

1 **Peripheral innate immunophenotype in neurodegenerative disease:**
2 **blood-based profiles and links to survival**

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46 **Abstract**

47
48 The innate immune system plays an integral role in the progression of many neurodegenerative
49 diseases. In addition to central innate immune cells (e.g., microglia), peripheral innate immune
50 cells (e.g., blood monocytes, natural killer cells, and dendritic cells) may also differ in these
51 conditions. However, the characterization of peripheral innate immune cell types across different
52 neurodegenerative diseases remains incomplete. This study aimed to characterize peripheral
53 innate immune profiles using flow cytometry for immunophenotyping of peripheral blood
54 mononuclear cells in n=148 people with Alzheimer's disease (AD), frontotemporal dementia
55 (FTD), corticobasal syndrome (CBS), progressive supranuclear palsy (PSP), Lewy body disease
56 (LBD) as compared to n=37 healthy controls. To compare groups, we used multivariate
57 dissimilarity analysis and principal component analysis across 19 innate immune cell types. We
58 identified pro-inflammatory profiles that significantly differ between patients with all-cause
59 dementia and healthy controls, with some significant differences between patient groups.
60 Regression analysis confirmed that time to death following the blood test correlated with the
61 individuals' immune profile weighting, positively to TREM2+ and non-classical monocytes and
62 negatively to classical monocytes. Taken together, these results describe transdiagnostic
63 peripheral immune profiles and highlight the link between prognosis and the monocyte cellular
64 subdivision and function (as measured by surface protein expression). The results suggest that
65 blood-derived innate immune profiles can inform sub-populations of cells relevant for specific
66 neurodegenerative diseases that are significantly linked to accelerated disease progression and
67 worse survival outcomes across diagnoses. Blood-based innate immune profiles may contribute
68 to enhanced precision medicine approaches in dementia, helping to identify and monitor
69 therapeutic targets and stratify patients for candidate immunotherapies.

70

71 **Introduction**

72
73 Despite the clinical and pathological heterogeneity characterizing the main dementias,
74 dysregulation of the innate immune system is identified as a common feature in all
75 neurodegenerative diseases (1). Centrally, activation of cerebral microglia is reported for
76 Alzheimer's disease (AD) and its prodromal mild cognitive impairment (MCI), Lewy body
77 disease (LBD) including Parkinson's disease dementia (PDD) and dementia with Lewy bodies
78 (DLB), and the syndromes associated with frontotemporal lobar degeneration (FTLD). The latter
79 includes the behavioral variant of frontotemporal dementia (bvFTD), non-fluent (nfPPA) and
80 semantic (svPPA) variants of primary progressive aphasia, corticobasal syndrome (CBS), and
81 progressive supranuclear palsy (PSP). Moreover, genome-wide association studies link
82 mutations in genes coding for proteins related to the immune system to the development of
83 multiple neurodegenerative conditions (2–7). For example, mutations in the gene for triggering
84 receptor expressed on myeloid cells 2 (TREM2, encoding a receptor on monocytes and
85 microglia) may cause FTLD-syndromes and AD, with an effect size comparable to the immune-
86 related APOE4 variant (2,8). Such genomic associations are complemented by evidence of
87 abnormalities in central and peripheral innate immune systems in neurodegenerative diseases (9).

88
89 Most evidence describing abnormal innate immunity in dementia to date concerns cerebral cells.
90 Specifically, microglial activation in the central nervous system is implicated in many forms of
91 dementia (10–16). For example, human post mortem studies report activated microglia in
92 association with the severity of amyloid and tau pathology in AD, FTLD-syndromes and LBD
93 (11,12,17,18). However, post mortem studies are not well suited to characterize microglial
94 changes in early disease stages. Instead, overexpression of the translocator protein 18 kDa

95 (TSPO), overexpressed in activated microglia, can be detected by positron emission tomography
96 (PET). Microglial activation presents early in people living with many types of dementia and
97 predicts the rate of cognitive decline (13–16,19). While TSPO PET is a powerful, informative
98 tool to visualize cerebral innate immune activation, it is expensive and not readily scalable.
99 Alternatively, peripheral blood-based markers of immune dysregulation are to be potentially
100 scalable and repeatable over time.

101

102 Cells of the innate immune system— including monocytes, dendritic cells, and natural killer
103 (NK) cells – rapidly and non-specifically initiate an immune response upon detection of
104 pathogens or cellular damage (20). While this process is beneficial in the short-term to eliminate
105 harmful stimuli, chronic or dysregulated activation can lead to disease. The peripheral immune
106 system may interact with the central nervous system indirectly via chemokine and cytokine
107 signaling or directly via infiltration into and meningeal surveillance of the parenchyma (21–27).
108 Elevated pro-inflammatory cytokine concentrations are reported in post mortem brain tissue and
109 cerebrospinal fluid (CSF) in people with AD, LBD, and FTLN-syndromes (28–31); while
110 peripheral infections can exacerbate the neuroinflammatory environment and accelerate
111 cognitive decline (26,32). Taken together, evidence implicates primary effector cells of the
112 peripheral innate immune system in the pathogenesis of neurodegenerative diseases.

113

114 Monocytes, NK cells, and dendritic cells are each implicated in the pathogenesis of dementias.
115 For example, TREM2 is expressed on the surface of peripheral monocytes (33) and may be a
116 marker of monocyte recruitment in AD (27). Elevated concentration of CSF soluble TREM2
117 (sTREM2), cleaved from the membranes of microglia or monocytes, is linked to disease progress

118 cognitive decline in AD (34,34) and FTLD (11). Beyond TREM2, CSF concentrations of
119 chemokine motif ligand 2 (CCL2), mediating the chemotaxis of monocytes, are also predictive
120 of cognitive decline in AD (35). Similarly, abnormal activation patterns of NK cells occur with
121 AD (36) and Parkinson's disease (closely related to DLB and PDD) (37). Finally, myeloid
122 dendritic cell frequency is reduced in people with AD and Parkinson's disease (38–40). Overall,
123 evidence involving monocytes, NK cells, and dendritic cells supports a more integrative
124 neurodegenerative disease processes than initially appreciated.

125

126 While the mechanisms of action of peripheral immune cells remain unclear, they are nonetheless
127 more readily accessible to quantify, compared to central innate immune cells, via phlebotomy.
128 The identification of innate cell types in the periphery of people with dementia may clarify links
129 between innate immunity and neurodegenerative disease, and yield clinically relevant, blood-
130 based biomarkers to support target identification and patient stratification for immune-targeting
131 therapies. However, current immunophenotyping data is limited to few diagnoses of dementia
132 and remains incomplete in cellular subtype characterization (38,39,41–43). To meet the needs for
133 diagnostic, prognostic and trialist use, there is a pressing need for improved profiling of
134 peripheral innate immunity in multiple neurodegenerative disorders.

135

136 This study therefore aims to characterize innate immune profiles in blood of people with diverse
137 neurodegenerative dementias, including AD, FTLD-syndromes and LBD using Flow Cytometry-
138 a well-established method of identifying cells via their unique expression of activation and
139 lineage markers on the cell surface. We test the hypothesis that innate immune cell profiles are
140 abnormal in people with any of these disorders, with at least partial commonality in the immune

141 profile for the neurodegenerative disorders investigated here (i.e. a transdiagnostic abnormality).
142 Given the multivariate nature of immune activation, we use data driven approaches to reduce
143 dimensionality (complexity) and test for (dis)-similarity between the clinical diagnostic groups.
144 We then highlight clinical relevance by the correlation of multivariate immune profiles with time
145 to death.

146

147 **Materials and Methods**

148

149 *Participants*

150 Patients (n =148) were recruited from clinics for cognitive and movement disorders at the
151 Cambridge University Hospitals NHS Trust as well as collaborating regional psychiatry and
152 neurology services.

153

154 We included participants with a clinical diagnosis that met the standardised criteria of MCI/AD
155 (44,45) (n=24; 5 MCI and 19 AD), probable or possible PSP (n=54, predominantly Richardson's
156 syndrome), CBS (46) (n=23), FTD (47)(n=18; 12 bvFTD and 6 PPA), PDD (n=3), or DLB
157 (n=26) (48,49). In a sub-group of participants (n=34), PET and/or CSF markers for amyloid were
158 obtained to confirm the presence or absence of β -amyloid (interpreted as AD pathology) in
159 conditions with weak clinicopathological correlations and either high likelihood of a significant
160 fraction with AD as main or co-pathology (CBS and DLB) or a high likelihood of clinical
161 diagnostic false positives (amnestic MCI). Of the CBS patients, n=12 underwent amyloid PET,
162 which confirmed amyloid positivity in n=4. Of the MCI patients, all were confirmed amyloid-
163 positive with PET imaging with the Pittsburgh compound B (PiB tracer at a cut-off of 19

164 centiloids (44)) and/or CSF Alzheimer's biomarkers at lumbar puncture (Amyloid-beta 1-42/40
165 ratio < 0.065 as recommended laboratory threshold from University College London Hospitals
166 reference Laboratory (50)). Of the DLB cohort, n=6 out of 17 patients resulted amyloid-positive
167 at PiB PET. We also included n=37 healthy controls given MMSE > 26/30, absence of memory
168 symptoms, no signs of dementia, or any other significant medical illnesses (51). Exclusion
169 criteria for both patient and healthy control cohorts included recent or current acute infection,
170 major concurrent psychiatric illness, other severe physical illness, or a history of other significant
171 neurological illness and/or autoimmune conditions.

172

173 All participants underwent baseline clinical and neuropsychological assessment, including the
174 revised Addenbrooke's Cognitive Examination (ACE-R, 0-100 points) and mini-mental state
175 examination (MMSE). The ACE-R is subdivided into five domains: Attention and Orientation
176 (18/100), Memory (26/100), Fluency (14/100), Language (26/100), and Visuospatial abilities
177 (16/100). In a subset of participants, an informant completed the 8 Item Clinical Dementia
178 Rating (CDR) FTLN NACC (Table 1.) Survival data were collected for all up to and including
179 the 17th of October 2023 (the census date).

180

181 *Ethics approval and consent to participate*

182 Participants with mental capacity gave their written informed consent to take part in the study
183 according to the Declaration of Helsinki. For those who lacked capacity, their participation
184 followed the consultee process in accordance with the UK law. The research protocols were
185 approved by the National Research Ethics Service's East of England Cambridge Central
186 Committee.

187 ***Blood collection and flow cytometry analyses***

188 At baseline, 18 ml of blood was drawn in EDTA and analyzed at the NIHR Cambridge Cell
189 Immunophenotyping Hub. Wherever possible, samples were processed within 2h (over 98% of
190 samples). Blood was layered onto sterile Ficoll (Cytiva, Cat#: 17144003) for peripheral blood
191 mononuclear cell (PBMC) isolation by a technician blind to group status. Two aliquots
192 containing $\sim 1 \times 10^6$ PBMCs were stained using the antibody cocktail shown in Supplementary
193 Table S1, using either TREM2 or a matched isotype control. At the end of staining, cells were
194 washed, and the data were acquired on live (i.e., non-fixed) cells with a BD LSR Fortessa
195 instrument.

196 Cell classifications were determined via manual gating in FlowJo (BD) by individuals
197 blind to participant group according to standards recommended for the Human Immunology
198 Project (52). Briefly, monocytes were identified as HLA-DR+/CD14+; populations were further
199 stratified based on CD16 and CD14 positivity where CD16-/CD14hi monocytes were labelled as
200 classical, CD16+/CD14+ monocytes labelled as intermediate, and CD16+/CD14lo monocytes
201 labelled as nonclassical. Each monocyte subpopulation was then gated to discern CCR5+,
202 CCR2+, and TREM2+ staining. Dendritic cells (DCs) were identified as HLA-DR+/CD14- and
203 further stratified based on CD123 and CD11c staining: CD123+/CD11c+ cells were double
204 positive (DC+/+), CD123+/CD11c- were plasmacytoid DCs, CD123-/CD11c+ were myeloid
205 DCs, and CD123-/CD11c- were double negative (DC-/-). NK cells were identified as CD56+ and
206 further subdivided into two groups as either CD16+ (which were CD56lo) or CD16- (which were
207 CD56hi). The full gating strategy is found in Supplementary Figure S2. Interrater validation
208 studies showed excellent concordance between different evaluators (Supplementary Figure S3;
209 Supplementary Table S2).

210

211 ***Plasma dementia-related biomarkers***

212 In a sub-cohort of 122 participants (Control = 27, AD = 17, CBS = 16, FTD =12, LBD = 22, PSP
213 = 28), plasma samples were stored at -70°C for further analyses at the Clinical Neurochemistry
214 Laboratory in Mölndal (Sweden). Plasma samples were thawed on wet ice, centrifuged at 500× g
215 for 5 min at 4°C. Calibrators (neat) and samples (plasma: 1:4 dilution) were measured in
216 duplicates. The plasma assays performed were the Quanterix Simoa Human Neurology 4-Plex E
217 assay (measuring Aβ40, Aβ42, GFAP and NfL, Quanterix, Billerica, MA) and the p-tau217
218 ALZpath assay measuring p-tau217 of the human tau protein associated with AD, as previously
219 described (53). Plasma samples were analyzed at the same time using the same batch of reagents.
220 A four-parameter logistic curve fit data reduction method was used to generate a calibration
221 curve. Two control samples of known concentration of the protein of interest (high-control and
222 low-control) were included as quality control. Intra-assay coefficients of variation were below
223 10%.

224

225 ***Statistical Analysis***

226 Analyses were performed using R (Version 2023.03.0+386). Non-parametric tests were used for
227 all pairwise comparisons given non-normal data distributions. For inference, p-values were
228 corrected for multiple comparisons using the Benjamini-Hochberg procedure to control the false
229 discovery rate (FDR) and considered significant with threshold p-FDR < 0.05. For transparency
230 and explorative analyses, uncorrected p-values are also reported. Statistical analysis was carried
231 out in three steps.

232

233 First, to understand similarity of innate immune cell profiles between diagnoses and controls,
234 absolute cell numbers from FlowJo were normalized to standardize across minimum and
235 maximum absolute cell count ranges. These normalized cell counts were averaged within each
236 diagnosis to yield an average, normalized 19-cell vector per diagnosis. To evaluate dissimilarity
237 between diagnoses, Euclidean distance was calculated on the normalized data based on a 19-cell
238 vector for all diagnoses followed by single linkage hierarchical clustering.

239

240 Second, a Principal Component Analysis (PCA) was applied on the 19 innate immune cell
241 classes. This reduced the dimensionality of our dataset to minimize multiple comparisons,
242 identifying a limited number of components that best explain the data variance. The PCA was
243 computed using cell counts calculated as a proportion of their parent population across all cell
244 types (Supplementary Figure S1). Local Outlier Factor (LOF) analysis with a hyperparameter of
245 $k=10$ identified 2 participants as outliers following PCA computation ($LOF = 3$) (54). These
246 outliers were excluded before re-computing the PCA. We retained 3 components based on
247 eigenvalues >1 , explained variance $> 10\%$, and application of the visual “elbow method” from
248 the scree plot for further analyses (55,56) (Supplementary Figure S4). Individual participant
249 loadings in each of the 3 selected components were included in group-comparison analyses using
250 Mann Whitney U tests to compare median values between patients and controls, and Kruskal
251 Wallace tests to compare across patient groups followed by Dunn’s post hoc analysis, where
252 applicable.

253

254 Finally, we tested for associations between the individual participant loadings and clinical
255 outcome. Individual component loadings were individually evaluated as a predictor of NfL, p-

256 tau217, A β 40/A β 42, GFAP, and ACE-R total scores using a linear regression, controlling for age
257 and sex. Similarly, we investigated each principal component loading in relationship to years of
258 survival following blood draw using a linear model, including age, sex, and biomarkers as
259 covariates.

260 **Results**

261 Participant summary clinical characteristics are in Table 1. There were 76 female and 109 male
262 participants. Most groups, apart from CBS, had more males than females. As expected, control
263 participants scored significantly higher on the ACE-R examination than each patient group ($p <$
264 0.001 for all comparisons); while people with AD had significantly lower ACE-R total scores
265 than people with PSP ($p = 0.002$).

266

267 Dissimilarity Analysis

268 Figure 1 shows dissimilarity values of the 19-cell vector measured by Euclidean distance
269 between groups (See Supplementary Figure S5 and S6, and Tables S7 and S8, for individual cell
270 pairwise comparisons). Hierarchical clustering via single linkage represented in the dendrogram
271 summarizes the group-wise differences in the pattern (rather than magnitude) of immune
272 profiles. Maximal relative distance was found between controls and all patient groups as
273 evidenced by the distinct cluster or branch from the dendrogram. The smallest distance (i.e. most
274 similarity) was identified within PSP and FTD groups, while PSP, CBS and FTD groups were
275 more similar to each other than to AD/LBD. Importantly, the distance value between CBS and
276 AD (2.14) and LBD (2.18) are smaller than the distance between AD, LBD, and other FTLD
277 conditions.

278

279 Principal Component Analysis

280 From the PCA on the 19 cell populations, three components were selected for further
281 analyses. Together, these components accounted for half of the total variance in the data.
282 Supplementary Table S3 shows the contribution of each cell type to the selected components.
283 The first principal component (PC1) accounted for 17.97 % of the variance and was strongly
284 positively weighted by TREM2+ monocytes and nonclassical monocytes (excluding CCR5+
285 nonclassical monocytes). PC1 was most negatively loaded onto classical monocytes, including
286 CCR2+ classical monocytes, as well as NK cells high in CD16 expression (CD16+ NK cells)
287 (Figure 2C). The second principal component (PC2) accounted for 16.02 % of the total variance.
288 PC2 was strongly, negatively weighted by intermediate monocytes and CCR5+ monocytes, and
289 strongly positively weighted by dendritic cells negative for CD11c and CD123 (Figure 3C). The
290 third principal component (PC3) accounted for 12.98% of the total variance and was most
291 strongly positively weighted by CD16- NK Cells, and most strongly, negatively weighted in
292 CD16+ NK cells and TREM2+ monocytes (Figure 4C). PC relationships to sex and age are
293 found in Supplementary Table S4. Regression analysis revealed that there was no significant
294 relationship between individual loadings in PC1, PC2, or PC3 and age ($p > .05$). There was no
295 significant relationship between individual loadings in PC1, PC2, or PC3 and sex ($p > .05$). We
296 identified 3 patients (2 FTD and 1 CBS) with hsCRP > 10 mg/L. Similar components and
297 individual component loadings were obtained when excluding these participants, which may be
298 indicative of either central or peripheral inflammatory conditions (see Supplementary Figure S7,
299 Table S5).

300

301 All-cause dementia patients showed significantly lower median loading values in PC1 (U =
302 3351, $p = 0.035$) as compared to controls (Figure 2A). Multi-group comparison did not identify a
303 significant difference in PC1 loading between controls and each patient group ($H = 10.4$, $p =$
304 0.065) (Figure 2B). There was no statistically significant difference in individual PC2 loading
305 between all-cause dementia and controls ($U = 2844$, $p = 0.767$) or between controls and each
306 patient group ($H = 3.88$, $p = 0.567$) (Figure 3A and B). All-cause dementia patients had
307 significantly reduced PC3 loading as compared to controls ($U = 2025$, $p = 0.0145$) (Figure 4A).
308 Multi-group comparison identified a significant difference in PC3 loading across groups ($H =$
309 15.9 , $p = 0.007$), and post hoc analysis revealed that patients with LBD showed significantly
310 lower median loading values in PC3 than controls ($p\text{-FDR} = 0.01$) (Figure 4B). See
311 Supplementary Table S6 for statistics from post hoc analysis group comparisons.

312

313 Clinical Outcomes

314 Individual patient loadings of PC1, PC2, and PC3 were each evaluated as a predictor of ACE-R
315 scores including the interaction of diagnostic group as an independent variable. Regression
316 analysis did not reveal significant predictive value of PC1, PC2, or PC3 for ACE-R total scores.
317 There was no interaction between diagnostic group, age, or sex, with any principal component in
318 terms of ACE-R score.

319

320 In the subset with biomarker data, patient loadings of PC1, PC2, and PC3 were each evaluated as
321 a predictor of plasma-based biomarker values (mg/L) including the interaction of diagnostic
322 group as an independent variable. Regression analysis did not reveal significant predictive value
323 of PC1, PC2, or PC3 for p-tau217, NfL, A β 40/A β 42 (57), or GFAP ($p < 0.05$). There was no

324 interaction between diagnostic group, age, or sex, with any principal component in terms of these
325 biomarkers.

326

327 We used our data to evaluate the sample size required for traditional Cox proportional hazards
328 regression. We completed a power calculation (power = 0.8, alpha = 0.05). To detect a hazard
329 ratio of 1 +/- 0.2 as seen in previous studies using this regression in immune data in dementia
330 (50), we would require 234 patients with confirmed death, or 835 total participants given our
331 current distribution of deceased to living patients (28%).

332

333 Thus, to carry out a primary survival analysis, we tested whether PC1, PC2 or PC3 were
334 predictive of years of time to death in the subset of 42 patients who died prior to our census date
335 (2 AD, 2 CBS, 10 LBD, 8 FTD, and 20 PSP). Higher individual loadings in PC1 predicted longer
336 time from the research visit to death ($y=0.4463x+2.0915$, $F = 10.24$, $p = 0.0027$, $r^2=0.184$). This
337 correlation remained after controlling for age, sex, and diagnosis ($F = 3.48$, $p = 0.00507$,
338 $\beta_{Age} = -0.025$, $\beta_{Male} = 0.13$). Importantly, PC1 predicted survival over and above all biomarkers
339 when including NfL, A β 40/A β 42, p-tau217, and GFAP as covariates in our model ($F = 1.443$, p
340 $= 0.035$, $\beta_{NfL} = -0.012$, $\beta_{p\text{-tau}217} = 1.23$, $\beta_{A\beta42/AB40} = -0.09$, $\beta_{GFAP} = 0.001$). There was no association
341 between PC2 ($F = 0.1043$, $p = 0.75$) or PC3 ($F = 2.983$, $p = 0.09$) and years of survival following
342 blood draw.

343 **Discussion**

344
345 The main outcome of this study is confirmation that peripheral blood-based innate
346 immunophenotypes are abnormal in people with AD, LBD, FTD, PSP, and CBS. The principal
347 components of the 19-cell class immunophenotype were similarly abnormal in each type of
348 neurodegenerative disease, and one component (or immune profile) predicted time to death. We
349 identified transdiagnostic similarity in the *magnitude* of this abnormality in PC1, and diagnostic
350 specificity in the magnitude and composition of this abnormality for LBD patients in PC3.
351 Importantly, the multivariate *pattern* of cell-types was dissimilar between controls and all-cause
352 dementia participants in this study. Taken together, these results indicate that even peripheral
353 immune profiles have diagnostic specificity and identify a cellular profile represented by a
354 redistribution of monocyte subtypes as being linked to survival across neurodegenerative
355 dementias.

356
357 In the introduction, we proposed that peripheral innate immune cells are directly related to the
358 pathogenesis of neurodegenerative diseases, based on genomics data and the physiological
359 integration between central and peripheral immune compartments (2–7,38–43,58,59). Rather
360 than a cell-by-cell account, we adopted a multivariate approach and identified three principal
361 components of interest. The first (PC1) was weighted positively to TREM2+ and nonclassical
362 monocytes and negatively to classical monocytes. The second (PC2) was weighted positively to
363 CCR5+ monocytes and intermediate subtypes, and negatively to DC-/- cells. The third
364 component (PC3) was positively weighted by CD16- NK cells and negatively weighted by
365 CD16+ NK cells. These multivariate (multi-cell type) patterns help to contextualize the results of
366 more selective prior studies.

367

368 For example, previous work demonstrated altered monocyte subpopulations in AD that varied
369 with disease stage (41), albeit without natural killer cell or dendritic cell data. While we do not
370 present data on disease severity, our data corroborate these findings by suggesting aberrant levels
371 of monocyte subtypes in neurodegenerative disease. In addition, monocyte subtype redistribution
372 may explain why total monocytes have not been found to be different in the MCI stage as
373 compared to controls (42). Similarly, our identification of greater CD16+ NK cell overexpression
374 in disease adds to existing literature identifying NK cell alterations in AD versus controls,
375 however, this study did not compare CD16+ and CD16- NK cell subtypes (43). Peripheral
376 myeloid DCs have previously been found to be altered in AD and Parkinson's disease versus
377 controls (38,39). While results from PC2 suggest AD may indeed be characterized by a trend of
378 reduced myeloid DCs (Figure 3B), these cells did not strongly correlate to PC1 or PC3, thus
379 underscoring the potential relative importance of monocytes and NK cells in all-cause
380 neurodegenerative dementia. In all, our work builds upon extant immunophenotyping literature
381 and confirms monocyte and NK cell population changes with multiple neurodegenerative
382 diseases.

383

384 Monocytes are a heterogeneous population of innate immune cells (60). They have diverse
385 functions, cytokine release, migration to damaged tissues, and differentiation into phagocytic
386 cells. Based on surface markers, monocytes can be divided into classical, nonclassical, and
387 intermediate subtypes. Classical monocytes are pro-inflammatory, potentially neurotoxic, and are
388 recruited to damaged tissue during inflammation (61). Nonclassical monocytes are anti-
389 inflammatory and may be neuroprotective (62,63). In addition, monocytes may express
390 functional receptors such as CCR2, a chemotaxis receptor controlling the recruitment of the cell,

391 or TREM2, a receptor controlling phagocytic and inflammatory function (64,65). In contrast, NK
392 cells in other diseases facilitate the death of infected cells, regulate the adaptive immune
393 response through cytokine production, and mediate autoimmunity. NK cells can be subdivided
394 based on their relative presentation of surface markers into CD16-, regulating cytokine
395 production, or CD16+, indicating cytotoxicity (66). NK cells have been implicated in many
396 disorders of the central nervous system, including neurodegenerative dementia (43,67,68).
397 Finally, DCs are monocyte-derived antigen presenting immune cells that have been linked to
398 Parkinson's disease and AD (38,39).

399

400 Although previous evidence suggested monocyte, NK, and DCs are each involved in
401 neurodegenerative diseases, prior evidence was mainly gleaned from investigation in
402 Alzheimer's disease. Our results identify abnormal features of the peripheral innate immune
403 system that are common across AD, LBD, FTD, CBS and PSP. By using data-driven analytic
404 methods, we explore multivariate peripheral features that may not be revealed by evaluating one
405 cellular type at a time. Innate immune patterns across the 19 cells investigated in this study
406 identify patient groups to be dissimilar from controls and exhibit a cluster of innate immune
407 patterns in FTLD as compared to LBD and AD. Interestingly, we confirmed cases of amyloid
408 pathology in CBS, AD, and LBD patients. A relatively smaller distance between CBS and
409 AD/LBD as compared to PSP and FTD suggests a shared innate immune pattern, at least in part,
410 may reflect amyloid pathology (Figure 1). Through the application of dimensionality reduction
411 techniques, our results imply neurodegenerative disease to be characterized by pro-inflammatory
412 and cytotoxic peripheral immune dynamics atypical to healthy aging.

413

414 We observed reduced PC1 loading in all-cause dementia patients versus controls (Figure 2A).
415 This suggests that reduced relative expression of TREM2+ on peripheral monocytes and
416 increased relative prevalence of classical monocytes may be a characteristic pro-inflammatory
417 and cytotoxic immune signature linked to neurodegenerative dementia. TREM2 is involved in
418 modulating inflammation, mediating phagocytosis, and promoting myeloid cell survival (64,69).
419 While the current study did not evaluate CSF levels of sTREM or CCL2, looking at TREM2 and
420 CCR2 expressed on peripheral monocytes enables the investigation of functional dynamics of
421 active peripheral cells accessible from blood. In the context of neurodegenerative diseases,
422 TREM2 expressed on microglia is linked to prevention or downregulation of tau
423 hyperphosphorylation and cited to harbor a protective effect in neurodegenerative disease
424 (70,71). However, this interaction has been investigated primarily in microglia of murine models
425 of AD rather than peripheral monocytes and human studies across the dementia spectrum. Our
426 results support claims of TREM2 as an important receptor acting from the periphery in
427 neurodegenerative disease (27). While TREM2 is often reported in relation to microglia,
428 transcriptional analyses in the human brain suggest the infiltration of TREM2 expressing
429 monocyte-derived immune cells in patients with AD (72). In addition, elevated levels of TREM2
430 transcript in peripheral blood cells is found to be protective in the clinical progression of AD
431 (73). Although the current study is correlational without evidence of causality, we build upon the
432 clinical relevance of peripheral TREM2 by analyzing peripheral monocyte dynamics, rather than
433 TREM2 expressing cells alone.

434

435 In contrast to the diagnostic generalizability of PC1, patients with LBD displayed lower median
436 individual loadings in PC3 versus controls (Figure 4B). This third component was weighted

437 positively by CD16⁻ NK cells and negatively by NK Cells and CD16⁺ NK cells. Given CD16⁺
438 NK cells contributed to the negative arm of both PC1 and PC3, this cell type is of interest in all-
439 cause dementia. However, LBD was particularly characterized by an increased number in NK
440 cells and a functional shift in NK cell subtypes. Indeed, human postmortem studies show
441 redistribution of NK cellular subtypes in patients with alpha-synuclein pathology, suggesting a
442 potentially strong effect in this disease group (37,74). Beyond this, reduced average PC3 loading
443 in LBD suggests this cohort may harbor TREM2 dynamics that are dissimilar to other dementias
444 as these cells comprise the negative end of PC3 (opposite to PC1 composition). The present
445 findings distinguishing LBD are in line with previous observed differences in microglial
446 activation and TREM2 genetic relationships in LBD as compared to other diseases (18,29,75).
447 However, because we did not identify relationships between PC3 and clinical or cognitive
448 outcomes, the relevance of NK cells in therapeutic target and monitoring of LBD patients must
449 be further investigated.

450

451 The results presented in this study are relevant to therapeutic development and application in
452 neurodegenerative disease. We identify inflammatory profiles that are transdiagnostic and
453 prognostic, pointing toward the application of successful inflammatory therapeutics across
454 neurodegenerative diseases. As there are an increasing number of immunomodulatory drugs
455 under evaluation in dementia (76,77), there is an increasing need to understand immune changes
456 in neurodegeneration so that these therapies may be accurately targeted and monitored with
457 appropriate biomarkers of response in clinical trials. This is particularly pertinent for our
458 understanding of the functional outcomes associated with TREM2, where animal models show
459 conflicting results (33). Further studies are needed to clarify the functional role of TREM2 as
460 expressed by different cells of the immune system in humans (72). Specifically, our results probe

461 further investigation into common features of immune profiles for the effective adaptation of
462 treatment and disease monitoring.

463

464 The clinical relevance of shifting peripheral monocyte patterns toward a protective, anti-
465 inflammatory phenotype is highlighted by the associations of PC1 individual loading with
466 survival across patient groups (Figure 4). Increased peripheral TREM2⁺ expression and
467 nonclassical monocytes combined with reductions in classical monocytes and CD16⁺ NK cells
468 may represent a protective effect in neurodegenerative disease, while the opposite patterns may
469 contribute to accelerated clinical decline. Our survival analysis revealed PC1 as a strong
470 predictor of survival over and above plasma dementia-relevant markers. This result suggests that
471 the cell types dominating PC1 may harbor important prognostic monitoring information to be
472 applied to clinical practice and clinical trials.

473

474 While further investigation is certainly required to establish effective PBMC biomarkers for
475 clinical use, akin to ongoing investigations in lymphocyte/monocyte ratios (78) , dynamic
476 changes in monocyte composition (TREM2⁺ and classical/nonclassical monocytes) may serve as
477 an accessible, transdiagnostic node for dementia monitoring and prediction. Moreover, the
478 present link between increased TREM2⁺ monocytes and prognosis is especially relevant in
479 light of ongoing clinical trials targeting TREM2 in people with AD (79). Given the
480 transdiagnostic loading of PC1, TREM2 mediated treatments may be effective in other
481 neurodegenerative conditions. Because we did not identify any relationships between immune
482 profiles and biomarkers of cerebral pathology measured in a subset of our participants, our
483 results do not suggest monocyte redistribution to be a strong diagnostic or pathological

484 biomarker for dementia, but rather may enable transdiagnostic enrichment of clinical trials and
485 practice.

486

487 Our study has several limitations. First, patient recruitment and cohort definition are based on
488 clinical criteria rather than pathology-confirmed cases, although clinic-pathological correlations
489 are high for PSP, FTD, LBD and AD. Next, the heterogeneity within cohorts (e.g., grouping
490 bvFTD with PPA) and co-pathologies (e.g., CBS underpinned by CBD and/or AD) may
491 complicate the interpretation of the results and reduce sensitivity to between-group differences.

492 In addition, group sizes are unbalanced, although the non-parametric tests used are relatively
493 robust to moderate variation in group size. Although the present cohort is representative of
494 patients who are referred in dementia clinics, future studies with larger sample sizes with more
495 pathology-specific markers and genotyping (including APOE) may be able to clarify the
496 interaction between innate immune system and co-pathologies across all syndromes. In addition,
497 our cohort was predominantly white/caucasian reflecting the ethnicity distribution of the over 65-
498 year-old population in the UK. Further studies are needed in ethnically diverse populations, to
499 test the generalisation of our results. Importantly, ACE-R was used as the primary assessment to
500 capture cognitive decline as this exam is widely implemented in memory clinics. However, this
501 screening-test varies in sensitivity to the domain-specific cognitive changes associated with
502 different diagnoses, and this may have reduced power to detect correlations with cognitive
503 deficits. Additionally, presently investigated peripheral cell types are not dementia-specific and
504 may capture comorbidities that are not directly linked to dementia. We have attempted to
505 mitigate the potential confound of co-occurring inflammatory conditions, or the use of specific
506 anti-inflammatory medication in several ways. First, the source study excluded patients with co-

507 morbid pro-inflammatory conditions, such as rheumatoid arthritis, inflammatory bowel disease,
508 psoriasis or other autoimmune disorders, and cancer; and all contributory cohorts excluded
509 recent systemic infections and current acute medical illness. Moreover, we demonstrate that
510 exclusion of the small number of patients with elevated hsCRP, indicative of systemic
511 inflammation, does not alter the main results of the study (Supplementary Figure S7). In this this
512 study, we were unable to include correlations with central measures of inflammation, as imaging
513 was not a requirement for recruitment and participant inclusion. However, recent studies have
514 shown strong associations between blood-based inflammatory markers (i.e. cytokines) and
515 central inflammation in dementia, as measured by TSPO PET(80). Linking innate immune
516 blood-based profiles with central inflammation will be a key step for future research. Finally,
517 data collection spanned several years, which may have led to batch variation in our
518 immunophenotyping analysis. To help control sample variability, sample gating was conducted
519 by a single person, and validated against the gating of two other experts on a sub-sample.

520

521 In conclusion, the present study provides a comprehensive characterization of the peripheral
522 innate immune system in multiple neurodegenerative dementias. We suggest dysfunctional
523 patterns of the innate immune system are characteristic of neurodegenerative diseases. Blood-
524 derived innate immune profiles can distinguish sub-populations of cells relevant to diverse
525 clinical cohorts and their prognosis. Further studies are needed to clarify interactions between the
526 peripheral innate immunity profiles and dementia-related events in the cerebrum, including
527 neuroinflammation. We hope that the identification of blood-based innate immune profiles can
528 contribute to enhanced precision medicine approaches dementia, to identify new therapeutic
529 targets and improve clinical trial design for immunotherapies.

530

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552

553 **Competing interest**

554 The authors have no conflicts of interest to report related to this work. Unrelated to this work, JTO
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570

571 **Supplementary materials**

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573 Supplementary materials are available at MPs website.

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580 **Data availability**

581 Anonymized processed data can be shared upon request to the corresponding author. Raw data
582 may also be requested but are likely to be subject to a data transfer agreement with restrictions
583 required to comply with participant consent and data protection regulations.

584

585 **Authors contributions**

586 AS, PS, SLK, RC, NS, LT, AGM, LC, NS, NJA, GS, WRBJ, AS, MM contributed to study
587 execution, data collection and/or analysis. AS and MM drafted the first version of the
588 manuscript. KB, HZ, MM oversaw the sample and data analysis. MM conceptualised the study,
589 JBR and JOB contributed to the study design. All authors reviewed the manuscript and approved
590 the final version of the manuscript.

591

592 **References**

593

- 594 1. Mason HD, McGavern DB. How the immune system shapes neurodegenerative diseases.
595 Trends Neurosci. 2022 Oct 1;45(10):733–48.
- 596 2. Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, et al. TREM2
597 Variants in Alzheimer’s Disease. N Engl J Med. 2013 Jan 10;368(2):117–27.
- 598 3. Beecham GW, Hamilton K, Naj AC, Martin ER, Huentelman M, Myers AJ, et al.
599 Genome-Wide Association Meta-analysis of Neuropathologic Features of Alzheimer’s Disease
600 and Related Dementias. PLOS Genet. 2014 Sep 4;10(9):e1004606.
- 601 4. Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buross J, et al. Common variants
602 at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer’s
603 disease. Nat Genet. 2011 May;43(5):436–41.
- 604 5. Fernández-Santiago R, Sharma M. What have we learned from genome-wide association
605 studies (GWAS) in Parkinson’s disease? Ageing Res Rev. 2022 Aug 1;79:101648.
- 606 6. Ferrari R, Hernandez DG, Nalls MA, Rohrer JD, Ramasamy A, Kwok JBJ, et al.
607 Frontotemporal dementia and its subtypes: a genome-wide association study. Lancet Neurol.
608 2014 Jul 1;13(7):686–99.

- 609 7. Jabbari E, Koga S, Valentino RR, Reynolds RH, Ferrari R, Tan MMX, et al. Genetic
610 determinants of survival in progressive supranuclear palsy: a genome-wide association study.
611 *Lancet Neurol.* 2021 Feb 1;20(2):107–16.
- 612 8. Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV, Snaedal J, et al. Variant of
613 TREM2 Associated with the Risk of Alzheimer’s Disease. *N Engl J Med.* 2013 Jan
614 10;368(2):107–16.
- 615 9. Bettcher BM, Tansey MG, Dorothée G, Heneka MT. Peripheral and central immune
616 system crosstalk in Alzheimer disease — a research prospectus. *Nat Rev Neurol.* 2021
617 Nov;17(11):689–701.
- 618 10. Alliot F, Godin I, Pessac B. Microglia derive from progenitors, originating from the yolk
619 sac, and which proliferate in the brain. *Dev Brain Res.* 1999 Nov 18;117(2):145–52.
- 620 11. Woollacott IOC, Toomey CE, Strand C, Courtney R, Benson BC, Rohrer JD, et al.
621 Microglial burden, activation and dystrophy patterns in frontotemporal lobar degeneration. *J*
622 *Neuroinflammation.* 2020 Aug 10;17(1):234.
- 623 12. Mackenzie IRA. Activated microglia in dementia with Lewy bodies. *Neurology.* 2000 Jul
624 12;55(1):132–4.
- 625 13. Malpetti M, Rittman T, Jones PS, Cope TE, Passamonti L, Bevan-Jones WR, et al. In
626 vivo PET imaging of neuroinflammation in familial frontotemporal dementia. *J Neurol*
627 *Neurosurg Psychiatry.* 2021 Mar 1;92(3):319–22.
- 628 14. Malpetti M, Passamonti L, Rittman T, Jones PS, Vázquez Rodríguez P, Bevan-Jones WR,
629 et al. Neuroinflammation and Tau Colocalize in vivo in Progressive Supranuclear Palsy. *Ann*
630 *Neurol.* 2020;88(6):1194–204.
- 631 15. Malpetti M, Kievit RA, Passamonti L, Jones PS, Tsvetanov KA, Rittman T, et al.
632 Microglial activation and tau burden predict cognitive decline in Alzheimer’s disease. *Brain.*
633 2020 May 1;143(5):1588–602.
- 634 16. Malpetti M, Cope TE, Street D, Jones PS, Hezemans FH, Mak E, et al. Microglial
635 activation in the frontal cortex predicts cognitive decline in frontotemporal dementia. *Brain.*
636 2023 Mar 8;awad078.
- 637 17. Hopperton KE, Mohammad D, Trépanier MO, Giuliano V, Bazinet RP. Markers of
638 microglia in post-mortem brain samples from patients with Alzheimer’s disease: a systematic
639 review. *Mol Psychiatry.* 2018 Feb;23(2):177–98.
- 640 18. Amin J, Holmes C, Dorey RB, Tommasino E, Casal YR, Williams DM, et al.
641 Neuroinflammation in dementia with Lewy bodies: a human post-mortem study. *Transl*
642 *Psychiatry.* 2020 Aug 3;10(1):1–11.
- 643 19. Finze A, Biechele G, Rauchmann BS, Franzmeier N, Palleis C, Katzdobler S, et al.
644 Individual regional associations between A β -, tau- and neurodegeneration (ATN) with microglial
645 activation in patients with primary and secondary tauopathies. *Mol Psychiatry.* 2023 Jul 26;1–13.
- 646 20. Roh JS, Sohn DH. Damage-Associated Molecular Patterns in Inflammatory Diseases.
647 *Immune Netw.* 2018 Aug 13;18(4):e27.

- 648 21. Møllgård K, Beinlich FRM, Kusk P, Miyakoshi LM, Delle C, Plá V, et al. A mesothelium
649 divides the subarachnoid space into functional compartments. *Science*. 2023 Jan
650 6;379(6627):84–8.
- 651 22. Louveau A, Harris TH, Kipnis J. Revisiting the Mechanisms of CNS Immune Privilege.
652 *Trends Immunol*. 2015 Oct;36(10):569–77.
- 653 23. Ferro A, Auguste YSS, Cheadle L. Microglia, Cytokines, and Neural Activity:
654 Unexpected Interactions in Brain Development and Function. *Front Immunol* [Internet]. 2021
655 [cited 2023 Jan 29];12. Available from:
656 <https://www.frontiersin.org/articles/10.3389/fimmu.2021.703527>
- 657 24. Litvin DG, Denstaedt SJ, Borkowski LF, Nichols NL, Dick TE, Smith CB, et al.
658 Peripheral-to-central immune communication at the area postrema glial-barrier following
659 bleomycin-induced sterile lung injury in adult rats. *Brain Behav Immun*. 2020 Jul 1;87:610–33.
- 660 25. Riazi K, Galic MA, Kuzmiski JB, Ho W, Sharkey KA, Pittman QJ. Microglial activation
661 and TNF α production mediate altered CNS excitability following peripheral inflammation. *Proc*
662 *Natl Acad Sci*. 2008 Nov 4;105(44):17151–6.
- 663 26. Wohleb ES, Fenn AM, Pacenta AM, Powell ND, Sheridan JF, Godbout JP. Peripheral
664 innate immune challenge exaggerated microglia activation, increased the number of
665 inflammatory CNS macrophages, and prolonged social withdrawal in socially defeated mice.
666 *Psychoneuroendocrinology*. 2012 Sep 1;37(9):1491–505.
- 667 27. Fahrenhold M, Rakic S, Classey J, Brayne C, Ince PG, Nicoll JAR, et al. TREM2
668 expression in the human brain: a marker of monocyte recruitment? *Brain Pathol*. 2017 Oct
669 30;28(5):595–602.
- 670 28. Amin J, Boche D, Clough Z, Teeling J, Williams A, Gao Y, et al. Peripheral
671 immunophenotype in dementia with Lewy bodies and Alzheimer’s disease: an observational
672 clinical study. *J Neurol Neurosurg Psychiatry*. 2020 Nov 1;91(11):1219–26.
- 673 29. Surendranathan A, Su L, Mak E, Passamonti L, Hong YT, Arnold R, et al. Early
674 microglial activation and peripheral inflammation in dementia with Lewy bodies. *Brain*. 2018
675 Dec 1;141(12):3415–27.
- 676 30. Rentzos M, Zoga M, Paraskevas GP, Kapaki E, Rombos A, Nikolaou C, et al. IL-15 Is
677 Elevated in Cerebrospinal Fluid of Patients With Alzheimer’s Disease and Frontotemporal
678 Dementia. *J Geriatr Psychiatry Neurol*. 2006 Jun 1;19(2):114–7.
- 679 31. Fernández-Botrán R, Ahmed Z, Crespo FA, Gatenbee C, Gonzalez J, Dickson DW, et al.
680 Cytokine expression and microglial activation in progressive supranuclear palsy. *Parkinsonism*
681 *Relat Disord*. 2011 Nov 1;17(9):683–8.
- 682 32. Perry VH, Cunningham C, Holmes C. Systemic infections and inflammation affect
683 chronic neurodegeneration. *Nat Rev Immunol*. 2007 Feb;7(2):161–7.
- 684 33. Jay TR, von Saucken VE, Landreth GE. TREM2 in Neurodegenerative Diseases. *Mol*
685 *Neurodegener*. 2017 Aug 2;12(1):56.
- 686 34. Ewers M, Franzmeier N, Suárez-Calvet M, Morenas-Rodriguez E, Caballero MAA,
687 Kleinberger G, et al. Increased soluble TREM2 in cerebrospinal fluid is associated with reduced

688 cognitive and clinical decline in Alzheimer's disease. *Sci Transl Med*. 2019 Aug
689 28;11(507):eaav6221.

690 35. Westin K, Buchhave P, Nielsen H, Minthon L, Janciauskiene S, Hansson O. CCL2 Is
691 Associated with a Faster Rate of Cognitive Decline during Early Stages of Alzheimer's Disease.
692 *PLOS ONE*. 2012 Jan 30;7(1):e30525.

693 36. Jadidi-Niaragh F, Shegarfi H, Naddafi F, Mirshafiey A. The Role of Natural Killer Cells
694 in Alzheimer's Disease. *Scand J Immunol*. 2012;76(5):451–6.

695 37. Earls RH, Lee JK. The role of natural killer cells in Parkinson's disease. *Exp Mol Med*.
696 2020 Sep;52(9):1517–25.

697 38. Ciaramella A, Salani F, Bizzoni F, Orfei MD, Caltagirone C, Spalletta G, et al. Myeloid
698 dendritic cells are decreased in peripheral blood of Alzheimer's disease patients in association
699 with disease progression and severity of depressive symptoms. *J Neuroinflammation*. 2016 Jan
700 25;13(1):18.

701 39. Ciaramella A, Salani F, Bizzoni F, Pontieri FE, Stefani A, Pierantozzi M, et al. Blood
702 Dendritic Cell Frequency Declines in Idiopathic Parkinson's Disease and Is Associated with
703 Motor Symptom Severity. *PLOS ONE*. 2013 Jun 11;8(6):e65352.

704 40. Ciaramella A, Sanarico N, Bizzoni F, Moro ML, Salani F, Scapigliati G, et al. Amyloid β
705 peptide promotes differentiation of pro-inflammatory human myeloid dendritic cells. *Neurobiol*
706 *Aging*. 2009 Feb 1;30(2):210–21.

707 41. Thome AD, Faridar A, Beers DR, Thonhoff JR, Zhao W, Wen S, et al. Functional
708 alterations of myeloid cells during the course of Alzheimer's disease. *Mol Neurodegener*. 2018
709 Nov 13;13(1):61.

710 42. Magaki S, Yellon SM, Mueller C, Kirsch WM. Immunophenotypes in the circulation of
711 patients with mild cognitive impairment. *J Psychiatr Res*. 2008 Feb 1;42(3):240–6.

712 43. Martins LCA, Rocha NP, Torres KCL, Santos RR dos, França GS, Moraes EN de, et al.
713 Disease-specific expression of the serotonin-receptor 5-HT_{2C} in natural killer cells in
714 Alzheimer's dementia. *J Neuroimmunol*. 2012 Oct 15;251(1):73–9.

715 44. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack Jr. CR, Kawas CH, et al.
716 The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National
717 Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's
718 disease. *Alzheimers Dement*. 2011;7(3):263–9.

719 45. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The
720 diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the
721 National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for
722 Alzheimer's disease. *Alzheimers Dement*. 2011;7(3):270–9.

723 46. Jabbari E, Holland N, Chelban V, Jones PS, Lamb R, Rawlinson C, et al. Diagnosis
724 Across the Spectrum of Progressive Supranuclear Palsy and Corticobasal Syndrome. *JAMA*
725 *Neurol*. 2020 Mar 1;77(3):377–87.

726 47. Boxer AL, Miller BL. Clinical Features of Frontotemporal Dementia. *Alzheimer Dis*
727 *Assoc Disord*. 2005 Dec;19:S3.

- 728 48. Matar E, Ehgoetz Martens KA, Halliday GM, Lewis SJG. Clinical features of Lewy body
729 dementia: insights into diagnosis and pathophysiology. *J Neurol*. 2020 Feb 1;267(2):380–9.
- 730 49. McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, et al.
731 Diagnosis and management of dementia with Lewy bodies. *Neurology*. 2017 Jul 4;89(1):88–100.
- 732 50. Keshavan A, O’Shea F, Chapman MD, Hart MS, Lunn MP, Paterson RW, et al. CSF
733 biomarkers for dementia. *Pract Neurol*. 2022 Aug 1;22(4):285.
- 734 51. Bevan-Jones WR, Surendranathan A, Passamonti L, Rodríguez PV, Arnold R, Mak E, et
735 al. Neuroimaging of Inflammation in Memory and Related Other Disorders (NIMROD) study
736 protocol: a deep phenotyping cohort study of the role of brain inflammation in dementia,
737 depression and other neurological illnesses. *BMJ Open*. 2017 Jan;7(1):e013187.
- 738 52. Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the
739 Human Immunology Project. *Nat Rev Immunol*. 2012 Mar;12(3):191–200.
- 740 53. Ashton NJ, Brum WS, Di Molfetta G, Benedet AL, Arslan B, Jonaitis E, et al. Diagnostic
741 Accuracy of a Plasma Phosphorylated Tau 217 Immunoassay for Alzheimer Disease Pathology.
742 *JAMA Neurol* [Internet]. 2024 Jan 22 [cited 2024 Jan 24]; Available from:
743 <https://jamanetwork.com/journals/jamaneurology/fullarticle/2813751>
- 744 54. Breunig MM, Kriegel HP, Ng RT, Sander J. LOF: identifying density-based local outliers.
745 *ACM SIGMOD Rec*. 2000 Jun;29(2):93–104.
- 746 55. Thorndike RL. Who belongs in the family? *Psychometrika*. 1953 Dec 1;18(4):267–76.
- 747 56. Syakur MA, Khotimah BK, Rochman EMS, Satoto BD. Integration K-Means Clustering
748 Method and Elbow Method For Identification of The Best Customer Profile Cluster. *IOP Conf*
749 *Ser Mater Sci Eng*. 2018 Apr;336(1):012017.
- 750 57. Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Doré V, et al. High
751 performance plasma amyloid- β biomarkers for Alzheimer’s disease. *Nature*. 2018
752 Feb;554(7691):249–54.
- 753 58. Zhang YR, Wang JJ, Chen SF, Wang HF, Li YZ, Ou YN, et al. Peripheral immunity is
754 associated with the risk of incident dementia. *Mol Psychiatry*. 2022 Apr;27(4):1956–62.
- 755 59. Prinz M, Priller J, Sisodia SS, Ransohoff RM. Heterogeneity of CNS myeloid cells and
756 their roles in neurodegeneration. *Nat Neurosci*. 2011 Oct;14(10):1227–35.
- 757 60. Sprangers S, Vries TJ de, Everts V. Monocyte Heterogeneity: Consequences for
758 Monocyte-Derived Immune Cells. *J Immunol Res*. 2016;2016(1):1475435.
- 759 61. Russo MV, Latour LL, McGavern DB. Distinct myeloid cell subsets promote meningeal
760 remodeling and vascular repair after mild traumatic brain injury. *Nat Immunol*. 2018
761 May;19(5):442–52.
- 762 62. Ziegler-Heitbrock L. Blood Monocytes and Their Subsets: Established Features and Open
763 Questions. *Front Immunol* [Internet]. 2015 [cited 2023 Jun 12];6. Available from:
764 <https://www.frontiersin.org/articles/10.3389/fimmu.2015.00423>
- 765 63. Berriat F, Lobsiger CS, Boillée S. The contribution of the peripheral immune system to
766 neurodegeneration. *Nat Neurosci*. 2023 Jun;26(6):942–54.
- 767 64. Colonna M. The biology of TREM receptors. *Nat Rev Immunol*. 2023 Feb 7;1–15.

- 768 65. Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial
769 infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol.* 2006
770 Mar;7(3):311–7.
- 771 66. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell
772 subsets. *Trends Immunol.* 2001 Nov 1;22(11):633–40.
- 773 67. Poli A, Kmieciak J, Domingues O, Hentges F, Bléry M, Chekenya M, et al. NK Cells in
774 Central Nervous System Disorders. *J Immunol.* 2013 Jun 1;190(11):5355–62.
- 775 68. Poli A, Michel T, Thérésine M, Andrès E, Hentges F, Zimmer J. CD56bright natural killer
776 (NK) cells: an important NK cell subset. *Immunology.* 2009;126(4):458–65.
- 777 69. Deczkowska A, Weiner A, Amit I. The Physiology, Pathology, and Potential Therapeutic
778 Applications of the TREM2 Signaling Pathway. *Cell.* 2020 Jun 11;181(6):1207–17.
- 779 70. Peng X, Guo H, Zhang X, Yang Z, Ruganzu JB, Yang Z, et al. TREM2 Inhibits Tau
780 Hyperphosphorylation and Neuronal Apoptosis via the PI3K/Akt/GSK-3 β Signaling Pathway In
781 vivo and In vitro. *Mol Neurobiol.* 2023 May 1;60(5):2470–85.
- 782 71. Gratuze M, Leyns CEG, Holtzman DM. New insights into the role of TREM2 in
783 Alzheimer’s disease. *Mol Neurodegener.* 2018 Dec 20;13(1):66.
- 784 72. Silvin A, Uderhardt S, Piot C, Da Mesquita S, Yang K, Geirsdottir L, et al. Dual ontogeny
785 of disease-associated microglia and disease inflammatory macrophages in aging and
786 neurodegeneration. *Immunity.* 2022 Aug;55(8):1448-1465.e6.
- 787 73. Casati M, Ferri E, Gussago C, Mazzola P, Abbate C, Bellelli G, et al. Increased
788 expression of TREM2 in peripheral cells from mild cognitive impairment patients who progress
789 into Alzheimer’s disease. *Eur J Neurol.* 2018;25(6):805–10.
- 790 74. Earls RH, Menees KB, Chung J, Gutekunst CA, Lee HJ, Hazim MG, et al. NK cells clear
791 α -synuclein and the depletion of NK cells exacerbates synuclein pathology in a mouse model of
792 α -synucleinopathy. *Proc Natl Acad Sci U S A.* 2020 Jan 21;117(3):1762–71.
- 793 75. Walton RL, Soto-Ortolaza AI, Murray ME, Lorenzo-Betancor O, Ogaki K, Heckman
794 MG, et al. TREM2 p.R47H substitution is not associated with dementia with Lewy bodies.
795 *Neurol Genet.* 2016 Jul 14;2(4):e85.
- 796 76. Albertini C, Petralla S, Massenzio F, Monti B, Rizzardi N, Bergamini C, et al. Targeting
797 Lewy body dementia with neflamapimod-rasagiline hybrids. *Arch Pharm (Weinheim).*
798 2024;357(6):2300525.
- 799 77. Cummings J, Zhou Y, Lee G, Zhong K, Fonseca J, Cheng F. Alzheimer’s disease drug
800 development pipeline: 2024. *Alzheimers Dement Transl Res Clin Interv.* 2024 Apr;10(2):e12465.
- 801 78. Mehta NH, Zhou L, Li Y, McIntire LB, Nordvig A, Butler T, et al. Peripheral immune cell
802 imbalance is associated with cortical beta-amyloid deposition and longitudinal cognitive decline.
803 *Sci Rep.* 2023 May 31;13(1):8847.
- 804 79. George J. TREM2 as an evolving therapeutic target in Alzheimer’s disease. *Neural Regen*
805 *Res.* 2023 Apr 10;18(12):2680–1.

806 80. Malpetti M, Swann P, Tsvetanov KA, Chouliaras L, Strauss A, Chikaura T, et al. Blood
807 inflammation relates to neuroinflammation and survival in frontotemporal lobar degeneration.
808 Brain. 2024 Aug 19;awae269.

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Tables

Table 1. Demographic and clinical summaries for patients and controls. Differences in sex were evaluated using Chi Squared test. Differences in cognitive/clinical test scores and biomarkers were evaluated using Kruskal Wallace Tests followed by Dunn’s Post hoc analysis giving H. * indicates statistical significance between groups at $p < 0.05$.

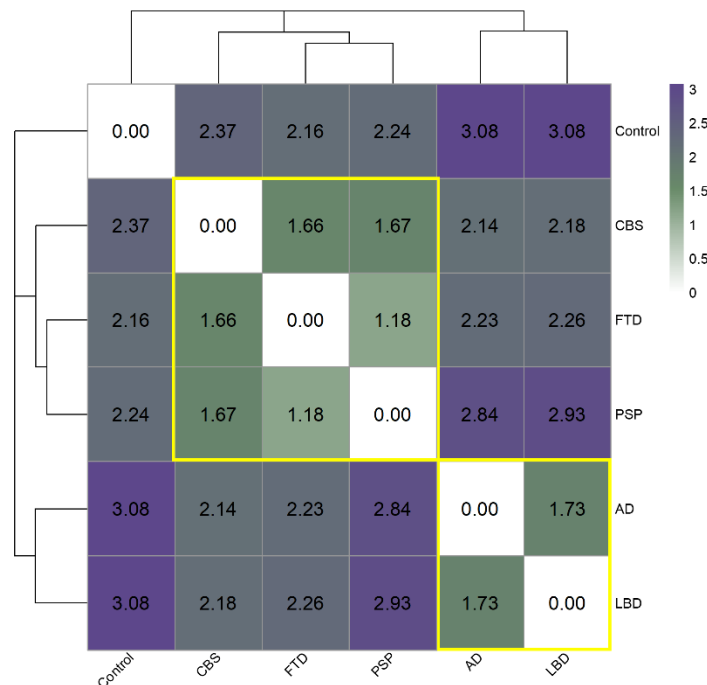
	Control	AD	CBS	LBD	FTD	PSP	Group difference
n	37	24	23	29	18	54	-
Sex, F/M	14/23	9/15	17/6	4/25	6/12	26/28	Chi Squared = 20.31, $p < 0.001^*$
Age, years, Mean (SD)	69 (7)	72 (7)	69 (9)	74 (7)	69 (8)	72 (7)	ns
MMSE, Mean (SD)	29 (1)	23(5)	25 (5)	25 (4)	21 (9)	27 (4)	H = 49.2, df = 5, $p < 0.001^*$
ACE-R, Mean (SD)	96 (3)	67 (16)	75 (18)	72 (15)	69 (26)	81 (13)	H = 76.8, df = 5, $p < 0.001^*$
CDR Sum of Boxes, Mean (SD) (n = 127)	0.038 (0.139)	5.12 (2.75)	5.83 (4.14)	8.45 (4.30)	12.2 (5.88)	7.29 (5.06)	H = 46.1, df = 5, $p < 0.001^*$
n Deceased by Census Date	0	2	2	10	8	20	-
n Biomarkers Collected	27	17	16	22	12	28	
ptau 217, pg/mL, Mean (SD)	0.331 (0.221)	0.924 (0.339)	0.667 (0.631)	0.666 (0.395)	0.412 (0.201)	0.456 (0.383)	H = 41.2, df = 5, $p < 0.001^*$
Nfl, pg/mL, Mean (SD)	18.33 (6.75)	32.5 (13.91)	57.133 (41.31)	34.223 (18.65)	58.367 (26.41)	51.272 (27.634)	H = 50.6, df = 5, $p < 0.001^*$
GFAP, pg/mL, Mean (SD)	107.27 (54.62)	192.8 (105.32)	238.48 (153.67)	161.2 (95.308)	174.50 (102.92)	164.99 (92.064)	H = 18.3, df = 5, $p = 0.002^*$
AB40/AB42, pg/mL, Mean (SD)	16.98 (2.703)	19.6 (2.38)	17.695 (2.66)	18.721 (5.029)	16.288 (2.21)	19.643 (5.32)	H = 16.0, df = 5, $p = 0.007^*$

Abbreviations: AD = Alzheimer’s disease, CBS = Corticobasal syndrome, LBD = Lewy Body Disease, FTD = frontotemporal dementia, PSP = progressive supranuclear palsy. AB = Amyloid beta, p-tau = phosphorylated tau, NfL = Neurofilament light chain, GFAP = Glial fibrillary acidic protein.

Figures

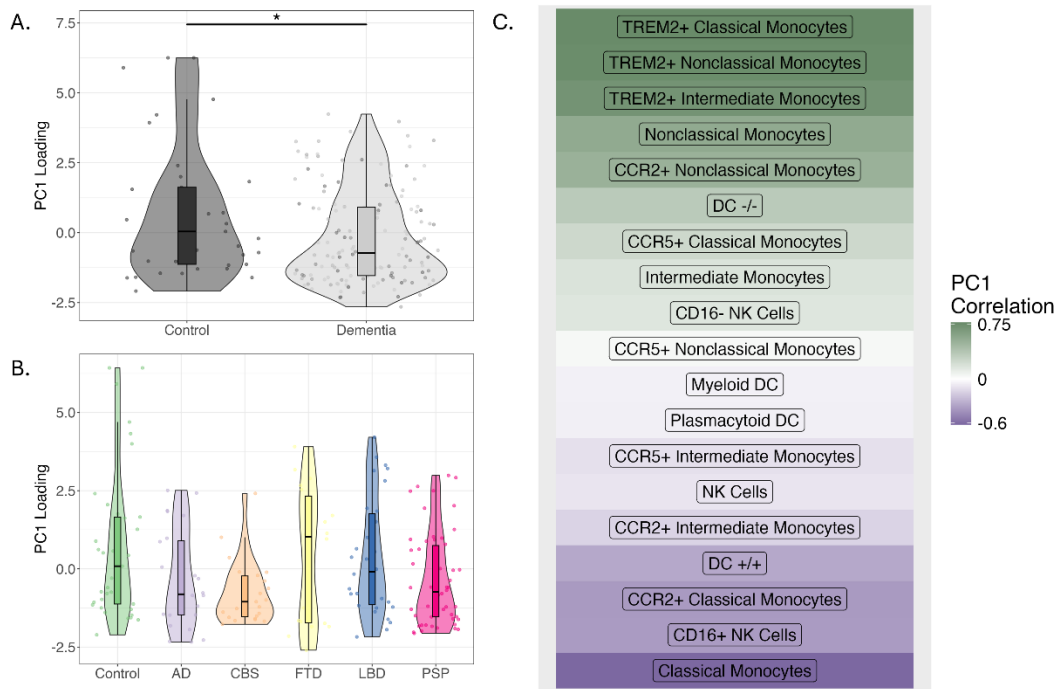
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825 Figure 1. Euclidean Distance dissimilarity analysis and hierarchical clustering. Colours and values
826 represent the Euclidean distance calculated from a 19-cell vector averaged across each group. The
827 value for the Euclidean distance is relative to the dataset, thus the number displayed represents the
828 length of the line segment between groups relative to the total group distance. Darker colors and
829 larger Euclidean distance values indicate greater dissimilarity between 19-cell immune profile
830 across diagnostic groups, while lighter colors indicate relative similarity. The dendrogram
831 represents single linkage hierarchical clustering. Note that all patients separate from controls
832 initially, and that the group of syndromes associated with frontotemporal lobar degeneration are
833 similar to each other (PSP, FTD, and CBS), in contrast to Alzheimer's disease (AD) and Lewy
834 Body Disease (LBD). These relative similarities are highlighted in yellow.



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836 Figure 2. Immune profile loading onto TREM2+ monocytes and nonclassical monocytes
 837 (positively) and classical Monocytes (negatively). A) Median individual loading onto PC1 between
 838 controls and all-cause dementia patients ($W = 3351$, $p = 0.02$). B) Median individual loading onto
 839 PC1 for each diagnostic group. Note the absence of significant differences between patient groups.
 840 C) Correlations of each cell type with PC1. Darker colors indicate stronger positive and negative
 841 correlations to PC1. Mann Whitney U and Kruskal Wallace tests were used to compare medians.
 842 Results were considered significant at $p < .05$ indicated with *.

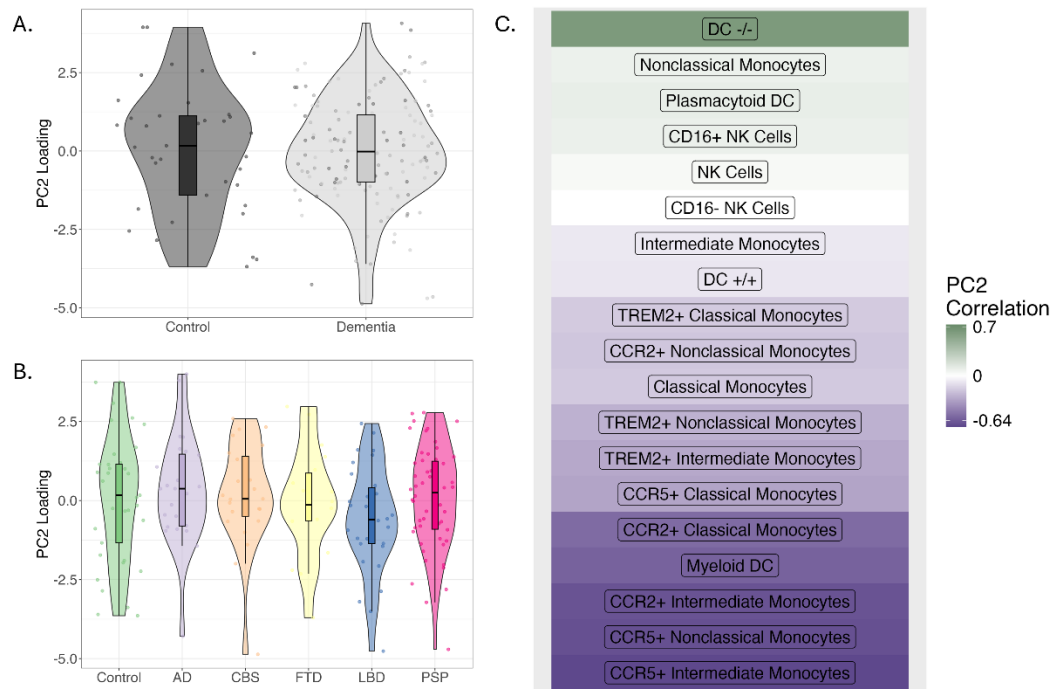


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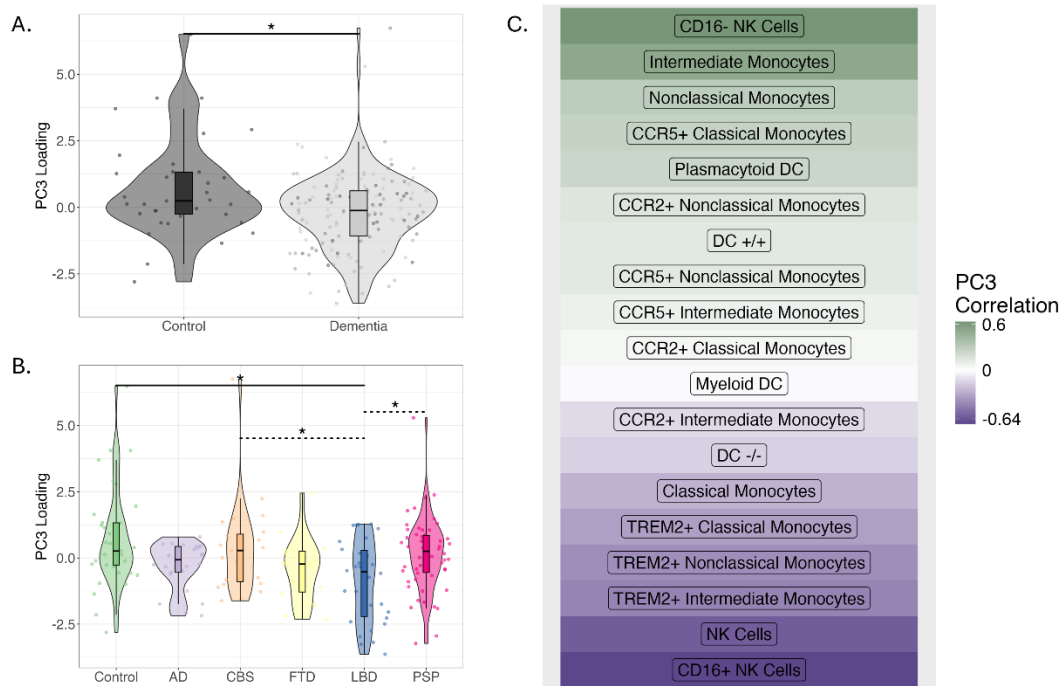
846 Figure 3. Immune profile loading onto DC-/- cells (positively) and CCR5+ monocytes
 847 (negatively). A) Median individual loading onto PC2 between controls and all-cause dementia
 848 patients. B) Median individual loading onto PC2 for each group. C) Correlations of each cell
 849 type with PC2. Darker colors indicate stronger positive and negative correlations to PC3. Mann
 850 Whitney U and Kruskal Wallance tests were used to compare medians. Results were considered
 851 significant at $p < 0.05$.



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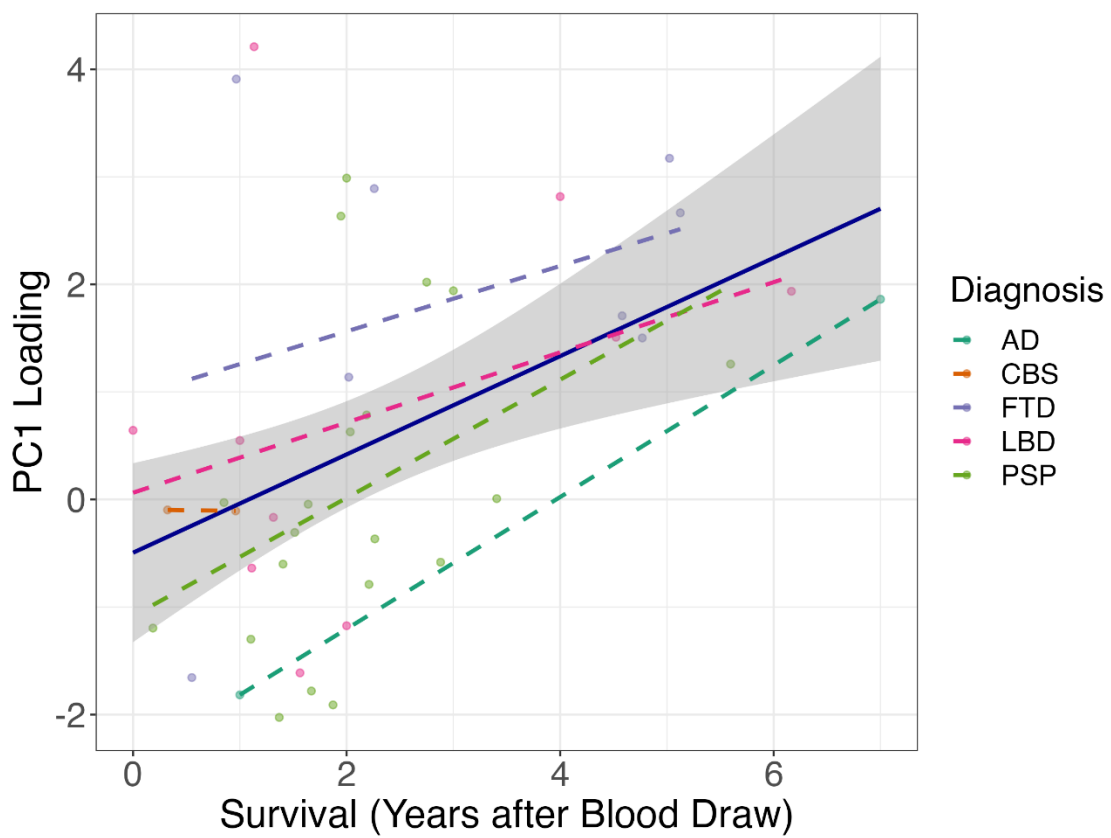
854 Figure 4. Immune profile loading onto CD16- NK cells (positively) and CD16+ NK cells
 855 (negatively). A) Median individual loading onto PC3 between controls and all-cause dementia
 856 patients, $W = 2025$, $p = 0.015$. B) Median individual loading onto PC3 for each group. Post hoc
 857 comparisons indicate LBD differs individually from controls, ($H = 3.38$, $p = 0.015$). C) Cellular
 858 correlations to PC3 extracted following PCA. Darker colors indicate stronger positive and
 859 negative correlations to PC3. Mann Whitney U and Kruskal Wallace tests were used to compare
 860 medians followed by Dunn's Post hoc analysis with FDR correction for multiple comparison. A
 861 dashed line indicates a result of statistical significance prior to FDR correction that did not
 862 maintain significance following correction.



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865 Figure 5. Years of survival following blood draw correlate with individual PC1 scores across
866 diagnostic groups. A linear regression was used to establish how years of survival following
867 blood draw was predicted by individual PC1 loading across all patient groups ($\beta = 0.450$, $F =$
868 10.098 , $p = 0.0026$, $r^2 = 0.184$), in a subset of 42 patients who had died by the census date.
869 Outcomes were considered significant at $p < 0.05$.



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