diseases and ageing mechanisms including cellular senescence in immune cells in animal and human studies (Fraser et al., 2022; Sayed et al., 2021; Yousefzadeh et al., 2021) and technical advancements in understanding cellular senescence in immune cells at single cell level (Zhou et al., 2021). These recent insights and methodological advancement in characterising ageing immune system should be acknowledged when interpreting our findings.

There may be several reasons for the null findings. First, as also observed in the Swedish NONA Immune Study (Nilsson et al., 2002), no association could be partly explained by a high prevalence of CMV seropositivity in the very old (e.g., 641 individuals were CMV+ or 85.6% of those tested), which has been recognised as a driving force in T cell immunosenescence, including in healthy older adults (Koch et al., 2007; Pawelec, 2014), making it difficult to disentangle the true correlation between immunosenescence and MLTC. We have found no difference in immune profiles when comparing participants with (≥ 2 chronic conditions) and without MLTC (0–1 chronic conditions) (detail not shown). Second, the healthy survivor effect, reflecting loss of participants with very impaired immune profile or poor health before the age of 85 years, may render T cells immunosenescence biomarkers as neutral or protective in extreme longevity (Derbovansian et al., 2018), despite the high prevalence of MLTC (by counts). Third, we used counts and patterns to describe MLTC that do not consider disease severity for overall disease burden. This may be higher than the sum of individual diseases (i.e., burden by counts) or disease groups in older adults (Diederichs et al., 2011). Thus, use of MLTC indices as an outcome in future work may provide important insights about the relationship between immunosenescence and MLTC. Fourth, because this is a cross-sectional study,
it is not known how changes in the immune system may have influenced the change in MLTC counts, patterns, and indices over time. Fifth, here we used an a posteriori, exploratory technique to derive both immunosenescence profiles and MLTC patterns from a list of lymphocyte compartments and diseases/ageing syndromes, respectively. Although this data reduction technique clustered participants into distinct patterns, categorisation of continuous data (i.e., two immunosenescence patterns) may have reduced the power to detect the associations. We have repeated data reduction procedure using PCA to derive summary variables of lymphocyte compartments and obtained similar results in multivariable associations. However, both methods that group either individuals (the Two-Step) with similar immune profiles or immune variables (PCA) depend on data at hand that were limited to 13 immune markers. Sixth, three major age-related diseases and ageing syndromes (sarcopenia, frailty, and depression) could not be included in MLTC counts because of missing data (>10%). This omission may have significantly affected how MLTC were defined. Seventh, there is a possibility that diseases with a common mechanism such as inflammation (discussed above) may have different associations with immunosenescence profiles, warranting further research. Lastly, a recent animal study using a mouse model with dysfunctional mitochondria in T cells owing to the lack of Tfam gene responsible for stabilisation and replication of mitochondrial DNA, has shown that T cell metabolic failure resulted in a surge of cytokines, inflammaging, and age-related changes in metabolic, cognitive, cardiovascular, and muscular systems (Desdín-Micó et al., 2020). Thus, loss of T cell immunometabolic control, and not only decrease and remodelling of T cell phenotypes, may have a role in age-related diseases (Rhoads et al., 2017), and potentially for the development of MLTC in older adults.

5.3. Strength and limitations

The strengths of our study include its sample size, a single year birth cohort to control for the effect of age, inclusion of participants with cognitive impairments and those living in institutions, as well as a range of immunosenescence biomarkers, chronic diseases and ageing syndromes (most being diagnosed by a doctor). We have derived MLTC patterns that have been described in other cohorts of older adults such as cardiometabolic pattern (Busija et al., 2019). The study has several limitations and some of them have been discussed above. The lack of associations could be due to the limitations in our outcome measures (e.g., counts and patterns versus indices), or in the biomarker selection and statistical method for data reduction. Specifically, although we evaluated the robustness of the cluster solution for both immunosenescence profiles (Gronic et al., 2020) and MLTC, the associations may have been affected by the choice of available immunosenescence biomarkers and conditions included and their groupings with three major age-related diseases/syndromes missing. We have included only 13 immune biomarkers of lymphocyte compartments which are limited in characterising the complexity of age-associated decline in immune function (Zhou et al., 2021). We used the Two-Step clustering, which is an a posteriori, exploratory technique dependent on data at hand and statistical parameters (e.g., importance factor) for the inclusion of biomarkers and diseases/ageing syndromes. Thus, some biologically relevant biomarkers may be excluded, and different dimension reduction techniques may result in slightly different MLTC patterns (Collerton et al., 2016). We aimed for robust, distinct MLTC clusters with acceptable cluster-to-cluster ratio, and other statistical parameters, and enough cases per cluster for multivariable analyses. A three-cluster solution met these requirements.

As described previously, we used a 4-colour flow-cytometry assay to describe T-cell compartments, which may be limited in characterising senescent cells. Utilisation of more sophisticated techniques at the single-cell level (Zhou et al., 2021) and the inclusion of other biomarkers related to immunosenescence (e.g., inflammatory markers, DNA damage) may have resulted in different immune profiles and affected their association with MLTC. Although we used a composite measure of immunosenescence, the utility of the individual biomarker approach should be acknowledged. We were limited in comparisons of the findings with other studies as there has been little research in this area to date. The results have limited generalisability and warrant further examination in a wide range of cohorts of the very old.

5.4. Conclusions

We used cross-sectional data to investigate the association between immunosenescence profiles derived from 13 biomarkers of lymphocyte compartments (‘Senescent-like phenotype’ and ‘Less senesence-like phenotype’), and MLTC (counts and patterns) in the Newcastle 85+ Study. We found no association between immunosenescence and MLTC in very old adults. A more senescent phenotype characterised by T-cell senescence and elements of the immune risk profile (e.g., CMV seropositivity) were not significantly associated with ≥ 5 MLTC group, MLTC counts, or patterns compared with the ‘Less senescent-like phenotype’. However, because of several important limitations in our study, the results need to be interpreted with caution. Further studies are needed to explore the relationship between immunosenescence and MLTC in late adulthood.

Declarations

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.

Data availability

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.19368833.

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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 analyses.
experiments and analysed flow cytometric data; critical review of the manuscript for intellectual/scientific content; validation of the results. LR contributed to: data analysis, manuscript editing, and critical review of the manuscript for intellectual/scientific content; RMD contributed to: critical review of the manuscript for intellectual/scientific content; validation and interpretation of the results. TBKL 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. LAR (The Newcastle 85+ Study PI) and IS 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; funding for the study. All authors read and approved to the final manuscript.

Declaration of interest

AG, CM-R, LR, RMD, LAR, IS, TBKL, TVz, AAS: none.

Data availability

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.19368833.

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Appendix A and B: Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mad.2022.111739.

References

Aiello, A., Farzaneh, F., Candore, G., Caruso, C., Davinelli, S., Gambino, C.M., et al., 2019. Immune system and its hallmarks: how to oppose aging strategically? A review of potential options for therapeutic intervention. Front. Immunol. 10, 2247.
Barb, L., Mercer, S.W., Norbury, M., Watt, G., Wyke, S., Guthrie, B., 2012. Deconstructing complex multimorbidity in the very old: the Newcastle 85+ study. BMJ 343, b4904.
Barnett, K., Collerton, J., Davies, K., Dodds, R.M., Jagger, C., King, L., et al., 2021. Multimorbidity measures, associated factors, and impact on health services–a systematic review on existing multimorbidity indices. J. Gerontol. A Biol. Sci. Med. Sci. 76, 528–534.
Busija, L., Lim, K., Szoeke, C., Sanders, K.M., McCabe, M.P., 2019. Do replicable profiles of immunosenescence exist? Front. Immunol. 10, 2247.
Chen, Y.J., Liao, Y.J., Tram, V.T.N., Lin, C.H., Liao, K.C., Liu, C.L., 2020. Alterations of specific lymphocytic subsets with aging and age-related metabolic and cardiovascular diseases. Eur. J. Immunol. 50, 545–557.
Chudasama, Y.V., Khunti, K., Davies, M.J., 2021. Clustering of comorbidities. Future Health J. 8, e224–e229.
Collerton, J., Barrans, K., Bond, J., Eccles, M., Jagger, C., James, O., et al., 2007. The Newcastle 85+ study: biological, clinical and psychological factors associated with healthy aging: study protocol. BMC Geriatr. 7, 14.
Collerton, J., Davies, K., Jagger, C., Kingston, A., Bond, J., Eccles, M.P., et al., 2009. Health and disease in 85 year olds: baseline findings from the Newcastle 85+ cohort study. BMJ 339, b4904.
Collerton, J., Martin-Ruiz, C., Davies, K., Hilkens, C.M., Jacobs, J., Kolenda, C., Parker, C., Dunn, M., Catt, M., Jagger, C., von Zglinicki, T., Kirkwood, T.B., 2012. Frailty and the impact of inflammation, immunosenescence and cellular aging in the very old: cross-sectional findings from the Newcastle 85+ Study. Mech. Ageing Dev. 133, 456–466.
Collerton, J., Jagger, C., Yadegefar, M.E., Davies, K., Parker, S.G., Robinson, L., Kirkwood, T.B., 2016. Deconstructing complex multimorbidity in the very old: Findings from the Newcastle 85+ Study. Biomed. Res. Int. 2016, 8745670.
Dong, H.J., Wressle, E., Marcussen, J., 2013. Multimorbidity patterns of and use of health services by Swedish 85+ year-olds: an exploratory study. BMC Geriatr. 13, 120.
Duggal, N.A., Niemiro, G., Harridge, S.D.R., Simpson, R.J., Lord, J.M., 2019. Can physical activity ameliorate immunosenescence and thereby reduce age-related multi-morbidity? Nat. Rev. Immunol. 19, 563–572.
Eccles, M.P., Berger, K., Bartlett, L., et al., 2011. The measurement of multiple chronic diseases–a systematic review on existing multimorbidity indices. J. Gerontol. A Biol. Sci. Med. Sci. 66, 301–311.
Ensinger, W., Sanz, H., Formiga, F., Ferrer, A., Pujol, R., Jagger, C., 2013. The contribution of diseases to the male-female disability-survival paradox in the very old: the Octabaix study. Eur. J. Epidemiol. 28, 1109–1113.
Fortin, M., Stewart, M., Poitras, M.E., Almirall, J., Maddocks, H., 2012. A systematic review of prevalence studies on multimorbidity: toward a more uniform methodology. Ann. Fam. Med. 10, 142–151.
Granic, A., Martin-Ruiz, C., Dodds, R.M., Robinson, L., Spyridopoulos, I., Kirkwood, T.B., 2020. Potential options for therapeutic intervention. Front. Immunol. 10, 2247.
Granic, A., Martin-Ruiz, C., Dodds, R.M., Robinson, L., Spyridopoulos, I., Kirkwood, T.B., 2020. et Immunosenescence profiles are not associated with muscle strength, physical performance and sarcopenia risk in very old adults: the Newcastle 85+ Study. Mech. Ageing Dev. 190, 111321.
Kingston, A., Davies, K., Collerton, J., Robich, L., Duncan, R., Bond, J., et al., 2014. The contribution of diseases to the female-female disability-survival paradox in the very old: results from the Newcastle 85+ male study. PLoS One 9, e88016.
Koch, S., Larbi, A., Oncizzle, D., Solana, R., Guzman-Castillo, C., Attrig, S., et al., 2007. Cytomegalovirus infection drives a driving force in human T cell immunosenescence. Ann. N. Y. Acad. Sci. 1114, 23–35.
Kudesia, P., Salmarouny, B., Stanley, M., Fortin, M., Stewart, M., Terry, A., et al., 2021. Biological mechanisms of aging predict age-related disease co-occurrence in patients. Aging Cell 21 (4), e13524.
Le Page, A., Dupuis, G., Larbi, A., Witkowska, J.M., Fülöp, T., 2018. Signal transduction changes in CD4+ and CD8+ T cell subpopulations with aging. Exp. Gerontol. 105, 129–139.
Lefvre, T., d'Ivernois, J.F., De Andrade, V., Crozet, C., Lombrail, P., Gagnayre, R., et al., 2014. What do we mean by multimorbidity? An analysis of the literature on multimorbidity measures, associated factors, and impact on health services organization. Rev. Epidemiol. Sante Publique 62, 305–314.
Marengoni, A., Angleman, S., Melis, R., Mangialasche, F., Karp, A., Garman, A., et al., 2011. Aging with multimorbidity: a systematic review of the literature. Ageing Res. Rev. 10, 430–439.
Mars-Ruiz, C., Jagger, C., Kingston, A., Collerton, J., Catt, M., Davies, K., et al., 2011. Assessment of a large panel of candidate biomarkers of ageing in the Newcastle 85+ study. Mech. Ageing Dev. 132, 496–502.
