



Trinity College Dublin

Coláiste na Tríonóide, Baile Átha Cliath

The University of Dublin

GETTING UNDER THE SKIN:

*How Social Adversity and Psychosocial Stress Affect the Immune
System in Health and Ageing*

Conor Reddy

B.A (Mod) Genetics

Department of Clinical Medicine

Under the Supervision of Prof. Clíona Ní Cheallaigh

**A Thesis Submitted to The University of Dublin, Trinity College for the
Degree of Doctor of Philosophy**

2026

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

The assistance and work of the following individuals has been included:

- Nicole Roche, Ailbhe Herity, Dr. Emma Connolly; who helped with recruitment, sample processing and data collection in PATH and AESS-Vax.
- Dr. Barry Moran, Prof. Jean Fletcher who provided initial guidance and a template for spectral cytometry assays, including the backbone of the immunophenotyping panel used in Chapter 5.
- Dr. Adam Dyer, who I collaborated with to optimise the immunophenotyping panel and protocol used in Chapter 5.
- Prof. Nollaig Bourke, who provided feedback on initial drafts of thesis sections and guidance through both studies.
- Prof. Clíona Ní Cheallaigh as PI who shaped and guided both PATH and AESS-Vax and provided feedback on drafts of this thesis.

I agree to deposit this thesis in the University's open access institutional repository or allow the library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgment. I consent to the examiners retaining a copy of my thesis beyond the examining period, should they wish to do so (EU GDPR May 2018).

Conor Reddy
.....

Acknowledgments

“Scholarship that is indifferent to human suffering is immoral.” Richard Lewontin

This thesis was written in the belief that science is not neutral. It reflects the world, but it can also shape it. When that world is marked by inequality and preventable suffering, research has a responsibility to engage and change it.

This work is dedicated to the marginalised and excluded, the homeless and the poor. It is grounded in the belief that inequality leaves a biological trace, that social exclusion and material deprivation are not just social facts, but immunological forces that profoundly affect health. But if health is shaped by social realities, then improving health outcomes must involve changing those realities. I hope this work contributes, however modestly, to the growing case for a science that more holistically recognises the social, and for public health grounded in justice.

To DePaul Ireland, thank you for your partnership, trust and support throughout my PhD journey. Without your commitment to this research, it would not have been possible. I look forward to continuing our relationship in research, and to supporting DePaul in advancing social justice, dignity, and meaningful change for those who need it the most.

To the homeless peer advisors who helped design the AESS-Vax and PATH studies, your clarity and honesty were invaluable. To all the participants in both studies, housed and unhoused alike, thank you for your generosity, for sharing your experiences, and for making this research possible. This work is stronger, and more meaningful, because of all of you. I hope that your voices are heard and reflected in this thesis, and in all of the research outputs that follow from it.

To the Lab 1.07 originals; Isabella Batten, Matt McElheron, Adam Dyer, and Liam Townsend. Thank you for the craic, the chaos, the memories, and of course, your support and friendship over the last five years. From Belfast to Berlin and the pipetting marathons that sometimes led late into the night, I couldn't have asked for better company in the trenches. You are all hugely talented scientists, and I feel privileged to have worked alongside you.

To Ailbhe Herity, Nicole Roche and Emma Connolly; although you've moved on to new chapters, your friendship and collaboration on these studies has been invaluable. To Ailbhe in particular, I was supposed to be a mentor to you in your MSc work on PATH, but I ended up learning far more from your insight and judgement than the other way around. Thank you.

To Dr. Aisling O'Halloran, Dr. Daniel Kit Hang Chu, Dr. Georgia Richards, Tara Kenny, and Jordy Smith for all your feedback, support and guidance over the years. If Carlsberg did lab mates...

To those who have *Lived, Labbed and Loved* in the TTMI since I came as a final year undergrad; to the Social Committee, to the lunch table, to the PIs and post-docs, research assistants and other staff members who make the TTMI the welcoming and academically stimulating place that it is.

To the members and executive of the Postgraduate Workers Organisation; I am immensely proud of what we have built together and the progress we have made for postgraduate researchers across this country. It was a privilege to serve as President of our union and to have made some great friends along the way.

To everyone in People Before Profit, particularly Dublin North West; for giving me community, deep friendships, purpose, and mentorship over the years. Thank you for reminding me, again and again, that change is possible when we organise together.

To my "kitchen cabinet", Aislinn Shanahan Daly, Alexandra Day, Clara McCormack, and Damian Kelly; your tireless work, honesty, and well-timed kicks up the backside won us a Council seat and helped get me over the line with this thesis when both elections were behind us. It's been a crazy year but only a beginning. I'm more than lucky to call you friends and comrades. Míle Buíochas.

To my parents, Ciara and Aidan for your care, encouragement and endless generosity, I can't thank you enough. And to my sister, Dr. Avril Reddy, for being a sounding board, editor and support when it mattered most.

To Bana Abu Zuluf, my partner, comrade and closest confidant. Your curiosity, compassion and patience pulled me through the hardest parts of this journey (your cooking over the past week played its part too!). I've learned so much from your *sumud* and your ability to find joy in the simplest things. I look forward to returning the favour next year when you're finishing your PhD, though I doubt my culinary skills will match yours!

To Nollaig, it's hard to know where to begin. Thank you for all you have done to support and inspire me over many years. Your guidance and the example you have set as a research leader and mentor has left its mark on me and so many others. I am hugely grateful to you, I would not have pursued or completed this PhD without you.

Finally, Clíona, thank you for showing me what science can look like when it's driven by empathy, integrity, and a refusal to look away. As a mentor and a supervisor, you have offered me unwavering support throughout this PhD. Your passion, humanity, and commitment have shaped both this work and my own path as a researcher. I look forward to the chapters yet to be written and to seeing our work make a difference.

Conor Reddy

March 2025

Table of Contents

.....	i
Declaration.....	i
Acknowledgments	ii
List of Figures	ix
List of Tables.....	x
List of Abbreviations	xi
Funding.....	xv
Oral Presentations	xv
Poster Presentations	xv
Outreach and Public Engagement	xvi
Abstract	1
Lay Abstract	3
1.0 Introduction	6
1.1 Homelessness - Social Determination at the Extremes	8
1.2 Allostasis and Allostatic Load.....	9
1.3 The Physiological Stress Response:.....	11
1.3.1 The Sympathetic Adreno-Medullary and Hypothalamic Pituitary Adrenal Axis:	14
1.3.2 Cellular Responses to SAM and HPA Axis Signalling.....	16
1.3.3 The Effects of Acute Stress on The Immune System:	19
1.4 Chronic Stress and the Immune System:	21
1.4.1. The Sympathetic-Adrenal-Medullary (SAM) Axis:.....	21
1.4.2 Immunological Consequences of Hypothalamic-Pituitary Adrenal Axis Dysregulation:	23
1.4.3 The Conserved Transcriptional Response to Adversity:.....	24
1.4.4 Stress-Induced Myelopoietic Shifts and Immune Dysregulation:.....	27
1.4.5 Mechanisms of Glucocorticoid Resistance	31
1.5 Linking Stress and Status Effects on Immunity to Health:	34
1.5.1 Respiratory Virus Challenge Studies.	34
1.5.2 Stress and the Reactivation of Latent Herpesvirus Infections.....	36

1.5.3 Vaccine Responses as a Model for Stress Effects on Immunity.....	39
1.6 Hypotheses:.....	46
1.6.1 Aims.	47
1.6.2 Specific Aims:	47
2.1 Equipment and Reagents	50
2.2 Kits.....	51
2.3 Fluorescent Antibodies for Flow Cytometry	51
2.4 Buffer Preparation	52
2.5 Software and Electronic Resources.....	52
2.6 AESS-Vax Participant Recruitment	53
2.7 PATH Participant Recruitment.....	54
2.8 AESS-Vax Questionnaire Methodology	54
Perceived Stress Scale	56
Holmes-Rahe Life Event Stress Inventory (HRLES).....	57
Adverse Childhood Experiences Questionnaire:.....	57
McArthurs' Ladder	58
2.9 PATH Survey Methodology	58
2.10 Biobanking of Samples for AESS-Vax Study:.....	59
2.10.1 – Isolation of Peripheral Blood Mononuclear Cells from Whole Blood:	59
2.10.2: Counting Peripheral Blood Mononuclear Cells and Assessing Cell Viability Using Hemacytometer:	60
2.10.3 Freezing PBMC Aliquots for AESS-Vax and PATH Biobanks.....	60
2.10.4 Biobanking Serum and Plasma Samples For AESS-Vax and PATH Biobanks	61
2.11 ELISA for Anti-CMV Antibodies:.....	61
2.12 Multiplex Cytokine and Chemokine Analysis Using MSD Immunoassay Platform	63
2.12.1 Human Custom Immune Biomarker Panel on U-Plex Platform	63
2.12.2 Human Proinflammatory (human) Biomarker Panel 1 on S-Plex Platform ..	65
2.13 Lymphocyte Immunophenotyping in Cryopreserved Peripheral Blood Mononuclear Cells.....	67
3.1 Introduction.....	72
3.1.1 Pathways Linking Social Exclusion to Poor Health	73

3.1.2 Psychosocial Stress and Trauma as Social Determinants of Health.....	75
3.1.3 The Homeless Population Has Grown and Become More Diverse.....	76
3.1.4 Studying the Health and Wellbeing of Socially Excluded and Homeless People Requires Appropriate Tools:.....	77
3.2 Hypothesis:	78
3.2.1 Specific Aims:	78
3.3 Specific Methods:.....	79
3.3.1 Overview of Statistical Approaches.....	79
3.3.2 Data Preparation and Transformation.....	79
3.3.3 Regression Models	80
3.3.4 Model Selection and Interpretation.....	80
Other Statistical Tests	81
3.3.6 R Packages Used	82
3.4 Results:.....	83
3.4.1 Homeless Adults in the AESS Vax Cohort are Older and Experience Poorer Health than Housed Participants - Review of Cohort Demographic and Health Data	83
3.4.2 Long Term Homeless Adults in the PATH Cohort Experience Premature Frailty and Poorer Health than the Housed Population.....	90
3.4.3 Long-Term Homeless Adults Experience a High Burden of Psychosocial Adversity and Trauma Throughout the Lifecourse	93
3.4.4 High SES Participants in AESS-Vax Were More Likely to Complete Psychosocial Stress Inventories and Questionnaires:.....	98
3.4.5 Stress Scales in AESS-Vax Assess Different Domains of Psychosocial Stress:	99
3.4.6 Psychosocial Stress and Trauma in the AESS-Vax Cohort is Experienced to an Increasing Extent as Socioeconomic Status is Lowered.....	102
3.4.7 Gender Influences the Experience and Perception of Psychosocial Stress and Trauma:	104
3.4.8 Relationship of Stress, Socioeconomic Status and Social Exclusion on Health	108
3.5 Discussion	113
1. Health Behaviours and the Social Gradient in Health in AESS-Vax and PATH ...	116

2. Impact of SES and Housing Stability on Participation and Bias in Data Interpretation	118
3. Psychosocial Stress and Health Outcomes: The Role of SES and Social Exclusion	120
4. Gendered Patterns in Psychosocial Stress and Health Outcomes	121
5. Implications for Health Policy and Interventions	122
3.6 Conclusion.....	123
4.0 Introduction:.....	127
4.1 Hypothesis:	127
4.1.1 Specific Aims:	128
4.2 Specific Methods:	129
4.2.1 Roche Elecsys Anti-Nucleocapsid and Anti-Spike Spike Assays	129
4.2.2 Interferon Gamma Release Assays:	130
4.2.3 Inclusion and Exclusion of Participant.....	131
4.2.4 Statistical Analyses and Modelling	131
4.3 Results:	133
4.3.1 Anti-Spike Antibodies are Diminished in Participants with Low Socioeconomic Status After Vaccination Against SARS CoV-2.....	133
No Significant Differences Between SES Groups or Homeless and Housed Participants up to 16 Weeks Post-Third Dose of Vaccine:	138
4.3.2 Modelling the Impact of Socioeconomic Status and Demographic Factors on Vaccine-Induced Humoral Immunity	140
4.3.3 Interaction Models Assessing SES and Demographic Influences on Vaccine - Induced Humoral Immunity:	144
4.3.4 Mediation Analysis of Stress as a Pathway Linking SES to Vaccine-Induced Humoral Immunity	146
V2 Antibody Titres	148
Power Analysis Results.....	151
Model Fit Indices	152
4.3.6: Cell-Mediated Vaccine-Induced Immunity is Diminished in Low SES Participants after Booster Vaccination:.....	153
4.3.7: Pairwise Analysis of Correlation Between Antibody Titres and Antigen-Specific IFN- γ Production After Third Dose of Vaccine	154

4.4 Discussion:	157
4.43 Public Health Implications of the Immunity Gap:	161
4.44 Contribution to the Literature and Future Directions:	162
4.5 Conclusion:	165
5.1 Introduction:	168
5.2 Hypothesis:	168
5.2.1 Specific Aims:	169
5.3 Specific Methods:.....	170
5.3.1 Sample and Data Acquisition:	170
5.3.2 Multiplex Cytokine and Chemokine Assays:	170
5.3.3 Univariate Statistical Analyses:	171
5.3.4 Multivariate Modelling	172
5.3.5 Cytokine Network and Module Analysis	172
5.3.6 Principal Component Analysis and PERMANOVA.....	172
5.3.7 Lymphocyte Immunophenotyping by Spectral Cytometry	173
5.3.8 Univariate Analyses of Immunophenotyping Data	175
5.3.9 R Packages Used:.....	176
5.4 Results:.....	177
5.4.1 Univariate Analyses of Circulating Cytokines and Chemokines Reveal Distinct Immune Profiles in Homeless and Low SES Participants:.....	177
5.4.2 Adjusted Regression Models Reveal Independent Associations Between Adversity and Cytokine Expression.....	183
5.4.3 Network and Hierarchical Clustering Analysis of Cytokine Profiles	191
5.4.4 Principal Component Analysis and PERMANOVA of Cytokine Variation	197
5.4.5 Low Socioeconomic Status is Associated with a More Mature, Terminally Differentiated Lymphocyte Immunophenotype	200
5.4.6 Adjusted Models Confirm Differences in Lymphocyte Immunophenotype by SES Group Persist After Controlling for CMV Status, Age and Sex	213
5.5 Discussion:	214
5.5.1 Soluble Signatures of Social Adversity – Cytokine and Chemokine Analyses	214
5.5.2 T Cell Immunophenotypes and Immune Ageing in Excluded Populations...	217

5.5.3 Novelty, Limitations, and Future Directions	219
5.6 Conclusion:.....	220
6.1 The Biology of Exclusion: Immune Function Across the Social Divide	225
6.2 The Other Vaccine Inequality: Vaccine-Induced Immunity is Socially Patterned:	227
6.3 Immune Composition and Function Across the Social Gradient	230
6.4 Future Directions.....	232
6.5 Conclusion:.....	234
References	236
Appendix 1 – AEES Vax Participant Questionnaire:	265
Appendix 2: PATH-I Participant Questionnaire:	280

List of Figures

Figure	Pg
Figure 1.1 Growth of Research in Psychoneuroimmunology Mentioning the Social Determinants of Health (2010-2024)	8
Figure 1.2 Fundamentals of the Physiological Stress Response	13
Figure 1.3 Integration of the Nervous, Endocrine and Immune System	19
Figure 1.4 Effects of Acute Stress on the Immune System	20
Figure 1.5 The Effects of Chronic Stress on the Immune System	28
Figure 1.6 Summary of Study by Snyder-Mackler et al (2016) Documenting the Effects of Social Rank on Immune Regulation and Responses to Infection in Female Rhesus Macaques	31
Figure 1.7 Schematic of Glucocorticoid Receptor Signalling	32
Figure 3.1 Psychological, Environment and Social determinants of Health in Socially Excluded Populations Including People Experiencing Homelessness	72
Figure 3.2 Distribution of Health and Behavioural Variables Collected in the AEES-Vax Study Across Socioeconomic Status Groups	87
Figure 3.3 Clinical Frailty Scores and Self-Rated Health in the PATH Cohort	89
Figure 3.4 Pair Plot of Stress Scales with Correlation Coefficients This Figure Displays Pairwise Relationships Between Four Stress-Related Variables	99
Figure 3.5 Distributions of Scores for Psychosocial Stress and Trauma Across Three Distinct Socioeconomic Groups in the AEES-Vax Study	101
Figure 3.6 Distribution of Stress Scale Scores by Gender Across SES Groups	103
Figure 3.7 Density Plots of Stress Scales by SES and Gender	105
Figure 4.1 Graphical Representation of Workflow and Aims for the AEES-Vax Study	125
Figure 4.2 Recruitment and Sample Collection Through the AEES-Vax Study	131
Figure 4.3 Total Anti-Spike Antibody Levels in High and Low SES Participants Post-Vaccination	132
Figure 4.4 Total Anti-Spike Antibody Levels in Socially Excluded and Housed Participants Post-Vaccination	134

Figure 4.5 Total Anti-Spike Antibody Levels in Participants by Socioeconomic Status and Social Exclusion Status Post-Third Dose	136
Figure 4.6 Interaction Models of SES, Age, and Sex on Vaccine-Induced Humoral Immunity	143
Figure 4.7 Path Diagram for the Full Models with Paths Annotated	145
Figure 4.8: Interferon Gamma Production in Incubated Whole Blood Samples	151
Figure 4.9 Pair plots of Antibody Titre and Interferon Gamma Production after Spike and Peptivator Spike Complete Stimulations	153
Figure 5.1 Basic Gating Strategy for Lymphocyte Immunophenotyping Panel	170
Figure 5.2 Identification of CD4+ and CD8+ T Lymphocyte Populations	172
Figure 5.3 Cytokines and Chemokines by Housing Status	175
Figure 5.4 Cytokines and Chemokines by Socioeconomic Status	178
Figure 5.5 Adjusted Effects of Homelessness and Covariates on Cytokine Levels	185
Figure 5.6 Adjusted Effects of Socioeconomic Status and Covariates on Cytokine Levels	186
Figure 5.6 Spearman Correlation Matrix of Log-Transformed Cytokine Levels	187
Figure 5.7 Cytokine Correlation Networks by Social Exclusion	189
Figure 5.8 Cytokine Correlation Networks by Socioeconomic Status	190
Figure 5.9 Hierarchical Clustering Dendrogram of Log-Transformed Cytokine Levels	191
Figure 5.10 Boxplots of Module-Level Z-Scores by SES and Social Exclusion	193
Figure 5.11 PCA Scatter Plots of Participants Coloured by SES	195
Figure 5.12 Innate-Like Lymphocytes as Proportion of Live CD45+ Cells in High and Low Socioeconomic Status Participants	200
Figure 5.13 CD4+, CD8+and T Regulatory Lymphocytes by Socioeconomic Status	202
Figure 5.14 CD4+ and CD8+ Lymphocyte Subsets Defined by Expression of CCR7 and CD45RA Shown by Socioeconomic Status	203
Figure 5.15 CD4+ and CD8+ Effector Memory Subsets as Proportion of TEMs and RA-TEMRA as a Proportion of TEMRA	205
Figure 5.16 Expression of Common Senescence and Exhaustion Markers on CD4+ and CD8+ T Lymphocytes by Socioeconomic Status	206
Figure 5.17 Co-expression of Senescence (CD57+ KLRG1) and Exhaustion Markers (PD1+ TIGIT+) on CD4+ and CD8+ T Cells by Socioeconomic Status	208

List of Tables

Table	Pg
Table 1.1 Adrenergic Receptor Subtype Expression on Immune Cells, Signalling Pathways, and Immune Functional Effects	16
Table 1.2 Summary of Vaccine Response Studies Considering Effects of Psychosocial Stress	41
Table 2.1 Equipment and Reagents	49
Table 2.2 Kits Used During the AESS-Vax and PATH Studies	50
Table 2.3 Fluorescent Antibodies for Flow Cytometry	50
Table 2.4 Buffers Used Throughout the AESS-Vax and PATH Studies	51
Table 2.5 Software and Electronic Resources Used in the AESS-Vax and PATH Studies	51

Table 2.6 Summary of Demographic and Health Information Collected in the AESS-Vax Study	54
Table 3.1 Demographic and Health Profile of the AESS-Vax and PATH Cohorts	82
Table 3.2 Health and Functional Characteristics of the PATH Cohort	90
Table 3.3 Prevalence of Selected Traumatic Life Events in the PATH cohort	93
Table 3.4 Subjective Loneliness Questionnaire by Gender	94
Table 3.5 Relationships and Social Interactions as Recorded in the PATH Cohort	95
Table 3.6 Number of Completed Tests, Non-completions (NA) and Percentage Completion for Each Stress Scale Included in the AESS-Vax Questionnaire	96
Table 3.7 Results of Negative Binomial Regression Models Examining the Relationship Between Stress and Key Health Outcomes in the AESS Vax cohort	106
Table 3.8 Results of Negative Binomial Regression Models Examining the Relationship Between Stress and Key Health Outcomes in the AESS Vax cohort	109
Table 4.1 Summary of Antibody Levels by Socioeconomic Status and Effect Sizes	133
Table 4.2 Summary of Antibody Levels by Housing Status and Effect Sizes	135
Table 4.3 Summary of Antibody Levels by Socioeconomic Status and Social Exclusion Status with Effect Sizes Post-Third Dose	137
Table 4.4 Regression Coefficients for Socioeconomic Status and Demographic Factors on Vaccine-Induced Humoral Immunity Across Timepoints	139
Table 4.5 Incidence Rate Ratios (IRRs) for the Impact of Socioeconomic Status, Age, and Sex on Antibody Levels Across Timepoints	140
Table 4.6 Standardised Path Coefficients and p-values for Structural Equation Models Developed to Analyse Stress as a Mediator of the Relationship Between SES and Log-Transformed Anti-Spike Antibodies at the V2 Timepoint	147
Table 4.7 Standardised Path Coefficients and p-values for Structural Equation Models Developed to Analyse Stress as a Mediator of the Relationship Between SES and Log-Transformed Anti-Spike Antibodies at the V3 Timepoint	149
Table 4.8 Model Fit Parameters for All Models Using the Original and Imputed Datasets	153
Table 5.1 Univariate Comparisons of Cytokine Levels Between Homeless and Housed Group	176
Table 5.2 Univariate Comparisons of Cytokine Levels Between Low and High SES Groups	179
Table 5.3 Adjusted Associations of Social Exclusion with Log-Transformed Cytokine Levels	181
Table 5.4 Adjusted Associations of Socioeconomic Status with Log-Transformed Cytokine Levels	183
Table 5.5 Cytokine Modules Derived from Hierarchical Clustering	191
Table 5.6 Summary of the First Five Principal Components in PCA of Log-Transformed Cytokines	194
Table 5.7 PERMANOVA Results for the First Five Principal Components of Log-Transformed Cytokine Levels	196
Table 5.8 Descriptive Statistics for the Major T cell, NK cell, and Innate T Cell Subsets Captured Using Spectral Cytometry	198
Table 5.9 Associations Between Socioeconomic Status and Immune Cell Phenotypes After Covariate Adjustment	210

List of Abbreviations

Abbreviation	Definition

ACE	Adverse Childhood Experience
ACTH	Adrenocorticotrophic Hormone
AIC	Akaike Information Criterion
AL	Allostatic Load
AP	Anterior Pituitary
AR	Adrenergic Receptor
AVP	Arginine Vasopressin
BIC	Bayesian Information Criterion
BMI	Body Mass Index
BSC	Biological Safety Cabinet
CAR	Cortisol Awakening Response
CFI	Comparative Fit Index
CFS	Clinical Frailty Scale
CMV	Cytomegalovirus
CNS	Central Nervous System
COI	Cut Off Index (Elecsys Assay)
CREB	cAMP Response Element-Binding Protein
CRH	Corticotrophin Releasing Hormone
CSV	Comma-Separated Values
CTQ	Childhood Trauma Questionnaire
CTRA	Conserved Transcriptional Response to Adversity
DC	Dendritic Cell
DMSO	Dimethyl Sulfoxide
EBV	Epstein-Barr Virus
ECLIA	Electrochemiluminescence Immunoassay
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
ETS	Erythroblast Transformation Specific (transcription factor family)
GC	Glucocorticoid
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
GR	Glucocorticoid Receptor
HCT	Hematopoietic Cell Transplantation
HIV	Human Immunodeficiency Virus
HPA	Hypothalamic-Pituitary-Adrenal
HPV	Human Papillomavirus
HRLES	Holmes-Rahe Life Event Stress Inventory
HRP	Horseradish Peroxidase
HSV	Herpes Simplex Virus
IFN	Interferon
IGRA	Interferon-Gamma Release Assay
IL-x	Interleukin: cytokine (abbreviation is followed by number and occasionally letter to denote specific cytokine)
IP-10	Interferon Gamma-Induced Protein 10
IQR	Interquartile Range

IRR	Incidence Rate Ratio
ITEM	International Trauma Exposure Measure
KSHV	Kaposi's Sarcoma-Associated Herpesvirus
LAT	Latency-Associated Transcript (Gene)
LPS	Lipopolysaccharide
MAIT	Mucosal-Associated Invariant T Cell
MAP	Mitogen-Activated Protein
MCP	Monocyte Chemoattractant Protein
MHC	Major Histocompatibility Complex
MHC-II	Major Histocompatibility Complex Class II
MIDUS	Midlife in the United States
MIP-1b	Macrophage Inflammatory Protein-1 Beta
MR	Mineralocorticoid Receptor
NE	Norepinephrine
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells (transcription factor)
NK	Natural Killer cell
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate-Buffered Saline
PC	Principal Component
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PEH	People Experiencing Homelessness
PERMANOVA	Permutational Multivariate Analysis of Variance
PMM	Predictive Mean Matching (Imputation technique)
PNI	Psychoneuroimmunology
PSI	Psychosocial Stress Index
PSS	Perceived Stress Scale
PTSD	Post-Traumatic Stress Disorder
PVN	Paraventricular Nucleus
RMSEA	Root Mean Square Error of Approximation
RSD	Repeated Social Defeat
RUDAS	Rowland Universal Dementia Assessment Scale
SAM	Sympatho-Adrenal Medullary
SEM	Structural Equation Modelling
SES	Socioeconomic Status
SIV	Simian Immunodeficiency Virus
SNS	Sympathetic Nervous System
SVR	Sustained Virologic Response
TEM	Effector Memory T Cell
TEMRA	Terminally Differentiated Effector Memory T Cell Re-expressing CD45RA
TLR	Toll-Like Receptor
TNF-α	Tumour Necrosis Factor Alpha

VIF	Variance Inflation Factor
VZV	Varicella-Zoster Virus
WHO	World Health Organization

Funding

This work was supported by a Government of Ireland Enterprise Partnership Scholarship from the Irish Research Council (now Research Ireland) with Depaul Ireland as enterprise partners and co-funders.

Oral Presentations

1. Is Premature Ageing in Long-Term Homeless Adults Immune Driven?
TCD Medical Gerontology Research Symposium, April 2024
Awarded Gold Medal, Department of Medical Gerontology PG Research Prize
2. Getting Under the Skin: How Socioeconomic Position and Psychosocial Stress Affect Ageing and Immune Function.
British Society of Immunology Congress, Belfast, December 2023
3. Getting Under the Skin: How Socioeconomic Position and Psychosocial Stress Affect Ageing and Immune Function.
Institut Pasteur-TCD Immunology Research Forum; Paris, July 2023
4. The Other Vaccine Inequality.
Trinity Faculty of Health Sciences Annual Research Symposium, June 2023
Awarded: (Joint) 1st Prize, Postgraduate Category
5. Does Psychosocial Stress Associated with Socioeconomic Status Affect Vaccine Induced Immunity to SARS CoV-2?
St James' Hospital Immunology Seminar, May 2022
6. Under the Skin: How Low Socioeconomic Status Affects Health Outcomes in Infectious Disease.
Trinity Translational Medicine Institute Conference Research Blitz, June 2021
Awarded: 2nd Place, Faculty Blitz Prize

Poster Presentations

1. Getting Under the Skin: How Socioeconomic Position and Psychosocial Stress Affect Ageing and Immune Function
European Congress of Immunology, Convention Centre Dublin, September 2024
2. The Effects of Socioeconomic Status and Psychosocial Stress on Vaccine-Induced Immunity to SARS-CoV-2: Early Results from the AESS-Vax Study
Young European Federation of Immunological Societies (yEFIS) Symposium, Berlin, November 2022
Awarded: Travel Prize by yEFIS-EFIS abstract judging panel; €500.

3. The Effects of Socioeconomic Status and Psychosocial Stress on Vaccine-Induced Immunity to SARS-CoV-2
Irish Society for Immunology Annual Meeting, Maynooth, September 2023
4. The Effects of Socioeconomic Status and Psychosocial Stress on Vaccine-Induced Immunity to SARS-CoV-2: Study Plan and Preliminary Results from the AESS-Vax Study
British Society of Immunology Congress, Edinburgh, December 2021

Outreach and Public Engagement

Science and Society – Dublin Digital Radio, October 2023

Live radio appearance discussing the relationship between science, inequality, and immunology.

Health vs Inequality – Tortoise Shack Live Podcast,

The Sugar Club Dublin, November 2021

Panel discussion on health inequalities in Ireland, featuring contributions from frontline clinicians, researchers, and inclusion health advocates.

Abstract

The stark disparities in health outcomes revealed during the COVID-19 pandemic have highlighted an urgent need to understand the biological consequences of social inequality. This thesis explores the overall hypothesis that many of the health outcomes caused by social inequality are due to alterations within the immune system; specifically, it investigates how socioeconomic status (SES), homelessness, and psychosocial adversity shape trajectories of immune ageing and impact on vaccine responsiveness using two linked human cohorts: Assessing Effects of SES-Associated Psychosocial Stress on Vaccine-Induced Immunity to SARS-CoV-2 (AESS-Vax, n=164) and Premature Ageing in long-Term Homeless Adults (PATH, n=59). Participants were stratified by SES and housing status, and provided detailed sociodemographic, clinical, and psychosocial data alongside biological samples. Immune parameters assessed included SARS-CoV-2 vaccine-induced humoral and cellular immunity, multiplex cytokine profiling, and spectral cytometry-based immunophenotyping of lymphocyte subsets.

In AESS-Vax, total anti-Spike antibody levels were lower in infection-naïve low SES participants at both 12–16 and 22–28 weeks post-vaccination. Similarly, antigen-specific IFN- γ production following stimulation of whole blood with Spike peptides and protein was diminished in low SES participants at both 12–16 and 22–28 weeks following a third, booster dose of BNT162b2. Structural equation modelling did not detect significant mediation by psychosocial stress; however, post-hoc power analyses indicated the study was underpowered to detect such effects, a limitation compounded by inconsistent collection of stress measures across participants. This is the first

study to comprehensively profile post-vaccine immune responses and broad cellular immunophenotypes in socially excluded and homeless individuals.

To explore the broader range of social effects on immunity, a combined cohort comprised of both PATH and AESS-Vax participants was used (n=173). In an immune proteome screen of serum samples from this cohort, eight cytokines were found to be elevated in homeless compared to housed individuals, including TNF- α , IL-1 β , IL-6, IL-10, IL-12p70, IL-18, IL-4, and IL-2. Differences were also observed by SES, though of lesser magnitude. Notably, the differences associated with homelessness persisted after adjusting for age, sex, smoking history, and alcohol consumption. These cytokine profiles are consistent with observations of the immune profile seen in "inflammageing", highlighting homelessness as a particularly strong immunological stressor. Furthermore, high-dimensional immunophenotyping revealed that participants from low SES backgrounds had reduced frequencies of naive CD4⁺ and CD8⁺ T cells. They also had expanded populations of CD57⁺, KLRG1⁺, and TEMRA cells, features associated with terminal differentiation, immune exhaustion, and cellular senescence. These differences were independent of age, sex, and CMV serostatus, and were most pronounced in those with histories of chronic psychosocial stress and social exclusion.

Together, these findings define an adversity-associated immunophenotype marked by persistent inflammation, altered lymphocyte composition, and diminished functional immune responses to vaccination. This social adversity associated immunophenotype may represent a mechanism by which social disadvantage becomes biologically embedded, accelerating immune ageing

and compromising host defence. These results underscore the importance of integrating social context into models of human immunity and ageing.

Lay Abstract

Poverty and homelessness have a profound impact on how long we live and how healthy we are. While the social effects of inequality are well known, we are only beginning to understand how social inequality gets "under the skin" to affect biology.

This research investigates how chronic exposure to poverty, homelessness, and psychosocial stress can shape the functioning of the immune system over time. We studied two groups drawn from two linked studies: AESS-Vax, which focused on socioeconomic status and COVID-19 vaccine responses, and PATH, which focused on premature ageing in long-term homeless people. We divided participants from both studies based on their housing status and socioeconomic background, allowing us to compare immune function across different levels of social advantage and exclusion. In AESS-Vax, participants received the same COVID-19 vaccine and samples were collected after vaccination to study immunity to the virus that causes COVID-19. In PATH, samples were collected independently of vaccination status. We also assessed participants' overall health and the levels of stress they experienced throughout their lives.

We found that individuals from lower socioeconomic backgrounds and those who were homeless exhibited weaker immune responses to the vaccine. Blood samples from these groups showed lower levels of protective antibodies and signs of reduced cellular immune activity; meaning their immune systems

responded less effectively to the vaccine. Their immune systems also showed signs of ageing; with increased inflammatory proteins and fewer of the young, adaptable immune cells the body needs to fight new infections and generate protection after vaccination.

These findings suggest that long-term exposure to poverty, unstable housing, and social stress may accelerate biological ageing and impair vaccine responsiveness. This is the first study to show that poverty and homelessness are linked to changes in the immune system that look like early ageing. Recognising these links is key to designing better public health responses and to understanding how life circumstances shape our biology.

Chapter 1: Introduction

“Health inequalities and the social determinants of health are not a footnote. They are the main issue... The cause of causes are the social determinants of health” - Michael Marmot (2018) – Inclusion Health: Addressing the Cause of Causes¹

1.0 Introduction

The quality of the lives we live, the ailments we face and the age we live to are as much socially as biologically determined. This basic truth has never been clearer. While we advance our scientific understanding of human biology and our technological capacity to diagnose and treat disease faster than ever before, health inequalities grow, and population health worsens. The gap between the healthiest and least healthy in society is widening, not narrowing. In wealthy, industrialised nations like Britain and the United States, life expectancy, once on a steady upward trajectory, has begun to stagnate and, in some places, decline, for the first time in generations. In the U.S., life expectancy fell for two consecutive years in the wake of the Covid-19 pandemic, marking the sharpest decline in a century². The wealthiest 1% of American men can now expect to live nearly 15 years longer than the poorest 1%³ ; a disparity that has doubled since the 1980s⁴. In Britain, similar trends are emerging, with life expectancy improvements grinding to a halt in the most deprived areas while continuing to rise for the most affluent⁵. The Covid-19 pandemic cast these disparities into sharp relief as the compounding effects of increased occupational exposure, a higher burden of comorbidities and reduced access to healthcare meant large gaps in Covid-19 incidence and outcomes between the rich and the poor. In Mexico, the poorest decile of

people diagnosed with Covid-19 were five times more likely to die from Covid-19 than the richest decile⁶.

These patterns are more than statistical curiosities; they reveal an important underlying truth - that social and economic conditions shape human biology, profoundly. Rudolf Virchow, pioneer of cell theory and a founding father of modern pathology understood the inseparability of social and biological determinants of health as far back as the 1840s, as he observed during the 1848 Upper Silesian Typhoid Epidemic, *“the local conditions of society must be thoroughly investigated as they determine the course of disease”*⁷. Yet, for much of the modern, molecular era, biologists have left the study of social determinants to epidemiologists, socially conscious medics, and statisticians, creating a major gap in our understanding of disease processes. Marmot’s landmark 2008 study for the World Health Organisation (WHO) estimated that core social determinants such as income, employment, and education account for 30-55% of health outcomes, independent of behavioural and environmental factors⁸ but there are a paucity of causal models linking these social determinants to outcomes through disease processes. However, as health inequalities soar and high-throughput human -omics based studies have become more feasible, there has been a renewed interest in the integration of social and biological determinants in models of health and disease. This interest is evident in the growth of psychoneuroimmunology (PNI) research over the past decade as researchers attempt to unravel the biological pathways linking social conditions with health outcomes (see figure 1 below).

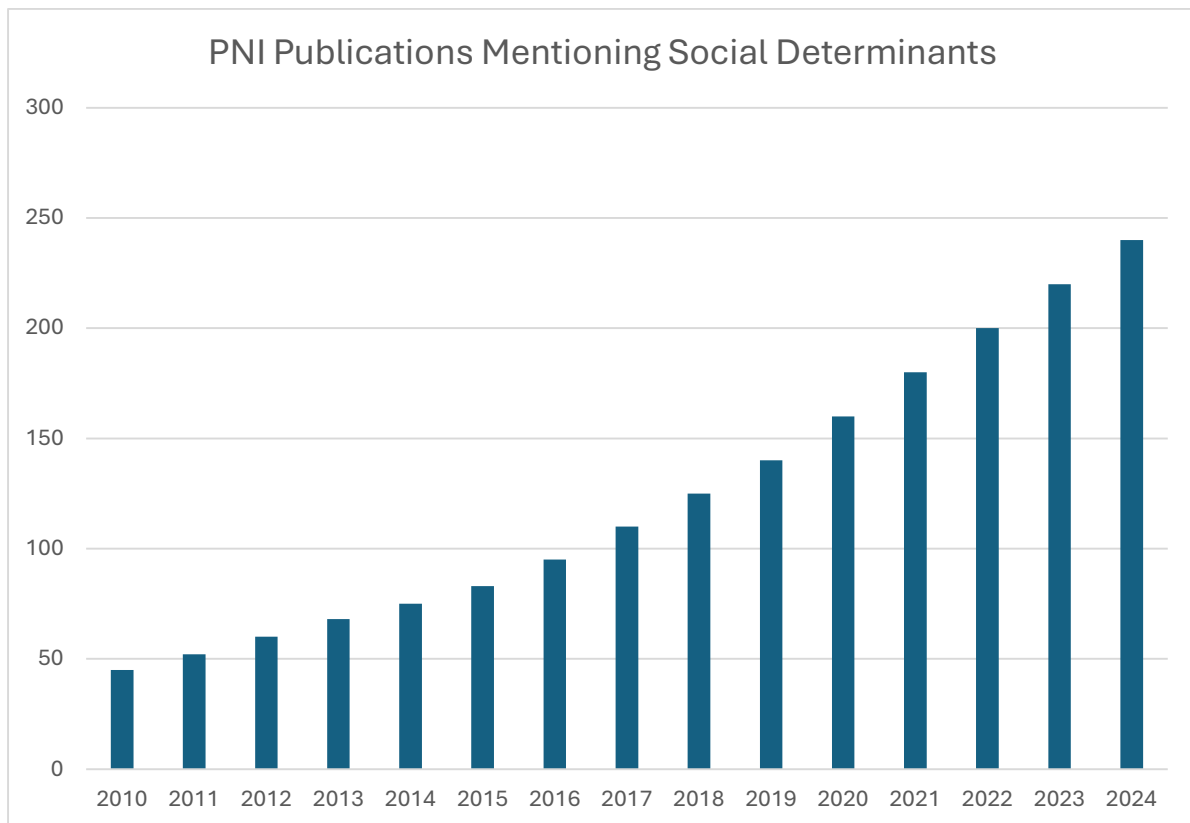


Figure 1.1 Growth of Research in Psychoneuroimmunology Mentioning the Social Determinants of Health (2010-2024) Publication data was retrieved from PubMed using the search terms ("psychoneuroimmunology" OR "psychoneuroendocrinoimmunology") AND ("social determinants" OR "socioeconomic" OR "psychosocial").

1.1 Homelessness - Social Determination at the Extremes

In Ireland, the median age at death for people experiencing homelessness (PEH) is just forty-two years⁹. Globally, PEH face disproportionately high rates of multimorbidity, premature frailty^{10,11}, and early mortality¹². While social determinants of health shape outcomes across the entire population, their effects are not linear, additive, or predictable. Most homeless adults in Ireland are "multiply excluded,"¹³ experiencing overlapping disadvantages where the compounding effects of material deprivation, chronic stress, and social

exclusion accelerate biological ageing and drive mortality rates that set PEH apart from the rest of society.

More than a condition of housing instability, homelessness is an extreme form of social exclusion¹³, marked not only by material deprivation but also by profound psychosocial adversity. PEH are significantly more likely to experience severe trauma and social isolation in adulthood, and these adversities are often compounded by a history of childhood adversity, with many having experienced neglect, abuse, or unstable family environments¹⁴. Homelessness, therefore, can be seen as a state where the fundamental determinants of health throughout the life course (economic security, social cohesion, and environmental stability) are profoundly disrupted. Given the outsized effects of social determinants in PEH, the study of health in the homeless population may serve as a natural experiment, shedding light on the causal pathways that allow social disparities to become biologically embedded. Recognising these dynamics is critical not only for understanding the limits of traditional socioeconomic models of health inequality but also for informing public health strategies that address the most marginalised populations. Health inequalities in PEH are discussed in more detail in Chapter 3.

1.2 Allostasis and Allostatic Load

In 1988, neurophysiologists Peter Sterling and Joseph Eyer introduced the concept of allostasis to better describe the physiological processes that allow organisms to adapt to ever-changing social and physical environments¹⁵. Unlike the earlier concept of homeostasis, which presumes optimal fixed

physiological set points and the maintenance of static equilibrium, allostasis recognises that biological regulation is inherently dynamic and context dependent, with internal physiology adjusting to meet varying internal and external demands.

The regulation of blood glucose levels serves as a clear example of an allostatic response. During prolonged fasting or caloric restriction when blood sugar is low, insulin receptors are upregulated to increase sensitivity to the effects of the hormone. In the context of high blood glucose levels, on the other hand, receptor expression is often downregulated to modulate sugar uptake by cells. Physiologically, there is not one “optimal” level of receptor expression; what is optimal is entirely dependent on environmental and physiological demands faced by the organism – in this case blood sugar levels at a particular moment. Through allostasis, biological systems reach physiological states which are adaptive in response to acute stressors. While allostasis is crucial for short-term adaptation, prolonged activation of these mechanisms can lead to cumulative strain, shifting physiological systems into states that become increasingly costly to maintain. Prolonged or repeated exposure to mediators of allostasis, including catecholamines, glucocorticoids (GCs), inflammatory cytokines, metabolic hormones, and autonomic nervous system modulators, can induce biological resistance to their effects, impairing transitions between allostatic states. Beyond their immediate effects, these mediators can also drive long-term structural changes in organs and tissues, such as stress-induced hypertrophy of vascular smooth muscle in hypertension¹⁶. When physiological systems are pushed beyond their evolved limits, they begin to accumulate physiological “wear and tear”; a phenomenon termed Allostatic Load (AL)¹⁷.

The dynamic capacity of physiological systems is evolutionarily advantageous, allowing organisms to efficiently manage energy and resources in response to environmental pressures. However, in modern environments characterised by chronic stressors rather than acute threats, these same adaptive mechanisms may contribute to long-term physiological dysregulation. Since AL was first proposed by stress biologist Bruce McEwen in 1993¹⁸, numerous methodologies have emerged to quantify it, often in composite scores incorporating biomarkers of neuroendocrine, immune, cardiovascular, and metabolic function (See McCrory, 2019 for example)¹⁹. Elevated AL scores indicate greater cumulative biological risk and have been associated with increased mortality risk²⁰, frailty²¹, and the incidence of various age-related diseases²².

Although the direct measurement of AL did not take place as part of the research presented in this thesis, the contribution of psychosocial stress to AL, the role of immune dysregulation in its manifestation, and its distinct social patterning²³ make it conceptually relevant. These concepts provide a framework for understanding how socioeconomic status (SES)-associated psychosocial stress affects immune function, reinforcing the necessity of integrating social and biological determinants in understanding health disparities.

1.3 The Physiological Stress Response:

Coordinating allostasis and underlying stress effects on the immune system are multiple physiological systems, including the brain, the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal-medullary (SAM) axes, which

play central roles in the physiological stress response. The physiological stress response itself is a key coordinator of both allostasis and homeostasis, dynamically regulating physiological processes to maintain internal stability while adapting to environmental demands. The physiological stress response is ancient and evolutionarily conserved, integrating the central nervous system (CNS), autonomic nervous system, endocrine system and less conventionally acknowledged, the immune system²⁴. Together, they communicate in a shared chemical language of hormones, cytokines, chemokines, and neurotransmitters, enabling these four systems to function as an integrated network that maintains allostasis and homeostasis in response to environmental challenges.

The physiological stress response is activated in conditions of physical, environmental, and psychosocial adversity, driving physiological adaptations that help meet the imposed demands of these stressors. Effects of the two arms of the stress response are highly pleiotropic. Epinephrine and norepinephrine (or adrenaline and noradrenaline), the mediators of the SAM axis component of the response, regulate cardiovascular, pulmonary, renal, hepatic, musculoskeletal and gastrointestinal systems in conditions of high stress and/or threat. Glucocorticoid hormones produced by HPA activation, can suppress inflammation, drive gluconeogenesis, mobilise energy reserves, and maintain metabolic homeostasis.

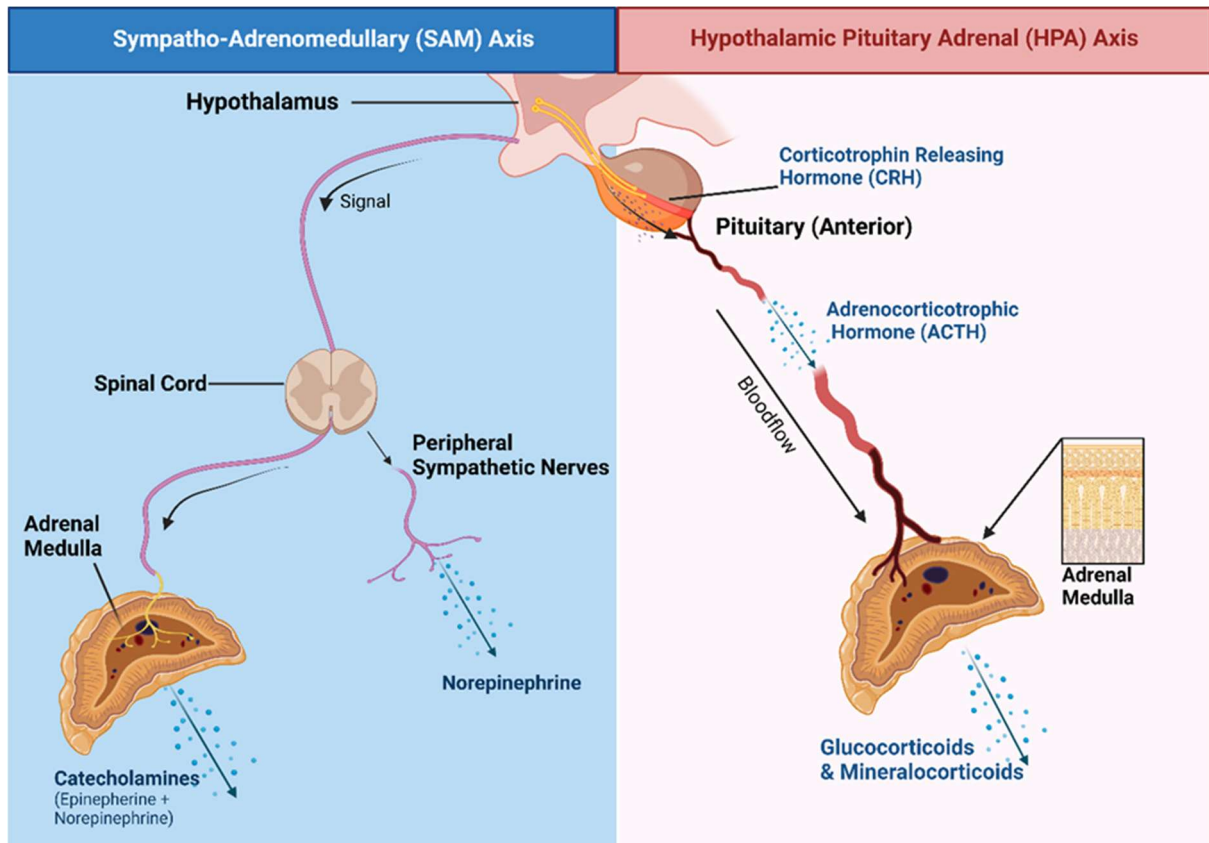


Figure 1.2 Fundamentals of the Physiological Stress Response

Responses to physical stressors are the fastest-acting and most primordial, coordinated by neural structures in the brainstem and hypothalamus (See Godoy et al 2018 for review of stress neurobiology²⁵) These include reflexive withdrawal from pain and shivering in response to cold, both of which involve direct neural circuits that bypass higher cortical processing, allowing for rapid adaptive responses to environmental stressors.

Psychosocial and cognitive stress responses, which arise when an organism evaluates environmental demands as exceeding its adaptive capacity²⁶, elicit similar responses to physical stressors but have added layers of complexity and regulation involving limbic circuits that connect the prefrontal cortex, amygdala, hippocampus, nucleus accumbens and other brain regions (see

Godoy et al., 2018²⁵ for review). This complexity allows for the integration of a diverse array of psychological stressors, from adversities shared across species, like social isolation²⁷ and threat of violence²⁸, to those specific to humans; varying between cultures and even individuals in the extent to which they cause stress (e.g. meaning and purpose in work²⁹, negative emotionality³⁰).

Importantly however, not all stress-relevant processing is accessible to conscious awareness or amenable to self-report. A substantial component of stress physiology is driven by rapid, non-conscious threat and safety appraisal systems centred on subcortical and brainstem-proximal circuitry, which can activate autonomic and HPA responses independently of conscious distress. In affective neuroscience, this distinction is often framed as a dissociation between the neural mechanisms that initiate defensive responding and the later, consciously reportable experience of stress or anxiety. In contexts characterised by chronic unpredictability and reduced environmental safety, these non-conscious “unsafety” signals may remain persistently engaged even when subjective reports of stress are modest ³¹ (see LeDoux, 2015 for an accessible book on the neuroscience and biology of stress and fear).

1.3.1 The Sympathetic Adreno-Medullary and Hypothalamic Pituitary Adrenal Axis:

Once the stress response is activated, its mediators are sympathetic neurons and catecholamines of the autonomic nervous system that project throughout the body and the components of the HPA axis. Activation of the sympathetic nervous system (SNS) in response to stress results in the secretion of

epinephrine into systemic circulation by the adrenal medulla and the release of norepinephrine both into systemic circulation (in smaller amounts than epinephrine) and at the terminals of SNS fibres to coordinate localised sympathetic activity in organs and tissues.

In contrast, HPA axis activation takes place across several sites and stages, beginning with stress-related signals relayed from the brainstem and limbic system to the paraventricular nucleus (PVN) of the hypothalamus. Activation of the PVN by these limbic and brainstem signals stimulates release of Corticotropin Releasing Hormone (CRH) into the hypophyseal portal system, a vascular network linking the hypothalamus to the anterior pituitary, where CRH - and in more intense stress, arginine vasopressin (AVP) - bind corticotroph cells that synthesise and release ACTH into systemic circulation. When ACTH reaches the suprarenal circulatory network around the adrenal glands, it binds melanocortin 2 receptors (MC2R) expressed in the adrenal cortex, triggering release of cortisol into the bloodstream completing the HPA axis cascade. As cortisol is secreted systemically, it exerts negative feedback on production of ACTH and CRH, meaning that under normal physiological conditions, the HPA axis auto-regulates³².

While the SAM and HPA axes operate in parallel, they are also interconnected, as sympathetic activation can enhance Corticotropin Releasing Hormone (CRH) secretion from the hypothalamus (by stimulating the paraventricular nucleus (PVN) of the hypothalamus), priming the HPA response, an example of feed-forward regulation of the stress response³³. These include responses to pain, inflammation and extremes of temperature and can be divided into

two phases. Firstly, an immediate sympathetic nervous response that drives rapid adaptations throughout the body and secondly, a hormonal response, involving HPA axis activation and the secretion of glucocorticoids which prolong physiological adaptations to stress³⁴.

1.3.2 Cellular Responses to SAM and HPA Axis Signalling

At the level of effects on cells and tissues, catecholamines and glucocorticoids work via dedicated cellular receptors. In the case of epinephrine and norepinephrine, signalling occurs through a variety of adrenoceptors, which are coupled to G-proteins and expressed at the membrane of target cells. In humans there are two classes of receptor, each with multiple subtypes, (i) alpha (α 1 (1A, 1B and 1D) and α 2(2A,2B,2C)) and (ii) beta (β 1, β 2 and β 3); subtypes differing in their affinities for epinephrine and norepinephrine, their expression, and the G-proteins they are coupled to³⁵. Adrenergic signalling has pleiotropic effects in the cell, regulating various aspects of cell physiology and metabolism, as well as affecting the expression of specific sets of genes (see Lohse (2015)³⁵ or Perez (2006)³⁶ for review of the fundamentals of adrenoceptor biology).

Table 1.1 Adrenergic Receptor Subtype Expression on Immune Cells, Signalling Pathways, and Immune Functional Effects

Receptor Subtype	Expression in Immune Cells	Signalling Mechanism	Immune Functional Effects	Notes	Main Endogenous Agonist
α_1 ³⁷	Detected at low-to-moderate levels on T cells, B cells, monocytes, macrophages, and dendritic cells	G α q/11 pathway (activates Phospholipase C (PLC), increases intracellular Ca ²⁺ , and activates PKC)	Promotes pro-inflammatory cytokine release and enhances immune cell migration and adhesion.	Expression is variable and inducible (generally low at baseline; more abundant on vascular smooth muscle)	Predominantly Norepinephrine (NE)
α_2 ^{38,39}	T and B cells, monocytes, macrophages, and platelets	G α i pathway (inhibits adenylate cyclase, reducing intracellular cAMP)	Mediates immunosuppressive effects; attenuates pro-inflammatory cytokine production and modulates immune cell migration and adhesion	Plays a role in feedback regulation of neuroimmune interactions	Predominantly NE
β_1 ³⁹	Limited expression on immune cells (predominantly found in cardiac and vascular tissues)	G α s pathway (stimulates adenylate cyclase, increasing cAMP)	Minimal direct immunomodulatory role: any effects are likely indirect via cardiovascular effects	Considered less significant in direct immune modulation compared to β_2	NE and epinephrine (Epi)

$\beta_2^{39,40}$	Highly expressed on a broad range of immune cells - T cells, B cells, monocytes, macrophages, dendritic cells, NK cells.	G α s pathway (increases cAMP and activates protein kinase A (PKA))	Exerts strong anti-inflammatory effect, suppressing inflammatory cytokine production, influencing T cell differentiation, and regulating immune cell trafficking	Primary mediator of catecholaminergic immunomodulation	Predominantly Epi

Glucocorticoids, signal through the intracellular complexes⁴¹, the glucocorticoid (GR) and mineralocorticoid (MR) receptors, which have dual functionality as trans-activators and repressors of gene expression. GR complexes also exert non-genomic effects on intracellular signalling pathways, including negative regulation of the Mitogen Activated Protein Kinase (MAP-K) pathway⁴². Trans-activation involves the translocation of GC-bound GR complexes to the nucleus where they bind GC response elements (GREs), driving expression of target genes. Trans-repression, on the other hand, occurs when activated GR complexes bind to other transcription factors in the cytosol (e.g., NF-kB in innate immune cells), preventing their translocation to the nucleus and consequently inhibiting the expression of their target genes, many of which are pro-inflammatory. See Kadmiel and Cidlowski (2013)⁴¹ and Oakley and Cidlowski (2013)⁴³ for comprehensive overviews of GR signalling.

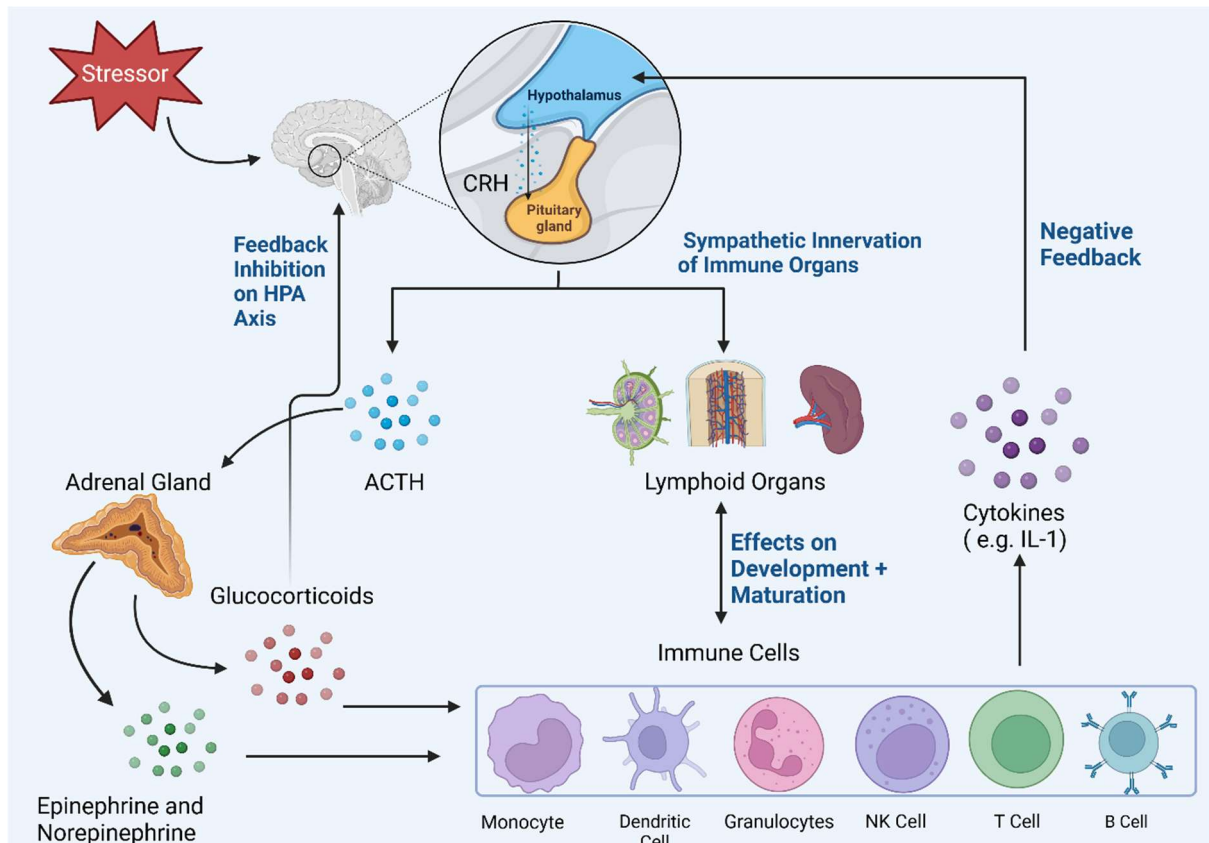


Figure 1.3 Integration of the Nervous, Endocrine and Immune System:

1.3.3 The Effects of Acute Stress on The Immune System:

The effects of acute stress on the immune system are biphasic. In the short term, on the order of minutes to hours, catecholamines enhance immune surveillance, redistribute leukocytes to sites of potential injury⁴⁴ and drive a transient increase in pro-inflammatory cytokine activity, likely serving an adaptive role in early wound healing and infection resistance⁴⁵. However, as the stress response develops, usually hours after initial activation, glucocorticoids like cortisol begin to exert anti-inflammatory and immune regulatory effects, suppressing excessive immune activation and preventing tissue damage. Through modulation of cytokine production and inhibition of immune cell trafficking, glucocorticoids play a crucial role in immune homeostasis and the resolution of inflammation.

As with allostasis, the physiological stress response is adaptive in the short term, helping to mobilise resources required to deal with threats and demands imposed by the physical and social environment. However, when stress becomes chronic or repeated, the same mechanisms that initially aid survival contribute to immune dysregulation and increased disease risk. The next section will explore the pathological effects of chronic stress on the immune system, which is one of the targets of the physiological stress response.

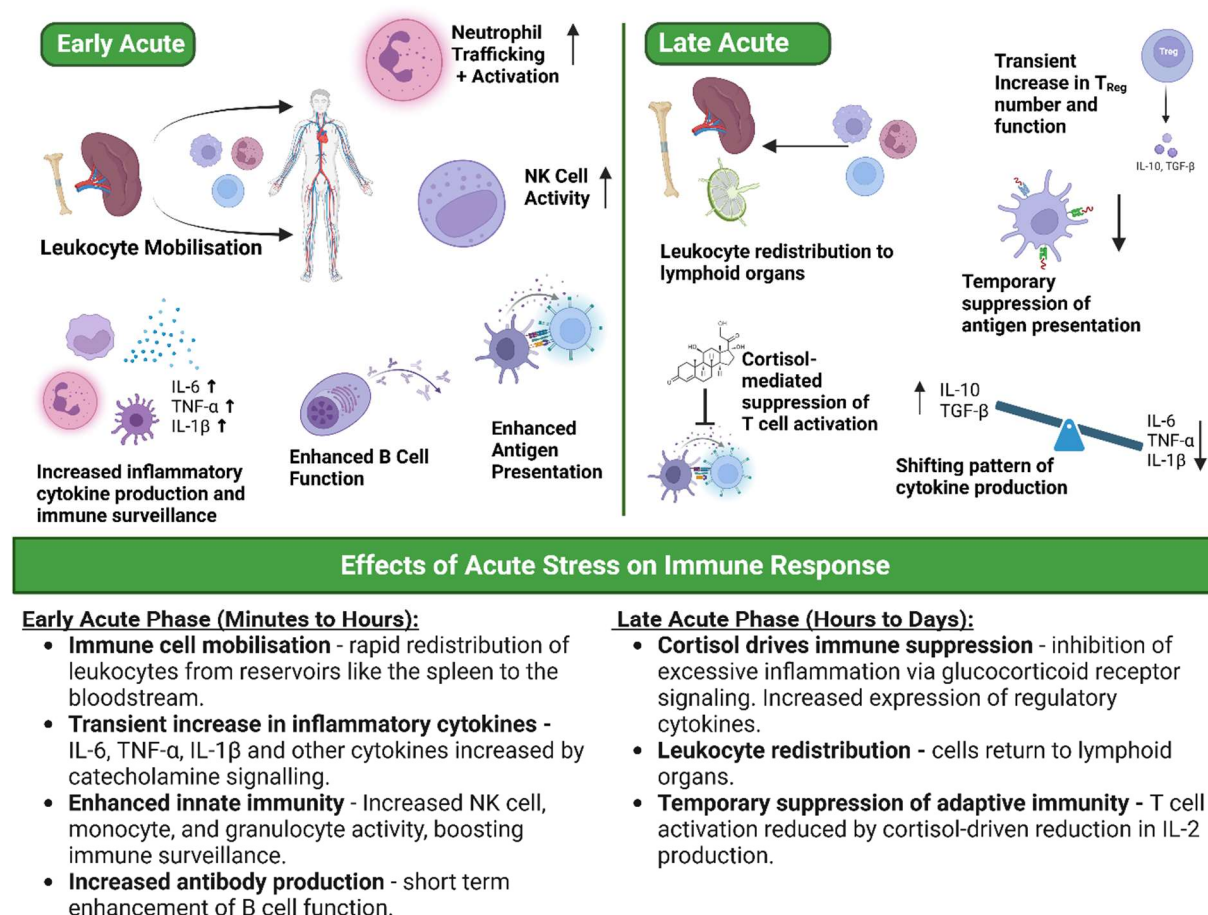


Figure 1.4 Effects of Acute Stress on the Immune System

1.4 Chronic Stress and the Immune System:

1.4.1. The Sympathetic-Adrenal-Medullary (SAM) Axis:

Among the most well characterised mechanisms explaining stress effects on the immune system is the activity of the SNS. Cells of the innate and adaptive immune system express adrenoceptors, particularly the $\beta 2$ receptor (ADRB2) which is most abundantly expressed, allowing them to respond directly to the catecholamines that mediate SNS signalling³⁹. Experiments carried out in the 1980s and 1990s began to cast light on the extent of connections between the SNS and immune system, showing the innervation of primary and secondary lymphoid tissues by norepinephrine (NE)-secreting sympathetic nerve fibres, and their direct association with immune cells^{46,47,47-49}. Early functional studies of SNS-signalling in immune cells suggested an immunosuppressive role for ADRB2; $\beta 2$ activation by NE (and other agonists) was shown to reduce production of inflammatory cytokines including Tumour Necrosis Factor Alpha (TNF- α), by macrophages and dendritic cells in response to challenge with lipopolysaccharide^{50,51} and then to suppress pro-inflammatory signalling through a variety of Toll-Like Receptor (TLR) pathways by rapidly inducing secretion of the anti-inflammatory cytokine, interleukin-10 (IL-10)⁵². $\beta 2$ activation has also been shown to reduce MHC-II expression on tissue-resident antigen presenting cells, potentially limiting T cell activation^{52,53} and shaping the adaptive immune response.

Activity is not constrained to innate cells either, ADRB2 activity has been shown to suppress *in vitro* IFN- γ production by Th1 cells⁵⁴, reduce IFN- γ and TNF- α secretion by activated CD8+ effector subsets⁵⁵ and to suppress cytolytic activity by CD8+ cytotoxic T lymphocytes (CTLs)⁵⁶. More recently, ADRB2

activation was demonstrated to inhibit lymphocyte egress from the lymph nodes by enhancing the expression of chemokine receptors that promote retention in the lymph nodes and to prevent migration to sites of inflammation; another mechanism for β 2 mediated suppression of inflammation⁵⁷.

In contrast, ADBR2 activity in the context of sustained inflammation has been shown to exert an opposing, proinflammatory effect, similar to that seen with alpha-adrenergic signalling in immune cells^{58,59}. This shift may be driven by receptor desensitisation, or changes in adrenergic receptor subtype expression which modulate downstream inflammatory responses.

Although less extensively studied in immune cells, α 1- and α 2-adrenergic receptors have been implicated in pro-inflammatory responses. α 1-AR signalling in monocytes and macrophages has been shown to enhance NF- κ B activation and cytokine production, while α 2-AR activation exhibits complex, context-dependent effects³⁷. While the precise mechanisms that determine the pro- or anti- inflammatory effects of β -adrenergic signalling are not well understood, it is likely that cellular context, NE concentration (lower in blood than in SNS-innervated immune organs) and signal duration and timing (autonomic activity is tonic and follows circadian and ultradian rhythms) all play a role (see Mueller et al. (2022)³⁴ for a more comprehensive review).

Regardless of mechanism, it is known that in conditions of chronic or extreme stress, SNS signalling is a major contributor to immune dysregulation, driving many of the stress-related pathologies of the immune system. This SNS-

induced dysregulated state emerges at two separate but interdependent levels, (i) by driving stress-associated patterns of gene expression in circulating immune cells and (ii) by influencing the developmental trajectories of immature myeloid cells, altering myelopoietic output, skewing towards pro-inflammatory, reactive phenotypes.

1.4.2 Immunological Consequences of Hypothalamic-Pituitary Adrenal Axis Dysregulation:

The SNS response to threat and stress enables rapid physiological adaptation in the face of challenge. In contrast, HPA axis-mediated adaptations to stress develop over time, often following SNS activation, deepening, sustaining, and expanding physiological changes. In the context of acute stress in humans, the glucocorticoid, cortisol acts on immune cells to suppress activation, particularly by inhibiting pro-inflammatory cytokine production in innate immune cells and downregulating interferon-regulated genes (IRGs) involved in antiviral responses. This suppression acts as a brake on immune activation, preventing pathological inflammation. However, with chronic stress, prolonged cortisol exposure can induce glucocorticoid resistance, reducing immune cells' sensitivity to cortisol's anti-inflammatory effects and leading to dysregulated immune function.

A pivotal study in 2002 by Sheldon Cohen's group provided early experimental evidence of chronic stress-induced glucocorticoid (GC) resistance in humans⁶⁰. Parents of paediatric cancer patients, who reported significantly higher stress levels than parents of healthy children, exhibited reduced sensitivity to dexamethasone-mediated suppression of cytokine production by peripheral

blood mononuclear cells (PBMCs). This demonstrated that chronic psychosocial stress impairs GC responsiveness, contributing to dysregulated immune activity.

1.4.3 The Conserved Transcriptional Response to Adversity:

In 2007, the stress biologist Steve Cole published a landmark paper, showing that the experience of loneliness was associated with alterations in genome-wide gene expression in circulating immune cells, with lonely individuals showing upregulation of genes involved in inflammation and immune cell proliferation, alongside downregulation of genes involved in type-1 IFN responses and antibody production²⁷. Taking this result as proof of concept that social adversity was capable of altering gene expression in immune cells, Cole and others began to probe other kinds of stress, from poverty and bereavement⁶¹ to unexpected job loss⁶² and chronic stress in caregivers^{63,64}. Across these diverse social contexts, and across cultures, what emerged was a common pattern of gene expression; upregulation of genes under the control of the proinflammatory AP-1, NF- κ B and β -adrenergic CREB-family of transcription factors and coincident downregulation of those under the control of type-1 IFNs and the glucocorticoid receptor. Studies of the effects of experimentally induced stress in other species reproduced this pattern too; in mice⁶⁵, in zebrafish⁶⁶ and in non-human primates⁶⁷, suggesting that response is evolutionarily conserved. With a convincing body of evidence assembled, Cole coined the term “Conserved Transcriptional Response to Adversity” (CTRA) to describe the pattern.

Since the CTRA was first conceptually established, research has been enhanced by technical advances in genomics that have allowed for analysis of whole transcriptomes (as opposed to pre-defined gene sets on microarrays) and for much larger studies. This has led to more sophisticated measurements of the CTRA including a contrast score, generated by taking quantitative read outs of expression for 53 genes commonly observed in the CTRA and for promoter-based bioinformatic analyses which first linked β -adrenergic signalling pathways to the emergence of the CTRA⁶⁸. Bioinformatic analyses integrating the expression of cell-type specific transcripts along with elements of the CTRA pattern (e.g., Transcript Origin Analysis) have also allowed for the identification of cells of the myeloid lineage (monocytes, macrophages, and dendritic cells, primarily) as the cell types that contribute most to the pattern observed in whole blood transcriptomic studies of the CTRA⁶⁹. Although myeloid-lineage cells dominate the CTRA signature, stress-related transcriptional shifts have also been observed in lymphocytes, where reduced IFN- γ production by NK and T cells, contributes to impaired antiviral immunity.

Recent research has applied an epidemiological approach to CTRA measurements, linking them to disease outcomes in large population-based health and aging studies, such as the Midlife in the United States (MIDUS) cohort⁷⁰. These efforts have been particularly fruitful, showing associations with increased cardiovascular risk⁷¹, cancer risk⁷², higher rates of cancer relapse⁷³, poorer response to hematopoietic cell transplant (HCT)⁷⁴ and worse outcomes following viral infections⁶⁹. Also significant in many of these studies is the association between CTRA contrast scores and low socioeconomic status, as documented in Cole et al. (2015)⁶⁹.

Beyond its association with disease states, emerging clinical trials have sought to pharmacologically mitigate CTRA-related immune dysregulation using β_2 antagonists like propranolol, in perioperative breast cancer patients⁷⁵, colorectal cancer⁷⁶ and to improve molecular risk markers in HCT recipients⁷⁷. Hiller et al (2020)⁷⁸ is a notable example – a randomized, placebo-controlled trial that investigated the effects of preoperative propranolol on metastatic biomarkers and immune infiltration in early-stage breast cancer patients (n=60). Propranolol significantly downregulated mesenchymal gene expression (P = 0.002) and reduced activity of pro-metastatic transcription factors NF- κ B, AP-1, and Snail/Slug (P < 0.01). It also enhanced tumour infiltration of NK cells, dendritic cells, and CD8⁺ T cells. These findings suggest that short-term β -blockade may attenuate metastatic potential and there are now multiple trials investigating the therapeutic utility of beta-blockade in cancer.

Beyond pharmacological intervention to ameliorate the CTRA, there have been a number of studies documenting reductions in CTRA scores following psychological and social interventions including meditation⁷⁹, yoga⁸⁰, Cognitive Behavioural Therapy⁸¹ and the practice of pro-social caring behaviour⁸². These findings, as well as the association between eudaimonic (but not hedonic) wellbeing and reduced CTRA signature⁸³ emphasise the value of psychosocial intervention in principle. Future research should explore whether structural interventions aimed at reducing poverty and social exclusion can mitigate CTRA-associated immune dysregulation, potentially offering a more sustainable approach to reducing health disparities.

1.4.4 Stress-Induced Myelopoietic Shifts and Immune Dysregulation:

The identification of monocytes, macrophages, and dendritic cells as the primary contributors to the CTRA gene expression profile through transcript origin analysis marked a major advancement in understanding stress effects on the immune system. Notably, the overrepresentation of specific monocyte subpopulations (e.g., CD14⁺ CD16⁻ classical monocytes) in individuals exhibiting the CTRA expression profile⁶⁹ raised the critical question of whether stress influences haematopoietic progenitor differentiation, thereby reshaping immune cell composition.

Earlier studies established associations between chronic stress and shifts in immune cell populations, including reductions in total B and T lymphocytes⁸⁴, diminished mononuclear cell counts, and more specifically, reductions in CD8⁺ T cells as well as decreased NK cell numbers and activity⁸⁵. Independently, sympathetic nervous system activity has been shown to mobilise haematopoietic stem cells (HSCs) from the bone marrow⁸⁶, activate HSCs⁸⁷, and alter myelopoiesis in response to systemic bacterial infections. The study of CTRA provided a conceptual bridge, linking stress-induced immune cell shifts to SNS regulation of myelopoietic niches, where sympathetic innervation and β -adrenergic signalling promote myeloid lineage commitment. These stress-driven changes prime the myeloid compartment for inflammatory responses, increasing monocyte and granulocyte availability and reactivity to immune challenges.

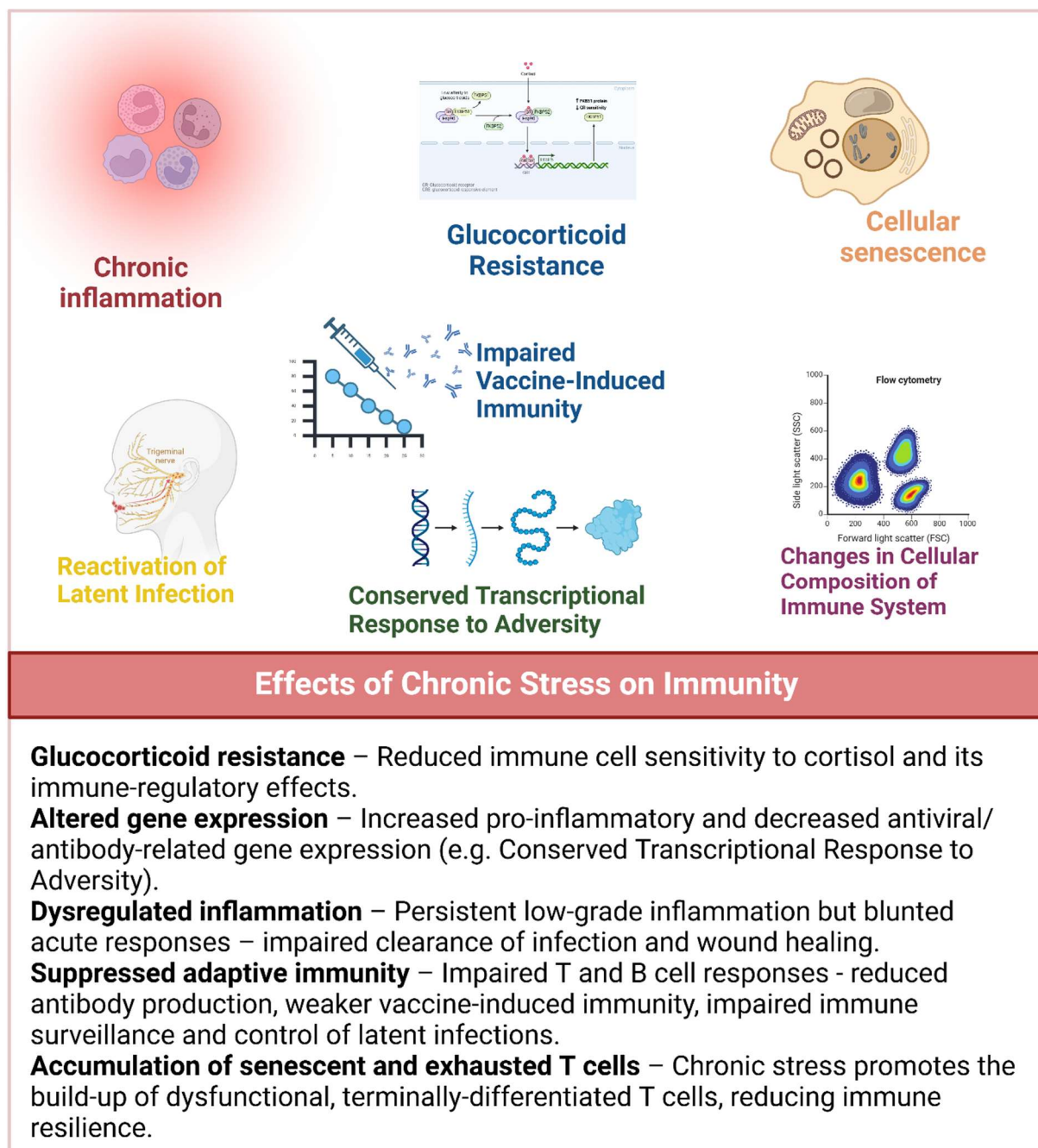


Figure 1.5 The Effects of Chronic Stress on the Immune System

Powell et al.⁸⁸ elegantly demonstrated this relationship, showing that repeated social defeat (RSD) stress elevated the output of immature, pro-inflammatory Ly6C^{Hi} monocytes (CD16⁻ in humans) in mice. This effect was reversed by propranolol, a β_2 -adrenoceptor antagonist. Extending these findings to humans, Powell's team examined a cohort stratified by

socioeconomic status (SES), reporting that (i) 32% of differentially expressed transcripts in PBMCs of low SES individuals overlapped with monocyte expression profiles in RSD-exposed mice; (ii) monocyte-associated transcripts were more prevalent in low SES individuals; (iii) absolute and proportional monocyte counts were elevated in low SES individuals; and (iv) low SES participants exhibited increased serum catecholamines and CREB transcription factor activity, consistent with chronic β -adrenergic signalling. These findings support the hypothesis that social adversity drives monocyte expansion and skews their function towards a pro-inflammatory phenotype, potentially contributing to the chronic low-grade inflammation characteristic of lower SES populations.

Building on this, Steve Cole and John Cacioppo (2015)⁶⁹ investigated perceived social isolation (PSI) in humans and macaques. They found increased circulating monocyte counts, particularly CD14⁺ CD16⁻ classical monocytes, in PSI-affected individuals, along with glucocorticoid resistance, elevated catecholamines, and a transcriptional profile resembling the CTRA. Experimentally infected socially isolated macaques exhibited impaired antiviral responses to simian immunodeficiency virus (SIV).

Beyond affecting haematopoiesis, chronic stress also induces splenic myelopoiesis as an alternative route for stress-driven immune cell production. McKim et al⁸⁶. demonstrated that SNS-derived norepinephrine mobilises haematopoietic stem and progenitor cells (HSPCs) from the bone marrow to the spleen, where sustained β -adrenergic signalling enhances proliferation and skews differentiation towards erythro-myeloid lineages. This

extramedullary haematopoiesis expands circulating monocyte and granulocyte pools, mirroring stress-induced immune shifts seen in bone marrow myelopoiesis. By recapitulating this process using the β -agonist isoprenaline and blocking β -adrenergic signalling with propranolol, McKim et al. confirmed the SNS dependence of stress-induced splenic haematopoiesis. Notably, they also documented an increase in immature neutrophils, a finding previously reported in only a handful of small-sample studies.

It is possible that these stress-induced changes in myeloid differentiation evolved to counter the increased risk of bacterial infections in animals exposed to wounding in social conflict. However, emerging evidence suggests a more complex relationship between social stress and immune activation, as dominance hierarchies and species-specific social structures modulate stress-related inflammatory responses. For example, in some primates, low social rank is associated with heightened inflammation⁸⁹, whereas in others, dominant individuals show greater inflammatory activity^{67,90}, highlighting the need for species- and context-specific interpretations of stress-immune interactions.

SOCIAL STATUS AND IMMUNE REGULATION IN RHESUS MACAQUES – Snyder-Mackler et al. (2016)

Background to Study:

- 45 Female Rhesus Macaques in captivity.
- Social rank determined by order of introduction to troupe – manipulated by reconfiguration of small troupes.
- **High rank** = More grooming by conspecifics, affiliative behaviour and social contact
- **Low rank** = harassment, exclusion, violence
- **Methods:** PBMCs isolated → Cell counts, cell sorting, stimulation with LPS and gene expression analysis

Summary of results:

- 1,622 genes showed rank-responsive differential expression across five immune cell types.
- 795 genes upregulated in Low rank in response to LPS (enriched for involvement in bacterial infection, inflammation and cytokine production), 439 upregulated in High rank (enriched for involvement in Type I IFN signalling).
- GC treatment of PBMCs before stimulation shows effects at least partly mediated by HPA Axis.

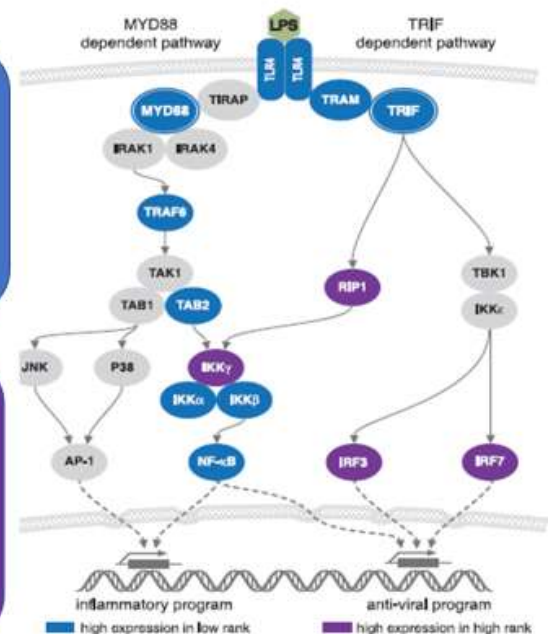


Figure 1.6 Summary of study by Snyder-Mackler et al (2016) documenting the effects of social rank on immune regulation and responses to infection in female Rhesus Macaques. In isolated monocytes in culture, social rank was shown to polarise TLR-4 signalling in response to LPS stimulation. High rank animals were polarised to an anti-viral programme of gene expression via a TRIF-TRIM dependent pathway, while low ranking animals were polarised towards an NF-κB mediated inflammatory programme of gene expression, dependent on MYD88. Figure of pathway reproduced from Snyder-Mackler et al (2016).

1.4.5 Mechanisms of Glucocorticoid Resistance

While the precise mechanisms underlying GC resistance are not fully understood, several pathways have been shown to contribute to its development. Transcriptional regulation plays a key role, with the glucocorticoid receptor (GR) complex binding to negative GC-response elements (nGREs) in the promoter region of NR3C1, the gene encoding the GR⁹¹. This binding reduces NR3C1 expression, either directly or in combination with transcription factors such as GC-responsive factor-1, c-ETS-1, c-ETS-2, and c-Myb⁶². Chronic stress, particularly via prolonged adrenergic receptor (AR)

signalling, further drives NR3C1 downregulation through the Conserved Transcriptional Response to Adversity, discussed earlier.

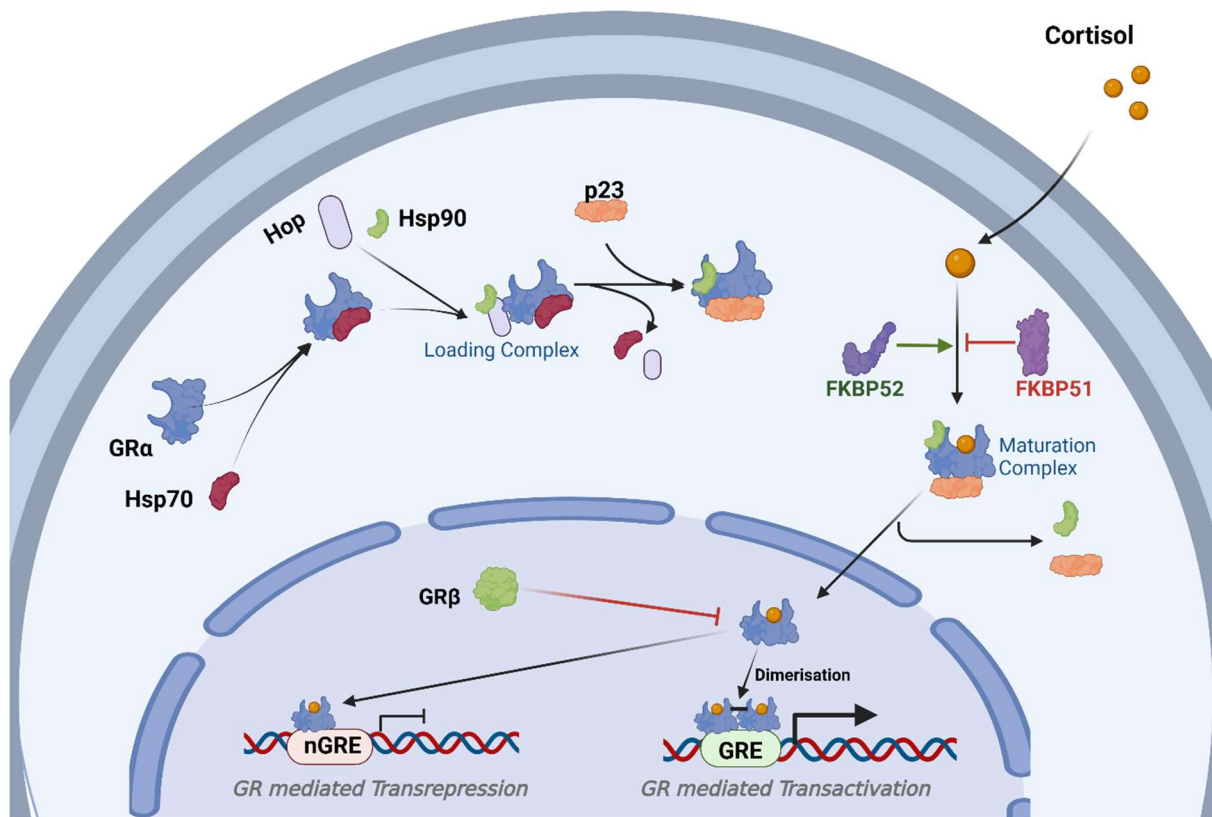


Figure 1.7 Schematic of Glucocorticoid Receptor Signalling: Key steps in GR signalling, adapted from Hori-Harvey et al. (2024) and created with BioRender. In the cytoplasm, the glucocorticoid receptor α (GR α) initially associates with Hsp70 and subsequently forms a loading complex with Hsp90, Hop, and p23. This complex undergoes further maturation through interaction with FKBP52, a process antagonised by FKBP51. Upon cortisol binding, the activated GR complex translocates to the nucleus where it exerts transcriptional effects via glucocorticoid response elements (GREs) to induce gene expression and via negative GREs (nGREs) for gene transrepression. GR β , a splice variant of the receptor, may act as a dominant-negative inhibitor, blocking GR α -mediated transcriptional activity.

At a post-transcriptional level, microRNAs (miRNAs) regulate NR3C1 stability and translation. For instance, miR-124 has been implicated in stress-induced glucocorticoid resistance by destabilising NR3C1 transcripts, reducing GR availability⁴². This phenomenon has been documented in models of sepsis and cancer-induced psychosocial stress (see Vandevyver et al. (2014) for review)⁹².

Beyond transcriptional and translational modifications, GR functionality is also regulated post-translationally, with modifications affecting receptor stability, nuclear translocation, and membrane expression.

Epigenetic mechanisms may further modulate glucocorticoid sensitivity. Methylation of GR target gene loci can restrict chromatin accessibility, thereby reducing transcriptional activation. A 2018 study in rhesus macaques demonstrated that PBMCs from lower-ranking animals exhibited closed chromatin configurations at GR target sites, impairing their transcriptional responsiveness. Treatment with dexamethasone triggered chromatin remodelling, increasing accessibility at previously closed sites and shifting gene expression patterns toward those observed in higher-ranking macaques⁹⁰.

In addition to GC resistance, prolonged and extreme stressors are associated with systemic dysregulation of the HPA axis. Chronic stress flattens the diurnal rhythm of cortisol secretion, with reduced secretion during the cortisol awakening response (CAR) and elevated evening levels⁹³. Such disruptions may have widespread physiological consequences, including effects on immune processes that follow circadian rhythms.

Extreme stress and early-life adversity have also been implicated in long-term alterations in HPA axis function. Adverse childhood experiences (ACEs) are linked to elevated serum cortisol levels in children and adolescents, and blunted stress responses in adulthood^{94,95}, an effect functionally similar to GC resistance. These alterations are further associated with persistent low-grade inflammation and immune priming, where prolonged stress exposure

heightens immune cell sensitivity to inflammatory stimuli. Such persistent alterations in stress responsivity and immune regulation increase disease susceptibility over the life course, underscoring the lasting impact of early-life adversity on immune health.

1.5 Linking Stress and Status Effects on Immunity to Health:

To date, most evidence linking social status and stress to impaired immune function has been observational; focused on alterations in immune function but not the pathologies these alterations lead to. Studies discussed so far have established links between low social status and sustained low-level inflammation, chronic stress and shifts in immune cell populations, and social adversity and the CTRA. However, fewer studies have integrated these findings into cohesive models capturing the full breadth of stress-related immune dysregulation and its downstream health consequences. Addressing this gap requires research that bridges molecular, cellular, and systemic immune responses to stress with clinical and epidemiological outcomes.

Two areas where integration has been successful are (i) studies of immune responses to infection in the context of stress and/or social adversity and (ii) vaccine response studies examining potential links between experiences of stress/low status and protection achieved by immunisation.

1.5.1 Respiratory Virus Challenge Studies.

One of the earliest and most prolific areas of research into the effects of stress on immunity has focused on upper respiratory virus susceptibility in individuals experiencing psychosocial stress. Studies spanning from the 1980s

to the present day have established robust associations between stress and increased susceptibility to respiratory viruses, more pronounced symptoms following exposure and prolonged duration of infection. Some studies have gone as far to establish mechanistic links with alterations to the respiratory mucosal immune environment and with GC resistance.

Due to the relatively low virulence of common cold viruses, many of these studies have employed experimental "challenge" designs, in which volunteers are deliberately exposed to respiratory viruses under controlled conditions. This research is best exemplified by Sheldon Cohen's Common Cold Project, which has been in operation since the early 1990s.

In Cohen's first major study on psychosocial stress and respiratory virus susceptibility⁹⁶, 394 participants completed questionnaires assessing recent stressful life events, perceived stress, and negative emotional states (e.g., anxiety, depression) before being exposed to one of five common cold viruses. Following six days of quarantine, participants were assessed for symptoms, infection status, and antiviral antibody responses. Those in the upper quartile of a compound stress index were found to be 2.16 times more likely to develop a cold post-exposure compared to those in the lowest quartile. Psychological stress was associated with an increased risk of clinical illness across all five viruses included in the study.

A follow-up study in 1998, involving 298 participants and two rhinoviruses, replicated these findings and further demonstrated a direct relationship between the duration of stressful life events and the probability of infection

following viral exposure⁹⁷. "Enduring interpersonal problems" and unemployment emerged as the most predictive stressors. Research throughout the 2000s continued to establish links between stress and both symptom severity and proinflammatory cytokine levels in nasal mucus⁹⁸. Additionally, studies identified associations between chronic stress, mucosal inflammation, symptom severity and elevated serum cortisol⁹⁹ following viral exposure.

Two mechanistic studies in 2012¹⁰⁰ and 2016¹⁰¹ provided key insights into the biology underpinning these effects. They demonstrated that stressful life events lasting over one month were associated with decreased GC sensitivity in nasal-resident immune cells, which in turn mediated the relationship between stress and both the risk of developing a cold and levels of proinflammatory cytokines in nasal mucus. Cohen's work provides compelling evidence that chronic stress disrupts immune regulation by inducing GC resistance in immune cells, impairing their ability to regulate inflammation. This failure to suppress inflammatory responses at sites of infection contributes to heightened symptom severity and prolonged illness duration. Moreover, excessive local inflammation in mucosal tissues may disrupt antiviral immune coordination, impairing viral clearance, and potentially increasing viral shedding.

1.5.2 Stress and the Reactivation of Latent Herpesvirus Infections.

There is a widespread belief that cold sores are more likely to occur during periods of high or sustained stress. While direct evidence for stress-induced HSV-1 reactivation in humans remains inconclusive, preclinical and

observational studies support a role for stress in reactivation of other latent herpesvirus infections.

Herpesviruses including Herpes Simplex Virus-1 (HSV-1), HSV-2, Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), and Varicella Zoster Virus (VZV), have a two-stage lifecycle: a lytic (active) phase, during which infections are symptomatic and contagious, and a latent phase, characterised by an absence of symptoms and persistence within host cells. The transition between these phases is controlled by a delicate balance between host immune activity and viral immune evasion (see Stoeger et al. 2019¹⁰² for review). Stress, through the action of glucocorticoids and catecholamines, is hypothesised to disrupt this balance and facilitate viral reactivation.

In HSV-1, virus-specific CD8⁺ T cells residing in the trigeminal ganglia play a crucial role in maintaining dormancy, primarily through interferon- γ (IFN- γ) secretion. CD4⁺ T cells also contribute by supporting CD8⁺ T cell function and sustaining antiviral cytokine signaling^{103,104}.

In EBV, natural killer (NK) cells provide early immune surveillance of infected B cells, while CD8⁺ T cells play a key role in long-term control of latently infected cells¹⁰⁵.

CD8⁺ T cells and NK cells are stress-responsive and express glucocorticoid (GR) and adrenergic (AR) receptors, making them susceptible to stress-induced modulation. However, stress effects on control of latent infections are not restricted to cytotoxic lymphocytes, chronic stress also impairs dendritic cell

(DC) function, reducing antigen presentation and weakening T cell priming, further compromising antiviral immunity.

The precise mechanisms underlying stress-induced herpesvirus reactivation remain unclear, but experimental evidence suggests that glucocorticoids impair the function of virus-specific CD8+ T cells and NK cells, reducing their capacity to suppress viral reactivation. Specifically, glucocorticoids modulate NK cell function, altering their phenotype, proliferation, and cytotoxicity, particularly when combined with cytokine signals such as IL-15¹⁰⁶, which may impact their ability to recognize^{107,108} and lyse infected cells. In CD8+ T cells, glucocorticoid signalling suppresses the production of IFN- γ and perforin^{107,108}, key molecules required for antiviral defence. Additionally, stress-induced glucocorticoid exposure can lead to a shift toward an exhausted T cell phenotype, characterized by reduced proliferation and impaired cytokine secretion.

Stress hormones also directly influence viral gene expression by interacting with host transcription factors that regulate latency-associated viral transcripts. In HSV-1, stress-responsive transcription factors modulate the expression of host latency-associated transcript (LAT), which helps maintain viral dormancy^{109,110}. In Kaposi's sarcoma-associated Herpesvirus (KSHV), β -adrenergic signalling can drive transcription by the lytic-phase-associated RTA transcription factor, also promoting reactivation¹¹¹.

A 2020 systematic review by Hayward et al.¹¹² identified 19 studies documenting stress-associated herpesvirus reactivation, covering HSV-1/2¹¹³,

EBV¹¹⁴, and CMV¹¹⁵. Most of these studies, dating back to the 1980s and 1990s, measured anti-viral antibody titres as markers of viral reactivation. These findings have been consistently referenced as evidence of the impact of stress on immunity and have significantly contributed to the development of psychoneuroimmunology.

Further investigation into CMV and EBV reactivation in response to stress is particularly important given their high prevalence in the population and their potential negative impact on immune ageing¹¹⁶. Their associations with degenerative autoimmune conditions, such as Multiple Sclerosis (MS)¹¹⁷, further underscore their significance. Additionally, CMV and EBV may influence the generation of immune memory in response to vaccines^{118,119}.

These findings highlight the necessity of integrating psychosocial variables into immunological research, particularly within the framework of precision medicine. Future research should explore whether interventions targeting stress reduction, whether through pharmacological means such as β -blockers or psychosocial approaches like mindfulness-based stress reduction, can enhance vaccine efficacy and improve immune resilience in high-stress populations.

1.5.3 Vaccine Responses as a Model for Stress Effects on Immunity.

Vaccine response studies represent an attractive experimental paradigm for investigating psychosocial effects on immunity. Exposure is standardised, outcomes have direct clinical relevance in terms of protection, and they serve as readouts of the activity of numerous immune cell types and processes,

bridging both innate and adaptive immune responses. One commonly assessed outcome in vaccine studies is vaccine-induced antibody titre. The production of antibodies following vaccination requires antigen processing and presentation by antigen-presenting cells (APCs), such as monocytes, dendritic cells, and macrophages. These cells signal to helper T cells, which recognise the antigen in the context of major histocompatibility complex (MHC) molecules on APCs. This process stimulates clonal expansion of cytokine-producing activated T helper (CD4⁺) cells, which, in turn, drive B cells to produce antibodies, proliferate, and differentiate into antibody-secreting plasma cells and memory B cells. Measuring variation in antibody titres across different social or stress gradients can therefore capture psychosocially mediated variations in these immune processes.

A smaller subset of studies suggest that chronic psychosocial stress not only dampens humoral responses but also the functionality of vaccine-induced T cells. Stress-associated increases in glucocorticoids have been linked to reduced proliferation and diminished effector functions, including lower IFN- γ and perforin production by CD8⁺ T cells, potentially undermining the overall efficacy of vaccination. Given the importance of vaccination in global public health, understanding how psychosocial factors influence vaccine responses is particularly relevant for populations experiencing chronic adversity. These findings highlight important considerations for vaccine deployment strategies, particularly in underserved communities where stress-related immune dysfunction may reduce vaccine effectiveness.

A systematic review by Hayward et al. (2021) identified 18 vaccine response studies examining the effects of stress on immune outcomes, including eight on influenza vaccines, as well as studies on hepatitis A, hepatitis B, human papillomavirus (HPV), and pneumococcal infection. Collectively, these studies illustrate how chronic stress influences both antibody production and broader immune activation post-vaccination. A summary of key findings is presented in Table 1.2 below. While these findings suggest that psychosocial adversity may impair vaccine-induced immunity, critical gaps remain. Few studies directly assess the biological mechanisms underlying these disparities, and none explicitly address SES or social exclusion as distinct forms of psychosocial adversity. The present research addresses this gap by explicitly capturing SES and engaging a socially excluded, homeless cohort to study effects on vaccine-induced immunity, immune competence and broader immune dynamics.

Table 1.2 A summary of vaccine response studies and their results drawn from Hayward et al (2020) and from other published literature.

Result	Notes
Higher stress and anxiety at time of vaccination associated with higher peak antibody titre.	No effect for social isolation when adjustment made for effect of stress.
Stress associated with lower antibody titres at 6- and 7- months post-vaccination in those with protective titres.	
Effect of stress is insignificant when covariates included in models (age, sex, BMI etc.)	
High stress predictive of lower antibody titre and influenza-specific IL-2 production by PBMCs at 2 weeks. Socialisation is predictive of IL-10 production.	

Result	Notes	Study	Vaccine	Sample	Psychosocial Exposure	Outcome
High life event stress associated with increased risk of inadequate protective titre after vaccination regimen completed.		Petry et al. (1991) ¹²⁰	Hepatitis B	82 University Students	Stress (Perceived), Anxiety	Antibody Titre (9 months post-vaccination (PV))
Perceived stress, anxiety and insomnia independently associate with lower antibody titres.	No significant associations for life event stress or social support.	Jabaaij et al. (1993) ¹²¹	Hep B (Low Dose)	95 University Students	Stress, social isolation	Antibody Titre (7 months PV)
Life event stress and perceived stress were higher in individuals with titres below threshold of protection at 5 months.		Jabaaij et al. (1996) ¹¹⁷	Hep B	68 University Students	Stress	Antibody Titre (7 months PV)
Sign Significant positive correlations between IL-10 and IFN-γ production and psychological wellbeing and relationship quality.		Kohut et al. (2002) ¹²²	Influenza A + B	56 Nursing Home Residents	Stress, social activities	Antibody titre, antigen specific cytokine production by PBMCs (IL-2, IFN-γ, IL-10) (2 weeks PV)

Result	Notes	Study	Vaccine	Sample	Psychosocial Exposure	Outcome
Stress associated with lower titres to New Caledonia strain, but not other strains included in vaccine.		Burns et al. (2002a) ¹²³	Hep B	259 University Students	Stressful life events	Antibody titre
Life event stress associated with lower titres against Shandong strain at 5 weeks and 5 months; and with lower titres against Meningococcal C antigens at 5 weeks.		Burns et al. (2002b) ¹²⁴	Meningitis C	60 University Students	Stressful life events, stress, anxiety, insomnia, social support	Antibody Titre
Social support associated with higher titres against Inf A Panama strain at 5 weeks and 5 months.		Burns et al. (2003) ¹²⁵	Influenza	31 University Students	Stressful life events, stress	Antibody titre (5 weeks and 5 months PV)
Smaller social networks and higher loneliness associated with lower titres against New Caledonia Inf A strain at 1 month and 4 months.	No effect on Inf A Panama strain or Inf B.	Hayney et al. (2003) ¹²⁶	Influenza, Hep A	18 adults from Wisconsin Longitudinal Study	Psychological wellbeing, relationships	Cytokines (IFN- γ , IL-10) produced by stimulated PBMCs isolated 28 days post vaccination.
Stress associated with lower titres to N.C strain at 1 month and 4 months.						

Result	Notes	Study	Vaccine	Sample	Psychosocial Exposure	Outcome
Bereavement in year prior to vaccination associated with lower titres against Inf A Panama strain and Inf B Shandong strain at 1 month post-vaccination. Marital satisfaction associated with higher titres against Panama strain at 1 month.		Miller et al. (2004) ¹²⁶	Influenza	83 University Students	Stress	Antibody titre (4 months PV)
		Phillips et al. (2005) ¹²⁷	Influenza, meningococcal A + C	57 University Students	Stressful life events, social support	Antibody titre (5 months PV)
Life event stress negatively associated with anti-Hep A titres at 18 weeks but not 4 weeks. Number of friends positively associated with anti-pneumococcal titres at 4 weeks. Functional social support associated with higher titres against pneumococcal serotypes 3 and 23 at 5 days.		Pressman et al. (2005) ¹²⁸	Influenza	83 University Students	Loneliness, social network size	Antibody titre (4 months PV)
No significant associations						

Notes	Study	Vaccine	Sample	Psychosocial Exposure	Outcome
Maternal stress influenced those infants who had not completed immunisation, suggestion that the effect of maternal stress is most pronounced in the context of in suboptimal antigen exposure. Antibody titres for all HPV strains were highest in lower and upper quartiles at 6 months, and highest in these groups for HPV-18 at 12 months (U-Shape). Analysis included effects of age, comorbidities and other sociodemographic covariates; highlights the mediating role of loneliness in vaccine response.	Phillips et al. (2006) ¹²⁹	Influenza	135 adults over 65 years	Stressful life events, social support, marital satisfaction	Antibody titre (12 months PV)
	Gallagher et al. (2008) ¹³⁰	Hep A, Pneumococcus	75 University Students	Stressful life events, social support	Antibody titre (18 weeks PV)
	Segerstrom et al. (2012) ¹³¹	Influenza	134 Adults over 65 years	Trait and state distress (inclusive of stress, depression and anxiety)	Antibody titre, cytokines (IL-6), microglobulin

Study	Vaccine	Sample	Psychosocial Exposure	Outcome	Result
O'Connor et al. (2013) ¹³²	Hep B, Tetanus	Pregnant women and infants (n=142 total)	Maternal anxiety	Antibody titre in infants, cytokines (IL-2, IL-4, IL-12), cytokine producing cell frequencies by EliSpot. (11 months PV)	In infants who had not completed 3 dose courses for Hep B, prenatal maternal anxiety is correlated with antibody titre at 6 months. Prenatal maternal anxiety is associated with reduced HepB-specific IFN- γ producing CD4 ⁺ T cells at six months, and with increased tetanus-specific IL-4 producing cells at four months post vaccination.
Wu et al. (2017) ¹³³	Human Papilloma Virus	204 men under 40 years	Stress	Antibody titre (6- or 12-months PV)	Association between stress and lower titres against HPV-6 and -16 at 6 months post-vaccination.
Gallagher et al. (2022) ¹³⁴	Covid-19 (mRNA)	676 British Adults	Social Cohesion, Loneliness	Antibody Titres (1 month PV)	Lower social cohesion was associated with significantly reduced antibody titres after 1 dose, with this relationship mediated by loneliness.

1.6 Hypotheses:

Psychosocial stress, which intensifies as socioeconomic status declines, contributes to immune dysregulation, potentially affecting (i) vaccine-induced

immunity and (ii) immune resilience, which may in turn influence longevity and overall health outcomes.

1.6.1 Aims.

To explore the connection between psychosocial stress, socioeconomic status, and immune function, with a particular focus on immune biomarkers, detailed immunophenotyping, and vaccine responses, in order to gain a deeper understanding of their role in health inequalities.

1.6.2 Specific Aims:

1. To investigate whether socioeconomic differences, particularly among socially excluded individuals, are linked to variations in *in vivo* immune responses to vaccines against a viral pathogen, SARS-CoV-2, and to examine the potential influence of psychosocial stress on these differences.
2. To assess the impact of socioeconomic status on immune function by analysing immune biomarkers and immune cell composition in blood samples from socially excluded, low SES, and high SES individuals, employing detailed immune characterisation methods while considering psychosocial stress as a relevant factor.
3. To evaluate the feasibility of conducting longitudinal, biologically intensive research within a socially excluded population by examining the collection and retention of biological samples over multiple time

points, alongside the completion of demographic, health, and psychosocial surveys across homeless, low SES housed, and high SES groups.

Chapter 2: Materials and Methods

2.1 Equipment and Reagents

Table 2.1 Equipment and Reagents

Equipment	Company
Centrifuge	Eppendorf Hamburg, Germany
2ml Centrifuge tubes	Sarstedt, Co. Wexford, Ireland
Cytek Aurora Spectral Cytometer (4 Laser)	Cytek Biosciences, California, USA
FACs tubes	Thermo Fisher Scientific, Loughborough, England
Light Microscope	Olympus Corporation, Japan
Multichannel pipette	Thermo Fisher Scientific, Loughborough, England
Pipettes (p2, p20, p200, p1000)	Thermo Fisher Scientific, Loughborough, England
pH Meter	Hanna Instruments, UK
Pipette Gun	VWR International Ltd., Ballycoolin, Dublin, Ireland.
Pipette tips p2/p200/p1000	Sarstedt, Co. Wexford, Ireland
Filter pipette tips p2/p200/p1000	Thermo Fisher Scientific, Loughborough, England
Serological Pipettes 5/10/25ml	Corning, New York, USA
MSD MESO Quickplex SQ 120mm	Meso Scale Diagnostics LLC, MD, USA
Vortex	
Lithium Heparin Vacuettes	Greiner Bio-One GmbH, Kremsmunster, Austria
KEDTA Vacuettes	Greiner Bio-One GmbH, Kremsmunster, Austria
Clot Activator[®] Vacuettes	Greiner Bio-One GmbH, Kremsmunster, Austria
Quantiferon Monitor (QFM) Blood Collection Tubes	Qiagen NV, Netherlands
QuantiFERON Monitor Test	Qiagen NV, Netherlands
FoxP3/Transcription Factor Fixation and Permeabilisation Concentrate/Diluent Kit	eBioscience/Thermofisher, Loughborough, England
RPMI 1640 Media	Sigma-Aldrich, Darmstadt, Germany
Iscove's Modified Dulbecco's Medium (IMDM)	Sigma-Aldrich, Darmstadt, Germany
Fetal Bovine Serum – Sterile Filtered	Sigma-Aldrich, Darmstadt, Germany

2.2 Kits

Table 2.2 Assay kits used with suppliers

Kit	Supplier
Human Proinflammatory (human) Biomarker Panel 1 on S-Plex Platform	Meso Scale Diagnostics, MD, USA
U-PLEX Custom Biomarker Group 1 (human) Assays	Meso Scale Diagnostics, MD, USA
Human CMV-IgG (cytomegalovirus-Immunoglobulin G) ELISA Kit (HUF103440)	Assay Genie, Dublin, Ireland
eBioscience™ Foxp3 / Transcription Factor Fixation/Permeabilization Concentrate and Diluent	Thermofisher Scientific, Loughborough England

2.3 Fluorescent Antibodies for Flow Cytometry

Table 2.3 Fluorescent Antibodies for Flow Cytometry

Fluorophore	Target	Clone	Manufacturer
BV421	Vα24Jα18	6B11	BD Biosciences
VIOBLUE	Vδ2	REA771	Miltenyi
BV480	Vα7.2	OF-5A12	BD Biosciences
BV510	CD69	FN-50	BD Biosciences
BV570	CD56	HCD56	Biologend
BV605	CD137	4B4-1	BD Biosciences
BV711	CD28	CD28.2	BD Biosciences
BV750	CCR7	3D12	BD Biosciences
PEFIRE-810	TIGIT	VSTM3	Biologend
BB515	CD27	M-T271	BD Biosciences
BB630	CD4	SK3	BD Biosciences
BB790	CD8	SK1	Biologend
SPARK BLUE 550	CD3	SK7	Biologend
PERCP-EF710	NKG2D	1D11	Thermo Fisher
PE	Vδ1	TS8.2	Thermo Fisher
PE-CF594	CD57	NK-1	BD Biosciences
PE-CY5	CD161	DX12	BD Biosciences
PE-CY5.5	CD45RA	MEM-56	Thermo Fisher
PE-CY7	CCR6	R6H1	Thermo Fisher
AF647	PD-1	MIH4	BD Biosciences
APC	KLRG1	14C2A07	Biologend
SPARK NIR 685	CD127	A019D5	Biologend
BV650	Ki67	B56	BD Biosciences
R718	FoxP3	259D/C7	BD Biosciences
SparkNIR 685	CD127		Biologend

		A019D5	
APC-Fire 810	CD45	Hi30	Biolegend

2.4 Buffer Preparation

Table 2.4 Buffers used in AESS-Vax and PATH studies and their composition

Buffer	Components
PBMC Freezing Solution	10% DMSO, FCS
PBMC Resuspension Media	10% FCS RPMI-1640
CMV IgG ELISA Wash Buffer	10% 10x PBS, 0.0005% Tween-20, dH ₂ O
FACS Buffer	0.025% FCS, 0.004% EDTA PBS
PBMC Incubation/Stim Media	10% FCS, 1% Penicillin, 1%Streptomycin, IMDM

2.5 Software and Electronic Resources

Table 2.5 Software and Electronic Resources Used in the AESS-Vax and PATH Studies

Software	Company
GraphPad Prism v10	GraphPad Software, San Diego, USA
R (version 4.4.3)	Free Software Foundation GNU Project, Austria
R Studio	Posit PBC, Austria
FloJo V10.10	BD Biosciences, USA
SpectroFlo®	Cytek Biosciences, California, USA
MSD Methodical Mind	Meso Scale Diagnostics, MD, USA
MSD Discovery Workbench	Meso Scale Diagnostics, MD, USA
Biorender 2024	Biorender, Canada
Typeform	Typeform, Barcelona, Cataluña
Microsoft Excel	Microsoft Corporation, USA

2.6 AESS-Vax Participant Recruitment

The AESS-Vax study was granted ethical approval as a substudy of the St James's and Tallaght University Hospital and Trinity College Dublin Allied Researchers (STTAR) Covid-19 Bioresource project (JREC 2020-05 List 19). Individuals fully vaccinated against SARS CoV-2, aged over eighteen and below sixty-five years old without (i) current or former PCR or Antigen-test confirmed SARS CoV-2 infection, (ii) an active blood-born viral infection (e.g. HIV, hepatitis A/B/C), (iii) recent or current immunosuppression as a result of medical treatment or disease, were deemed eligible for participation.

Potential participants were recruited in two groups, housed and socially excluded. Housed participants were initially recruited at St. James' Hospital and in the Trinity Centre for Health Sciences at St. James' Hospital via emails circulated to staff members and via information stalls set up on the hospital campus during the roll out the National Covid-19 Vaccination Programme in December 2020 and during the roll out of "booster" vaccinations in December 2021. Homeless participants were recruited beginning June 2021, in collaboration with SafetyNet Primary Care and Depaul Ireland.

All participants were designated a study identification (CV) number on completion of Informed Consent Forms to pseudonymise all further data collected. Once informed consent was obtained, participants were required to complete a STTAR Bioresource Case Report Form – collecting basic health and demographic information. Case Report and Informed Consent Forms were returned to the STTAR Bioresource Data Manager in the St. James'-Wellcome Clinical Research Facility after collection and stored separately to data generated in the AESS-Vax study.

2.7 PATH Participant Recruitment

Participants for the PATH study was recruited between March 2022 and March 2023 across six hostels in central Dublin, in collaboration with Depaul Ireland and Focus Ireland hostel staff and St James' Hospital Inclusion Health. Eligible participants were aged between 20 and 70 years old and were all residing in hostels at time of recruitment with the exception of one participant who had recently returned to rough sleeping. Active blood-borne viral infection or untreated infection of any kind (including respiratory infections) were exclusion criteria for study enrolment for health and safety reasons and to eliminate the potentially confounding effects of infection on immunological analyses.

Prior to providing written informed consent, all participants were guided through the study's aims, procedures, potential risks, and their right to withdraw. Each participant was assigned a study ID to pseudonymise subsequent data collection. The study protocol, including the participant information leaflet and researcher approach to recruitment, was co-designed in consultation with a panel of individuals with lived experience of homelessness recruited by St James' Inclusion Health and Depaul Ireland.

2.8 AESS-Vax Questionnaire Methodology

In addition to filling in the STTAR Bioresource Consent and Case Report Forms at enrolment in the study, all participants completed the AESS-Vax participant questionnaire. Questionnaires were administered using a combination of paper forms and digital surveys. Both paper forms and the digital survey were self-administered, with help available on request for participants with literacy or numeracy deficits. Digital surveys were administered using TypeForm, an

online survey platform with secure EU server storage of responses. Responses from the online survey were downloaded in CSV format, while responses generated on paper were input into a spreadsheet. At the end of recruitment, the survey response spreadsheet and online survey CSV file were merged to generate a single CSV file.

The AESS-Vax Participant Questionnaire was designed to capture additional health, demographic and lifestyle data not already recorded in the STARR Bioresource Case Report form. Specific to AESS-Vax, the questionnaire collected data on psychosocial stress and wellbeing using a set of standardised and pre-validated tools including the Perceived Stress Scale¹²⁷ Holmes-Rahe Life Event Stress Inventory¹²⁸, Adverse Childhood Experiences-10 and McArthurs' Ladder¹²⁹.

Table 2.6 Summary of Demographic and Health Information Collected in the AESS-Vax Study

Item Collected	Responses Recorded
Age	Self-reported
Sex and Gender	Sex at birth and gender identity
Ethnicity	White, Black, East Asian, South Asian, West Asian, Arab, Latin American, Aboriginal or First Nations, Roma, Minceir/Traveller, Other
Height (cm)	Self-reported
Weight (kg)	Self-reported
Highest Level of Education	Primary, Some Secondary, Junior/Inter Certificate, Leaving Cert, FETAC Level 5 or 6, Bachelors Degree (Level 7 or 8), Masters Degree or Higher Diploma, PhD or other NFQ Level 10, Other
Relationship Status	Married, Single, Co-habiting, Separated, Divorced, Other
Housing Status	Homeowner, Council/Approved Housing Body, Private Rental, Private Rental (>2yr same address), Short Term Rental or Subletting, Homeless, Living with Family/Friends, Formerly Homeless, Other
Residents at Current Address	0-2 others, 3-4 others, 4-5 others, 6+ others

Homelessness Situation*	Own-Door/Supported Housing, Own Room in Hub/Hostel, Shared Room or Dorm in Emergency Accommodation, Rough Sleeper, "Couch-Surfing", Other
Years Homeless*	Self-reported
History of Incarceration	Never, Once (>5yrs), Once (>5yrs), Twice, More than Twice Years of incarceration
Long-term or Chronic Conditions	Self-reported list
Medications	Self-reported list
Smoking Status and History	Yes, currently, Yes, Previously, No Cigarettes per day, Years Smoking (Pack year history)
Alcohol Consumption	Yes, No, In the past but not now <u>Weekly Units:</u> 1-5, 6-10, 11-15, 16-20, 20-30, Over 30 units
Sleep	Sleep per night: Less than 4 hours, 4-5 hours, 6-7 hours, 8-9 hours, More than 9 hours
Drug Use	Yes, currently, Yes, occasionally, Yes, previously, No <u>Drugs Used:</u> Cannabis, Cocaine (Powder), Crack Cocaine, Heroin, Methamphetamine, Amphetamines, MDMA/pills, Ketamine and other dissociatives (e.g. PCP, DXM, NOS), Psychedelics (e.g. LSD, Mushrooms, 2CB, Mescaline), Opioid Substitutes, Other

Perceived Stress Scale

The Perceived Stress Scale (PSS), developed by Cohen et al (1983) is a ten item self-reported measure of "the degree to which situations in one's life are appraised as stressful", with questions on the scale referring to the 6–8-week period prior to testing. The PSS has shown strong test-retest consistency and its factorial validity been demonstrated across different age groups,

populations and languages¹³⁰. Questions are scored on a 5-point Likert Scale (Never =0, Once or Twice=1, Sometimes=2, Often=3, Very Often=4), with scoring reversed for three questions and a maximum score of 40.

Holmes-Rahe Life Event Stress Inventory (HRLES)

The Holmes-Rahe Life Event Stress Inventory (HRLES) was first developed by Holmes-Rahe in 1967 as the Social Readjustment Ratings Scale, as part of a study interrogating the effects of stressful life events on the health of US Naval personnel¹³¹. The HRLES includes a list of 43 “life events” which participants may have experienced over the previous year, with scores assigned to each of the events, ranging from 100 to 11. Participants add the scores from events they have experienced for a total personal score. Higher scores are predictive of poor health outcomes, with the original HRLES predicting an 80% chance of illness within two years for those scoring ≥ 300 and a 50% chance of illness for those scoring ≥ 150 to ≥ 299 (Noone, 2017). While intercultural variability in the interpretation of certain events included on the inventory has been noted, the HRLES has shown good overall consistency across populations and social groups.

Adverse Childhood Experiences Questionnaire:

ACES-10 is a ten-item questionnaire profiling Adverse Childhood Experiences (ACEs) across two major domains (i) child maltreatment (e.g., physical, and sexual abuse) and (ii) household challenges (e.g., having a parent or guardian with an alcohol or drug dependency)^{132,133}. In this study, the questions from ACES-10 were taken and broken down into 18 distinct questions (separating questions containing “or” scenarios) and scored on a 5-point Likert Scale (Never =0, Once or Twice=1, Sometimes=2, Often=3, Very Often=4). Scoring in

the original 10-item scale was binary (No=0, Yes =1) and was modified here by TypeForm, the survey platform used for the questionnaire. In the analysis of results from the ACES questionnaire, results are presented as a Likert-scale score and in a binary format, where answers in the affirmative (Once or Twice, Sometimes, Often and Very Often) were scored as 1 and the negative (Never) as 0.

McArthurs' Ladder

McArthur's ladder is a tool used to measure subjective social status on a scale from 1-10. Initially developed by Adler et al, McArthur's ladder uses an image of a ladder with ten steps which participants are encouraged to take as representative of society. Participants are invited to mark the step that they feel best reflects their relative social position, taking those on the top step to have the highest social status and wealth, and those at the bottom to have the lowest social status and security. McArthur's ladder displays strong test-retest reliability and consistency across population groups¹³⁴.

2.9 PATH Survey Methodology

Data collection for the PATH study was conducted through structured, face-to-face interviews carried out by trained researchers. Interviews were conducted in private settings within the hostels and included a combination of standardised and adapted instruments. The survey captured self-reported sociodemographic information (including age, gender, biological sex at birth, highest level of education completed, occupational history, history of incarceration), physical and mental health status, and current medication use. Participants were also asked about their use of alcohol, tobacco, and other drugs.

Frailty was assessed using the Clinical Frailty Scale (CFS), a 7-point measure based on global clinical judgement (for review see Church et al, 2020)¹³⁵. A trained research nurse assigned frailty scores following completion of each interview before blood draw and health assessment.

Adverse childhood experiences were assessed using an adapted version of the ACEs questionnaire, capturing exposure to physical, sexual, and emotional abuse, neglect, and household dysfunction before the age of 18¹³². Current psychosocial wellbeing was captured across a number of questions, including frequency of loneliness, frequency of contact with family and friends, perceptions of social value, shame and locus of control.

2.10 Biobanking of Samples for AESS-Vax Study:

2.10.1 – Isolation of Peripheral Blood Mononuclear Cells from Whole Blood:

15ml of Ficoll-Paque® Plus (Cytivia) was added to a 50ml centrifuge tube in a class II biological safety cabinet (BSC). The tubes were centrifuged at 1000g for one minute to disperse separation media below the tube membrane. Fresh whole blood collected in a lithium heparin tube was transferred to the Leucosep tube in the BSC before centrifugation at 1500g for ten minutes with deceleration set to 1. The entire contents of the tube above the membrane were transferred to a new, labelled 50ml centrifuge tube in BSC and 30ml of RPMI 1640 Glutamax™ (ThermoFisher Scientific) media was introduced to wash cells in suspension. The tube was centrifuged at 500g for ten minutes with the brake on to capture cells in a pellet. The supernatant was discarded into liquid waste in BSC in one fluid motion. 30ml of RPMI 1640 media was added to the pellet and the pellet resuspended with gentle pipetting. The tube was centrifuged for five minutes at 500g with brake on and the supernatant

discarded into liquid waste. 1ml of 10% FCS-RPMI was added to the remaining pellet and the pellet resuspended with gentle pipetting.

2.10.2: Counting Peripheral Blood Mononuclear Cells and Assessing Cell Viability Using Hemacytometer:

Viability and number of isolated PBMCs per participant sample were determined using trypan blue staining and a glass hemacytometer. 2µl of resuspended cells were added to one well of a 96 well round bottom plate, to which 38µl of trypan blue solution were added for a 1-in-20 dilution. Trypan blue dye and cells were mixed by gentle pipetting. 10µl of dyed cell suspension were pipetted carefully under a glass coverslip placed on top of a glass hemacytometer. Cells were viewed on the hemacytometer under a light microscope. Live (did not stain with Trypan Blue) and dead cells (stained dark blue) in each 4x4 corner grid of the hemacytometer slide were counted using a handheld counter and an average calculated using the four counts. The total live cell count for the sample was calculated as follows:

$$\text{Cells ml}^{-1} \text{ per sample} = (\text{Average Count} \times 10^4) \times \text{Dilution Factor}$$

Dead cell counts were taken following the same protocol to assess viability (expected to be below 5% dead).

2.10.3 Freezing PBMC Aliquots for AESS-Vax and PATH Biobanks

A 10% DMSO-FCS freezing solution was prepared using heat inactivated fetal calf serum and Dimethyl Sulfoxide (DMSO). Based on cell count, an appropriate volume of freezing solution was added to each resuspended PBMC sample to yield a suspension containing $1.5\text{-}2 \times 10^6$ cells ml^{-1} (e.g. for sample containing 10×10^6 cells ml^{-1} , 4ml of DMSO-FCS solution was added to

1ml of PBMCs suspended in FCS-RPMI). 1.5ml of resuspended PBMCs in FCS-DMSO were added to each cryovial and cryovials were transferred to a Mr Frosty® (Nalgene) controlled-rate freezing container. The container including PBMC samples was placed in a -80°C freezer overnight. Frozen samples were transferred to storage boxes and arranged according to a box plan for ease of future recovery and use.

2.10.4 Biobanking Serum and Plasma Samples For AESS-Vax and PATH Biobanks

9ml red-topped clot activator and 9ml purple-topped EDTA blood collection tubes containing participant blood were centrifuged at 3,400rpm for 10mins in a bench top centrifuge. Identical tubes containing 9ml of water were used to balance the centrifuge when sample number was uneven. Following centrifugation, both tubes had yellow-straw coloured serum or plasma layers on top of layers of erythrocytes, leukocytes and other blood components. In a biological safety cabinet, sterile, labelled 2ml centrifuge tubes were taken and 250ul serum or plasma was added to each tube. Care was taken to avoid addition of blood to tubes. 4-6 aliquots of serum and plasma were stored for each participant in clearly labelled boxes according to a box plan for ease of future recovery and use.

2.11 ELISA for Anti-CMV Antibodies:

Anti-Cytomegalovirus (CMV) IgG levels in serum samples from AESS-Vax and PATH were quantified using an ELISA kit with pre-coated plates (AssayGenie), following the manufacturer's guidelines. TMB substrate was equilibrated to 37°C in a water bath for 30 minutes before use.

The plate was washed twice with 100µl of wash buffer per well and tapped dry to remove any unbound material. 100µl of positive and negative control solution was dispensed into the four designated control wells (three negative controls and one positive). 100µl of sample dilution buffer was added to two wells directly under the control wells. 10µl of sample serum was added to the remaining wells according to pre-designated plate plans. All samples were run in duplicate to ensure consistency in readings. 100µl of sample dilution buffer was added to all wells for a 1:10 dilution of sample serum. Plates were sealed with adhesive tape and incubated for 30 minutes at 37° C in a shaker incubator set to low speed (~100rpm).

The plate was washed five times with 100µl wash buffer per well and 100µl proprietary HRP-anti-human IgG solution was added to each well before a further 30-minute incubation at 37° C. The plate was washed five times with 100µl wash buffer per well and 1 minute between washes.

50µl TMB Substrate solution was added to each well and the plate was covered with tinfoil to block out light before incubation at 37°C for 15 minutes. At this point stop solution was added, turning blue wells to a yellow colour. Absorbance at 450nm was measured across the plate on a microplate reader.

The cutoff for seropositivity was determined as the mean absorbance of the negative controls plus 0.5.

2.12 Multiplex Cytokine and Chemokine Analysis Using MSD Immunoassay Platform

2.12.1 Human Custom Immune Biomarker Panel on UPlex Platform

For this Custom Electrochemiluminescence Assay (ECLIA), 10 targets were analysed using multiplex plates.

Cytokines and Chemokines Analysed: GM-CSF, IL-13, IL-5, G-CSF, IL18, IL23, IL-33, IP-10, MCP-1, MIP1 β

Target cytokines were assigned specific MSD linkers (numbered 1-10) and the linker-cytokine pairs were marked on an assay spot map (in kit). For each target, 200 μ L of biotinylated antibody was added to 300 μ L of assigned linker solution, the mixtures were vortexed at high speed briefly before incubation at room temperature for thirty minutes. At the end of the thirty-minute incubation, 200 μ L of proprietary stop solution was added to each mixture before vortexing and a further thirty-minute incubation at room temperature. Coating solution for the plates was then made up by combining 600 μ L of each antibody-linker mixture in a single tube and making up to a total volume of 6ml with MSD stop solution before vortexing.

MSD U plex plates were washed three times with 90 μ L proprietary wash buffer per well and the plates tapped dry on absorbent paper. 50 μ L of multiplex coating solution was added to each well and the plates sealed with optical tape. The plates were incubated in a shaker incubator (700rpm) at room temperature for one hour. Plates were washed a further three times with 150 μ L wash buffer per well.

Standards for the assays came in Calibrator mixtures. For the single plex YKL-40 plate, 50µL of calibrator was added to 200µL assay diluent in a tube before vortexing to generate the top standard (highest concentration). For the multiplex plate, 50µL of each calibrator was taken in an Eppendorf tube and the total volume was made up to 250µL with assay diluent before vortexing. With each top standard, six four-fold dilutions were performed to give seven 300µL standards (e.g. 75µL standard added to 225µL assay diluent with mixing, 75µL of this mix added to another tube containing 225µL assay diluent etc.). 25µL of assay diluent was added to each well in the first two columns of the plate, where standards were plated in duplicate, starting with the top standard in the first row and finishing with standard #7 in the 7th row. 25µL of assay diluent was added to the remaining two wells in the 8th row, making this the “zero” standard”.

In remaining wells, 25µL of sample serum was added per well, with samples assayed in singlicate. In addition to standards (run in duplicate), two control serum samples were run across plates to ensure inter-assay consistency. Plates were sealed with optical adhesive tape and placed on a shaker incubator (700rpm) for four hours at room temperature.

Plates were washed three times with 90µL MSD wash buffer per well. After tapping dry, 25µL of detection antibody was added to each well and plates were incubated on a shaker (700rpm) for two hours at room temperature.

Plates were washed three times before the addition of 40µL MSD Gold Read Buffer B to each well. Readings were acquired on an MSD MESO QuickPlex SQ 120mm instrument using the Methodical Mind proprietary software.

2.12.2 Human Proinflammatory (human) Biomarker Panel 1 on S-Plex Platform

Prior to running the assay, MSD Diluent 100 and S-Plex Proinflammatory Panel 1 Coating Solution were allowed to thaw and come to room temperature. For each plate being run, 4,500 μ L of Diluent 100 was added to 1,500 μ L Coating Buffer in a 15ml centrifuge tube and the mixture vortexed. Plates were washed three times with 150 μ L MSD Wash Buffer per well and the plates tapped on absorbent paper until dry. 50 μ L diluted coating solution was added to each well and plate(s) sealed with adhesive tape before incubation on a shaker incubator (700rpm) for one hour at room temperature.

While the plates were incubating with coating buffer, MSD Diluent 58, Blocker S1 and Blocker S2 were allowed to thaw and come to room temperature. Once at room temperature, 3440 μ L Diluent 58 was added to 35 μ L Blocker S1 and 35 μ L Blocker S2 (per plate), unused Blocker solution was frozen immediately.

Next, 1000 μ L of Diluent 58 was added to the glass vial containing lyophilised Calibrator Blend. The plastic plug was replaced in the vial and the vial inverted three times to resuspend the lyophilised Blend. The reconstituted solution was left to come to room temperature (~ 30 minutes) and then vortexed at medium speed using short pulses. 50 μ L of reconstituted solution was added to 150 μ L of diluent 58 in a 2ml Eppendorf tube and the tube was vortexed. Standards 3 to 7 were prepared by serial dilution from standard 2 (Eppendorf tube), taking 50 μ L at a time from the previous standard and adding to 150 μ L Diluent 58. Unused reconstituted calibrator (Standard 1) was immediately taken to -80°C freezer for storage through up to three freeze-thaw cycles. Samples were diluted in a 96 well ELISA plate using Diluent 58.

On completion of the one-hour incubation with coating solution, the plate(s) were washed three times with 150µL MSD wash buffer per well and the plates were tapped dry. 25µL blocking mixture was added to each well. 25µL of calibrator (Standards 1-7) were added to the first 7 wells of the first two columns on each plate, 25µL Diluent 58 was added to the last well in each of the first two columns (zero standard). 25µL of sample was added to the remaining wells according to plate plans, including two replicate samples which were included on each plate run. The plates were sealed with adhesive and placed in a shaker incubator (700rpm) for 90 minutes at room temperature.

During the 90-minute incubation, detection antibody vials were thawed and allowed to come to room temperature. For each plate, 5730µL Diluent 59 was added to a 15ml centrifuge tube. Additionally, 30µL of Turbo-Boost® Antibody Solution for each target was added to the centrifuge tube(s) and the tube(s) were vortexed.

Following 90-minute incubation and preparation of Turbo-Boost antibody solution, the plate(s) were washed three times with 150µL MSD wash buffer per well. 50µL of antibody solution was added to each well, the plate(s) sealed with an adhesive seal and incubated on shaker incubator (700rpm) at room temperature for 1 hour. Meanwhile, 2,970µL molecular grade water was added to a 15ml centrifuge tube along with 1,500 µL of S-Plex Enhance E1, 1,500µL S-Plex E2 and 30µL S-Plex E3 solution. After incubation, the plate(s) were washed three times as before and 50µL Enhance solution was added to wells. The plate(s) were sealed and incubated as before for 30 minutes.

During the 30-minute incubation, Turbo-Tag® Detectors D1 and D2 were vortexed and then spun down in a microcentrifuge at 1500rpm. To a new 15ml centrifuge tube, 4,470µL molecular grade water, 1,500µL S-Plex Detect D1 and 30µL S-Plex Detect D2 were added and the mixture(s) vortexed.

The plate was washed as in previous steps and 50µL detection solution was added to each well. The plate was sealed and placed on a shaker incubator (700rpm) for incubation at 27°C for one hour. The plate was washed three times as before and 150µL MSD Gold Read Buffer B was added to each well. Readings were acquired on an MSD MESO QuickPlex SQ 120mm instrument using the Methodical Mind proprietary software.

2.13 Lymphocyte Immunophenotyping in Cryopreserved Peripheral Blood Mononuclear Cells

This flow cytometry panel and protocol was designed to profile lymphocyte immunophenotype, particularly in T lymphocyte subsets, using cryopreserved PBMCs from study donors.

- (i) **Evening Day 1:** PBMCs were thawed in a water bath set to 37°C. Meanwhile in a biosafety cabinet, 15ml centrifuge tubes and FACS tubes were labelled with sample numbers. Once thawed, PBMC samples were pipetted up and down before being transferred to labelled 15ml centrifuge tubes. To each 15ml tube, 10ml of cIMDM was added and lids closed. The tubes were briefly vortexed before being centrifuged at 500g in a bench top centrifuge for five minutes, leaving a pellet containing cells. In one fluid motion, liquid was poured from the tubes into waste. The pellets were resuspended in the remaining liquid using a P1000 pipette before being transferred

to labelled FACS tubes. Lids were placed loosely on the FACS tubes before tubes were placed in an incubator set to 37°C for one hour. During the incubation, Zombie-NIR live/dead stain (0.5µl stain to 500µl sterile PBS to make stain for five samples) and surface antibody cocktails (see table below for amounts of each stain per sample) were prepared in the biosafety cabinet.

Following incubation 2.5µl human Fc Block and 100µl Zombie-NIR solution were added to each sample. Tubes were vortexed and placed in the dark at room temperature for fifteen minutes. 1ml of FACS buffer was added to wash each sample before brief vortexing and centrifugation at 500g for five minutes, leaving cell pellets. Supernatants were discarded in one fluid motion into waste. 50µl of surface antibody cocktail was added to each tube and pellets were re-suspended by pipetting. FACS tubes were placed in fridge at 4°C overnight.

- (ii) **Day 2:** Samples were washed with 1ml FACS Buffer, centrifuged for five minutes at 500g and the supernatant discarded once more. The pellet was resuspended in 200µl Fix Solution (1:3 dilution of Fix concentrate in Fix Buffer; Thermofisher) and the samples were left to incubate in the dark for twenty minutes. Meanwhile, perm solution (Thermofisher) and intracellular antibody cocktail (see table below) were made in the biosafety cabinet. Following incubation, the cells were washed in 1ml Perm solution (Thermofisher) and centrifuged at 500g for five minutes. The supernatant was again

discarded. 50µl intracellular antibody cocktail was added to each sample and the samples were incubated in the dark for thirty minutes. Samples were washed a final time with 1ml perm solution and the supernatant discarded. The pellets were resuspended in 100µl FACS buffer and stored at 4°C until ready for acquisition on a Cytex Aurora spectral cytometer.

Table 2.7 Targets and Fluophores for the Lymphocyte Immunophenotyping panel used in the AESS-Vax and PATH cohorts.

(I) Surface Stain		
Marker	Fluorophore	0.1x
Vα24Jα18	BV421	0.6
Vδ2	VIOBLUE	0.12
Vα7.2	BV480	0.6
CD69	BV510	0.6
CD56	BV570	3
CD137	BV605	0.3
CD28	BV711	0.3
CCR7	BV750	0.3
CD25	BV786	0.6
TIGIT	PEFIRE-810	0.72
CD27	BB515	0.3
CD4	BB630	0.3
CD154	BB700	3
CD8	BB790	0.3
CD3	SPARK BLUE 550	0.3
NKG2D	PERCP-EF710	0.3
Vδ1	PE	0.3
CD57	PE-CF594	0.6
CD161	PE-CY5	6
CD45RA	PE-CY5.5	0.3
CCR6	PE-CY7	0.6
PD-1	AF647	0.3
KLRG1	APC	0.6
CD127	SPARK NIR 685	0.6
CD45	APC-FIRE 810	0.3
BS Buffer		6
FACS Buffer		32.8

(II) Intracellular Stain		
Marker	Fluorophore	0.1x
Ki67	BV650	3
FoxP3	R718	1.5
Perm Buffer		49.5
BS Buffer Plus		6

Chapter 3:

Socioeconomic Status, Social Exclusion and Homelessness as a Model for Understanding Social Determination of Health

3.1 Introduction

Poverty, socioeconomic status, and social exclusion are among the strongest determinants of health outcomes throughout the lifecourse. In the 2019 OECD Health at a Glance Report¹³⁶, it was found that only 73% of Irish adults in the lowest income quintile reported being in good health, compared to 93% in the highest income quintile, revealing a 20% gap. While these disparities in health outcomes across socioeconomic groups are striking, they are neither new nor unique to any one country. However, an underlying and often understudied trend within the social gradient in health is that individuals who are maximally and *multiply* excluded (those experiencing multiple forms of social exclusion simultaneously) suffer the poorest health outcomes of all. These individuals not only face premature mortality⁹ but also exhibit an early onset of age-associated conditions¹³⁷. Importantly, these adverse health outcomes occur for reasons that extend beyond the intensification of material poverty's general effects on health.

This underscores the need to delve deeper into the concept of multiple exclusion and its impact on health, particularly in the context of homelessness. Homelessness serves as an example of severe multiple exclusion, where individuals are not just economically disadvantaged but are also marginalised through a combination of social, psychological, and structural factors that compound to deteriorate health outcomes significantly.

Additionally, homelessness can be both a cause and a consequence of extreme social exclusion, further differentiating the homeless population from other socioeconomically disadvantaged groups. Unlike individuals who may struggle with poverty but still maintain some level of social integration

(through family, employment, or community tie) many homeless individuals experience a complete separation from these support networks. For instance, someone who loses their job and can no longer afford rent may initially turn to friends or family for help. But as financial instability persists, relationships strain, and without a stable address or phone number, securing new employment becomes increasingly difficult. Over time, this individual may find themselves not only homeless but also socially invisible; barred from job opportunities, health services, and even the basic dignity of everyday interactions. In this way, homelessness doesn't just stem from social exclusion; it deepens it, creating a cycle that is uniquely isolating compared to other forms of economic hardship.

In Ireland, homeless individuals have a median age at death of just forty-two years⁹, while globally it is known that homeless individuals display greatly increased rates of multimorbidity and premature frailty¹³⁷. People Experiencing Homelessness (PEH) are therefore an important model population for studying the origins of health inequalities and understanding the effects of maximal and multiple exclusion as distinct from the social gradient in health. However, in such contexts, it is important to recognise that homelessness is often embedded within complex cognitive, behavioural, and social processes that may both precipitate housing loss and mediate its downstream effects on health.

3.1.1 Pathways Linking Social Exclusion to Poor Health

There are many complex and overlapping causal pathways linking social exclusion and poor health outcomes, some of which are referenced in fig 3.1 below.

At the most basic level, socially excluded people often lack the material resources required for good health – access to quality nutrition, access to appropriate healthcare or to shelter, for example. As a consequence of their material circumstances, socially excluded individuals may also experience increased exposure to environments that present a risk to health. Poorer people are more likely to work in dangerous and precarious roles, sometimes in the informal economy where there are few if any protections in place to offer physical or financial security. Those living in communal or substandard accommodation are more likely to be exposed to infection, pollutants or other environmental hazards such as moulds, to violence or to the harmful effects of the elements.

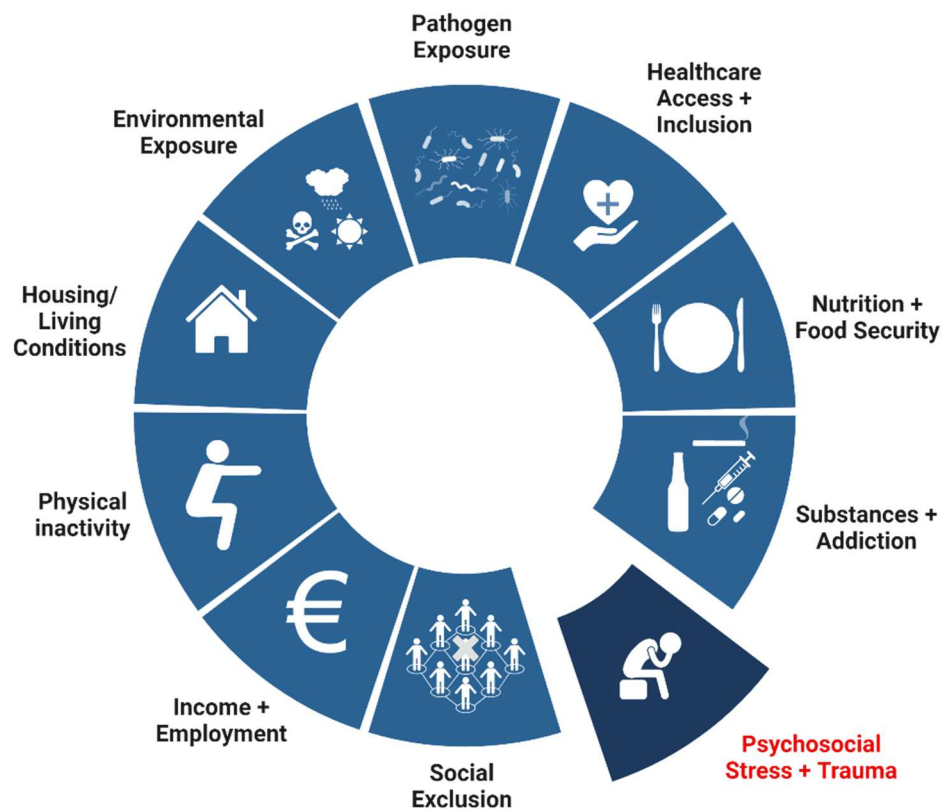


Figure 3.1 Psychological, Environment and Social determinants of Health in Socially Excluded Populations including people experiencing homelessness. *Psychosocial stress and trauma are highlighted as the major focus of this research.*

3.1.2 Psychosocial Stress and Trauma as Social Determinants of Health

Central to this research, however, are the effects of psychosocial stress and trauma which become more prevalent and more intense as socioeconomic status decreases. Psychosocial stress and trauma affect health directly and indirectly. Indirectly, stress can drive negative health behaviours like smoking, drinking and drug use¹³⁸, as individuals experiencing stress turn to these vices to self-medicate or buffer the effects that psychosocial stress and trauma have on their lives^{139–141}. Similarly, psychosocial stress and trauma often makes it harder for individuals to engage in health promoting behaviour. The negative impacts of stress on mental health can make it more difficult to find the

motivation to exercise, sleep well, adhere to a healthy diet or to maintain relationships with friends, family, and partners.

Psychosocial factors are frequently neglected for more tangible mediators of the effects of poverty on health outlined above, however a wide body of experimental and observational evidence exists for a direct effect of psychosocial stress and trauma on the body. Psychosocial stress and trauma are processed and biologically integrated by the neuro-immune axis (nervous, endocrine and immune systems), which plays a critical role in the maintenance of organism-wide homeostasis^{25,142}. In the short term, integrated, multisystem physiological responses to psychosocial stress are adaptive, maximising chances of survival in stressful times. When psychosocial stress or trauma becomes chronic or is experienced to an extreme extent in critical developmental periods like early-life or adolescence, however, effects can be maladaptive, driving pathophysiological processes like hypertension and chronic inflammation^{100,143,144}.

3.1.3 The Homeless Population Has Grown and Become More Diverse

Homelessness and social exclusion present a serious and growing concern to global health. In the Dublin region, the homeless population has more than doubled over the last decade - from 4,149 individuals at the end of 2014¹⁴⁵ to 10,291 individuals at the end of May 2024¹⁴⁶. The changing face of homelessness in Ireland is not only seen in the dramatic growth in numbers of PEH but in the changing demographics seen within the homeless population. In July 2014 PEH were mainly single males, and there were 344 families living in emergency accommodation nationally. By November 2023, there were over 2000 families – a 480 percent increase in family homelessness in less than a decade¹⁴⁷.

Similar increases in homelessness have been seen across the Global North since the 2007/8 Global financial crisis ^{13,15,16}. The growth and diversification of homeless populations in Ireland and around the world magnifies the challenge of homelessness to public health research and policy. Children, young people, and workers are now homeless in significant numbers as a result of insufficient housing supply, low wage growth compared to housing cost inflation and instability in the rental sector. This expanded breadth within homeless populations necessitates a more nuanced approach in health research. The diversity within homeless cohorts means that generalisations can obscure critical differences in health outcomes and needs. Consequently, it is imperative that research focused on the health of homeless people clearly defines and considers the specific sub-cohorts or mix of homeless individuals being studied. By doing so, we can ensure that findings are relevant and that interventions are appropriately targeted to address the distinct challenges faced by different segments of the homeless population.

3.1.4 Studying the Health and Wellbeing of Socially Excluded and Homeless People Requires Appropriate Tools:

Studying the health and wellbeing of socially excluded and homeless people necessitates the use of appropriate tools and methodologies that can accurately capture the unique social determinants of health at play in these populations. Moreover, working with multiply excluded populations demands careful ethical and methodological considerations to avoid harm to participants and the erection of additional barriers to participation. These populations are often marginalised and may have limited trust in research processes, necessitating culturally sensitive and trauma-informed approaches to data collection.

Furthermore, PEH and other multiply excluded populations are frequently excluded from health research, resulting in a lack of understanding of their specific health needs and challenges. This under-representation means that there is a significant moral and ethical imperative to include these groups in research to ensure that health interventions and policies are inclusive and effective. By addressing these gaps, researchers can contribute to a more equitable understanding of health and wellbeing, ultimately improving health outcomes for some of the most vulnerable individuals in society.

3.2 Hypothesis:

Social gradients in health and in experiences of psychosocial and traumatic stress are well established. Homeless individuals lie at the sharpest end of these gradients, dying younger and becoming frail far earlier in life than their housed counterparts^{148–150}. Homeless people experience extremes of psychosocial and traumatic stress throughout their lives, from adverse childhood experiences in early life to greater incidence of traumatic events and daily psychosocial stress in adulthood. We hypothesised that in the AESS-Vax and PATH cohorts, despite differences in demographic profiles from the overall homeless population in Ireland and from published studies of stress and health in PEH worldwide, both psychosocial and traumatic stress, and measures of health would be worse than those seen in housed participants in both studies.

3.2.1 Specific Aims:

1. To provide detailed demographic profiles of the AESS Vax and PATH cohorts, comparing homeless, housed, low socioeconomic and high

socioeconomic status participants internally and with measures taken in other contexts in Ireland and around the world.

2. To document the overall health status of homeless, housed, high SES and low SES participants in AESS Vax and PATH.
3. To measure psychosocial stress and adversity experienced by AESS-Vax and PATH participants using a variety of measurement tools and to compare levels of stress experienced by homeless, housed, low SES and high SES participants.
4. To compare the health status and psychosocial stress measures between socioeconomic groups.

3.3 Specific Methods:

3.3.1 Overview of Statistical Approaches

The statistical analyses in this chapter were conducted using R. All analyses were performed using R version (Version 4.1.3) and R Studio (2023.06.0 Build 421) with various packages specifically designed for data manipulation, statistical modelling, and visualisation.

3.3.2 Data Preparation and Transformation

Before analysis, datasets were thoroughly examined for missing values, outliers, and inconsistencies. Continuous variables were scaled where appropriate, particularly when interaction terms were included in

statistical models. This scaling ensures that interaction effects are interpretable and that the resulting coefficients are on a comparable scale.

3.3.3 Regression Models

To investigate the relationships between socioeconomic status (SES), psychosocial stress/trauma and health outcomes, we employed multiple regression models. These models were chosen for their ability to estimate the influence of several predictors on a continuous outcome while controlling for potential confounding variables.

- **Model Specification:** Each outcome variable (e.g., Meds (number of medications currently prescribed), HealthCond (number of current diagnoses)) was analysed by regressing it on a set of predictor variables (e.g., PSS, HRLES, ACESLikert, ACESBina, SES, Sex, Age, PackYrs (Pack-Year smoking history), AlcMp (Alcohol consumption per week)). The general structure of the model was: Outcome $\sim$ Predictor + Covariates + Interaction Terms. Interaction terms were included to explore whether the effect of one predictor variable on the outcome differed depending on the level of another variable (e.g., SES, Sex, Age). This was particularly important for understanding how demographic factors might modify the relationship between stress and health outcomes.

3.3.4 Model Selection and Interpretation

Models were selected based on theoretical relevance and statistical criteria. Initially, all main effects were included in the models, followed by the addition of interaction terms. The inclusion of interaction terms was validated by statistical significance testing (p-values < 0.05).

- **Statistical Significance:** Coefficients were considered significant if the p-value was below 0.05. For interaction terms, a significant coefficient indicated a modification of the relationship between the primary predictor and the outcome by the interacting variable.
- **Model Fit and Diagnostics:** Model fit was assessed using adjusted R-squared values, residual plots, and tests for multicollinearity (Variance Inflation Factor, VIF). Residual plots were examined to ensure homoscedasticity, and any deviations from normality were addressed through transformations.

Other Statistical Tests

- (i) **Kruskal-Wallis Test:** Non-parametric test employed to determine whether there were statistically significant differences in the distribution of stress scale scores across more than two independent groups (e.g., High SES, Low SES Housed, and Low SES Homeless).
- (ii) **Mann-Whitney U-Tests (Wilcoxon Ranked Sum):** For non-parametric pairwise comparisons between groups (low vs high SES, homeless versus housed), the Wilcoxon rank-sum test was used, particularly where normality assumptions were not met.
- (iii) **Fishers' Exact Test:** Fisher's Exact Test was utilised to assess the significance of the association between categorical variables. This test was used to analyse the distribution of missing data across groups to ensure the robustness of comparisons despite the limitations of sample size. Due to the size and sparseness of some contingency tables, Monte

Carlo simulations were employed with 10^7 replicates to ensure stable and accurate p-value estimates.

- (iv) **Correlation Analysis:** Pearson correlation coefficients were calculated to examine the relationships between different stress scales, determining both the strength and direction of linear relationships between continuous variables (e.g. PSS and HRLES).

3.3.6 R Packages Used

The primary packages used in this analysis included:

- (i) **dplyr and other tidyverse packages:** For data manipulation and cleaning, ensuring that the dataset was properly prepared for analysis.
- (ii) **ggplot2:** For creating high-quality visualisations, including scatter plots with regression lines, violin plots, and correlation matrices.
- (iii) **ggpubr:** used with ggplot2 to annotate plots with statistics and to improve the appearance of plots.
- (iv) **rstatix:** For statistical tests and pairwise comparisons, particularly the Wilcoxon test for non-parametric data.
- (v) **broom:** For tidying model outputs and facilitating the extraction of coefficients, confidence intervals, and p-values for reporting.
- (vi) **FSA:** Used to carry out Dunn's post-hoc pairwise tests after Kruskal-Wallis tests.
- (vii) **MASS:** Used for statistical modelling functions such as negative binomial regression (with functions like `glm.nb`), which are applied to model count data like Health Conditions and Medications.

- (viii) **GGally**: Used for creating pair plots via the `ggpairs` function, which provides a comprehensive view of variable relationships.
- (ix) **Corrplot**: Employed to visualize correlation matrices (e.g., with the `corrplot` function), making it easier to interpret the strength and direction of correlations among stress scales.

3.4 Results:

3.4.1 Homeless Adults in the AESS Vax Cohort are Older and Experience Poorer Health than Housed Participants - Review of Cohort Demographic and Health Data

In total 143 individuals completed demographic questionnaires upon recruitment to the AESS-Vax study. A demographic profile of these 143 individuals is presented below in table 3.1 alongside data from the PATH cohort. At a gross demographic level, high SES participants were significantly younger than Low SES participants (homeless and housed) with a mean age of 38.12 years versus 46.11 years and 47.54 years in homeless AESS-Vax

All groups in AESS-Vax, with the exception of the homeless group, were over 50% female but differences in gender between groups in AESS-Vax were not statistically significant ($p=0.0712$). All groups were predominantly of white European ethnicity; 87.7% of the High SES group, 82.3% of the Low SES group 66% of the AESS-Vax homeless group all of the PATH Cohort.

While both AESS-Vax and PATH cohorts included individuals experiencing homelessness, they represent distinct segments of the homeless population. PATH participants were recruited from medium-

to long-term supported accommodation services for adults who have experienced chronic homelessness. These participants in some cases may be accommodated in supported accommodation because of their higher medical or social care needs. In contrast, AESS-Vax participants were recruited from vaccination clinics and were living in a mix of accommodation types, including emergency and temporary housing. They are individuals with less overall experience of homelessness and typically, better health and lower care needs than PATH participants.

Table 3.1 Demographic and Health Profile of the AESS-Vax and PATH Cohorts: Participants were divided into six groups to allow for analysis by socioeconomic status (High SES, Low SES) and by housing status (High SES, Low SES Housed, Homeless), PATH was taken as a standalone category. Standard deviations are shown in rounded brackets. Interquartile range is shown in square brackets. p-values were generated by Kruskal-Wallis tests for continuous variables and by either Chi-Squared or Fisher's Exact Tests for categorical variables. Test used for categorical variables is displayed with p-value on table

	High SES	Low SES AESS-Vax Total	Low SES Housed	AESS-Vax Homeless	PATH Homeless	p-value
Total Sample Size (n)	81	62	35	27	59	
Age	38.12 (10.5)	46.11 (13.4)	45.08 (13.7)	47.54 (13.2)	49.77 (10.3)	p= 1.06e-7
Gender, %Female (n)	59.26 (48)	61.29 (38)	75 (27)	42.3 (11)	23.73 (14)	p = 1.2e-6 (Chi Squared Test)

Ethnicity							
(I)	White (Irish, British, European)	71	51	33	18	All White Irish	p = 0.0447 (Fishers' Exact Test)
(II)	East Asian	4	2	1	1		
(III)	South Asian	3	2	0	2		
(IV)	Irish Traveller/ Minceir	0	3	1	2		
(V)	Latin	1	1	0	1		
(VI)	Arab	1	1	0	1		
(VII)	Black	1	1	0	1		
(VIII)	Roma	0	1	0	1		
BMI		25.32 (4.08)	26.37 (4.52)	26.57 (4.18)	26.09 (5.16)	Not Collected	p = 0.3814 (On collected data)
Health Conditions [IQR]		0 [0-0]	0 [0-1]	0 [0-0]	0 [0-2]	4.26 [3-6]	p = 7.87e-24
Medications [IQR]		0 [0-0]	0 [0-1]	0 [0-0.25]	0 [0-3]	3.65 [1-6]	p = 4.04e-15
Smoker							
(I)	Current	4	14	7	7	46	p = 1e-7 (Fishers' Exact Test)
(II)	Previously	18	17	12	5	4	
(III)	Non-Smoker	59	27	16	11	9	
(IV)	N/A	1	4	0	4	0	
Pack Year History (Current and Previous Smokers)		4.6 (6.02)	14.84 (16.75)	16.27 (20.36)	12.78 (10.32)	33.85 (30.67)	p = 9.67e-8

Alcohol Consumption						$p = 6e-7$
(I) Current	53	21	14	7	32	(Fishers' Exact Test)
(II) Previous	5	6	5	1	15	
(III) Non-Drinker	7	11	5	6	10	
(IV) N/A	17	23	11	13	2	
Units Alcohol Per Week	7.13 (7.12)	7.48 (10.36)	3.84 (4.14)	14.4 (14.79)	52.6 (90.79)	$p = 8.92e-4$
Drug Use						$p = 1e-7$
(I) Current	1	6	2	4	8	(Fishers' Exact Test)
(II) Occasionally	4	0	0	0	11	
(III) Previously	7	44	3	1	17	
(IV) Non-User	66	3	26	18	22	
(V) NA	3	8	4	4	1	
Number of Drugs Consumed in Last Week [IQR]	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0]	1.24 [0-2]	$p = 2.83e-8$
Daily Sleep Duration	6.8 (1.13)	5.8 (1.35)	6.25 (1.21)	5.04 (1.27)	5.55 (1.71)	$p = 4.28e-7$

In examining health and health-related behaviours across socioeconomic groups within the AESS-Vax and PATH cohorts, sharp social gradients in both overall health and health behaviours were evident, with PATH homeless participants emerging as an extreme outlier (Table 3.1 and Figure 3.2).

Health Conditions and Medication Use: Homeless participants in the AESS-Vax cohort reported a higher number of health conditions on average

compared to both the high SES and low SES housed groups (Figure 3.2D, $p < 0.01$ and $p < 0.05$, respectively). This pattern was even more sharply observed in the PATH cohort, a maximally excluded, long-term homeless group who reported more health conditions than AESS Vax homeless ($p_{\text{adj}} = 0.0004$) and high SES ($p_{\text{adj}} = 3.17 \times 10^{-17}$) groups (not shown in Figure 3.2, Dunn's Test for pairwise comparisons).

Similarly, the number of medications used, a proxy for chronic illness management, was significantly higher in the PATH homeless group compared to all AESS-Vax groups, including AESS-Vax homeless ($p_{\text{adj}} = 4.00 \times 10^{-4}$). Within the AESS-Vax cohort, homeless participants were prescribed significantly more medications than high SES participants ($p = 0.0427$), though differences with the low SES housed group were not significant ($p = 0.8997$).

Health Behaviours: The analysis of health behaviours revealed striking disparities, particularly in smoking and sleep patterns. The number of pack years, reflecting cumulative tobacco exposure, was significantly higher among AESS-Vax homeless individuals compared to both high SES and low SES housed participants. PATH homeless participants reported the highest pack years overall, with significant differences compared to all AESS-Vax groups, including AESS-Vax homeless ($p_{\text{adj. Fisher}} = 3.97 \times 10^{-5}$).

Alcohol consumption patterns were more nuanced. While there was no significant difference between the AESS-Vax homeless group and either the high SES or low SES housed groups, a significant difference was observed between high SES and low SES housed groups ($p < 0.05$). PATH homeless participants consumed significantly more alcohol compared to high SES ($p_{\text{adj}} =$

9.30×10^{-4}), low SES housed ($p_{\text{adj}} = 1.42 \times 10^{-2}$), and AESS-Vax homeless participants ($p_{\text{adj}} = 8.16 \times 10^{-6}$).

Interestingly, no significant differences were found in drug use across the AESS-Vax groups, which might indicate that drug use behaviours in these cohorts are not as strongly differentiated by SES or housing status as other health behaviours. PATH homeless participants on the other hand, showed higher drug use compared to high SES ($p_{\text{adj}} = 5.15 \times 10^{-2}$), low SES housed ($p_{\text{adj}} = 4.19 \times 10^{-4}$) and AESS-Vax homeless participants ($p_{\text{adj}} = 1.15 \times 10^{-3}$).

Sleep Patterns: In AESS-Vax there were pronounced differences in sleep duration between homeless participants and both high SES and low SES housed groups (fig 2G, $p < 0.0001$ and $p < 0.01$, respectively). PATH homeless participants report a slightly higher mean sleep duration per night than AESS-Vax homeless participants (5.5 hours versus 5.04 hours), but this difference was not significant ($p_{\text{adj}} = 1.00$). PATH homeless participants slept significantly less than High SES ($p_{\text{adj}} = 0.0005$) but not low SES participants ($p_{\text{adj}} = 0.9746$).

Body Mass Index (BMI): Despite the other health disparities observed, BMI did not differ significantly across the AESS-Vax groups. BMI was not recorded consistently in the PATH study.

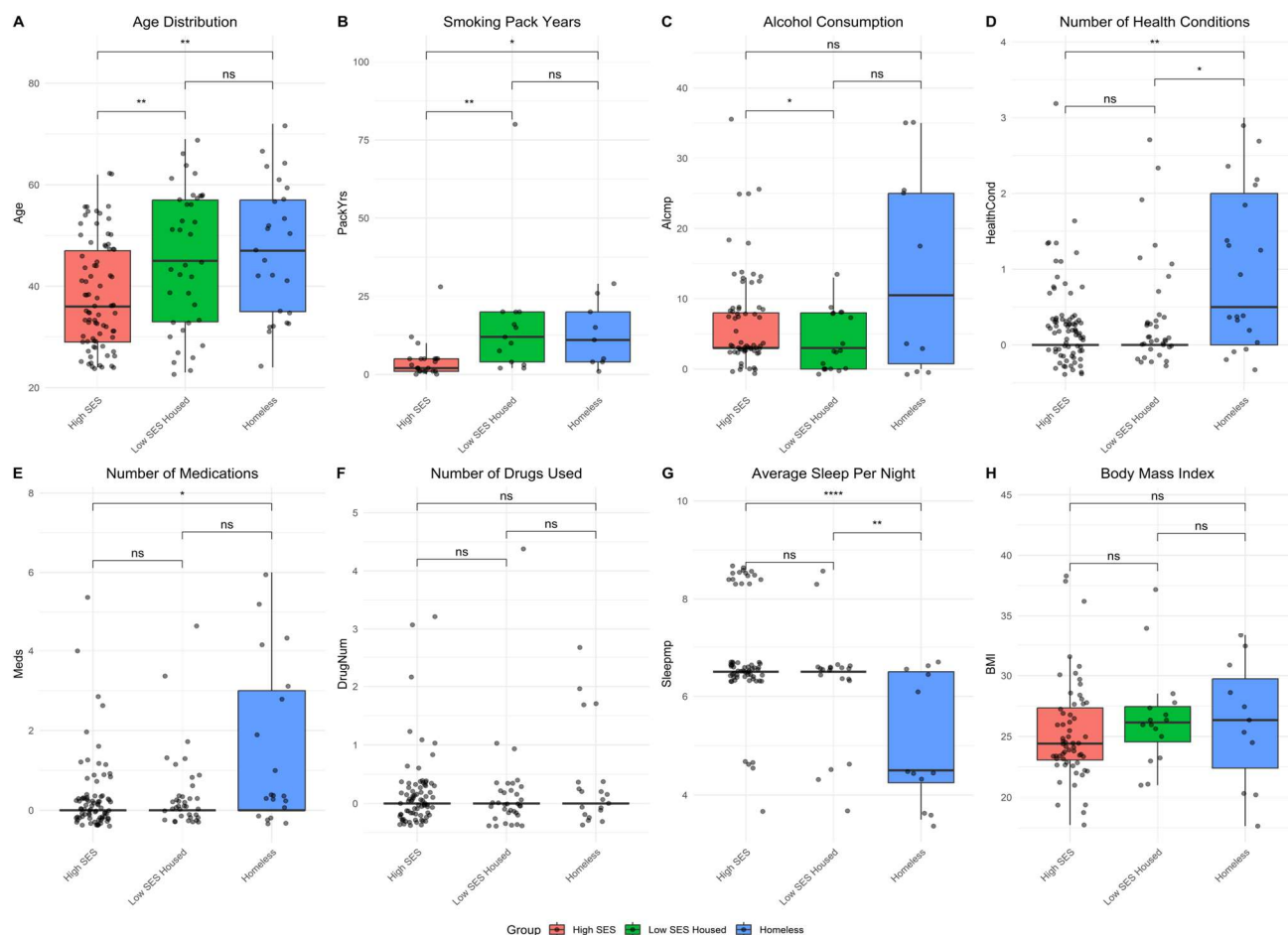


Figure 3.2 Distribution of health and behavioural variables collected in the AESS-Vax study across socioeconomic status groups. **A. Age:** Low SES housed, and homeless groups were both older than the high SES group ($p < 0.01$). **B. Pack Years:** The number of pack years was significantly higher in the Homeless group compared to High SES ($p < 0.05$) and Low SES Housed ($p < 0.01$). **C. Alcohol Consumption (AlcMp):** There were no significant differences between the homeless group and either the Low SES housed or High SES group. There were significant differences between the High SES and Low SES Housed group ($p < 0.05$). **D. Number of Health Conditions:** The Homeless group reported significantly more health conditions than the High SES ($p < 0.01$) and Low SES Housed ($p < 0.05$) groups. Differences between the Low SES Housed and High SES group did not reach significance. **E. Number of Medications:** The Homeless group reported taking a higher number of medications than the High SES group but not the Low SES Housed Group. Differences between High and Low SES Housed groups were not significant. **F. Number of Drugs Used:** No significant differences were observed between groups for the number of drugs used. **G. Average Sleep per Night:** The Homeless group reported significantly less sleep per night compared to High SES ($p < 0.0001$) and Low SES Housed ($p < 0.01$) groups. Differences between housed groups were not significant. **H. Body Mass Index (BMI):** No significant differences were observed in BMI across the groups. After carrying out Kruskal-Wallis tests on distribution of continuous variables, Mann-Whitney U-tests were conducted to make pairwise comparisons between groups - * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.4.2 Long Term Homeless Adults in the PATH Cohort Experience Premature Frailty and Poorer Health than the Housed Population

At the gross level, PATH homeless participants appear as an outlier across comparable domains with the three AESS-Vax groups. PATH participants are homogenously white, predominantly male, significantly more ill (as indicated by number of reported health conditions and prescribed medications) and more likely to drink heavily, smoke and take illicit drugs.

The PATH study was designed to provide a more holistic assessment of health than the AESS-Vax study, with data unique to its 59 participants further illustrating the deep health challenges faced by maximally excluded or long-term homeless individuals. This is most obviously observed in Clinical Frailty Score data (Fig 3.3, left), where 92% of participants were assigned a score indicative of some level of frailty. However, this observation is in contrast to participant's self-perceived health status (Fig 3.3, right), showing 34% of participants rated their health as either "good" or "excellent".

Participants may be framing their health in relative terms, comparing themselves to peers within their immediate social context rather than to broader population norms. This perspective could reflect a process of normalisation, wherein persistent adversity and chronic health challenges become internalised as the status quo, particularly in a population accustomed to long-term hardship and instability. The CFS, while valuable for capturing visible and functional frailty, may not fully encompass the lived experience or internal health perceptions of this group, underscoring the complexity of evaluating health in socially excluded populations.

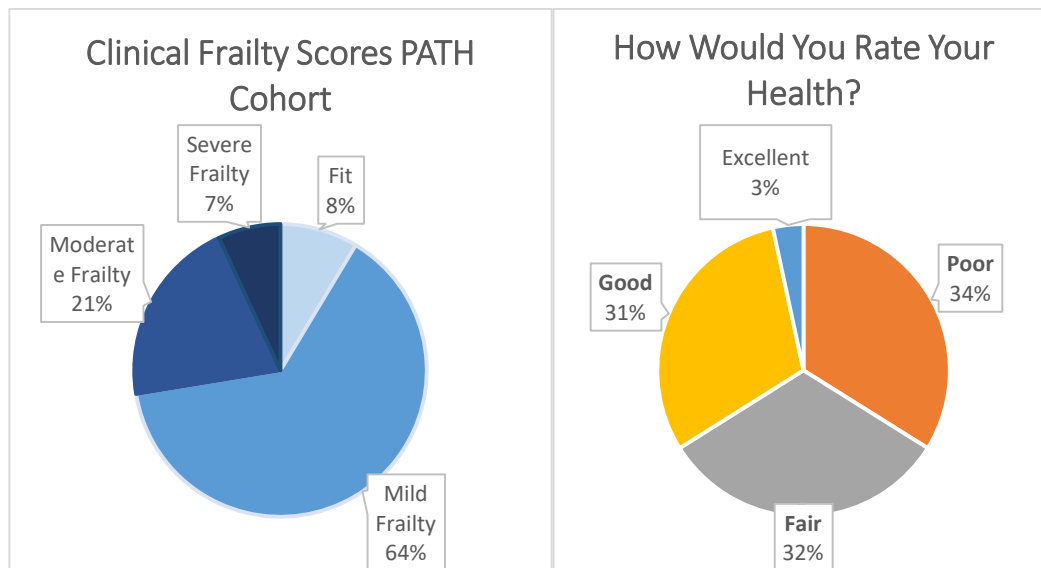


Figure 3.3: Clinical Frailty Scores and Self-Rated Health in the PATH Cohort: The left pie chart illustrates the distribution of clinical frailty scores within the PATH cohort. Frailty categories include Fit-Manging Well (score 1-3; 8%), Very Mild-Mild Frailty (Score 4-5; 64%), Moderate Frailty (score 6; 21%), and Severe Frailty (score 7-9; 7%). The right pie chart shows participants' self-rated health, categorised as Poor (34%), Fair (32%), Good (31%), and Excellent (3%).

The Barthel Index (Table 3.2 I), which captures levels of functional dependence in activities of daily living gives a more objective picture of health within the cohort, with an average score of 93.47 (SD = 12.91) out of 100. Most participants (66.10%) were classified as functionally independent, while 23.73% reported moderate dependence, 6.78% slight dependence, and 3.39% severe dependence.

Cognitive assessment using the Rowland Universal Dementia Assessment Scale (RUDAS; table 3.2 II) was conducted in full on 19 participants, with an average score of 20.89 (SD = 7.34). Scores below 22 indicate a risk of cognitive impairment requiring further evaluation ^{15,16}. In PATH, 36.84% (7/19) of participants scored below this threshold. One participant failed to complete all tasks included in the scale scoring a total of 7 on tasks completed (participant fell asleep).

Multimorbidity was prevalent, with 27.12% (16/59) of participants reporting five or more chronic health conditions, while 62.71% (37/59) reported two to five conditions. Chronic pain (Table 3.2 IV) was also common, with 74.58% of participants (44/59) reporting ongoing pain, and 23.73% (14/59) indicating they did not experience chronic pain.

Table 3.2 Health and Functional Characteristics of the PATH Cohort: (I) Barthel Index: A measure of functional independence, assessing activities of daily living (ADLs) with scores ranging from 0 to 100. Categories include Total Dependence (0–20), Severe Dependence (21–60), Moderate Dependence (61–90), Slight Dependence (91–99), and Independent (100). **(II) Rowland Universal Dementia Assessment Scale (RUDAS):** A cognitive screening tool assessing six domains, including memory with a total score out of 30. A score of ≤ 22 suggests potential cognitive impairment. The RUDAS was administered to the final 20 PATH participants. **(III) Health Conditions:** Prevalence of the top eight diagnosed health conditions reported by participants, including Hepatitis C (achieving sustained virologic response [SVR] or cure) and HIV (virologically suppressed with undetectable = untransmissible). **(IV) Self-Reported Chronic Pain:** Participants were asked whether they currently experience chronic pain, with responses categorised as "Yes," "No," or "No Answer."

	Percent	Count		Percent	Count
(I) Barthel Index			(III) Health Conditions		
Total Dependence	0.00%	0	Hepatitis C (SVR/Cured)	25.42%	15
Severe Dependence	3.39%	2	Depression	23.73%	14
Moderate Dependence	23.73%	14	Addiction	20.34%	12
Slight Dependence	6.78%	4	Asthma	20.34%	12
Independent	66.10%	39	COPD	15.25%	9
No Answer	0.00%	0	Hypertension	15.25%	9
(II) RUDAS (n =19)			HIV (Virologically Suppressed/U=U)	11.86%	7
Score >22	57.89	11	(IV) Chronic Pain?		
Score <22	36.84	7	Yes	74.58%	44
			No	23.73%	14
No Answer	5.26	1	No Answer	1.69%	1

3.4.3 Long-Term Homeless Adults Experience a High Burden of Psychosocial Adversity and Trauma Throughout the Lifecourse

(I) Adverse Childhood Events are Substantially More Common in Long Term Homeless Adults

Although the PATH questionnaire captures experiences of psychosocial adversity in early life and adulthood, there is no overlap in the tools used to profile psychosocial adversity in the AESS-Vax study. Unlike AESS-Vax, PATH does not capture psychosocial stress in the short to medium term (Perceived Stress and Holmes-Rahe Life Event Stress Inventory). However, in both studies, early-life adversity is captured, but using two different tools (ACES questionnaire, Childhood Trauma Questionnaire).

The PATH cohort exhibits a pronounced burden of childhood adversity, as reflected in their responses to the ACEs questionnaire. The mean ACE score in this cohort was 4.36 (SD = 1.63), with scores ranging from 1 to 10 out of a maximum of 10. Notably, three quarter of participants scored at least 3 ACEs (first quartile = 3), while one in four scored more than 5 ACEs (third quartile = 5). Only one participant reported a single ACE, further highlighting the extent of adversity faced by this population.

These findings are striking when compared to general populations in high-income countries like Ireland and the United States where it is estimated that 10-15% experience four or more ACEs¹⁵¹. In the PATH cohort, most participants fell into this higher-risk category, consistent with patterns observed in other homeless populations. For example, research from the Cork Simon Community revealed that 77% of homeless individuals surveyed had experienced four or more ACEs¹⁵². Such comparisons underline the

exceptional levels of early-life adversity among individuals experiencing homelessness.

Specific categories of ACEs were particularly prevalent in this cohort. Nearly half (45.83%) reported physical abuse, 31.25% reported sexual abuse, and 26.53% reported emotional abuse. Experiences of neglect were also common, with 20% of participants indicating they often lacked sufficient food and 16.67% reporting that no caregiver was consistently available. Reflecting the cumulative impact of these adversities, 62.79% of participants stated they did not have a happy childhood.

(II) Trauma Begets Trauma - Long Term Homeless Adults in Ireland Experience Extreme Adversity

Participants were also surveyed on seven distinct traumatic experiences encountered in adulthood, adapted from the International Trauma Exposure Measure (ITEM) and modified based on feedback from the Inclusion Health Research Group and homeless peer supporters (results in Table 3.3). The response rate for this section of the questionnaire was 92.74%, with 7.26% of participants refusing to answer any of the questions.

Experiences of trauma in adulthood were notably prevalent in the PATH cohort. Over half of the participants (57.6%) reported being the victim of a serious physical attack or assault during their lifetime. In the first and only nationally representative study of trauma exposure in Ireland, 31.1% of participants reported physical assault from someone other than a parent or guardian; if these figures reflect the true prevalence of assault in the Irish population, PATH participants were almost twice as likely to have suffered an

assault (worded as “serious physical assault or attack” in our questionnaire but as “physical assault” in Hyland et al¹⁵³.).

61% of participants reported experiencing a life-threatening illness or accident, making it the most frequently reported traumatic experience in this survey. In Hyland et al. 22.6% reported experiencing a life-threatening accident, while 14.1% reported diagnosis with a life-threatening condition – both experiences were captured in a single question in the PATH study. Participants were also asked whether their spouse, partner, or child had experienced a life-threatening illness or accident, with 30.5% affirming this compared to 51.9% in Hyland et al.

Exposure to other types of trauma was also significant. Over a quarter of participants (27.1%) reported that a close family member or loved one, such as a spouse, partner, or child, had been addicted to drugs. One in five participants (22%) had experienced a fire, flood, or natural disaster, while 11.9% reported having a child of their own die. Finally, over half of the participants (50.8%) indicated that they had been imprisoned at some point in their lives.

Table 3.3 Prevalence of Selected Traumatic Life Events in the PATH cohort

Question	% Answered Yes (n)	% Not Answered (n)
Have you ever experienced a fire, flood or natural disaster?	22.03 (13)	5.08 (3)
Has your spouse, partner or child ever been addicted to drugs?	27.12 (15)	6.78 (4)
Where You the Victim of a serious physical attack or assault in your life?	57.63 (34)	3.39 (2)
Have You ever had a life-threatening illness or accident?	61.20 (36)	1.69 (1)

Spouse/partner or child ever had a life-threatening illness or accident?	30.51 (18)	5.08 (3)
Has a child of yours ever died?	11.87 (7)	3.39 (2)
Have you ever been in prison?	50.85 (30)	6.78 (4)

(III) Long Term Homeless Adults Experience Loneliness and Isolation:

Social isolation and loneliness were reported frequently in the PATH cohort; captured in the single question, “How often do you feel lonely” and in a 5-item questionnaire asked to all participants. Over one third of respondents reported feeling lonely “often” (11.86%) or “very often” (22.03%) (Table 3.4). Conversely, only 15.25% of participants reported that they never felt lonely. Women in the cohort reported higher levels of loneliness overall, with 28.57% stating they felt lonely “very often,” compared to 20% of men, and only 7.14% of women indicating that they “never” feel lonely, compared to 17.78% of men.

Table 3.4 Subjective Loneliness Question showing a breakdown by gender.

How Often Do You Feel Lonely?	Total	Male	Female
Very Often	22.03 (13)	20.00 (9)	28.57 (4)
Often	11.86 (7)	13.33 (6)	7.14 (1)
Sometimes	25.42 (15)	24.44 (11)	28.57 (4)
Rarely	11.86 (7)	15.56 (7)	0.00 (0)
Never	15.25 (9)	17.78 (8)	7.14 (1)
Refused to Answer	13.56 (8)	8.89 (4)	28.57 (4)

Regarding interpersonal relationships (Table 3.5), 42.37% of participants reported not having any close friends, and 47.46% stated that they do not get to meet friends regularly. Women were less likely to report having close

friends, with 50% of female participants stating they had none, and 57.14% reporting they do not get to meet friends regularly. Familial relationships were more common, with 62.71% of participants reporting that they had close family members. However, 40.68% reported that they do not get to meet family members regularly. Women reported similar figures for close relatives to men, but 28.57% stated they do not get to meet them, compared to 44.44% of men.

Participation in group activities was the least common form of social interaction, with only 22.03% of participants engaging in any kind of group activity.

Table 3.5 Relationships and Social Interactions as Recorded in the PATH Cohort: Participants were asked five questions about social supports and interactions in their lives. Responses are reported as a percentage of the responses for the question, with numbers of participants answering yes in brackets and percentages of non-answers recorded per question.

Question	% Answered Yes (n)	%Not Answered
Do you have any close friends?	52.54 (31)	5.08
Do you get to meet any close friends?	45.76 (27)	6.78
Do You Have Any Close Family or Relatives?	62.71 (37)	5.08
Do you get to meet any Close Family or Relatives?	54.24 (32)	5.08
Do You Participate In Any Groups?	22.03 (13)	3.39

(IV) PATH Participants Show Poor Life Satisfaction and Poor Self-Rated Mental Health:

Life satisfaction among the PATH cohort was notably low, as assessed using Cantril's Ladder, a measure of subjective well-being, and another standalone

question (satisfied/ dissatisfied/ neither satisfied nor dissatisfied with life). Only 6.78% of participants reported a Cantril's Ladder score indicative of thriving (8 or higher), while the majority fell within the "struggling" (45.76%; scores between 4-7) or "suffering" (32.2%; scores below 4) categories. In the standalone question, 62.71% said they were dissatisfied, 18.64% satisfied and the remainder neither satisfied nor dissatisfied.

Mental health difficulties were prevalent within the cohort, with 33.90% rating their mental health as "poor" and an additional 32.20% rating it as "fair." Depression was the most common condition, reported by 23.73% of participants (see table 2), while 30.51% were currently prescribed antidepressants. Anxiety, schizophrenia, and suicidal ideation were each reported by 8.47% of participants, and PTSD was reported by 3.39%. Despite the high rates of mental health challenges, no participants were actively seeing a psychologist, and only 8.47% were seeing a psychiatrist, highlighting unmet mental health needs in this population.

3.4.4 High SES Participants in AESS-Vax Were More Likely to Complete Psychosocial Stress Inventories and Questionnaires:

Table 3.6 *Number of completed tests, non-completions (NA) and percentage completion for each stress scale included in the AESS-Vax questionnaire.*

	PSS Complete	PSS Completion	HRLES Complete	HRLES Completion	ACES Complete	ACES Completion
High SES	63	77.8% (18 NA)	62	76.5% (19 NA)	61	75.3% (20 NA)
Low SES Total	35	56.4% (27 NA)	28	45.2% (34 NA)	30	48.3% (32 NA)
Low SES Housed	22	59.4% (15 NA)	17	45.9% (20 NA)	20	54.1% (17 NA)
Homeless	13	52% (12 NA)	11	44% (14 NA)	10	40% (15 NA)

Collection of health and demographic information was successful and almost all participants provided answers to most questions asked on the AESS-Vax questionnaire. Completion for stress inventories included at the end of the AESS-Vax questionnaire pack was markedly lower, particularly in Low SES participants (table 6). We conducted Fisher's Exact Tests to examine differences in the likelihood of completion. The results revealed significant differences in completion rates between the High SES group and both the Low SES Housed group and the Homeless group for several stress indices. Specifically, participants in the High SES group were more than twice as likely to complete the PSS compared to those in the Low SES Housed group ($p = 0.0483$, odds ratio = 2.37), and more than three times as likely compared to the Homeless group ($p = 0.0208$, odds ratio = 3.19). Significant differences were also observed for the HRLES, where the High SES group had significantly higher completion rates compared to both the Low SES Housed group ($p = 0.0015$, odds ratio = 3.79) and the Homeless group ($p = 0.0032$, odds ratio = 4.09). For the ACES questionnaire scored on a Likert scale, completion rates were significantly higher in the High SES group compared to both the Low SES Housed group ($p = 0.0317$, odds ratio = 2.57) and the Homeless group ($p = 0.0016$, odds ratio = 4.50). However, no significant differences were found between the Low SES Housed and Homeless groups across any of the stress indices.

3.4.5 Stress Scales in AESS-Vax Assess Different Domains of Psychosocial Stress:

When designing the AESS-Vax study, it was decided that multiple domains of psychosocial stress would be captured - perceived stress, with the PSS; adverse childhood experiences or childhood trauma with the ACES-10

questionnaire; and stressful life-events, a more objective measure of psychosocial stress in adults, with the HRLES.

To better understand the relationships between these stress measures, a pairs plot was generated, displaying the distributions of scores for each of the stress indices and Pearson correlation coefficients between them (Figure 3.4). This plot offers insights into whether these scales are capturing similar or distinct aspects of psychosocial stress.

The Pearson correlations between the scales reveal moderate to strong relationships, suggesting that while these tools measure related constructs, each provides unique information about the participants' experience of stress. For instance, the Perceived Stress Scale (PSS) and the Holmes-Rahe Life Event Stress Scale (HRLES) are moderately correlated ($r=0.488$, $p<0.001$), indicating that individuals reporting higher levels of perceived stress tend to have experienced more stressful life events in the year prior to recruitment. However, the ACES-10 Questionnaire shows a weaker correlation with the PSS ($r=0.285$, 0.323 ; $p<0.01$, for binary and Likert scored versions) and HRLES ($r=0.318$, 0.370 ; $p<0.01$, $p<0.001$, for binary and Likert scored versions) highlighting that childhood adversity, as captured by the ACES-10, represents a distinct dimension of stress that does not necessarily align with current perceived stress or recent life events.

The ACES-10 questionnaire, originally scored as a binary scale was printed with a Likert scale in survey packs so two scores exist. The Likert score reported here was generated using a 5-point scale from “never” (0) to “very often” (5). The second, binary score took “never” responses as “no” (0) and scored all other answers as “yes” (1). Both scores are shown here as the Likert scale may

also capture information about the extent and/or frequency of specific traumatic events in childhood, a potential benefit over the binary scored version of the tool. Both scores were highly correlated, following a similar distribution among participants ($r=0.955$, $p<0.001$).

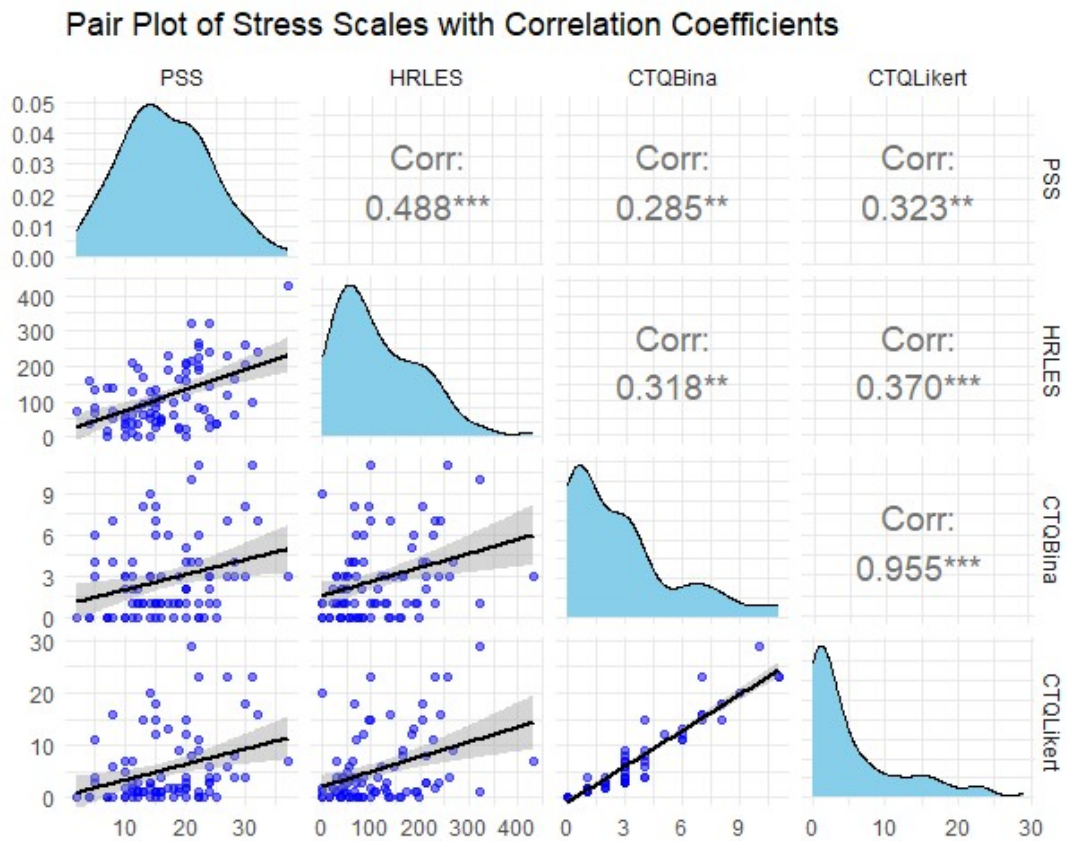


Figure 3.4 Pair Plot of Stress Scales with Correlation Coefficients This figure displays pairwise relationships between four stress-related variables: PSS (Perceived Stress Scale), HRLES (Holmes-Rahe Life Event Stress), CTQBina (ACES-10 Binary), and CTQLikert (ACES-10 Likert). Scatter plots in the lower triangle illustrate individual relationships, while histograms in the diagonal show the distribution of each variable. The upper triangle presents Pearson correlation coefficients with significance levels indicated: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.4.6 Psychosocial Stress and Trauma in the AESS-Vax Cohort is Experienced to an Increasing Extent as Socioeconomic Status is Lowered

Figure 3.5 illustrates the distributions of scores for psychosocial stress and trauma across three distinct socioeconomic groups in the AESS-Vax study: High SES, Low SES Housed, and Low SES Homeless. The violin plots provide a visual representation of the distribution and density of scores within each group.

Perceived Stress Scale (PSS, Fig 3.5A): The PSS captures the degree to which individuals perceive their life circumstances as stressful. The analysis reveals a clear gradient in stress levels across the socioeconomic spectrum with significant differences apparent ($p < 0.0001$; Kruskal-Wallis). Homeless participants reported experiencing significantly higher perceived stress compared to both High SES ($p < 0.0001$; Mann-Whitney) and Low SES Housed ($p < 0.01$) participants. This suggests that as socioeconomic stability decreases, the perception of stress intensifies, particularly among those experiencing homelessness. The absence of significant differences between the High SES and Low SES Housed groups may indicate that housing stability may serve as a critical buffer against perceived stress, even within lower socioeconomic strata ($p = 0.114$).

Holmes-Rahe Life Event Stress (HRLES, Fig 3.5B): The HRLES assesses the cumulative impact of significant life events in the twelve months prior to testing, which can contribute to overall stress. The violin plot for HRLES scores similarly demonstrates that homeless participants endure a significantly higher burden of stressful life events compared to their High SES counterparts ($p = 0.002$) but not housed Low SES participants ($p = 0.057$).

Housed Low SES and High SES participants do not differ significantly in their experiences of stressful life events, again suggesting that housing may act as a critical buffer or protective factor against psychosocial stress ($p=0.212$).

Adverse Childhood Experiences Questionnaire 10-Item (ACES-10, Fig 3.5C and 3.5D): The ACES-10, scored both as a binary and on a Likert scale, measures the prevalence and severity of adverse childhood experiences. The results indicate that childhood trauma is more prevalent among individuals in the Low SES Homeless group, with significantly higher scores compared to both High SES ($p<0.001$ for binary, $p<0.0001$ for Likert) and Low SES Housed participants ($p=0.006$ for binary, $p=0.003$ for Likert). There were no significant differences between the High SES and Low SES Housed groups ($p=0.612$ for binary, $p=0.313$ for Likert). Important to note is that all homeless participants reported becoming homeless in adulthood, after traumatic experiences captured in the ACES-10 would have taken place.

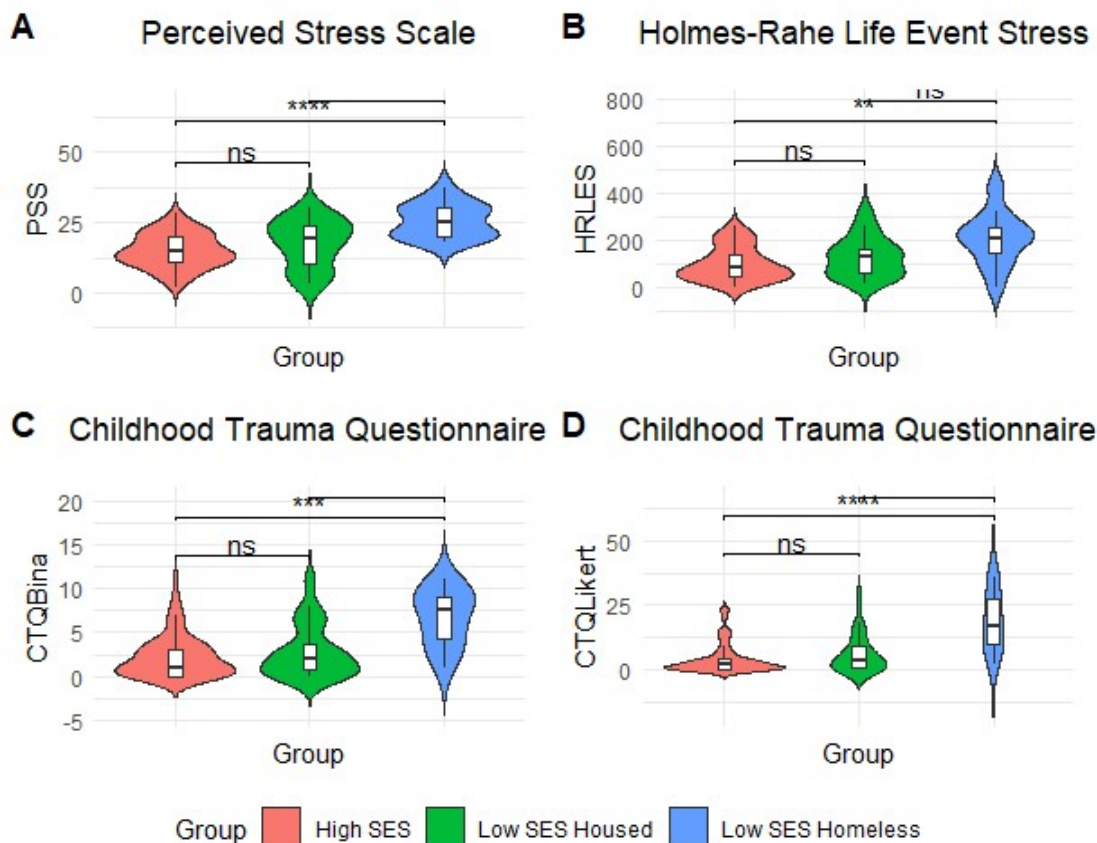


Figure 3.5 Violin plots depicting the distribution of scores on stress scales in the AESS-Vax study. A. Perceived Stress Scale (PSS): Kruskal Wallis revealed significant differences between the three groups ($p < 0.0001$). **B. Holmes-Rahe Life Event Stress (HRLES):** Significant differences were found between groups ($p = 0.0065$). **C. ACES-10 Questionnaire Binary (CTQBina; named “Childhood Trauma Questionnaire here):** Kruskal-Wallis showed significant differences between groups (0.0012). **D. ACES-10 Questionnaire Likert (CTQLikert):** Groups differed significantly ($p = 0.0003$). Kruskal-Wallis tests were used to detect overall significant differences between the three groups and Mann-Whitney U-Tests were deployed for pairwise tests between groups when Kruskal-Wallis tests found significant differences.

3.4.7 Gender Influences the Experience and Perception of Psychosocial Stress and Trauma:

Poverty and social exclusion are experienced differently by women and men^{154,155}. Following the initial exploration of the relationships between the stress scales through correlations and differences by SES group, the distributions of scores for each stress measure were further examined in

different contexts: first by socioeconomic status (SES) and gender (Figure 3.6), and then by housing status and gender (Figure 7).

Figure 3.6 provides a detailed visualisation of how scores for the four scales are distributed among High SES and Low SES participants, stratified by gender. Consistent with the results shown in 3.4.4, average and maximum scores are higher in low SES men and women, although there are some interesting, gendered patterns seen when men and women from either SES group are compared separately.

In the case of the PSS, there is a notable difference between PSS scores in low and high SES women that is not reflected in HRLES scores. Here it is possible that faced with a similar burden of adversity to high SES women, the subjective experience of stress is more intense in low SES women because of reduced psychosocial resources and social buffers to stress^{156,157} and/or because of increased impact of difficulties due to their caring role within their extended family.

Distributions of ACES-10 scores among low SES participants are much broader than those seen in the high SES groups, revealing stratification within the low SES group, where a number of participants score particularly high on both the binary and Likert scored versions of the inventory. Although average scores appear higher for the low SES males versus low SES females, numbers of low SES women reporting scores at the extreme high end of the distributions were higher than seen among low SES men.

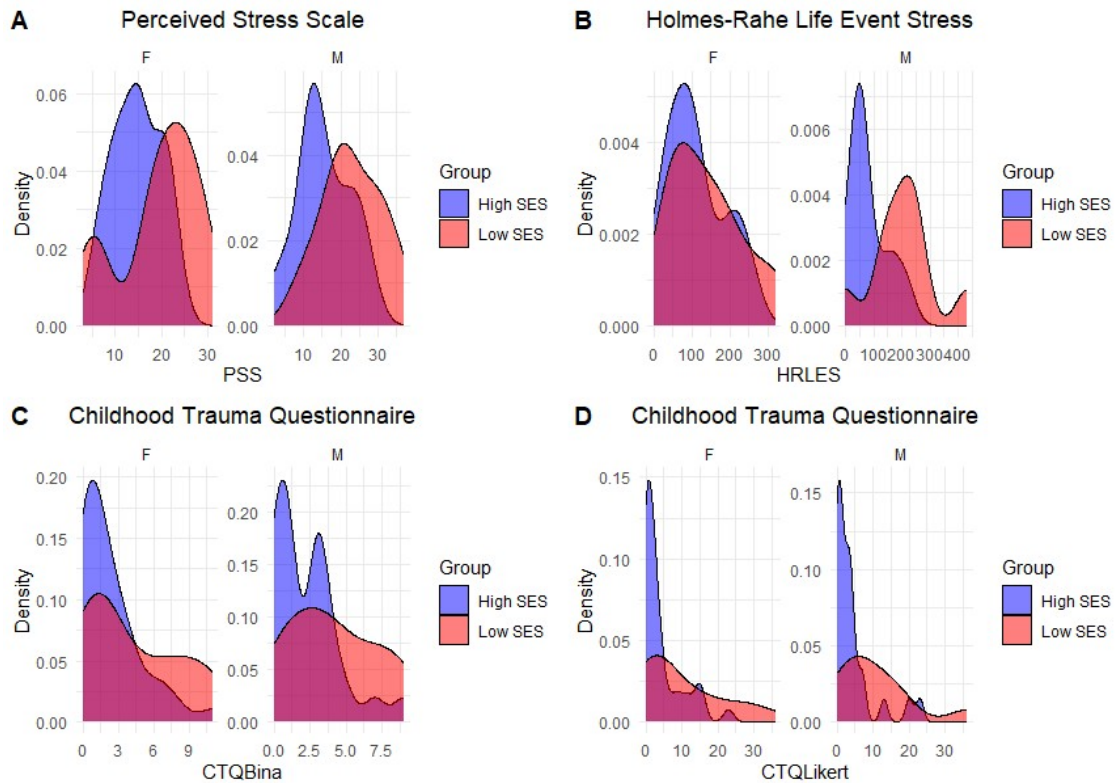


Figure 3.6 Density plots illustrating the distribution of scores on four stress scales (PSS, HRLES, CTQBina, and CTQLikert) stratified by gender (F and M) and socioeconomic status (SES: High SES vs. Low SES). These plots provide a visual comparison of stress levels across groups, highlighting the distributional differences between men and women within SES categories. In each plot, the distribution of scores is shown for High SES and Low SES individuals, with density curves representing the frequency of scores across the respective scales. All stress scales show distinct patterns of variability by SES and by gender.

In Figure 3.7, the focus shifts to housing status, with comparisons between housed and homeless participants across the same stress indices. The distributions reveal a deep contrast between these two groups. Homeless participants, particularly females, show markedly higher stress levels across all scales. The PSS scores in the homeless group are uniformly higher than those seen in the housed cohort, indicating that higher perceived stress is a feature of homelessness.

Similarly, the HRLES scores seen in homeless individuals, especially women, suggest a heavier burden of stressful life events in recent years.

The ACES-10 (referred to as “childhood trauma questionnaire” here) distributions for the binary and Likert scored questionnaires further highlight how pervasive early life adversity is within the homeless cohort. Homeless females in particular report much higher scores on both the binary and Likert scored questionnaires, perhaps explaining the pattern observed in low SES women versus low SES men.

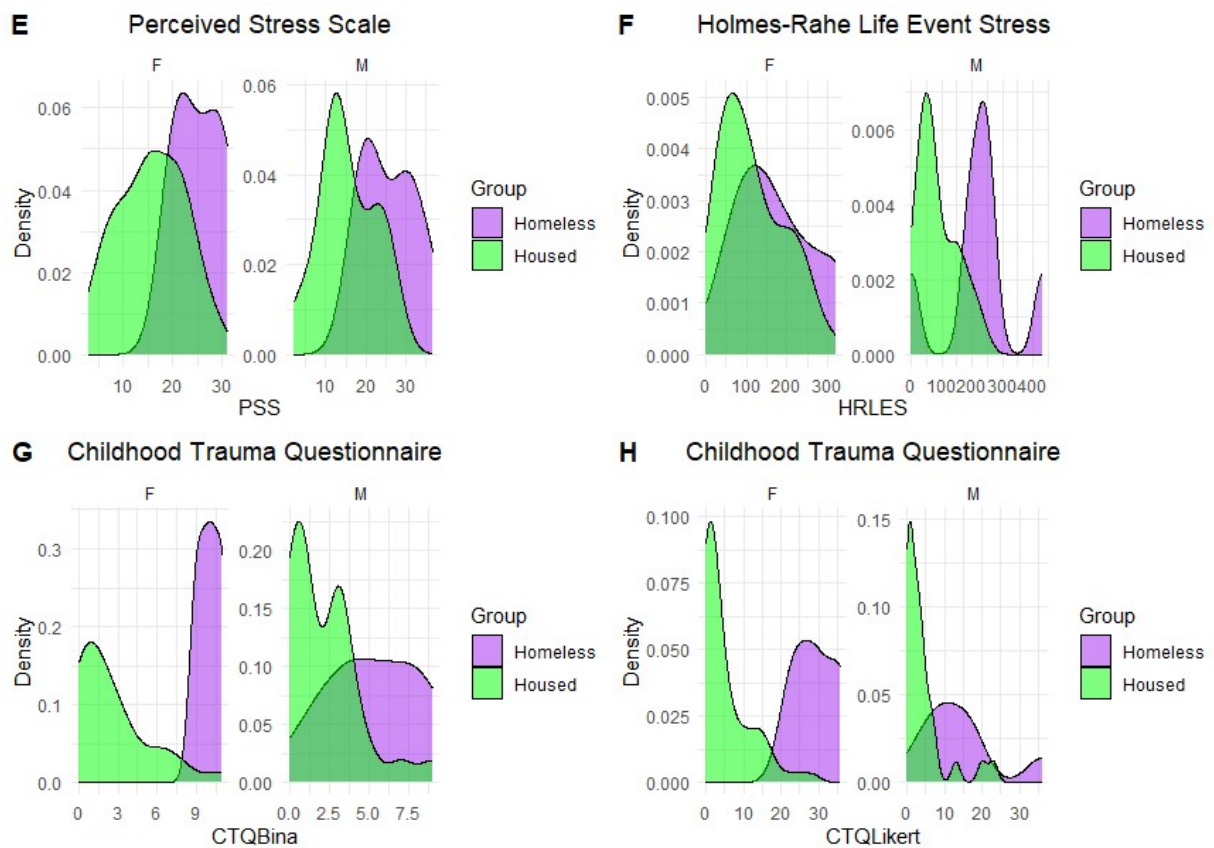


Figure 3.7 Density plots illustrating the distribution of scores on four stress scales (PSS, HRLES, CTQBina, and CTQLikert) stratified by gender (F and M) and housing status (Homeless vs Housed): These plots provide a visual comparison of stress levels across groups, highlighting the distributional differences between men and women, housed and homeless. In each plot, the distribution of scores is shown for homeless and housed individuals with density curves representing the frequency of scores across the respective scales. All stress scales show distinct patterns of variability by housing status and by gender with these patterns differing markedly from those seen in Fig 3.3

3.4.8 Relationship of Stress, Socioeconomic Status and Social Exclusion on Health

In this section, we examined the effects of stress, as measured using the four indices, on key health outcomes, including the number of health conditions and medication use. The analysis also considers how socioeconomic status (SES), and social exclusion modify these relationships when controlling for age and sex.

Table 3.7 Results of negative binomial regression models examining the relationship between stress (measured by PSS, HRLES, and ACES-10 (aka CTQ)), SES (low SES), and key health outcomes (number of health conditions and medication use) in the AESS Vax cohort. The models control for age and sex. The table includes estimates, incidence rate ratios (IRR), standard errors, z values, and p-values for the main effects and interactions, highlighting *significant and near-significant predictors (italicised).**

Outcome Variable	Regression Parameters	Predictor	Estimate	Incidence Rate Ratio (IRR)	Std. Error	z value	p-value
(I) Number of Health Conditions							
	Main Effects	(Intercept)*	-2.585	0.0754	1.199	-2.156	0.0311
		PSS*	0.1104	1.1167	0.0541	2.039	0.0414
		SESLOW	1.7297	5.6375	1.3448	1.286	0.1984
		HRLES	-0.0057	0.9943	0.0047	-1.219	0.2229
		CTQLikert	0.0491	1.0503	0.0448	1.095	0.2735
		Age	-0.0131	0.9871	0.0217	-0.6	0.5484
		SexM	0.1995	1.221	0.4739	0.421	0.6737
	Interactions	PSS: SESLOW	-0.1187	0.8882	0.0679	-1.748	0.0804
		HRLES: SESLOW	0.0107	1.0107	0.0058	1.846	0.0649
		CTQLikert: SESLOW	-0.0287	0.9717	0.0577	-0.498	0.6187
(II) Number of Medications							
	Main Effects	(Intercept)	-1.5132	0.2196	1.2128	-1.248	0.2121
		PSS	0.0876	1.0914	0.0541	1.621	0.1051
		SESLOW	0.7556	2.1292	1.5231	0.496	0.6198
		HRLES	-0.0061	0.9939	0.0048	-1.268	0.2049

		CTQLikert	0.0296	1.0301	0.051	0.581	0.5609
		Age	-0.0131	0.987	0.0247	-0.533	0.5942
		SexM	0.3059	1.358	0.5173	0.591	0.5544
	Interactions	<i>PSS: SESLOW.</i>	<i>-0.1389</i>	<i>0.8705</i>	<i>0.0799</i>	<i>-1.738</i>	<i>0.0822</i>
		HRLES: SESLOW*	0.0149	1.015	0.0068	2.186	0.0288
		CTQLikert: SESLOW	0.0054	1.0054	0.0723	0.075	0.9403

(I)Health Conditions: Stress and SES

The analysis of health conditions as an outcome revealed that all three stress measures were associated with an increased number of reported health conditions, though the magnitude and significance of these effects varied across the different models.

PSS: The PSS, which measures self-perceived current stress, was significantly associated with a higher incidence of health conditions in the SES model (IRR = 1.1167, $p = 0.0414$). This indicates that for every one-unit increase in perceived stress, the expected number of health conditions increases by approximately 12%, controlling for SES, age, and sex.

HRLES: The HRLES, which measures the impact of stressful life events, had a positive but non-significant relationship with health conditions across both SES and social exclusion models (IRR = 0.9943, $p = 0.2229$ in SES). This suggests that recent life events might not have as immediate or direct an impact on the number of health conditions as perceived stress.

ACES-10: In the SES model, ACES-10 showed a small positive coefficient (IRR = 1.05), but this association did not reach statistical significance ($p = 0.27$), indicating no clear evidence of an independent relationship with health conditions in this analysis.

Interactions with SES:

The interaction between HRLES and low SES showed a small positive coefficient (IRR = 1.01) and approached statistical significance ($p = 0.065$).

The interaction between PSS and low SES was negative (IRR = 0.89) and similarly approached statistical significance ($p = 0.080$).

(II) Medication Use: Stress and SES

The analysis of medication use revealed similar patterns, with some distinctions:

PSS: In the SES model, higher stress as measured by the PSS was associated with increased medication use, though the effect was not statistically significant (IRR = 1.0914, $p = 0.1051$).

CTQ: The relationship between childhood trauma and medication use was again positive but not statistically significant (IRR = 1.0301, $p = 0.5609$), indicating that while there may be a relationship, its effects were not independently detectable in this model after adjustment for SES, age, and sex.

HRLES: Recent life-event stress, as measured by the HRLES, was not independently associated with medication use across the cohort (IRR = 0.9939, $p = 0.2049$), suggesting that exposure to stressful life events alone does not uniformly translate into increased medication burden.

Interactions with SES: The interaction between PSS and low SES indicated a trend towards a reduced association between perceived stress and medication use in lower SES groups (IRR = 0.8705, $p = 0.0822$), though this did not reach conventional thresholds for statistical significance.

In contrast, a significant interaction was observed between HRLES and low SES (IRR = 1.015, $p = 0.0288$), indicating that recent life-event stress was associated with increased medication use specifically among low SES participants. This suggests that the health consequences of objective stress exposures are socially patterned, with equivalent life-event stress translating into greater medication burden in socioeconomically disadvantaged individuals, but not in their high SES counterparts.

Table 3.8 Results of negative binomial regression models examining the relationship between stress (measured by PSS, HRLES, and CTQ/ACES-10), Social Exclusion (Homelessness) and key health outcomes (number of health conditions and medication use) in the AESS-Vax cohort. The models control for age and sex. The table includes estimates, incidence rate ratios (IRR), standard errors, z values, and p-values for the main effects and interactions, highlighting *significant and near-significant predictors (italicised).**

Outcome Variable	Regression Parameters	Predictor	Estimate	Incidence Rate Ratio (IRR)	Std. Error	z value	p-value
(III) Health Conditions							
	Main Effects	(Intercept)	-2.307	0.0995	1.0098	-2.284	0.0224
		PSS	0.0475	1.0487	0.0376	1.263	0.2067
		SocialExclusionY	-2.291	0.1012	3.1034	-0.738	0.4604
		HRLES	-0.0015	0.9985	0.0034	-0.458	0.6468
		<i>CTQLikert.</i>	<i>0.0618</i>	<i>1.0637</i>	<i>0.0321</i>	<i>1.922</i>	<i>0.0546</i>
		Age	0.0017	1.0017	0.0192	0.09	0.9283
		SexM	0.167	1.1818	0.4541	0.368	0.713
	Interactions	PSS: SocialExclusionY	0.104	1.1095	0.1165	0.892	0.3722
		HRLES: SocialExclusionY	0.0014	1.0014	0.0063	0.216	0.8291
		CTQLikert: SocialExclusionY	-0.0756	0.9272	0.0853	-0.887	0.3753
(IV) Medications							
	Main Effects	(Intercept)	-1.1472	0.3176	1.0886	-1.054	0.292
		PSS	0.0309	1.0314	0.0418	0.739	0.46
		SocialExclusionY	0.5496	1.7324	3.167	0.174	0.862
		HRLES	-0.0034	0.9966	0.004	-0.846	0.398
		CTQLikert	0.0541	1.0556	0.0417	1.297	0.195
		Age	-0.0054	0.9946	0.0226	-0.239	0.811
		SexM	0.2001	1.2214	0.5214	0.384	0.701

	Interactions	PSS: SocialExclusionY	-0.0088	0.9912	0.1279	-0.069	0.945
		HRLES: SocialExclusionY	0.0081	1.0081	0.0074	1.09	0.276
		CTQLikert: SocialExclusionY	-0.119	0.8879	0.0967	-1.231	0.218

(III) Health Conditions: Stress and Social Exclusion

In the context of social exclusion, the relationships between stress measures and health conditions displayed different patterns:

PSS: While still positive, the relationship between PSS and health conditions was weaker than seen in the SES model (i) and not statistically significant (IRR = 1.0487, $p = 0.2067$). This may indicate that the impact of perceived stress on health outcomes is less pronounced when social exclusion is considered.

ACES-10 (CTQ): The ACES-10 questionnaire had a stronger association with health conditions in the social exclusion model (IRR = 1.0637, $p = 0.0546$), suggesting that the effects of childhood trauma on health conditions might be exacerbated by experiences of social exclusion.

Interactions with Social Exclusion:

None of the interactions between stress measures and social exclusion were statistically significant, indicating that the relationship between stress and health conditions does not differ substantially based on whether an individual is socially excluded.

(IV) Medication Use: Stress and Social Exclusion

The social exclusion model showed weaker associations between stress and medication use:

PSS: The relationship between PSS and medication use in the social exclusion model was not significant (IRR = 1.0314, $p = 0.460$), indicating that perceived stress does not strongly predict medication use when considering social exclusion.

HRLES and ACES-10: Like the findings for health conditions, neither HRLES nor ACES-10 had significant main effects on medication use, nor did their interactions with social exclusion.

Interactions with Social Exclusion:

No significant interactions were observed between stress measures and social exclusion for medication use, suggesting that social exclusion does not significantly alter the relationship between stress and the likelihood of using medications.

3.5 Discussion

Analysis of health and demographic data from the AESS-Vax and PATH studies provides novel insights into the "dose effect" of socioeconomic status (SES) on health, with long-term homeless participants representing the extreme end of a gradient along which health status deteriorates as socioeconomic status declines. While the association between poverty and poorer health outcomes is well-documented, we show a clear and consistent pattern of worsening health status as SES decreases, even *within* a low SES cohort. Homelessness, as an indicator of extreme socioeconomic disadvantage, appears to exacerbate the negative health effects associated with low SES, contributing to a distinctly elevated health burden in this group. This is seen most clearly in the PATH cohort, who are long term homeless and often frail or pre-frail regardless of their age. In PATH, the

average participant had 4.26 diagnosed long-term conditions and was prescribed 3.65 medications. In the AESS-Vax High SES group, on the other hand, the average participant had no diagnosed long term health issues or prescribed medications. Here it is important to highlight the difference between PATH and AESS-Vax study cohorts, which represent opposite ends of the spectrum of homelessness. PATH participants were recruited from supported long-term homeless accommodation while AESS-Vax homeless participants were recruited through SARS CoV-2 vaccination clinics run by SafetyNet Primary Care. SafetyNet vaccinated the most stable homeless individuals with the lowest support needs, while those with higher support needs (homeless individuals similar to those in the PATH cohort) were vaccinated in-situ, with a single dose vaccine regimen.

Importantly, a parallel gradient in psychosocial stress measures observed in this study suggests that stress may play a role in mediating the relationship between SES and health. Homeless individuals in the AESS-Vax study reported the highest levels of perceived stress, life event stress, and childhood trauma, suggesting that psychosocial adversity is more common and more intensely experienced in the homeless population. Significant associations between perceived stress and the number of diagnosed health conditions in the models presented provide some support for the stress-health link but the absence of significant interactions between perceived stress and low SES suggests that it is not the subjective and current experience of stress (as captured by the PSS) alone mediating the stress-SES-health relationship.

The differential impact of childhood trauma on health outcomes observed in homeless versus housed individuals suggests that the long-term health consequences of early-life adversity may be amplified in the context of severe

social exclusion. In the PATH Cohort, every participant had at least one ACE, with 25% experiencing more than 5 ACEs. ACEs are strongly associated with increased risks of poor health outcomes and negative health behaviours. People with more than four ACEs are four times more likely to experience depression, three times more likely to suffer from a chronic respiratory disease and twice as likely to suffer from cardiovascular disease as individuals with no ACEs²⁴.

Whether effects of psychosocial adversity on health are direct (e.g. because of the physiological stress response) or indirect (e.g. stress/adversity increases rates of smoking, alcohol consumption etc.), there is strong rationale for health interventions targeting psychosocial adversity and its causes. The psychosocial adversity faced by socially excluded or marginalised groups (as documented here) is specific, with material and systemic roots - the destabilising realities of poverty, the internalised and systemic shame of exclusion and the heightened vulnerability to acute and chronic traumas felt from early life to death. While individually focussed psychosocial (e.g. stress reduction protocols, therapy) and material interventions (e.g. supported housing) may be effective strategies to improve the immediate health and wellbeing of socially excluded people, deeper, systemic interventions are what will make the most difference overall. Only by creating inclusive, equitable societies do we reduce exposure to pathological adversity, breaking generational cycles and laying the foundations for long term health and stability for all.

1. Health Behaviours and the Social Gradient in Health in AESS-Vax and PATH

The link between psychosocial adversity and negative health behaviours is robust and well documented across a range of behaviours and for a variety of different adverse experiences^{158–160}.

Rates of smoking in AESS-Vax and PATH were notably higher among homeless and low SES participants, with long term homeless individuals reporting the highest rates of smoking overall (84.8% current or previous smokers, average pack year history of 33.85 years). In contrast, just over a quarter of High SES participants were current or previous smokers with an average pack year history of 4.6 years. While differences in age profile of the cohorts may account for some of the observed differences (in pack year history in particular), these general trends are in keeping with what has previously been observed in the context of low SES and in alignment with the hypothesis that psychosocial adversity may drive propensity to smoke.

Similarly, alcohol consumption was markedly higher in the PATH homeless cohort, with an average consumption of 52.6 units per week compared to 14.4 in the AESS-Vax homeless group, 7.13 in the high SES group and 3.84 in the low SES housed group. In Ireland the Health Service Executive's Low Risk Weekly Alcohol Guidelines state that women should consume no more than 11 units of alcohol per week and men, no more than 17 units¹⁶¹.

A similar pattern to smoking and alcohol consumption was seen with sleep. Low SES participants in AESS-Vax slept for an average of 5.8 hours per night, compared to 6.8 hours for High SES participants, while homeless AESS-Vax participants reported average sleep of just 5.04 hours per night. Sleep is vital

for general health and wellbeing, these disparities between SES groups show just how pervasive health inequalities can be, affecting almost every aspect of health and life. In the case of homeless participants, reduced sleep may be explained by unsafe sleeping conditions, noise, and the psychological stress of living without stable housing. Dunn's tests with Bonferroni Corrections were conducted to assess the significance of differences between groups homeless and housed groups beyond the Kruskal-Wallis analysis reported in Table 1. These tests revealed statistically significant differences in sleep duration between high SES and AESS-Vax homeless participants ($p_{adj}=0.0011$) and between high SES and the PATH homeless group ($p_{adj}=0.0005$). Biologically, it is known that individuals sleeping less than 5 hours per night face a 40% higher risk of all-cause mortality compared to those sleeping 6-8 hours¹⁶². Additionally, short sleep duration is associated with a 48% increased risk of developing or dying from coronary heart disease¹⁶³.

Finally, despite the common perception that drug use is highly prevalent among homeless populations, our findings did not show significant differences in drug use across socioeconomic and housing status groups in the AESS-Vax study. This suggests that, within this specific cohort of homeless participants, drug use behaviours may not be as pronounced as other health-related disparities. However, it is important to note that the AESS-Vax homeless cohort is not representative of all homeless individuals. The homeless participants in this study were patients of SafetyNet, a healthcare network that administered anti-SARS CoV2 vaccines to a more stable subset of the homeless population. PATH participants, a more vulnerable subpopulation of long-term homeless adults were more likely to use illicit drugs and more likely to report addiction than any other group.

It is also possible that drug use was underreported in both studies due to privacy concerns, lack of trust, and the mode of data collection. High SES participants were predominantly health workers who could face professional consequences if they were to be confirmed as drug users. Homeless participants often completed surveys with the help of a researcher rather than filling them out pseudonymously using an online questionnaire or on a tablet with a unique identifier as other participants were able to. This method may have influenced disclosure, resulting in lower reported rates of drug use. Future studies should consider these factors when designing data collection methods to ensure that the most marginalised and vulnerable groups are accurately represented.

2. Impact of SES and Housing Stability on Participation and Bias in Data Interpretation

Differential completion rates of psychosocial stress inventories by SES and housing status in AESS-Vax reflect a crucial methodological challenge when engaging socially excluded populations in research. High SES participants were significantly more likely to complete stress inventories than their low SES and homeless counterparts. This discrepancy likely results from underlying differences in literacy and numeracy, discomfort sharing personal experiences, and power imbalances between researchers and socially excluded participants. Such biases may lead to an overrepresentation of more stable individuals within the study, potentially skewing the findings and limiting the generalisability of the results.

Participation in the housed groups was almost certainly aided by the inclusion of an online survey option, which allowed participants to complete the AESS-

Vax questionnaire privately in their own time using a pseudonymised study ID. When trialled with homeless participants, take up was low (2/27 responses in AESS-Vax), possibly due to issues with literacy, numeracy and access to the internet. In the homeless group, participation was often facilitated by researchers who would take time to complete questionnaires with participants, explaining parts of the questionnaire and recording answers where necessary. While this aided participation in general, it may also have affected the answers and honesty of participants, particularly when answering sensitive questions about adverse childhood experiences, drug use and current trauma. During consultation with homeless people and members of the Inclusion Health team at St James' Hospital before the AESS-Vax study commenced, some of these potential challenges were noted. Unfortunately, as a result of the disruption caused by the pandemic and the need for a swift start to the AESS-Vax study before the National Vaccine Rollout in Winter 2020/21, it wasn't possible to implement all the recommendations we received for AESS-Vax before recruitment commenced. In future, the use of automated digital questionnaires that read and record verbal answers could be trialled to maximise participation in the homeless cohort.

PATH participants were recruited after the AESS-Vax homeless group and our approach was modified slightly. Here, we conducted more extensive PPI to shape the PATH questionnaire, recruitment and sample collection were moved to the community (hostels) in collaboration with established homeless service providers and researchers had support of a research nurse who assisted in sample collection and recruitment. These changes most likely explain the higher response rates across all areas of the study, particularly for more sensitive questions, like the ACEs questionnaire (79.66%) and lifetime

trauma (ITEM) questions (response rate: 92.74%). In contrast, just 40% of homeless participants in AESS-Vax completed the Childhood Trauma Questionnaire in its entirety (zero values were assigned for questions not answered).

Understanding the factors that influence participation of socially excluded people in health and biomedical research is essential for improving the inclusivity and accuracy of research findings. Efforts to provide assistance during survey completion, build trust, and address literacy barriers can help reduce these disparities in participation and ensure that the experiences of more marginalised individuals are adequately captured in future studies (see Emmel et al. 2007 for review)¹⁶⁴.

3. Psychosocial Stress and Health Outcomes: The Role of SES and Social Exclusion

The analysis of psychosocial stress measures, including the Perceived Stress Scale (PSS), Holmes-Rahe Life Event Stress Scale (HRLES), Adverse Childhood Events (ACEs-10) questionnaire and Lifetime trauma inventory (ITEM), reveals that the burden of stress and trauma increases as socioeconomic status decreases. Homeless individuals reported the highest levels of perceived stress, cumulative life event stress, and childhood trauma. The regression models further demonstrate that perceived stress has a consistent and significant association with the number of health conditions. This suggests that current perceived stress and the experience of childhood trauma are critical determinants of health outcomes.

Interestingly, the relationship between childhood trauma and health conditions was more pronounced in the context of social exclusion, suggesting that experiences of homelessness may amplify the long-term health effects of early-life stressors. However, the weaker associations observed between other stress measures and health outcomes in the context of social exclusion indicate that the impact of stress on health may be less direct or may interact with other factors (e.g. smoking, alcohol or substance use) in socially excluded populations.

4. Gendered Patterns in Psychosocial Stress and Health Outcomes

Gender was found to be a significant modifier of stress experiences in AESS-Vax, with women in low SES and homeless conditions reporting higher levels of perceived stress and trauma than their male counterparts. This suggests that gender intersects with poverty and housing instability to shape the lived experience of stress and psychosocial adversity. Reduced social buffers and greater exposure to stressors may contribute to the more intense subjective experience of stress reported by low SES women. Additionally, the gendered burden of care and care-related stress was not specifically considered or captured in the questionnaire – though it is known and widely recognised that women share a disproportionate burden of the labour required for social reproduction (see Holborow, 2024¹⁶⁵ for review). It is possible that caring responsibilities disproportionately shouldered by women (especially low SES women) account for some of the gender differences in experiences of stress. Such findings highlight the need for gender-sensitive approaches when addressing the health and psychosocial needs of socially excluded populations.

5. Implications for Health Policy and Interventions

The compounded vulnerabilities experienced by low SES and homeless individuals call for targeted health interventions that address the specific needs of these populations throughout the lifecourse. Efforts should be made to improve access to healthcare, provide psychosocial support, and address the broader social determinants of health. The disproportionately high burden of ACEs and childhood trauma in homeless participants in PATH and AESS Vax highlights the importance of attention to critical developmental periods, especially early-life. It is not possible to confirm in the present study but there is ample evidence suggesting that early life adversity not only affects health across the lifecourse^{22,151,166} but social status and the ability of an individual to cope with subsequent stress and trauma, psychologically^{167,168} and physiologically^{169,170}.

Sadly, the most marginalised individuals are often badly under-represented in research outside of epidemiology and public health. There are a variety of reasons for this under-representation but the power of the effect of poverty and deprivation on health compels us as researchers to find new ways of including the most marginalised in our research. Not only will participation of marginalised people influence practice and policy, but it will also enhance our understanding of human biology, particularly stress biology, as stress effects are experienced to the most extreme extent in socially excluded populations. For these reasons and more, it will be crucial to develop and introduce new strategies to enhance research engagement with marginalised populations, building trust, providing assistance with participation, and addressing literacy, numeracy, and sociocultural barriers. The learnings from PATH, AESS-Vax and

an allied research project conducted by members of our group (Cognitive Impairment in People experiencing long-term Homelessness, Evidence and Relationships (CIPHER)) will be distilled in a publication with the hope that it enables others to carry out research inclusive of those who are most marginalised (Richards, Reddy and Ní Cheallaigh, *in preparation*)¹⁷¹.

3.6 Conclusion

Homelessness is more than the endpoint of a spectrum of socioeconomic disadvantage; it represents a distinct and extreme form of social exclusion with profound consequences for health. While social gradients in health are well established, the data presented here indicate that those at the very margins experience health inequalities that are not merely cumulative or incremental but qualitatively different, shaped by psychosocial trauma, chronic stress, and social exclusion. What emerges most strikingly from these findings is the deeply embedded nature of adversity in homeless populations, beginning in early life and persisting across the lifecourse. Data from the PATH cohort show with particular clarity that homelessness is more than a transient condition but a state of profound and prolonged vulnerability, where the biological imprints of past and present stress shape health outcomes in ways that are both predictable and extreme.

In AESS Vax and PATH we present evidence for a “dose effect” of socioeconomic status on psychological and physiological wellbeing. Homeless individuals consistently reported the worst health, most intense stress and most extreme, rooted psychosocial trauma. Critically, however, we showed distinctions within the homeless groups recruited, with the long-term

homeless PATH participants reporting the worst outcomes of all, followed by the AESS-Vax homeless group and then by low SES housed participants.

Beyond affirming our initial hypotheses about health and wellbeing among homeless individuals, these studies demonstrate that it is possible to engage maximally excluded groups in high-quality health and biomedical research. They also highlight the structural and methodological barriers that have historically prevented this from happening. In the design and execution of AESS-Vax and PATH we have identified what works (building trust, engaging potential participants in the community and adapting data collection methods to make them as accessible as possible) and, just as importantly, we have identified what does not work. In particular, these studies highlight the limitations of clinic-dependent sampling models, inflexible follow-up schedules, and methodological assumptions that rely on participant stability, which disproportionately constrain participation among the most socially excluded, especially people experiencing homelessness. As demonstrated in AESS-Vax, such structural barriers become most visible in post-booster and later pre-booster follow-ups, where attrition limits the interpretability of results.

The exclusion of the most marginalised from research has tangible consequences, it leaves gaps in knowledge, weakens public health responses, and perpetuates cycles of neglect. To address health inequalities, we must first ensure that those affected by them are seen, heard, and counted in the research that informs policy and intervention. The experiences of people at the sharpest end of inequality are often underrepresented in research, yet they offer the clearest insight into how social, psychological, and biological factors interact to shape health. By centring these populations in future work,

we not only address a critical gap in the literature but also move towards a more equitable and inclusive understanding of health itself.

Chapter 4: Low Socioeconomic Status and Homelessness are Associated with Poorer Vaccine-Induced Immunity to SARS CoV-2

Results from the AESS Vax Study

4.0 Introduction:

Vaccine response studies are an attractive experimental paradigm for studies of human immune function, particularly studies of the functional consequences of immune ageing. In vaccine response studies all participants receive the same dose of the same vaccine and are subjected to the same battery of serological and immunological tests. The generation and maintenance of vaccine induced immunity involves cells of the innate and adaptive immune system as well as an array of different immune processes from antigen processing and presentation to the T_h cell driven process of antibody production by B cells. Measurements of correlates of protection after vaccination therefore capture variability in complex immune processes and reflect differences in overall immune function between individuals.

The AESS Vax study was designed to measure the effects of psychosocial stress associated with low socioeconomic status and social exclusion on vaccine-induced immunity to SARS CoV-2. Chapter 3 detailed some of the health, demographic and psychosocial data captured as part of the study. In this chapter, the focus will turn to biological data captured during AESS Vax, with a special focus on vaccine-induced humoral and cellular immunity to SARS CoV-2 Spike protein.

4.1 Hypothesis:

We hypothesised that key correlates of protection, (i.e. indicators of vaccine-induced humoral and cell mediated immunity), would be reduced in low SES and homeless participants compared to their housed, high SES counterparts. Furthermore, we hypothesised that psychosocial stress negatively impacts

vaccine-induced immunity, perhaps mediating the relationship between socioeconomic status and protection after vaccination.

4.1.1 Specific Aims:

1. To measure vaccine induced *humoral* immunity to SARS CoV-2 Spike protein at a variety of post-vaccine timepoints in the AESS Vax Study cohort.
2. To measure vaccine-induced *cell-mediated* immunity to SARS CoV-2 Spike protein at two post-vaccine timepoints in the AESS Vax Study.
3. To explore the role of psychosocial stress and adversity as potential mediators of the relationship between socioeconomic status/social exclusion and vaccine-induced immunity to SARS CoV-2.

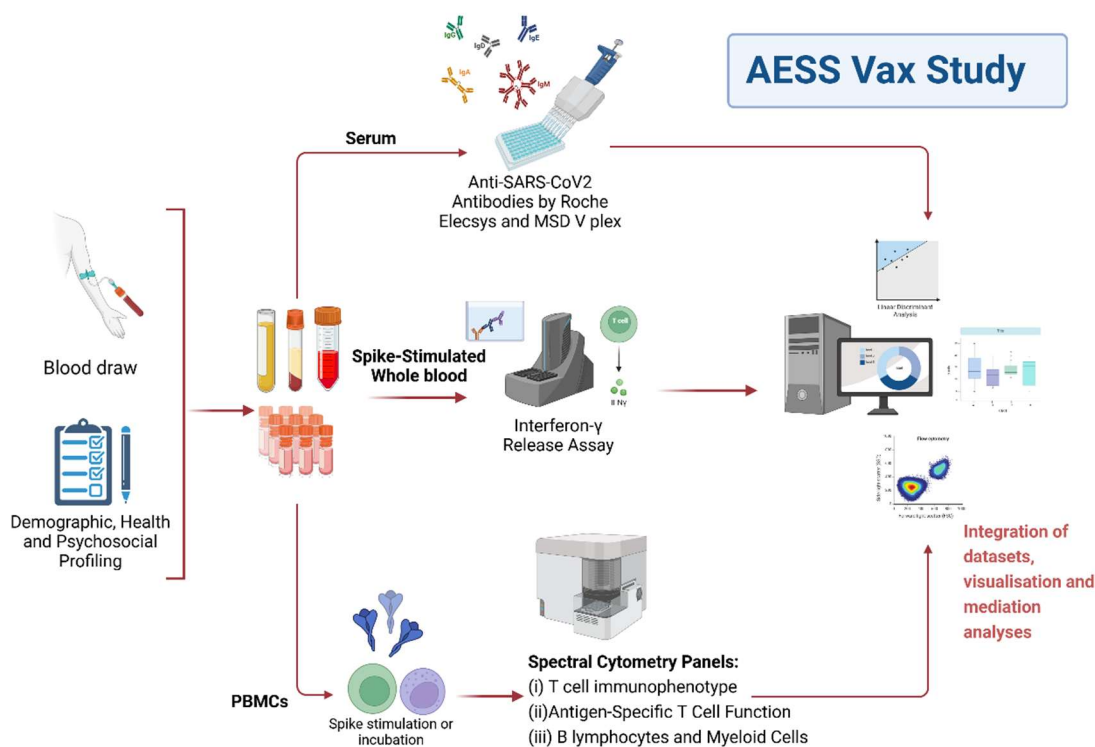


Figure 4.1 Graphical Representation of Workflow and Aims for the AESS-Vax Study: Note that panel (ii) and (iii) are not included in this thesis as methodology is under review.

4.2 Specific Methods:

4.2.1 Roche Elecsys Anti-Nucleocapsid and Anti-Spike Assays

To measure anti-viral antibodies in participant serum samples, two assays on the Roche Elecsys ECLIA platform were used. First, a calibrator-based qualitative anti-Nucleocapsid assay which assigns a Cut Off Index (COI) value to each sample assayed. Samples with a COI >1 are assigned as non-reactive, indicating with strong likelihood, no recent SARS CoV-2 infection. Second, a semi-quantitative assay that measures total anti-Spike antibodies in serum or plasma in Units/ml (U/ml), a measurement shown to be equivalent to the WHO standard BAU(Binding Antibody Unit)/ml measurement^{172,173}. In the semi-quantitative assay, samples with a U/ml reading below 0.8U/ml are determined to be non-reactive to Spike.

Sample Preparation:

Banked serum samples were taken and aliquoted at 150µl neat into fresh barcode labelled cryovials for the anti-N assay and initial anti-S assays. Separately, additional aliquots were diluted 1:20 with Roche Multiassay Diluent for repeat analysis of total anti-spike antibodies. Aliquots were stored at -20°C until use.

Anti-N Assay:

20µl undiluted serum was incubated with a proprietary mix of biotinylated and ruthenylated Nucleocapsid Antigen and allowed to incubate at room temperature for nine minutes. Next, streptavidin-coated microparticles were added, binding biotinylated antigen. The mixture was transferred to a well of a Cobas measuring plate. Once all samples were transferred onto the plate,

the plate was read using a Cobas e411 Analyser in St James' Hospital Biochemistry Department. Returned values were in COI.

Anti-S Assay:

Similar to the Anti-N assay, 20µl serum (undiluted or diluted depending on plate plan) was added to biotinylated and ruthenylated antigens and the mix allowed to incubate for nine minutes at room temperature. At this point, streptavidin-coated microparticle solution was introduced and the samples allowed to incubate for a further nine minutes. Finally, the mixtures were transferred to Cobas measuring plates and data acquired on e411 Analysers in the St James' Hospital Biochemistry Department. Final values were in U/ml.

4.2.2 Interferon Gamma Release Assays:

Whole blood samples collected in Lithium Heparin tubes were taken in a biological safety cabinet and 5ml of blood was transferred from each tube to a 15ml sterile centrifuge tube. 1ml aliquots of blood were then transferred to five QuantiFERON® tubes labelled with participant number and (i)Unstimulated, (ii)Cytostim (positive control; a T cell activation reagent that functions as a superantigen-like stimulus, inducing antigen-independent T-cell activation via APC-dependent TCR–MHC class II crosslinking), (iii)Spike, (iv)Peptivator S Complete, (v) Peptivator Prot N + Prot M. (Miltenyi BioTec, 2025)

Tubes were capped and transported to the St James Hospital Central Pathology Laboratory. Stock solutions of Cytostim, Spike protein, Peptivator S complete and Peptivator Prot N and Prot M were prepared and added to corresponding Quantiferon tubes giving concentrations of 0.25ug/ml for

Peptivator S Complete, 1ug/ml for Spike protein and 0.25ug/ml for the Prot M and N mixture. Tubes were placed in an incubator at 37°C for 18 hours.

After incubation tubes were centrifuged at 500g for 10 minutes and the straw-coloured supernatants were carefully pipetted into 2.5ml storage tubes for freezing at -20°C.

Interferon Gamma in supernatants was then measured per manufacturers' protocol using the QuantiFERON SARS-CoV-2 ELISA kit.

4.2.3 Inclusion and Exclusion of Participant

Participants with serological evidence of, or self-reported history of, SARS CoV-2 infection were excluded from the study, as the study aim was to determine vaccine-induced rather than infection-induced immunity, as well as reduce confounding effects of infection-induced immune responses in the context of vaccination.

4.2.4 Statistical Analyses and Modelling

Statistical analyses were performed using R. Before analysing any data, the distributions of key measures were inspected visually. Based on initial visual assessment of the distributions of antibody titres, Shapiro-Wilk tests for normality were conducted, confirming significant deviations from normality at all timepoints.

Informed by the results of the normality tests, non-parametric Mann–Whitney U-tests (aka Wilcoxon rank-sum tests) were used for univariate comparisons of antibody titres by socioeconomic status and housing status (coded as SocialExclusion (Y/N) in R).

To better understand the origin of observed differences in titres by SES/housing status, robust regression models with SES/housing status were developed with age and sex included as covariates. Antibody titres were log-transformed to normalise the underlying distributions before models were fit. Robust regression using M-estimators was chosen over conventional ordinary least-squares regression, as it mitigates against the effects of outliers the impact of heteroscedasticity. Models were generated separately for each timepoint, and incidence rate ratios (IRRs) were calculated to provide a multiplicative interpretation of effect sizes on the original scale.

To specifically address the contribution of psychosocial stress to trends in vaccine-induced humoral immunity at specific post-vaccine timepoints, structural equation modelling (SEM) was employed. SEM allows for analysis of mediation – here the analysis of stress as a mediator of the relationship between SES and antibody titres. However, SEM is most effective when missingness is minimal. To address missingness in stress measures, missing measures were imputed using predictive mean matching (PMM), an imputation technique that ensures imputed values retain the distribution of the experimentally observed data. SE Models were then developed using the original and imputed datasets. Following generation of model summaries, observed effect sizes were saved and used to conduct post-hoc power-analyses for the SE models at both timepoints.

Finally, pairwise correlation analyses were conducted to examine the relationship between humoral and cellular memory responses to vaccine antigen at the two post-booster timepoints, B1 (4-6 weeks post-third dose) and B2 (12-16 weeks post-third dose). Pearson correlation coefficients were

reported on Pair-plots to show both numeric and visual representations of correlation.

R packages used:

- dplyr, tidyr, and tidyverse for data manipulation.
- ggplot2 and ggpubr for data visualisation.
- broom, knitr, and gt for tidying and summarising model outputs.
- GGally for pair-plot generation.
- effsize for effect size calculations.
- MASS and rlang for robust regression modelling.

4.3 Results:

4.3.1 Anti-Spike Antibodies are Diminished in Participants with Low Socioeconomic Status After Vaccination Against SARS CoV-2

In total 143 participants reported no previous Covid infection and returned a below threshold anti-nucleocapsid antibody titre, indicating that they had not experienced asymptomatic infection prior to blood sample collection.

Total anti-spike antibody titres were quantified in serum at four post-immunisation timepoints (after two doses of BNT162b2 (Comirnaty®), usually administered two weeks apart) and at two further timepoints after the administration of a third, “booster” dose. Numbers of participants at each timepoint varied throughout the study (see figure 4.2) and participants did not consistently return for follow up blood draw (either uncontactable or unable to attend St James’ Hospital Clinical Research Facility for blood draw).

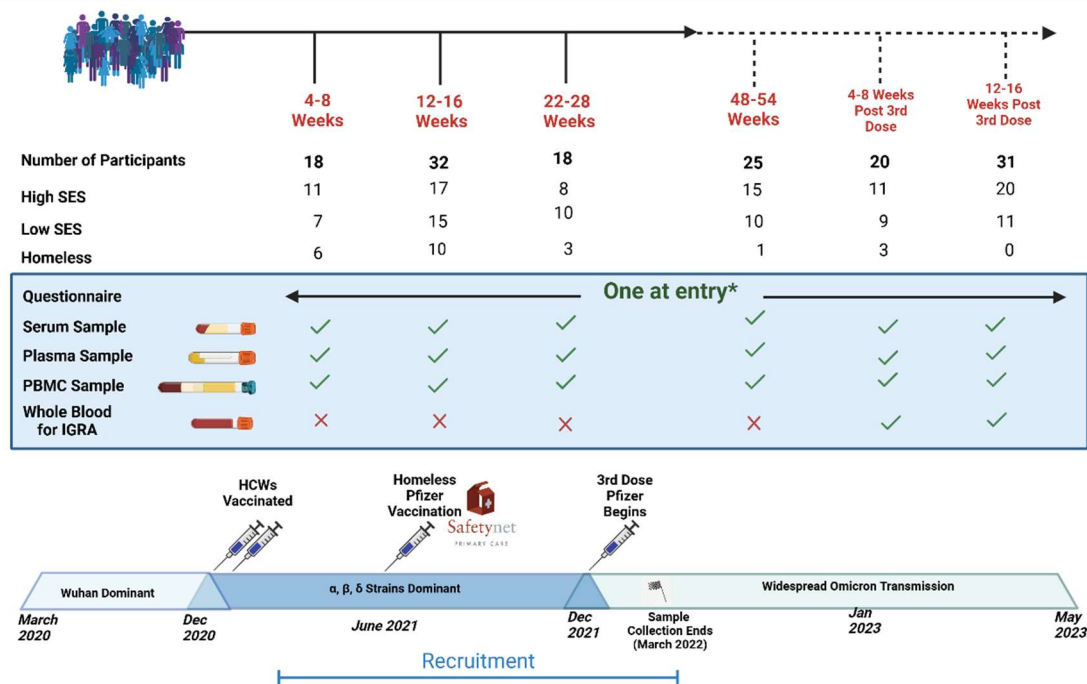


Figure 4.2 Recruitment and Sample Collection Through the AESS-Vax Study: The rollout of the National Immunisation Strategy commenced in late December 2020, with the first participants to AESS-Vax recruited in early March 2021. Recruitment continued through to March 2022, with new participants joining at several points through the study. *Follow-up questionnaires with repeat stress questionnaires and additional data collection were administered inconsistently due to time and space limitations in our follow-up clinic.

Vaccine-Induced Total Anti-Spike Antibodies are Diminished in Low SES Individuals up to One Year Post-Vaccination:

Total anti-spike antibody levels were lower in low SES participants for all timepoints prior to booster administration (Fig 4.3 and Table 4.1). Mann-Whitney U-tests revealed significant differences between high and low SES groups at the 12-16 week ($p= 0.0004$) and 22-28 week timepoints ($p= 0.0051$) but not at the 4-8 week ($p=0.1473$) or 48-54 week ($p= 0.3599$) timepoints.

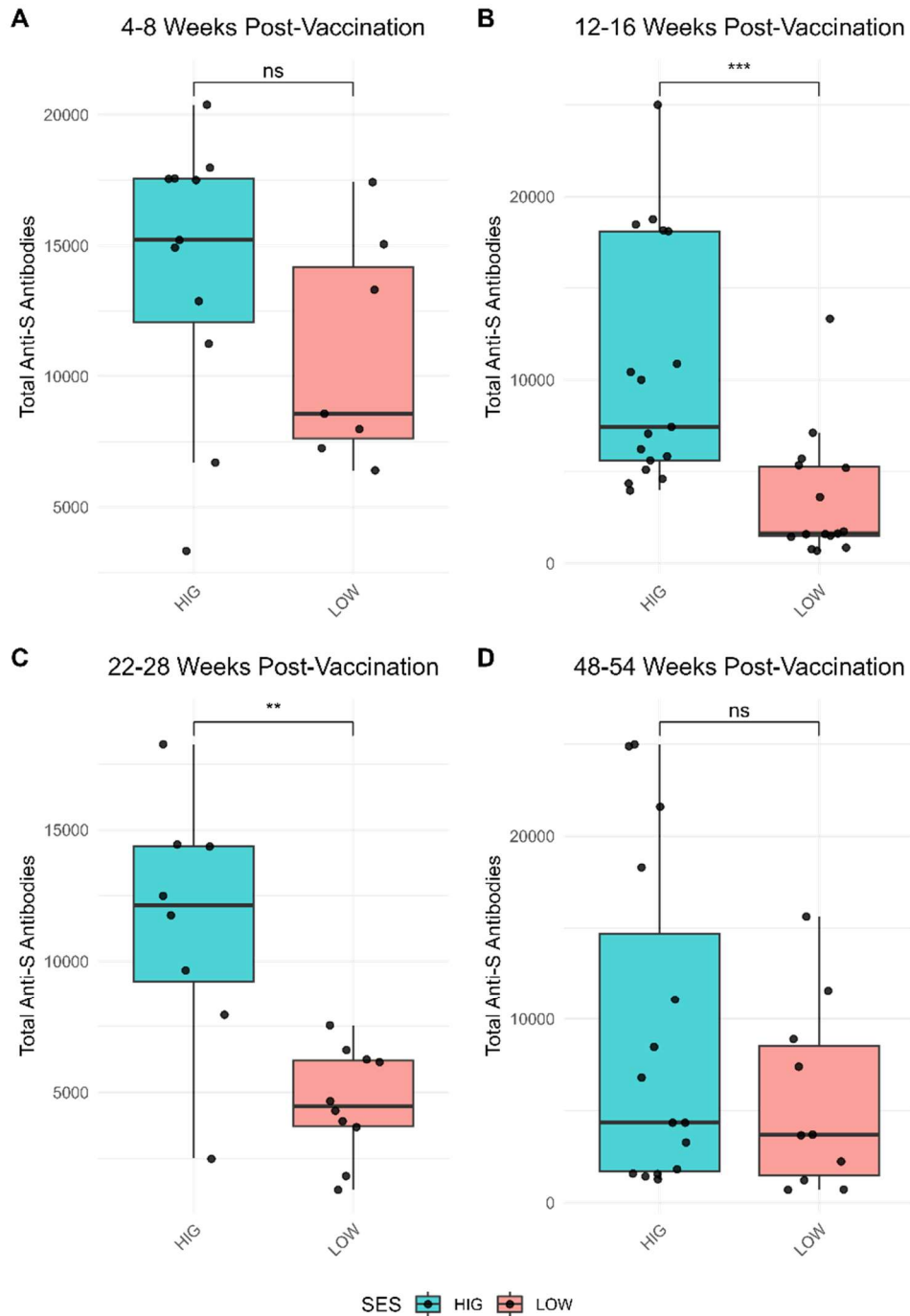


Figure 4.3 Total Anti-Spike Antibody Levels in High and Low SES Participants Post-Vaccination. Boxplots show the distribution of total anti-spike antibody levels (U/ml) for individuals from high and low socioeconomic status (SES) groups at four timepoints after administration of two doses of the BNT162b2 vaccine (A) 4-6 weeks ($p = 0.1473$), (B) 12-16 weeks ($p = 0.0004$), (C) 22-28 weeks ($p = 0.0051$), and (D) 48-54 weeks ($p = 0.3599$). Each point represents an individual's antibody titre. The black horizontal lines within the boxes represent the median values, while the upper and lower limits of the boxes show the interquartile range. The whiskers extend to the smallest and largest values within 1.5 times the interquartile range. Significant differences in antibody levels between SES groups are observed at 12-16 weeks and 22-28 weeks post-vaccination (Mann-Whitney U Test).

Table 4.1 Summary of Antibody Levels by Socioeconomic Status and Effect Sizes. This table presents the mean total anti-spike antibody levels (U/ml) for high and low SES groups at each timepoint post-vaccination, along with the corresponding standard deviations (SD), pooled standard deviations, Cohen's d effect sizes, and interpretation of effect size magnitude. Pooled SD accounts for variability across SES groups, and Cohen's d quantifies the magnitude of differences between the groups. Effect sizes are **bolded** for timepoints where statistically significant differences between SES groups were observed. Larger effect sizes suggest more pronounced differences, with statistical tests indicating a "large" or "very large" effect size.

Time-point	Mean (High SES)	Mean (Low SES)	SD (High SES)	SD (Low SES)	N (High SES)	N (Low SES)	Pooled SD	Cohen's D	Effect Size
V1	14,116	10,859	5,219	4,344	11	7	4,909	0.66	Medium
V2	10,591	3,477	6,553	3,437	17	15	5,330	1.33	Large
V3	11,428	4,611	4,790	2,045	8	10	3,520	1.94	Very Large
V4	9,050	5,571	8,939	5,118	15	10	7,674	0.45	Small

Vaccine-Induced Total Anti-Spike Antibodies are Diminished in Homeless Individuals up to One Year Post-Vaccination:

Next, it was investigated whether homelessness, as an extreme form of social exclusion, distinct from low socioeconomic status could affect vaccine-induced humoral immunity. Differences were seen consistently for up to one year between homeless and housed participants (fig 4.4). Differences only reached statistical significance at the 12–16 week timepoint ($p=0.0006$), however it should be noted that there was substantial variability in the number of homeless participants included from one timepoint to the next and that there were relatively few homeless participants due to national vaccination policy to use the Ad26.COV2.S (Jcovden; Janssen) vaccine in large sections of the homeless population (see table 4.3).

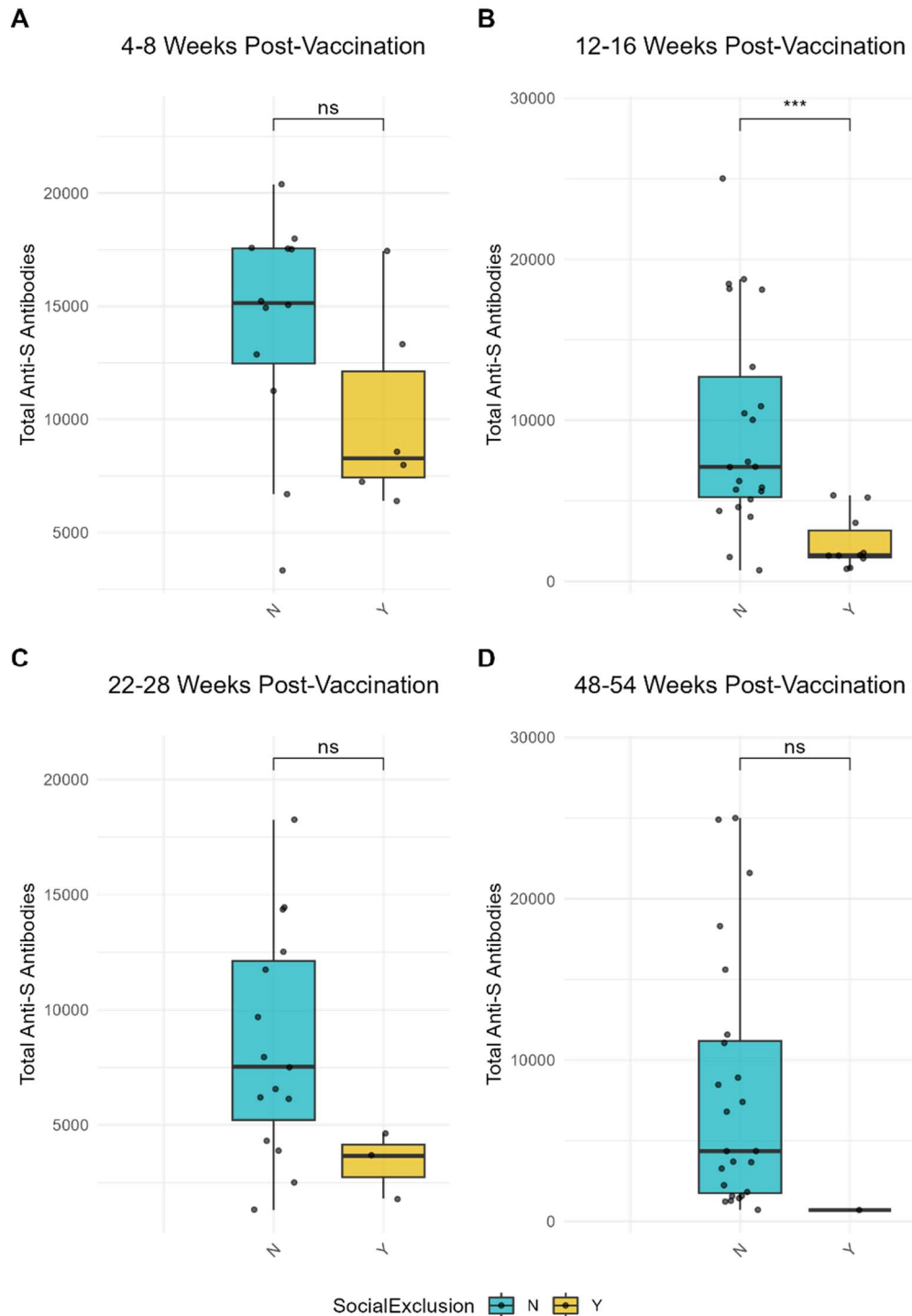


Figure 4.4 Total Anti-Spike Antibody Levels in Socially Excluded and Housed Participants Post-Vaccination. Boxplots display the distribution of total anti-spike antibody levels(U/ml) for individuals categorized as socially excluded or housed at four timepoints following administration of a two-dose vaccine regimen: (A) 4-6 weeks after second dose ($p = 0.1012$), (B) 12-16 weeks ($p = 0.0006$), (C) 22-28 weeks ($p = 0.0756$), and (D) 48-54 weeks ($p = 0.7799$). Each point represents an individual's antibody titre. The black horizontal lines within the boxes represent the median values, while the upper and lower limits of the boxes show the interquartile range. The whiskers extend to the smallest and largest values within 1.5 times the interquartile range. A significant difference in antibody levels between socially excluded and housed participants is observed at the 12-16 week timepoint post-vaccination (Mann-Whitney U Test).

Table 4.2 Summary of Antibody Levels by Housing Status and Effect Sizes: Mean total anti-spike antibody levels (U/ml) for housed and homeless participants at each timepoint post-vaccination, along with the corresponding standard deviations (SD), pooled standard deviations, Cohen's d effect sizes, and interpretation of effect size magnitude. Pooled SD accounts for variability across housing status groups, and Cohen's d quantifies the magnitude of differences between the groups. Effect sizes are bolded for timepoints where statistically significant differences between housed and homeless groups were observed. Larger effect sizes suggest more pronounced differences, with statistical tests indicating a "large" or "very large" effect size. For timepoints where data were not available or calculations could not be performed, cells are marked as "NA".

Time Point	Mean (Housed)	Mean (Homeless)	SD (Hd)	SD (Hs)	N (Hd)	n (Hs)	Pooled SD	Cohen's D	Effect Size
V1	14195	10158.33	4983.54	4303.91	12	6	4781.55	0.84	Large
V2	9472.59	2380.20	6485.70	1714.58	22	10	5506.99	1.29	Very Large
V3	8494.67	3370	4891.52	1437.12	15	3	4603.72	1.11	Large
V4	7948.42	700	7736.18	NA	24	1	NA	NA	NA

No Significant Differences Between SES Groups or Homeless and Housed Participants up to 16 Weeks Post-Third Dose of Vaccine:

In contrast to the trend seen from 4 up to 54 weeks, differences in total anti-spike antibodies by SES and housing status were not as apparent in *infection-naïve* participants after they received a third, booster dose of BNT162b2. Mean and median titres were higher in low SES (n=8) compared to high SES participants (n=11) 4-8 weeks post-third dose (Fig 4.5A, $p=0.9671$) but lower 12-16 weeks after a third dose of vaccine (Fig 4.5B, $p=0.1796$) – it is worth noting here that only one low SES participant with an antibody measurement at 4-6 weeks also had an antibody measurement at the 12-16 weeks timepoint. Also notable was the loss of serum samples for several participants due to experimental error, resulting in a disparity between the number of participants with antibody data at these timepoints and the number of participants recorded in the study log as having participated at these timepoints.

Homeless participation at the post-booster timepoint was low, with three participants at 4-6 weeks and none at 12-16 weeks. Mean and median titres

of the small homeless group at this timepoint were higher than observed in the housed group but not significantly so (Fig 4.5C).

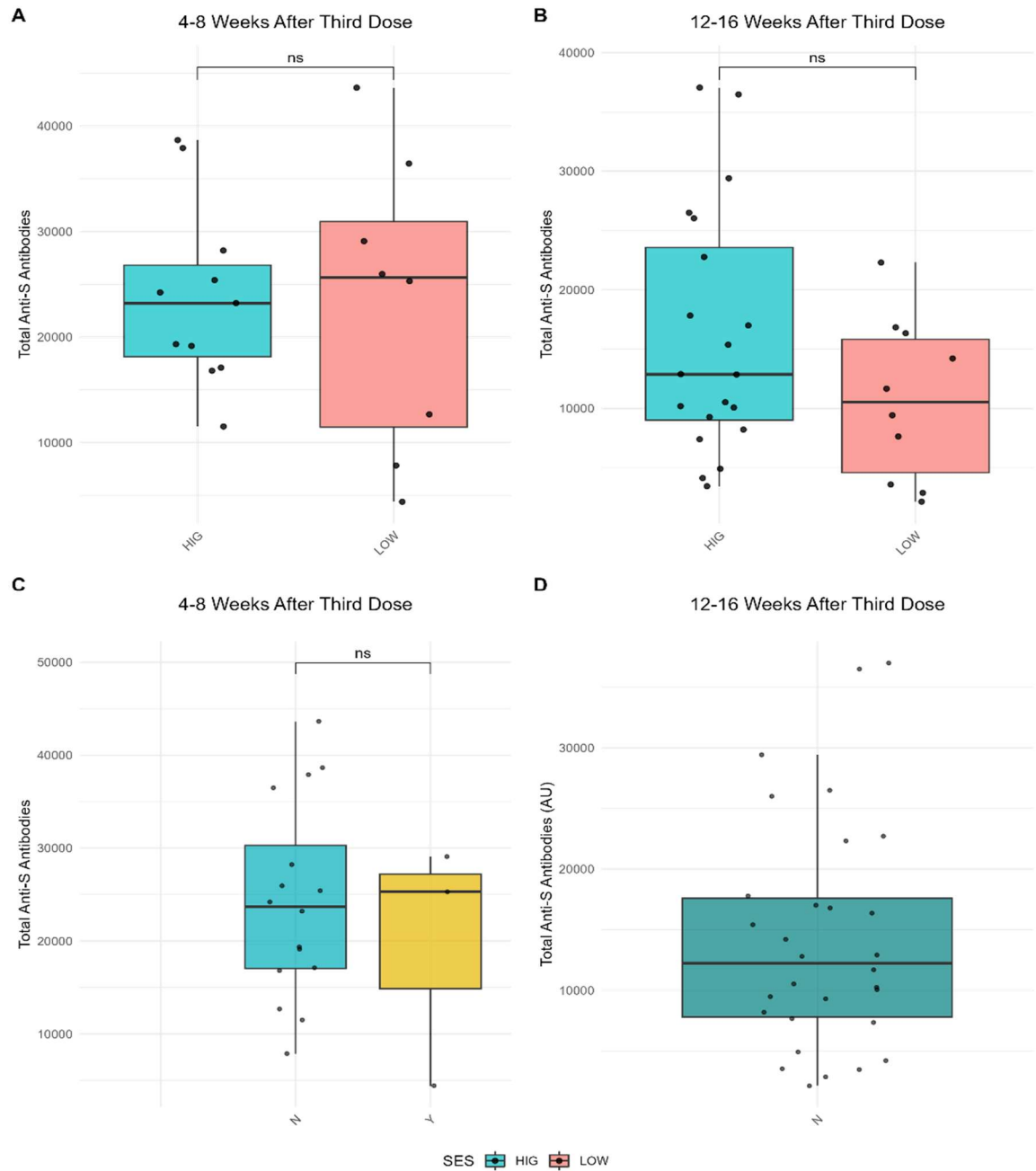


Figure 4.5 Total Anti-Spike Antibody Levels in Participants by Socioeconomic Status and Social Exclusion Status Post-Third Dose. Boxplots display the distribution of total anti-spike antibody levels at two timepoints post-third dose: 4-8 weeks and 12-16 weeks. Panels (A) and (B) compare high and low socioeconomic status (SES) groups, with p-values of 0.9671 and 0.1796, respectively. Panels (C) and (D) compare socially included (housed) and socially excluded (homeless) participants, with p-values of 0.7799 and NA, respectively. Each point represents an individual's antibody titre. The black horizontal lines within the boxes represent the median values, while the upper and lower limits of the boxes show the interquartile range. The whiskers extend to the smallest and largest values within 1.5 times the interquartile range. Note that for panel (D), data is only available for housed participants at this timepoint, hence no p-value is reported.

Table 4.3 Summary of Antibody Levels by Socioeconomic Status and Social Exclusion Status with Effect Sizes Post-Third Dose. This table presents the mean total anti-spike antibody levels for high and low SES groups, as well as housed and homeless participants, at each timepoint post-third dose, along with the corresponding standard deviations (SD), pooled standard deviations, Cohen's d effect sizes, and interpretation of effect size magnitude. Pooled SD accounts for variability across SES and housing status groups, and Cohen's d quantifies the magnitude of differences between the groups. Effect sizes are bolded for timepoints where statistically significant differences were observed. For timepoints or groups where data are not available or calculations could not be performed, cells are marked as "NA"

Time-point	Mean (High SES)	Mean (Low SES)	SD (Hi)	SD (Lo)	N (Hi)	N (Lo)	Pooled SD	Cohen's D	Effect Size
B1	23,785	23,178	8,535	13,827	11	8	11,026	0.06	Negligible
B2	16,121	10,704	6,785	6,785	20	10	9,323	0.58	Medium
Time-point	Mean (Housed)	Mean (Homeless)	SD (Hd)	SD (Hs)	N (Hd)	N (Hs)	Pooled SD	Cohen's D	Effect Size
B1	24624	19613	10522	13293	16	3	10884	0.43	Small
B2	14195	NA	9522	NA	30	0	NA	NA	NA

4.3.2 Modelling the Impact of Socioeconomic Status and Demographic Factors on Vaccine-Induced Humoral Immunity

To investigate the independent effects of SES, age and sex on vaccine-induced humoral immunity, robust regression models were deployed. A log transformation was applied to the antibody titre data prior to modelling to stabilise variance and better approximate normality, addressing the skewed distribution observed in the raw data. This transformation was implemented in these regression analyses, in contrast to the basic univariate analyses of SES and social exclusion/homelessness, which were conducted on untransformed antibody titres. Given the potential influence of outliers and heteroscedasticity, robust regression using M-estimators was favoured over conventional ordinary least-squares regression, as it down-weights extreme observations and yields more reliable results. Models were generated separately for each log-transformed timepoint, and incidence rate ratios (IRRs) were subsequently calculated to provide a multiplicative interpretation of the effect sizes on the original scale. Regression coefficients, standard errors, t

values, and IRRs derived from these analyses are presented in tables 4.4 and 4.5.

Table 4.4 Regression Coefficients for Socioeconomic Status and Demographic Factors on Vaccine-Induced Humoral Immunity Across Timepoints. This table presents the estimated regression coefficients for the effects of socioeconomic status (SES), age, and sex on total anti-spike antibody levels at various timepoints post-vaccination and post-third dose. Each model's intercept, as well as the coefficients for low SES (compared to high SES), age, and male sex (compared to female), are provided with standard errors and t values. Negative coefficients indicate a decrease in antibody levels relative to the reference group, while positive coefficients indicate an increase. These coefficients highlight the effect of demographic and socioeconomic factors on antibody responses at distinct post-vaccination intervals.

4-6 Weeks Post-Vaccination	Estimate	Std. Error	t Value
(Intercept)	9.827732	0.415435	23.65647
SESLOW	-0.27824	0.218237	-1.27494
Age	-0.00686	0.010261	-0.66893
SexM	-0.01569	0.205382	-0.07639
12-16 Weeks Post-Vaccination	Estimate	Std. Error	t Value
(Intercept)	10.19865	0.440168	23.16992
SESLOW	-0.74861	0.30872	-2.42488
Age	-0.03212	0.01113	-2.88614
SexM	0.146361	0.254285	0.575578
22-28 Weeks Post-Vaccination	Estimate	Std. Error	t Value
(Intercept)	9.215395	0.50691	18.17957
SESLOW	-1.02131	0.247458	-4.12722
Age	0.00582	0.013049	0.445998
SexM	-0.25908	0.28118	-0.9214
48-54 Weeks Post - Vaccination	Estimate	Std. Error	t Value
(Intercept)	9.990163	0.828465	12.05864
SESLOW	0.328303	0.542117	0.605595
Age	-0.04592	0.019677	-2.33372
SexM	0.50685	0.479166	1.057776
4-8 Weeks Post Third Dose	Estimate	Std. Error	t Value
(Intercept)	27797.85	8217.8548	3.3826
SESLOW	2334.9269	5620.7737	0.4154
Age	-198.0844	190.9314	-1.0375
SexM	5677.4851	5284.0195	1.0745
12-16 Weeks Post Third Dose	Estimate	Std. Error	t Value
(Intercept)	12637.7832	6791.3966	1.8609
SESLOW	-4780.6426	3716.6079	-1.2863
Age	62.7761	1543.8177	0.4055
SexM	177.7459	3782.7263	0.0470

To facilitate the interpretation of the regression coefficients, Incidence Rate Ratios (IRRs) were calculated for each robust model (table 4.5). The IRRs provide a multiplicative measure of the effect size on the original scale of antibody levels. An IRR greater than 1 indicates a positive association with

antibody levels, whereas an IRR less than 1 suggests a negative association. The significant negative IRRs for Low SES at 12-16 weeks (IRR = 0.47) and 22-28 weeks post-vaccination (IRR = 0.36) indicate that individuals with lower socioeconomic status (SES) have 53% and 64% lower antibody levels, respectively, compared to those with higher SES at the same timepoints. The IRRs for SES at other timepoints (e.g., 4-6 weeks and 48-54 weeks) are not statistically significant but show trends that could become significant in larger sample sizes, warranting further investigation. In contrast to Low SES, the effect of age, while significant at 12-16 weeks (IRR = 0.97) and 48-54 weeks post-vaccination (IRR = 0.96), is smaller, indicating a 3% and 4% decrease in antibody levels for each additional year of age, respectively. This suggests that older individuals experience a faster decline in immunity during these periods, making them more vulnerable to waning antibody levels over time.

Table 4.5 Incidence Rate Ratios (IRRs) for the Impact of Socioeconomic Status, Age, and Sex on Antibody Levels Across Timepoints. *This table displays the Incidence Rate Ratios (IRRs) for low SES, age, and male sex on total anti-spike antibody levels at each post-vaccination and post-third dose timepoint. An IRR above 1 indicates a positive association with antibody levels, while an IRR below 1 suggests a negative association.*

Timepoint	SES Low	Age	Sex Male
V1	0.7569	0.9931	0.9844
V2	0.4731	0.9684	1.1576
V3	0.3602	1.0058	0.7717
V4	1.3887	0.9552	1.66
B1	1.1090	0.9917	1.2818
B2	0.6393	1.0057	1.1886

4.3.3 Interaction Models Assessing SES and Demographic Influences on Vaccine -Induced Humoral Immunity:

To further explore whether the relationship between SES, age, and sex varies depending on their combined effects, interaction terms were added to the models using the same robust regression framework as the previous analyses.

The IRRs for these interaction terms are displayed in a forest plot (Fig 4.6). Although no significant interactions were observed, there were some trends suggesting that the impact of SES on antibody levels may differ across specific age groups at specific timepoints, particularly at 12-16 weeks post-vaccination. The non-significance of most SES-by-age interactions, however, suggests that the effect of SES on antibody levels is generally consistent across age groups. Similarly, SES-by-sex interactions were not significant, indicating that the influence of SES on antibody levels does not differ significantly between males and females. Nonetheless, these trends warrant further investigation in larger sample sizes, however, this shows that the effect of SES is not likely to be due to age- or sex differences between the cohorts.

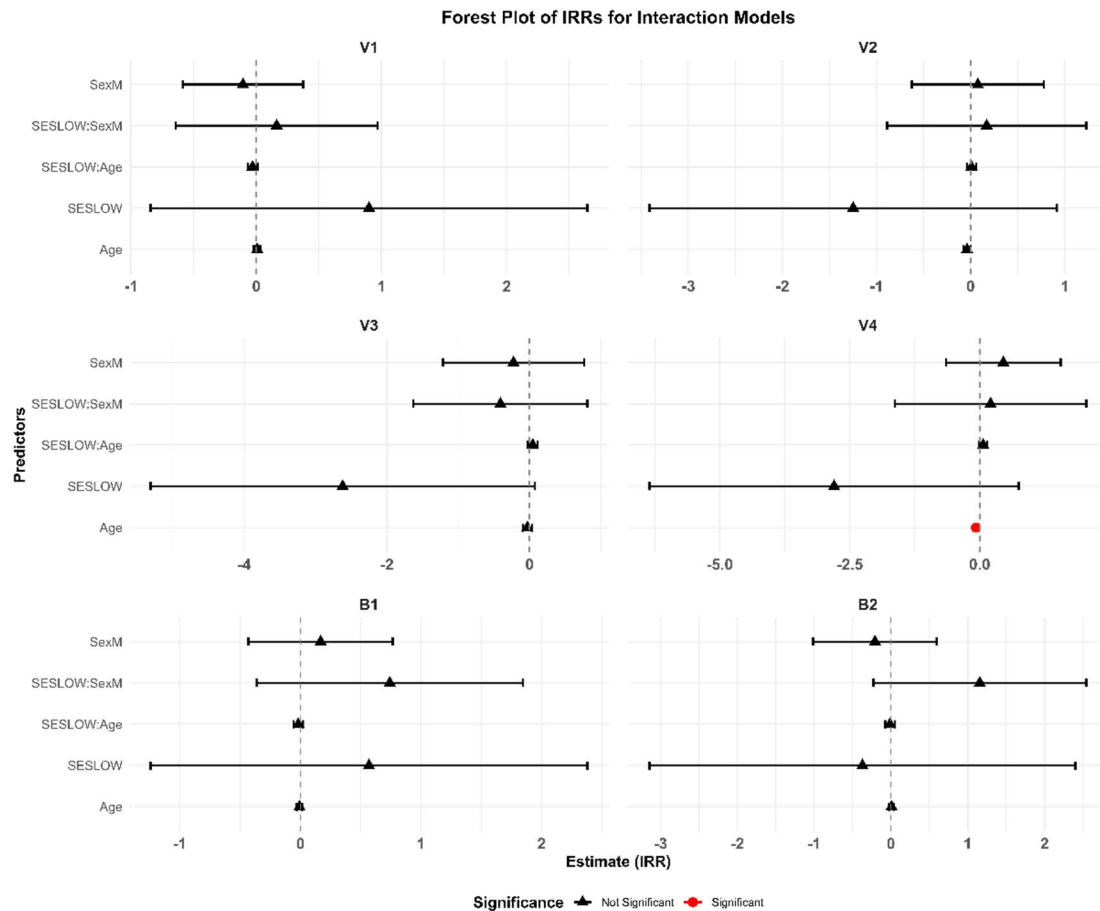


Figure 4.6 Interaction Models of SES, Age, and Sex on Vaccine-Induced Humoral Immunity: Incidence Rate Ratios (IRRs) are shown for models including interaction terms between socioeconomic status (SES), age, and sex at various post-vaccination timepoints.

4.3.4 Mediation Analysis of Stress as a Pathway Linking SES to Vaccine-Induced Humoral Immunity

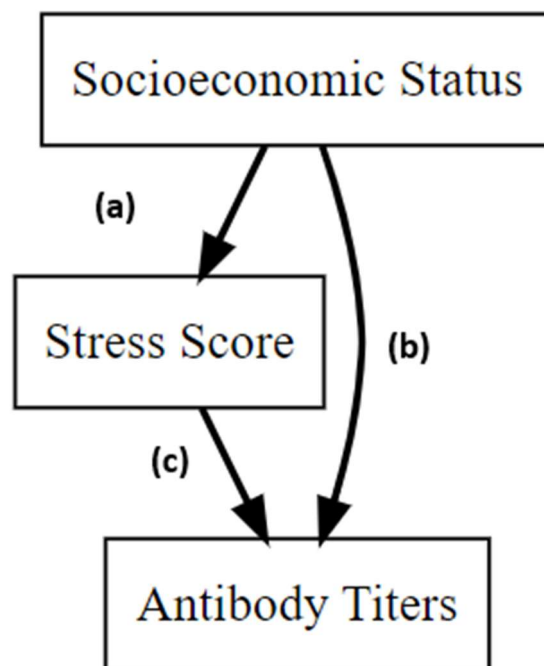
To more directly investigate the associations between SES, psychosocial stress and vaccine-induced anti-spike antibodies, Structural Equation Modelling (SEM) was deployed. Specifically, SEM was used to test whether stress (here, a composite of PSS, HRLES and CTQ) mediated the relationship between SES and antibody titres at the V2 and V3 post-vaccination timepoints, where statistically significant differences in total antibodies were observed between SES groups.

Model Specification and Fit

To look at the effects of stress in their totality, a composite of scores on the three stress indices (Perceived Stress Scale, Holmes-Rahe Life Event Stress Inventory and Childhood Trauma Questionnaire) was generated by standardising scores and taking their sum. This composite was used in the generation of the models, along with log transformed V2 and V3 antibody levels.

Given the degree of missingness in stress score data (e.g. 13/18 participants at V3 had all three scores recorded), stress scores were imputed using *predictive mean matching* (PMM), where missing values were replaced with values calculated to be like those observed in other participants of similar age, sex, SES, and housing status. This method ensures that imputed values retain the distribution observed in the data.

Two SEM models were developed for imputed and original data: a full model and a reduced model for each. The full models included direct and indirect paths from SES to antibody titres through stress, while also adjusting for age and sex. The reduced model included only SES and stress without the covariates.



c

Figure 4.7 Path Diagram for the Full Models with Paths Annotated (a)-(c)

V2 Antibody Titres

Summaries of associations estimated using SEM at V2 are included in table 4.6.

- (I) **Effect of SES on Stress (a Path):** SES was not significantly associated with stress in any model at the V2 timepoint, whether the models were run using the imputed or original datasets. The association was slightly stronger in the reduced model (original dataset), where the association between SES and stress most closely approached significance ($p=0.116$). This is in contrast to the overall associations between SES and stress scores across the full cohort reported in Chapter 3.
- (II) **Effect of Stress on Antibody Titres (b Path):** Stress was not a significant direct predictor of antibody titres at V2. The association

was weak across all models ($p > 0.68$), including the reduced models, where less covariates were included.

- (III) **Direct Effect of SES on Antibody Titres (c Path):** SES was consistently a strong predictor of V2 antibody titres, with significant effects observed for all models. The effect sizes were comparable across imputed and non-imputed datasets, suggesting that the relationship is robust to the inclusion of additional participants via imputation.
- (IV) **Indirect (Mediated) Effect (a*b Path):** Mediation was not supported in any model, as the indirect effect of SES on antibody titres through stress was non-significant ($p > 0.69$ across all models).

Table 4.6 Standardised Path Coefficients and p -values for Structural Equation Models Developed to Analyse Stress as a Mediator of the Relationship Between SES and log-transformed Anti-Spike Antibodies at the V2 Timepoint

Model	SES → Stress (a)	Stress → Ab (b)	SES → Ab (c)	Mediation (a* b)
Full (Imputed)	0.736 ($p=0.528$)	-0.001 ($p=0.983$)	-0.888 ($p=0.002$)	-0.001 ($p=0.983$)
Reduced (Imputed)	1.152 ($p=0.251$)	-0.006 ($p=0.890$)	-1.324 ($p<0.001$)	-0.007 ($p=0.891$)
Full (original)	1.221 ($p=0.380$)	-0.017 ($p=0.799$)	-0.896 ($p=0.037$)	-0.021 ($p=0.807$)
Reduced (original)	1.854 ($p=0.116$)	-0.028 ($p=0.684$)	-1.250 ($p=0.001$)	-0.052 ($p=0.694$)

V3 Antibody Titres

Summaries of associations using SEM at V3 are included in table 4.7.

- (I) **Effect of SES on Stress (a Path):** In the full imputed model, the association between SES and composite stress scores approached statistical significance ($p = 0.063$), while no association was observed in the reduced model ($p = 0.239$). In the original dataset, effect sizes

were similar but again did not reach statistical significance ($p = 0.061$ in the full model and $p = 0.196$ in the reduced model)

- (II) **Effect of Stress on Antibody Titres (b Path):** In the imputed dataset, stress was negatively associated with antibody levels, with a marginally significant effect in the full model ($p=0.074$) and a statistically significant effect in the reduced model ($p=0.050$). However, in the original dataset, stress showed a positive but non-significant relationship with antibodies ($p > 0.52$). This suggests some inconsistency in the role of stress, potentially influenced by imputation or sample size constraints.
- (III) **Direct Effect of SES on Antibody Titres (c Path):** SES was a significant predictor of antibody levels across all models, showing strong negative associations with antibody titres ($p \leq 0.010$ across all conditions). This reinforces the conclusion that lower SES is directly linked to diminished antibody responses, independent of stress.
- (IV) **Indirect (Mediated) Effect (a*b Path):** Mediation was not supported in any model, as the indirect effect remained non-significant ($p > 0.05$ across all models). While statistically insignificant, models using the imputed dataset found a slightly larger negative mediation effect than the original dataset.

Table 4.7 *Standardised Path Coefficients and p-values for Structural Equation Models Developed to Analyse Stress as a Mediator of the Relationship Between SES and log-transformed Anti-Spike Antibodies at the V3 Timepoint.*

Model	SES → Stress (a)	Stress → Ab (b)	SES → Ab (c)	Mediation (a* b)
Full (Imputed)	1.616 (p=0.063)	-0.127 (p=0.074)	-0.763 (p=0.007)	-0.205 (p=0.197)
Reduced (Imputed)	1.027 (p=0.239)	-0.127 (p=0.050)	-0.0773 (p=0.002)	-0.131 (p=0.313)
Full (original)	1.185 (p=0.061)	0.066 (p=0.578)	-0.783 (p=0.010)	0.078 (p=0.593)
Reduced (original)	0.890 (p=0.196)	0.067 (p=0.523)	-0.827 (p=0.003)	0.060 (p=0.567)

Stress does not appear to mediate the effects of SES on vaccine-induced anti-spike antibody titres at either the V2 or V3 timepoints, However, small sample sizes (n=18 for V3, n=32 for V2) may prevent the detection of mediation. Post-hoc power analyses were conducted to assess the robustness of the models.

Post-Hoc Power Analysis

Using the standardised effect sizes obtained from SEM with imputed stress scores, power analyses were carried out to determine the sample size requirement for detection of these effects with alpha = 0.05 and 80% power.

Power Analysis Results

V2 (n = 32, effect size = 0.669): Observed power = 0.75

V3 (n = 18, effect size = 0.536): Observed power = 0.35

- **Required Sample Size for 80% Power:**
 - **V2:** Required n = **36** per group (total **72** participants)
 - **V3:** Required n = **56** per group (total **112** participants)

These findings suggest that the study was underpowered to detect mediating effects of stress at both timepoints.

Model Fit Indices

To further evaluate model fit, key indices including the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), Root Mean Square Error of Approximation (RMSEA) and Comparative Fit Index (CFI) are presented in table 4.8.

Comparing AIC and BIC values of full versus reduced models, reduced models display slightly better fit as indicated by lower values. This is true of the models with imputed stress scores and of the models without imputation suggesting that the addition of age and sex to models does not improve fit.

Models without imputation show lower AIC/BIC values, suggesting better fit and that imputation may have introduced noise into the data. However, with small sample sizes and the degree of missingness, there is a trade-off; without imputation the robustness and generalisability of findings may be limited.

RMSEA values of zero and CFI values of 1 for all models indicate no discernible model misfit and very high fit, respectively. In this case, small sample sizes are likely to explain the low RMSEA and high CFI values, potentially indicating overfitting too. This is another limitation of the relatively small sample sizes assembled in AESS-Vax at each timepoint.

Table 4.8 Model Fit Parameters for All Models Using the Original and Imputed Datasets

Model	AIC	BIC	RMSEA	CFI
V2 Full	236.012	249.203	0	1
V2 Reduced	238.178	245.506	0	1
V2 Full (No Imputation)	159.733	169.134	0	1
V2 Reduced (No Imputation)	155.551	160.773	0	1
V3 Full	114.331	122.344	0	1
V3 Reduced	109.668	114.119	0	1
V3 Full (No Imputation)	73.435	78.520	0	1
V3 Reduced (No Imputation)	69.548	73.372	0	1

4.3.6: Cell-Mediated Vaccine-Induced Immunity is Diminished in Low SES Participants after Booster Vaccination:

Whole blood samples taken from participants 4-6 weeks (n=30) and 12-16 weeks (n=20) after a third dose of vaccine were stimulated under four conditions for eighteen hours – (i) Positive control: Cytostim, (ii)Peptivator Prot M/N 0.25µg ml⁻¹, (iii) Peptivator Prot S Complete 0.25µg ml⁻¹, (iv) whole trimeric Spike protein 1µg ml⁻¹.

Following incubation, Interferon Gamma (IFN-γ) was measured in supernatants by ELISA. Participants with IFN-γ above 0.1IU/ml after stimulation with Peptivator Prot N and Prot M were designated as previously infected, in line with manufacturer cutoffs.

After accounting for previous infection (n=14 at 4-6 week timepoint, n=7 at 12-16 week timepoint) and non-reactivity to CytoStim (n=2 at 4-6 weeks, n=1 at 12-16 weeks), there were 14 individuals included in analyses of antigen-induced IFN-γ production at 4-6 weeks and 12 individuals at the 12-16 week timepoint.

There were no significant differences in IFN-γ production by SES at either timepoint in the unstimulated (p=0.9999, p=0.9080) or CytoStim positive control (p=0.5829, p=0.610) conditions, as expected.

At 4-6 weeks, IFN- γ production in response to Peptivator spike peptides and whole spike protein were lower in low SES participants but differences failed to reach statistical significance ($p=0.7445$ and $p=0.8720$, respectively).

At 12-16 weeks however, IFN- γ production was significantly lower in low SES participants in response to spike peptides ($p<0.0001$) and spike protein ($p=0.0217$), indicating a more robust cell-mediated immune memory response to vaccine antigen in high SES participants at this timepoint.

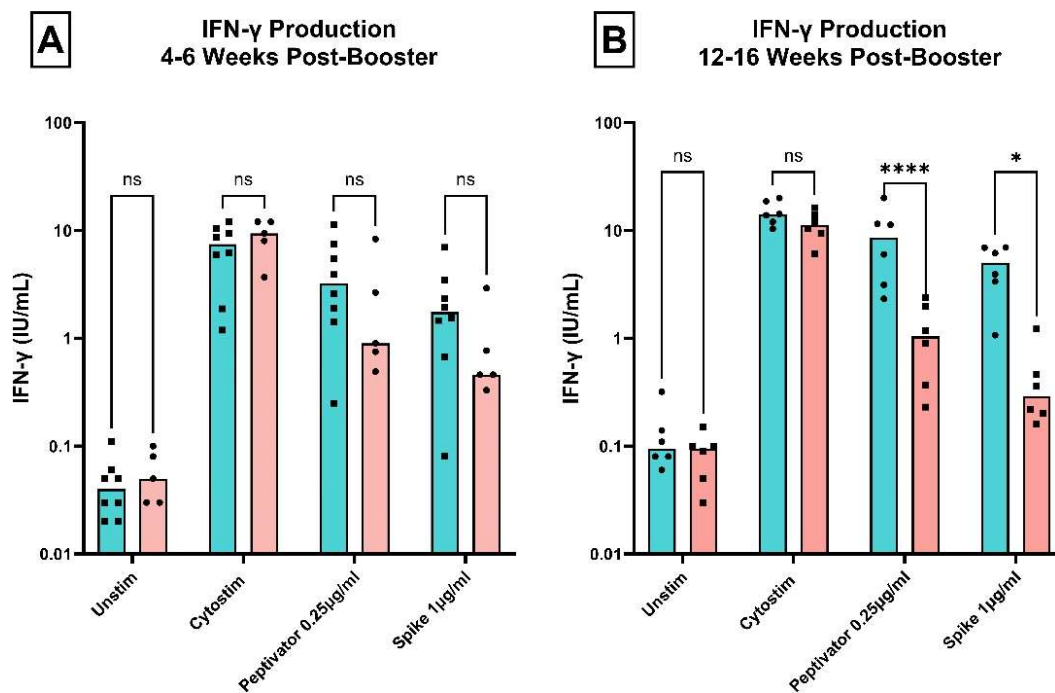


Figure 4.8: Interferon Gamma Production in Incubated Whole Blood Samples: High SES in teal blue and low SES in pink. (A) 4-6 weeks post-third dose BNT162b2 US; $p=0.9999$, CS; $p=0.5829$, Pep $p=0.7445$, S; $p=0.8720$ (B) 12-16 weeks post-third dose BNT162b2 US; $p=0.90802$, CS; $p=0.6100$, Pep; $p<0.0001$, S; $p=0.0217$.

4.3.7: Pairwise Analysis of Correlation Between Antibody Titres and Antigen-Specific IFN- γ Production After Third Dose of Vaccine

While there was no statistically significant difference in antibody titres between SES groups at the 12-16 week (B2) post-booster timepoint, the trend

mirrored the reduced cell-mediated memory response to spike and spike peptides (Fig 4.8). To further investigate the relationship between cellular and humoral responses post-third dose, pairwise analyses were conducted. Pair plots were generated (fig 4.9) for antibody and IGRA measures post-third dose. Pearson correlation coefficients were calculated for the paired measurements, revealing moderate to large correlations (e.g. -0.59 and -0.99 at B2) but not reaching statistical significance. These sizeable but non-significant correlations are likely the result of the limited sample size available after recruitment and elimination of previously infected participants. Here, small numbers yield wide confidence intervals and insufficient power to confirm association between humoral and cellular immune memory after vaccination.

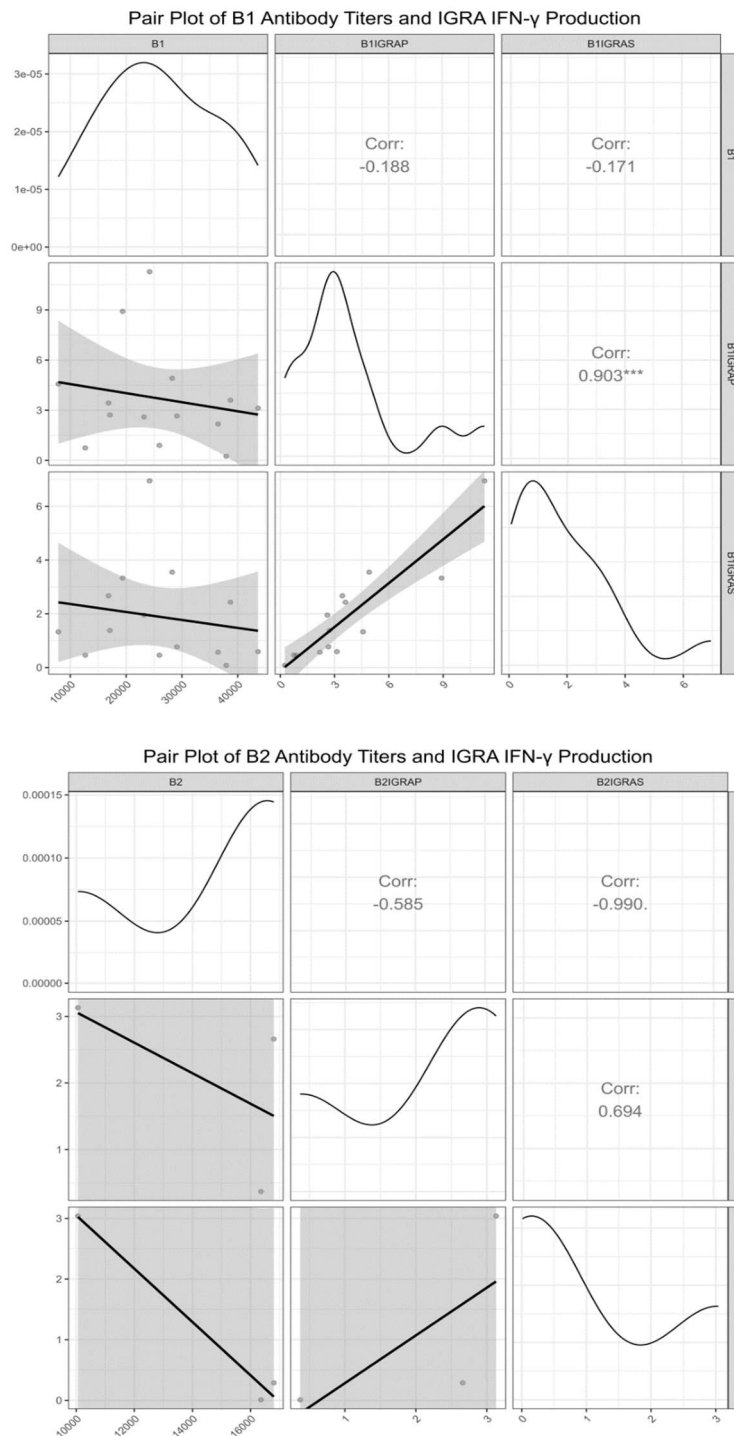


Figure 4.9 Pair plots illustrating the relationship between antibody titres and antigen-specific interferon- γ (IFN- γ) production at 4–6 weeks (B1, top panels) and 12–16 weeks (B2, bottom panels) following a third dose of BNT162b2. Diagonal panels display kernel density plots showing the distribution of antibody titres or IFN production after stimulation. Panels off the diagonal are pairwise scatterplots with fitted linear regression lines and shaded confidence intervals, representing relationships between antibody titres and IFN- γ production following stimulation with Peptivator Spike Complete (IGRAP) and whole trimeric Spike Protein (IGRAS). Numerical values (corr) indicate Pearson correlation coefficients (r), summarising the strength and direction of linear associations between paired measures. Antibody titres are expressed in assay-reported units per millilitre (U/mL), while IFN- γ concentrations are expressed in international units per millilitre (IU/mL).

4.4 Discussion:

This study provides evidence of an "immunity gap" between individuals from low and high socioeconomic status backgrounds after vaccination against SARS-CoV-2. Specifically, participants of low SES exhibited significantly lower humoral immune responses, as measured by anti-Spike antibody titres at multiple post-vaccination timepoints. After booster vaccination, this immunity gap narrowed. In addition to the immunity gap seen in humoral responses, a similar trend was observed for vaccine-induced, cell-mediated immunity to SARS CoV-2, suggesting the effects of socioeconomic status on immunity are felt across multiple arms of the adaptive immune response and particularly relevant in relation to primary response to vaccination.

Examining multiple timepoints post-vaccination provided an insight into the trajectories of vaccine-induced immunity in people from low vs high SES. A consistent trend in antibody titres was observed across the study period, with statistically significant differences noted at two timepoints, and the disparity persisting for up to 12 months post-immunisation. Notably, a similar trend was observed when comparing homeless and housed participants; however, this analysis was somewhat limited by the number of homeless participants included throughout the study.

Obstacles regarding recruitment and retention of homeless participants included national policy to vaccinate the majority of homeless people with a different vaccine due to it requiring only a single dose, difficulty with blood-draw, difficulty contacting participants for follow-up and the logistical challenge of arranging appointments for blood draws and interviews, which took place at St James' Hospital. Willingness to participate was high among

homeless individuals who were approached at vaccine clinics, but this did not always translate to participation. This may have been improved significantly if follow-ups were arranged in more convenient locations (e.g. PATH blood draws and CIPHER assessments took place in homeless accommodation and inclusion healthcare settings – engagement with homeless populations through AESS-Vax, PATH and CIPHER will be discussed at length in Richards G*, **Reddy C*** et al, *manuscript in preparation*).

After the administration of a third dose (booster) of vaccine, differences in antibody levels between high and low SES participants appeared to narrow, with no statistically significant differences observed at the post-booster timepoints. Effect size estimates (Cohen's D) suggest that the magnitude of the difference was reduced following booster vaccination. However, given the limited sample size at these timepoints, these findings should be interpreted with caution.

In contrast, and as mentioned at the outset, cell mediated immune memory after booster vaccination remained diminished in low SES participants. IFN-gamma production in antigen-stimulated whole blood was lower in low SES participants 4-6 weeks and 12-16 weeks post-third dose, although differences were only significant 12-16 weeks post-booster. While no direct association was found between psychosocial stress and antigen-specific IFN- γ production, previous research has reported psychosocial modulation of cell-mediated vaccine-induced immunity, including positive correlations between IFN- γ production and psychological wellbeing following hepatitis A vaccination¹²⁶ and reduced IFN- γ producing CD4⁺ T cells in infants born to mothers with high prenatal anxiety following hepatitis B immunisation¹⁷⁴

These results suggest that while booster-vaccination improves the socioeconomic gap in humoral immunity to SARS CoV-2, it may not be as effective in attenuating cell-mediated immune memory. However, further analysis of vaccine-induced, cell-mediated immunity before administration of a third dose would be required to confirm this hypothesis. Analysis of antigen-stimulated, banked PBMCs from pre-booster timepoints was initially planned as part of this study but technical issues prevented inclusion of any results in this thesis.

4.42 The Role of Psychosocial Stress in the “Immunity Gap”

Going beyond univariate analysis of humoral immunity by group, robust regression analyses confirmed the strength of the association between SES and antibody titres across timepoints as associations remained after the consideration of age, sex, smoking history and weekly alcohol consumption as covariates. Across timepoints, age was a significant predictor only twice (12-16 weeks and 48-54 weeks) and with a modest effect size compared to SES (IRRs of 0.97 and 0.96 respectively). This was despite the greater average age of the low SES (46.11 years versus 38.12 years) sub-cohort overall. Sex was not associated with antibody titre at any timepoint.

Mediation models assessing the role of psychosocial stress in shaping vaccine responses yielded null results. Beyond statistical and logistical limitations, these null findings may also reflect a more fundamental issue of measurement validity. Not all stress-relevant neural processing is accessible to conscious awareness or amenable to self-report (as briefly discussed in Chapter 1). A substantial component of stress physiology is driven by rapid, non-conscious threat and safety appraisal systems centred on subcortical and brainstem-

proximal circuitry, which can activate autonomic and HPA responses independently of conscious distress. In neuroscience, this distinction is often framed as a dissociation between neural mechanisms that initiate defensive responding and the later, consciously reportable experience of stress or anxiety. In contexts characterised by chronic unpredictability and reduced environmental safety, such non-conscious “unsafety” signals may remain persistently engaged even when subjective reports of stress are modest³¹.

While the findings here do not support a mediating role for stress in the current dataset, several factors may explain the null findings. These include insufficient power for mediation analysis, as confirmed by post-hoc power calculations; substantial missingness in data that was collected and suboptimal timing of stress measurements, which were often limited to a single assessment at recruitment.

At a higher level, the choice of stress scales deployed may have been unsuitable for the study's aims. Firstly, the Perceived Stress Scale captures subjective stress in the six to eight weeks prior to survey completion - follow-up administration of PSS questionnaires was not consistent for participants returning for blood-draws for logistical reasons, so only one PSS measurement is included per participant in the dataset used in analyses described here. To more reliably interrogate the effects of subjective stress throughout the study period, more consistent administration of the PSS or use of another longer-term measure would have been required. Secondly, the Holmes-Rahe inventory was designed in the US for the general population. While the inventory's validity in other populations has been demonstrated, it may have failed to capture the full extent of stressful life events experienced by socially excluded/homeless participants who often reported multiple distressing

events in their lives in the 12 months prior to recruitment (e.g. assault, bereavement, harassment, serious illness). More fundamentally still, both instruments capture consciously appraised stressors rather than perceived safety. Neither capture non-conscious threat detection processes that are more proximal to autonomic and neuroendocrine regulation. From this perspective, the absence of stress mediation alongside robust direct SES effects may reflect a mismatch between the stress constructs measured and the biological processes shaping vaccine-induced immunity. There is undoubtable value in the development and validation of bespoke measurement tools to capture psychosocial stress in excluded populations.

Beyond the use of more appropriate tools, more consistent measurement of psychosocial stress, larger sample sizes, and incorporation of biological indices of stress (e.g. serial serum or salivary cortisol, hair cortisol, or circulating catecholamines) should be considered in future studies aiming to address the causal pathways underlying stress-effects on vaccine responses.

4.43 Public Health Implications of the Immunity Gap:

The AESS-Vax study was conducted against the backdrop of global pandemic that was initially perceived by some as a “great leveller” – a crisis that would affect all strata of society equally. The reality, however, was that Covid-19 revealed and deepened pre-existing social inequalities. Poor and marginalised groups were disproportionately impacted. They were more likely to be exposed to the virus and become infected, more likely to experience severe disease and death, and more likely to suffer long-term health and economic consequences because of disease or the pandemic generally. Access to vaccines globally was grossly inequitable, with the Global North having access to vaccines long before the Global South¹⁷⁵. The results of this study add

another layer to this pattern of pandemic-associated inequity – an “immunity gap” in vaccine-induced protection.

While this study did not directly investigate the significance or consequences of diminished vaccine-induced immunity, it is known that lower correlates of protection translate to greater vulnerability to breakthrough infection, weakened protection in the face of novel pathogenic variants and higher likelihood of transmitting infection when infected^{176–178}. This study not only has relevance for understanding the pandemic’s unequal toll retrospectively, but a value for action facing into future epidemics or pandemics. While the structural drivers of inequality must ultimately be addressed through political and systemic change, the immunity gap described here presents a more clinically actionable dimension of inequality – one that offers the opportunity to mitigate the unequal burden of infectious disease risk and harm. Booster vaccinations appear to attenuate socioeconomic disparities in immunity observed in AESS-Vax - warranting further investigation into prioritised access to booster vaccination or altered dosing regimens for socially excluded groups – not only because they face heightened exposure and risk but because their immunological protection appears to wane more quickly or be suboptimal initially.

4.44 Contribution to the Literature and Future Directions:

This study represents a significant addition to the growing body of literature on stress effects on vaccine responses¹¹². It is the first longitudinal cohort study to focus specifically on the effects of socioeconomic status and social exclusion on vaccine-induced immunity, and among the largest studies of stress effects on vaccine responses to have been conducted (see table 1.2, Chapter 1 for review of studies to date). While the study did not detect a direct

association between psychosocial stress as measured by the scales used in the study and immune outcomes, it confirmed that individuals from low SES and socially excluded backgrounds report substantially higher levels of perceived stress, childhood trauma and exposure to adverse life events; factors that remain important targets for future investigation. Crucially, the study also identified a parallel social gradient in both humoral and cellular measures of vaccine-induced immunity.

Compared to previous studies that have reported SES-related variation in vaccine-induced immunity, studies from the Netherlands^{179,180} and South Africa¹⁸¹, and of rotavirus vaccine efficacy in poorer regions of the global South¹⁸² - AESS-Vax differs substantially in design, population, and depth of immunological profiling. Hoes et al.¹⁸⁰ and den Boogaard et al.¹⁷⁹ used national serosurvey data and cross-sectional analyses to explore SES associations with IgG levels for various childhood vaccines. While they identified some SES-related differences in antibody titres, these were pathogen-specific, modest in size, and largely inconsistent across markers. The South African study by Ezkenazi (2025)¹⁸¹ highlighted stronger associations between early-life undernutrition and impaired antibody responses to measles and tetanus vaccines, indicating how developmental disadvantage can compromise immunity. While Lopman (2012)¹⁸² examined global disparities in rotavirus vaccine efficacy, reporting that immunogenicity and protection were consistently lower in low-income settings, most probably due to environmental (e.g. prevalence of enteropathogenic pathogens) and nutritional stressors.

These studies offer valuable evidence of SES-related differences in vaccine outcomes, but they are largely cross-sectional, primarily focused on global

inequality (situated in the global south) or childhood. They often rely on single timepoint sampling and analyse humoral markers in isolation. AESS-Vax, on the other hand, tracked participants over multiple timepoints for up to a year and uniquely incorporated both humoral and cellular read outs. It also the first study to intentionally recruit socially excluded individuals, who are often overlooked in immunological research. This design offers a more detailed and inclusive view of how social inequalities are biologically embedded in vaccine responses.

While some of the study's original design ambitions were only partially realised, particularly regarding longitudinal stress profiling and pre-booster T cell data collection, AESS-Vax still delivers important methodological and empirical contributions. It highlights both the need and feasibility of integrating marginalised populations into immunological research, and underscores the value of sustained, inclusive cohort studies.

These findings reinforce the necessity of interdisciplinary research that links immunology, social epidemiology, and public health. Future studies should deepen this approach to better characterise the mechanisms through which social inequality influences immunity, refine psychosocial exposure measures, and contribute to the development of vaccine policies that promote equity in both access and outcome.

In sum, AESS-Vax demonstrates that equal access to vaccines does not equate to equal immunological benefit. Addressing this inequity requires more than universal vaccine coverage, it calls for deeper biological understanding, more inclusive research, and a systemic commitment to health justice.

4.5 Conclusion:

AESS-Vax contributes important new evidence to our understanding of how social disadvantage can shape vaccine-induced immunity. By following a diverse cohort across multiple timepoints, AESS-Vax demonstrates a clear and sustained disparity in humoral and cellular immune responses to SARS-CoV-2 vaccination among individuals from low socioeconomic backgrounds and those experiencing social exclusion. These disparities, evident even after adjusting for demographic and behavioural covariates, support the existence of an "immunity gap", a biologically measurable expression of broader health inequalities.

While booster vaccination appears to reduce some of the differences in antibody responses, the limited IFN- γ Release Assay data available post-booster did not show a similar pattern for cell-mediated immunity. Given the small sample sizes and absence of pre-booster T cell data, definitive conclusions about disparities in cell-mediated responses over time cannot be drawn. These findings nonetheless highlight the importance of further investigation into the temporal dynamics and determinants of cellular immunity in disadvantaged groups, and the potential value of tailored vaccination strategies to address unequal outcomes in future pandemics.

The study's limitations, including sample size constraints, incomplete data on stress exposure, and the absence of pre-booster T cell measurements, should inform the design of future work. Despite these challenges, AESS-Vax stands out for its methodological ambition, inclusive recruitment, and detailed immunological profiling, providing the first ever data showing a gradient effect

between social status and antibody and cell-mediated responses to *in vivo* immune challenge.

Ultimately, these findings call for a more nuanced approach to vaccine policy: one that goes beyond equal access to ensure equitable protection. This will require closer integration of social, health and biological sciences, improved tools for capturing lived experience of stress and exclusion, and a renewed commitment to addressing the structural determinants of health. In the context of vaccination, as in broader public health, equity must be measured not just by who gets the intervention, but by who truly benefits.

Chapter 5: The Adversity-Associated Immunophenotype

5.1 Introduction:

This thesis has examined how social exclusion and chronic psychosocial adversity shape immune function and contribute to disparities in health.

Chapter 1 outlined theoretical models including the Conserved Transcriptional Response to Adversity (CTRA), which describes stress-related immune shifts characterised by elevated pro-inflammatory and reduced antiviral signalling. Although gene expression was not directly assessed here, prior research has linked these transcriptional patterns to changes in circulating cytokines such as IL-6, TNF- α , and IL-10.

Empirical data from Chapters 3 and 4 showed that socially excluded and low SES participants in the AESS-Vax and PATH studies experience elevated psychosocial stress, poorer health outcomes and weaker vaccine-induced immunity than their housed, high SES peers. One possible explanation for these findings is the emergence of an "adversity-associated immunophenotype", characterised by dysregulated patterns of circulating cytokines and chemokines, alongside shifts in immune cell frequency, phenotype, and function.

By linking cytokine production patterns and lymphocyte phenotypes, this chapter provides a cellular and molecular framework that may help explain the poorer health outcomes and diminished vaccine responses observed in individuals exposed to chronic social adversity. This approach builds directly on the models introduced in previous chapters, aiming to cast light on the cells and regulatory processes underlying differences already observed.

5.2 Hypothesis:

We hypothesised that individuals exposed to psychosocial adversity; specifically, those of low socioeconomic status or experiencing social exclusion, would exhibit broad immune dysregulation. This dysregulation

would be evident in circulating cytokine and chemokine profiles, characterised by persistent low-grade inflammation, and in alterations to the cellular composition of the immune system. We further hypothesised that adversity-exposed individuals would demonstrate a skewed lymphocyte immunophenotype, marked by a higher prevalence of mature, terminally differentiated, and senescent populations.

5.2.1 Specific Aims:

- 1.** To characterise differences in circulating cytokine and chemokine levels across socioeconomic and housing status groups in a merged AESS-Vax and PATH cohort.
- 2.** To examine associations between low socioeconomic status and homelessness/social exclusion, as distinct forms of social adversity, and cytokine levels, using multivariate models that adjust for covariates including age, sex, alcohol use, and smoking history.
- 3.** To characterise differences in immune cell composition and lymphocyte immunophenotypes across social adversity groups using flow cytometry data.
- 4.** To examine associations between adversity exposures, immune measures (cytokine profiles and/or flow cytometry data) and reported health conditions, to explore whether observed immune dysregulation has clinically relevant correlates in this cohort.

5.3 Specific Methods:

5.3.1 Sample and Data Acquisition:

For cytokine analysis, biobanked samples from the PATH and AESS-Vax study were taken from storage at -80°C. Further detail on processing and storage are reported in Chapter 2, Materials and methods. Demographic, health and stress exposure data from AESS-Vax and PATH were integrated in a single comma-separated value (CSV) file – common measurements with separate names in source files were integrated in the new file with appropriate renaming (e.g. “HealthCond” in AESS-Vax and “Comorbidities” in PATH, both numerical reports of the number of diagnosed health conditions, were amalgamated into a single new column, “NumHealthCond”).

5.3.2 Multiplex Cytokine and Chemokine Assays:

Cytokines and chemokines were quantified in serum samples using the Meso Scale Discovery (MSD) electrochemiluminescence platform. Full assay protocols are described in Chapter 2 (Sections 2.14.1 and 2.14.2). Briefly, 250µL aliquots of frozen serum were thawed at room temperature on the bench and pipetted into 96-well plates according to plate plans for dilution with MSD assay diluent and aliquoting into separate U-Plex and S-Plex assay plates.

The U-Plex panel included the following 10 cytokines and chemokines: GM-CSF, IL-13, IL-5, G-CSF, IL-18, IL-23, IL-33, IP-10, MCP-1, and MIP-1β, with concentrations reported in pg/mL.

The S-Plex Proinflammatory Panel 1 included high-sensitivity measurements of IL-1 β , IFN- γ , TNF- α , IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-17A, and IL-18, with concentrations reported in fg/mL.

All samples were run in singlicate, with standards run in duplicate and control sera included on each plate to assess inter- and intra-assay consistency. Plates were read using the MSD QuickPlex SQ 120 instrument, and analyte concentrations were determined using the Methodical Mind software.

5.3.3 Univariate Statistical Analyses:

Initial group comparisons (e.g., by SES or housing status) were conducted using GraphPad Prism (v10). Raw cytokine and chemokine concentrations were log-transformed and then tested for normality using a built in Shapiro-Wilk test function. For normal data, uncorrected unpaired t-tests were used to test for group differences in cytokine levels, while for non-normal data, two-tailed Mann–Whitney U tests were deployed. These results are presented as violin plots throughout the chapter.

To account for potential errors introduced via multiple testing, all univariate analyses were subsequently repeated in R (v4.3.1) with corrections applied. Shapiro–Wilk tests were again used to assess normality. Depending on distribution, either t-tests or Wilcoxon rank-sum tests (equivalent to Mann-Whitney U-tests) were applied. False discovery rate correction was by the Benjamini–Hochberg procedure. Results include effect sizes (Cohen’s d or rank-biserial correlation) and group medians on the original (non-log) scale.

5.3.4 Multivariate Modelling

Multivariate linear regression was used to test associations between cytokine levels and adversity exposures (homelessness, SES), adjusting for age, sex, log-transformed weekly alcohol use, and log-transformed smoking history. All cytokine outcomes were log-transformed. Models were specified as:

$$\log(\text{Cytokine}) \sim \text{Exposure} + \text{Age} + \text{Sex} + \log(\text{AlcMP}) \\ + \log(\text{PackYrs})$$

Effect sizes, standard errors and confidence intervals were extracted from model output and visualised in forest plots.

5.3.5 Cytokine Network and Module Analysis

Pairwise Spearman correlations were calculated between log-transformed cytokines where missingness was below 50%. Correlation matrices were visualised, and hierarchical clustering (average linkage) was applied to define cytokine modules based on shared expression patterns. Modules were extracted from the dendrogram structure, and participant-level module scores were calculated by averaging standardised (z-scored) cytokine values within each cluster.

5.3.6 Principal Component Analysis and PERMANOVA

PCA was performed on scaled, log-transformed cytokine concentrations. The top five principal components (PCs) were extracted for analysis. Group differences in multivariate cytokine profiles were assessed via PERMANOVA with 999 permutations using Euclidean distances. Separate

models tested the effects of SES and homelessness. Additional PERMANOVA tests were conducted on each individual PC.

5.3.7 Lymphocyte Immunophenotyping by Spectral Cytometry

For immunophenotyping, cryopreserved PBMCs were stained and analysed by spectral flow cytometry to assess immune cell composition and lymphocyte subsets. Full details of the staining protocol are provided in Chapter 2. In brief, single use cryovials of PBMCs were thawed at 37°C in a water bath. Once thawed, cells were transferred to labelled 15 mL centrifuge tubes and washed with complete IMDM (cIMDM). Samples were centrifuged at 500g for five minutes and the resulting cell pellets were transferred to labelled FACS tubes containing fresh cIMDM. Cells were then incubated at 37°C for one hour.

Following incubation, samples were stained with Zombie-NIR viability dye, then surface antibody cocktails, which were incubated overnight at 4°C. On the following day, cells were fixed, permeabilised, and stained with intracellular antibody cocktails. After final washes, cells were resuspended in FACS buffer and stored at 4°C until acquisition.

Samples were run on a Cytex Aurora spectral cytometer. Compensation and spectral unmixing were performed using SpectroFlo software, and FCS files were exported for downstream analysis in FlowJo (v10.8). Gating and population identification were performed in FlowJo, with representative plots shown in Figure 5.1.

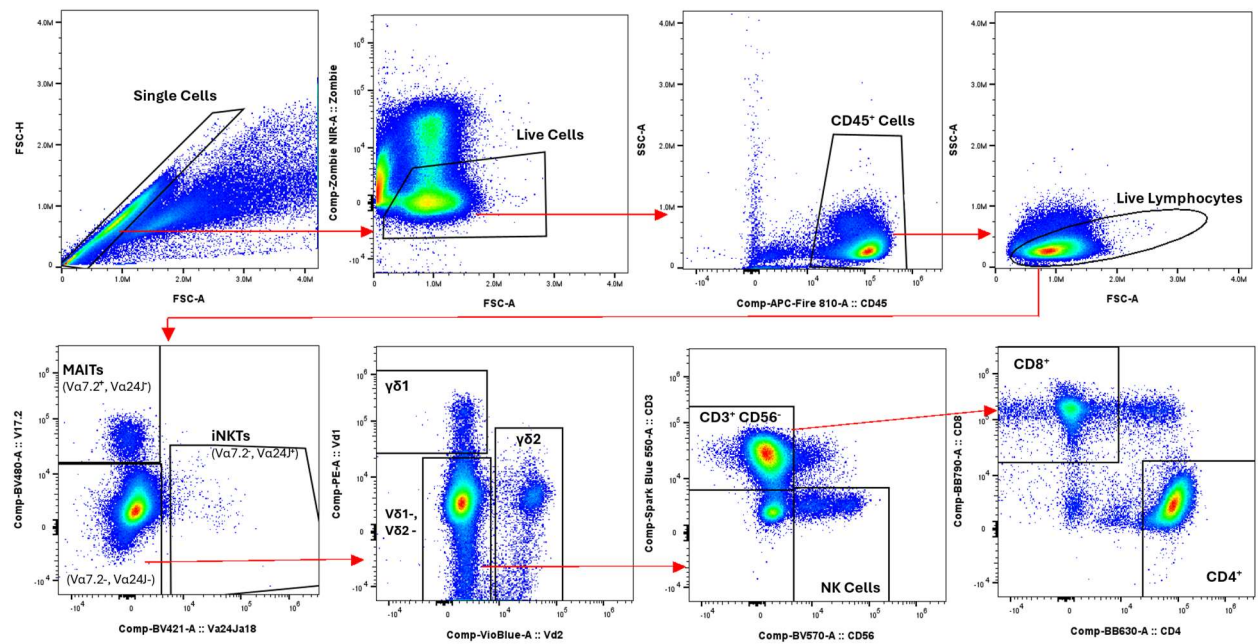


Figure 5.1 Basic Gating Strategy for Lymphocyte Immunophenotyping Panel: Shown here is the gating strategy for identification of main T cell subpopulations (MAITs, iNKTs, $\gamma\delta$ T cells, NK cells, $CD4^+$ and $CD8^+$ T cells) and NK cells. Not shown are a time versus CD45 plot and an FSC-A versus SSC-A plot immediately preceding the first plot shown here.

Further gating was performed to characterise differentiation states within $CD4^+$ and $CD8^+$ T cell populations. As shown in Figure 5.2, CCR7 and CD45RA expression was used to classify T cells into naïve, central memory, effector memory, and TEMRA subsets. Within these memory compartments, co-stimulatory markers CD27 and CD28 were used to delineate early, intermediate, and terminally differentiated effector populations. This gating strategy, alongside separate gating for surface marker expression enabled the identification of immunophenotypic features associated with T cell maturation and senescence.

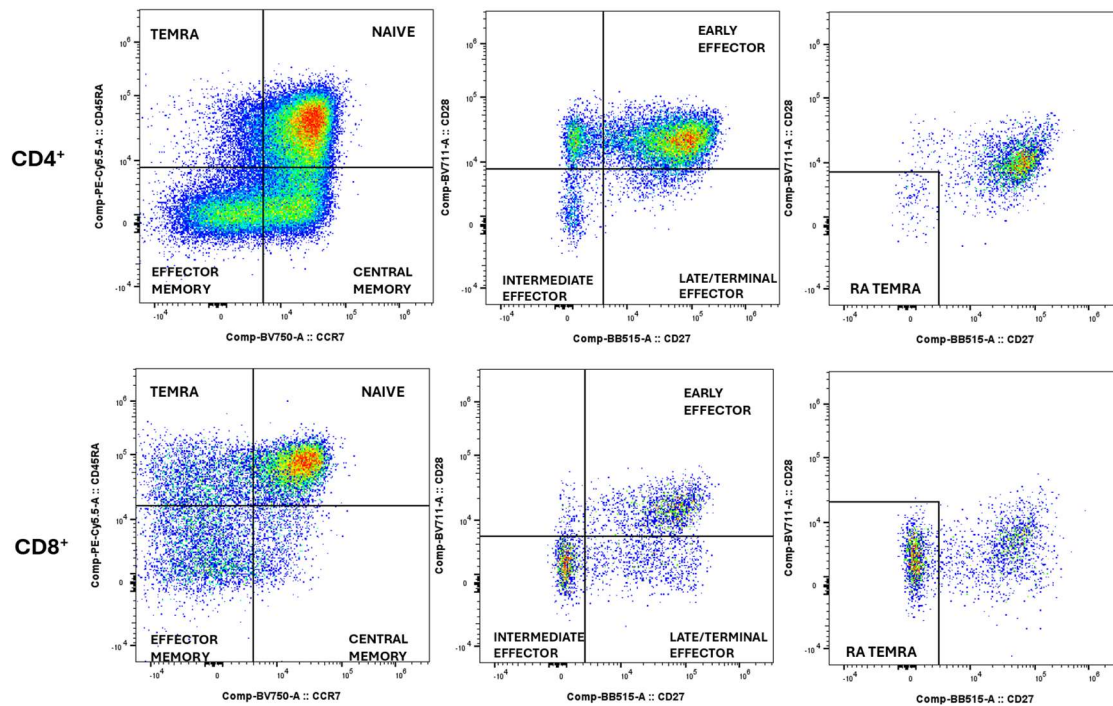


Figure 5.2 Identification of CD4⁺ and CD8⁺ T Lymphocyte Populations: From left to right; (I) CCR7 and CD45RA were used to separate naïve (CCR7⁺CD45RA⁺), effector memory (CCR7⁺, CD45RA⁻), central memory (CCR7⁺, CD45RA⁺) and TEMRA (CCR7⁺, CD45RA⁺) CD4⁺ (top) and CD8⁺ (bottom) populations, (II) within the effector memory subpopulations CD27 and CD28 were used to separate early (CD27⁺ CD28⁺), intermediate (CD27⁺ CD28⁻) and late/terminal effector (CD27⁺ CD28⁻) subsets, and (III) within the TEMRA subpopulations CD27 and CD28 were used to identify the most terminally differentiated “RA TEMRA” cells that have lost expression of both co-stimulatory receptors.

5.3.8 Univariate Analyses of Immunophenotyping Data

Statistical comparisons of lymphocyte subset frequencies between socioeconomic groups were conducted using GraphPad Prism (v10). Frequencies were as percentages of parent populations (e.g., %CD4⁺ of live CD45⁺ cells, %CD57⁺ of CD8⁺ T cells). In-built normality tests (D’Agostino–Pearson and Shapiro–Wilk) were used to assess normality. Depending on the result of normality tests, either unpaired t-tests or Mann–Whitney U tests (two-tailed) were applied.

Graphs were displayed as histograms showing group medians and individual data points for each participant. P-values are reported without

correction for multiple comparisons, consistent with the exploratory nature of the analysis.

Descriptive statistics for all populations and for marker expression were computed in R and presented in Table 5.8.

5.3.9 R Packages Used:

- **dplyr** – for data wrangling and manipulation
- **tidyr** – for data reshaping and long-to-wide format transformations
- **ggplot2** – for creating visualisations including violin plots, forest plots, and PCA scatterplots
- **stats** – for standard statistical modelling and hypothesis testing functions (e.g., t-tests, Wilcoxon tests, linear regression, Shapiro–Wilk tests)
- **ggpubr** – for adding statistical comparisons and annotations to ggplot figures
- **broom** – for extracting and formatting regression model outputs
psych – for principal component analysis and descriptive statistics
- **corrplot** – for visualising cytokine correlation matrices
- **qgraph** – for constructing cytokine correlation networks
- **vegan** – for conducting PERMANOVA and multivariate analyses
- **moments** – for assessing skewness in cytokine and covariate distributions
- **kableExtra** – for generating formatted tables of regression results

5.4 Results:

5.4.1 Univariate Analyses of Circulating Cytokines and Chemokines Reveal Distinct Immune Profiles in Homeless and Low SES Participants:

To examine whether experiences of homelessness were associated with altered immune profiles, univariate comparisons of serum cytokine and chemokine concentrations were conducted between participants. These comparisons included 19 immune markers measured in serum, spanning pro- and anti-inflammatory cytokines and chemokines involved in immune regulation and immune cell trafficking. Given the non-normal distribution of several analytes, data were log-transformed and non-parametric tests were carried out where appropriate. Distributions were visualised using violin plots and group comparisons were assessed using Mann-Whitney U-tests (aka Wilcoxon Rank Sum tests), depending on the distribution of each variable. Figure 5.3 and Table 5.1 detail these analyses.

After correction for multiple comparisons using the Benjamini–Hochberg method, eight cytokines were significantly elevated in homeless participants compared to their housed counterparts. These included key pro-inflammatory cytokines, IL-1 β , TNF- α , and IFN- γ , as well as IL-4 and IL-12p70, which are involved in Th2 and Th1-type immune responses respectively. In addition, the regulatory cytokine IL-10 and pleiotropic cytokines IL-2 and IL-18 were also significantly elevated.

The magnitude of the differences between groups was substantial. Non-parametric Wilcoxon rank-sum tests yielded large test statistics for the cytokines that differed significantly between groups, with standardised test statistics (Z values) ranging from 3.12 (IL-10) to 3.51 (IL-2). Similarly

high values were observed for IL-1 β (3.23), IFN- γ (3.18), TNF- α (3.18), IL-4 (3.38), and IL-12p70 (3.48). While these statistics reflect the strength of evidence against the null hypothesis rather than an effect size, their consistently high values indicate clear separation between the cytokine distributions of homeless and housed participants. For example, homelessness was associated with a more than two-fold increase in IL-1 β levels, with a median concentration of 1141.6 pg/mL in homeless individuals compared to just 535.3 pg/mL in the housed group.

The concurrent elevation of both inflammatory and regulatory cytokines suggests a pattern of broad immune dysregulation, rather than a simple pro-inflammatory shift. It is possible that the increase in IL-10 may reflect a compensatory response to persistent immune activation. This co-activation of opposing arms of the immune response is consistent with the immune profile observed in chronic stress^{52,183} and immune ageing^{184,185}, where homeostasis is disrupted, and regulatory feedback mechanisms fail to fully resolve inflammation.

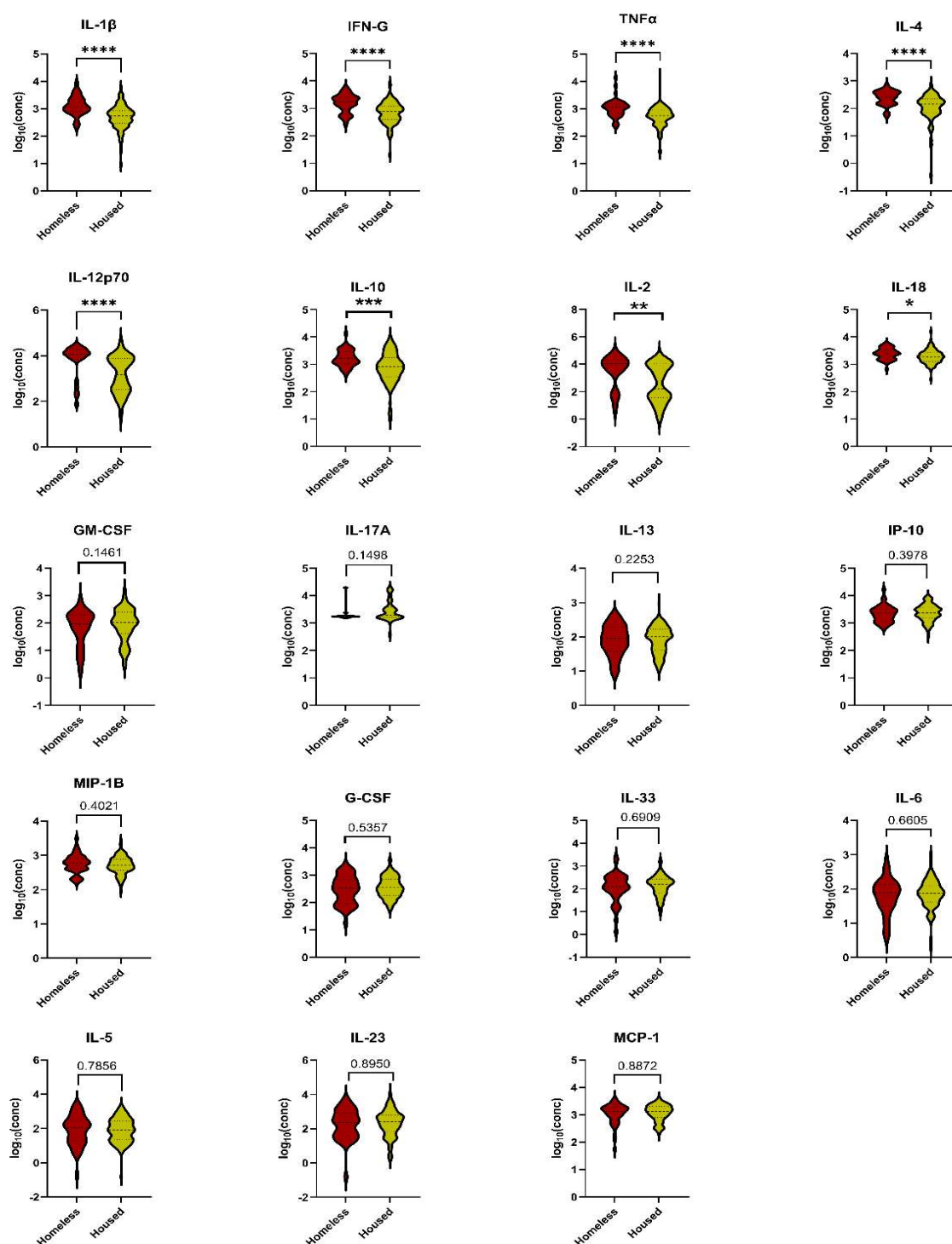


Figure 5.3 Cytokines and Chemokines by Housing Status: Violin plots display the distribution of log-transformed cytokine concentrations among participants who are Homeless (red; AESS-Vax and PATH) versus Housed (gold). Each violin shape represents the distribution of the data, with horizontal lines indicating median values. Statistical tests were performed using Mann–Whitney/Wilcoxon tests for non-normal data or unpaired *t* tests for normally distributed data ($p < 0.05$ considered significant). Where $p > 0.05$, the corresponding *p* values are indicated.

Table 5.1 Univariate Comparisons of Cytokine Levels Between Homeless and Housed Group: results of univariate tests comparing log-transformed cytokine concentrations between Homeless (n=47) and Housed (n=120) participants. Depending on data distribution, either the Mann–Whitney/Wilcoxon test (Wilcox) or unpaired t test (t-test) was used. All p values were adjusted for multiple comparisons using the Benjamini–Hochberg method. The test statistic, effect size, and median values (on the original scale) for each group are shown. Statistical significance was evaluated at $p < 0.05$.

OCytokine	Test	p value	Adjusted p value	Test Stat	Effect Size	Median (Homeless)	Median (Housed)
IL-1 β	Wilcox	1.00E-07	1.91E-06	981.5	3.23	1141.56	535.32
IFN- γ	Wilcox	6.57E-07	6.24E-06	1110.5	3.18	1544.94	730.46
TNF- α	Wilcox	1.23E-06	7.80E-06	1118	3.18	1140.35	553.05
IL-4	Wilcox	2.14E-05	1.02E-04	537.5	3.38	261.31	123.14
IL-12p70	Wilcox	5.08E-05	1.93E-04	270.5	3.48	12102.6	1192.8
IL-10	Wilcox	1.74E-04	5.52E-04	1251	3.13	1461.28	769.62
IL-2	Wilcox	0.00772	0.02096	177.5	3.51	11369.1	78.65
IL-18	t-test	0.02186	0.05191	-2.34	0.39	2475.04	1916.8
GM-CSF	Wilcox	0.14641	0.28468	2758	2.6	91.36	108.08
IL17A	Wilcox	0.14983	0.28468	412.5	3.43	1748.32	2828.7
IL-13	Wilcox	0.22526	0.38908	2698	2.62	68.81	104.43
IP-10	t-test	0.39784	0.58768	0.85	-0.15	2319.45	2583.1
MIP-1 β	t-test	0.4021	0.58768	-0.84	0.15	593.36	510.07
G-CSF	Wilcox	0.53572	0.72705	2549.5	2.67	342.83	350.92
IL-33	Wilcox	0.69095	0.8205	2494	2.69	122.84	156.48
IL-6	Wilcox	0.66058	0.8205	2052	2.85	80.35	76.03
IL-5	t-test	0.7856	0.87803	0.27	-0.05	107.58	97.85
IL-23	Wilcox	0.89501	0.89501	2360.5	2.74	203.64	174.03
MCP-1	Wilcox	0.88721	0.89501	2430	2.71	1451.89	1382.3

Next, to assess whether broader socioeconomic gradients were associated with similar immune alterations, the same 19 serum cytokines and chemokines were compared between low and high SES participants. As in the previous analyses, values were log-transformed, and group differences were evaluated using either Mann–Whitney U or unpaired t tests. Figure 5.4 and Table 5.2 display the distributions and statistical comparisons. This allowed for the identification of shared and distinct immunological signatures associated with homelessness and low socioeconomic status.

After adjusting for multiple comparisons using the Benjamini–Hochberg method, nine cytokines were significantly elevated in the low SES group. These included IL-1 β , IL-18, IFN- γ , MIP-1 β , IL-10, G-CSF, IP-10, and TNF- α . Non-parametric Wilcoxon rank-sum tests and unpaired t tests yielded large test statistics for these cytokines, reflecting clear separation between the cytokine distributions of low and high SES participants.

This pattern reflects a similar immune phenotype to that seen in the homelessness comparisons, characterised by elevations in both pro-inflammatory markers (IL-1 β , TNF- α , IFN- γ), chemokines involved in leukocyte recruitment (MIP-1 β , IP-10), and regulatory cytokines (IL-10). The co-occurrence of elevated inflammatory and regulatory markers again suggests broad, wide-ranging dysregulation.

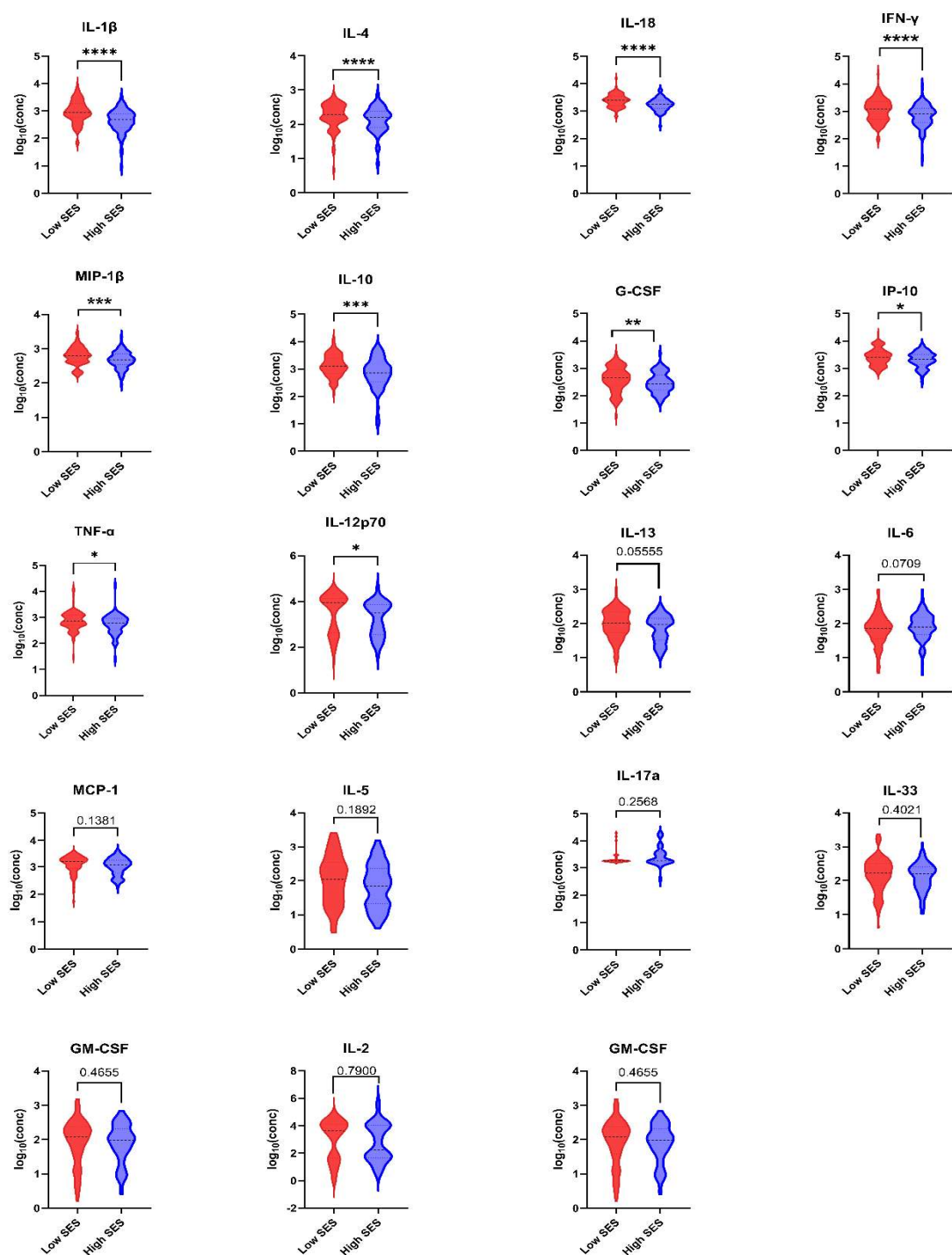


Figure 5.4 Cytokines and Chemokines by Socioeconomic Status: Violin plots display the distribution of log-transformed cytokine concentrations among low SES (red; including homeless from AESS-Vax and PATH) and high SES participants (blue). Each violin shape represents the kernel density of the data, with horizontal lines indicating median values. Statistical tests were performed using Mann–Whitney/Wilcoxon tests for non-normal data or unpaired t tests for normally distributed data ($p < 0.05$ considered significant). Where $p > 0.05$, the corresponding p values are indicated.

Table 5.2 Univariate Comparisons of Cytokine Levels Between Low and High SES Groups: Results of univariate tests comparing log-transformed cytokine concentrations between participants with Low SES (n=87) and High SES (n=85). Depending on the distribution of the data, either the Mann–Whitney/Wilcoxon test (Wilcox) or an unpaired t test (t-test) was used. All p values were adjusted for multiple comparisons using the Benjamini–Hochberg method. The table displays the test statistic, effect size, and the median cytokine values (on the original scale) for each SES group. Statistical significance was evaluated at the conventional $p < 0.05$ level.

Cytokine	Test	p value	Adjusted p value	Test Statistic	Effect Size	Median (Low SES)	Median (High SES)
IL-1 β	Wilcox	2.05E-07	3.90E-06	1551	1.62	867.5	420.97
IL-4	Wilcox	2.14E-05	0.13456	1045.5	1.75	162.57	132.55
IL-18	t-test	1.39E-04	0.00132	-3.91	-0.62	2524.66	1841.78
IFN- γ	t-test	7.26E-04	0.00460	-3.45	-0.55	1203.51	731.57
MIP-1 β	t-test	0.00160	0.00608	-3.21	-0.51	639.8	476.22
IL-10	Wilcox	0.00149	0.00608	1935.5	1.51	1252.45	719.80
G-CSF	Wilcox	0.00698	0.02211	2409	1.38	461.76	270.95
IP-10	t-test	0.01514	0.04031	-2.46	-0.39	3007.64	2227.53
TNF- α	Wilcox	0.01700	0.04030	2400.5	1.39	709.52	586.44
IL-12p70	Wilcox	0.04510	0.09530	566.5	1.88	7500	1486.00
IL-13	Wilcox	0.05555	0.10554	2638.5	1.32	103.64	96.44
IL-6	t-test	0.07086	0.12239	1.82	0.3	67.1	78.34
MCP-1	Wilcox	0.13813	0.20189	2765	1.29	1567.95	1247.32
IL-5	t-test	0.18921	0.25678	-1.32	-0.21	127.13	90.68
IL17A	Wilcox	0.25679	0.32527	430	1.92	1749.35	2849.59
IL-33	Wilcox	0.40212	0.47751	2954	1.24	162.2	148.83
GM-CSF	Wilcox	0.72393	0.80179	3096	1.2	121.03	99.84
IL-2	Wilcox	0.79000	0.80200	425.5	1.92	1110.96	96.30
IL-23	Wilcox	0.80179	0.80179	3274	1.15	164.13	236.20

5.4.2 Adjusted Regression Models Reveal Independent Associations Between Adversity and Cytokine Expression

To further assess the contribution of homelessness to the immune profile observed, multivariate linear regression models were developed with homelessness as the main predictor and age, sex, smoking history (pack years) and weekly alcohol consumption as covariates. This approach allowed for

analysis of association between homelessness and immune profile independent of the effects of age, sex, smoking and alcohol consumption. Table 5.3 contains β -coefficients, standard errors, t values and p values for homelessness as a predictor of each cytokine/chemokine.

After adjustment, six cytokines remained significantly associated with homelessness. These included IL-1 β (p = 0.0035), IL-4 (p = 0.0005), IL-10 (p = 0.0218), IL-12p70 (p = 0.0102), and IL-17A (p = 0.0334), all of which were elevated in homeless participants, except IL-17A which was reduced. GM-CSF was also significantly lower in the homeless group (p = 0.0004), despite not showing a significant difference in univariate comparisons. This suggests that the relationship between GM-CSF and homelessness may have been masked by confounding influences, such as smoking or alcohol use, which are more prevalent among homeless individuals in the PATH and AESS-Vax cohorts and may act in the opposite direction.

The magnitude of these associations was substantial. Because cytokine concentrations were log-transformed prior to modelling, the β -coefficients can be interpreted as approximate percentage changes on the original scale using the formula:

$$\text{Percentage Change in Marker} = (e^{\beta} - 1) \times 100$$

For example, homelessness was associated with an estimated 313% increase in IL-12p70, a 274% increase in IL-4, and an 84.5% decrease in GM-CSF.

These findings indicate persistent and directionally consistent associations between homelessness and several immune markers even after adjustment for behavioural and demographic covariates. The combination of increased pro-inflammatory (IL-1 β , IL-12p70), regulatory (IL-10), and Th2/Th17-related

(IL-4, IL-17A) signals, alongside decreased GM-CSF, further supports a link between homelessness and broad immune dysregulation.

Table 5.3 Adjusted Associations of Social Exclusion with Log-Transformed Cytokine Levels: *Estimated regression coefficients (β), standard errors, t values, and p values for the effect of Social Exclusion (Y vs. N, with N as the reference) on log-transformed cytokine concentrations. All models were adjusted for age, sex, log-transformed smoking history (PackYrs), and log-transformed alcohol consumption (Alcmp).*

Cytokine	Coefficient	Standard Error	t value	p value
GM-CSF	-1.8599	0.4931	-3.7714	0.0004
IL-4	1.3195	0.3439	3.8366	0.0005
IL-1β	1.0003	0.3274	3.0555	0.0035
IL-12p70	1.4195	0.5105	2.7806	0.0102
IL-10	0.7002	0.2958	2.3672	0.0218
IL-17A	-0.5419	0.2341	-2.3148	0.0334
IFN-γ	0.652	0.3326	1.9606	0.0552
IL-5	-1.1452	0.6087	-1.8814	0.0653
IL-2	2.8693	1.4539	1.9736	0.0685
G-CSF	-0.6475	0.3681	-1.759	0.0842
MIP-1b	-0.3534	0.2382	-1.4837	0.1437
TNF-α	0.3714	0.2695	1.3782	0.174
IL-13	-0.5001	0.4938	-1.0128	0.3157
MCP-1	-0.2338	0.2782	-0.8407	0.4042
IP-10	-0.1833	0.2208	-0.8299	0.4102
IL-6	-0.2521	0.3674	-0.686	0.4959
IL-18	0.1005	0.1794	0.5606	0.5774
IL-33	0.1388	0.5291	0.2622	0.7941
IL-23	0.2359	0.9266	0.2546	0.8

A parallel set of adjusted models was developed to estimate the independent associations between SES and cytokine/chemokine concentrations. As with the previous models, adjustments were made for age, sex, alcohol use and smoking history. Table 5.4 shows the estimated coefficients, standard errors, t values, and p values for each cytokine.

IL-1 β was the only cytokine to remain significantly associated with SES after adjustment ($p = 0.0076$), with lower concentrations observed in high SES participants. The magnitude of this effect was notable: the coefficient of -0.877 suggests that low SES was associated with a 58% elevation in IL-1 β levels, after accounting for behavioural and demographic covariates. Other cytokines, such as IL-12p70 and IL-10, trended towards higher concentrations in the low SES group but did not reach statistical significance. These findings suggest a more modest but still measurable immune impact of low SES compared to the more pronounced effects observed with homelessness.

Table 5.4 Adjusted Associations of Socioeconomic Status with Log-Transformed Cytokine Levels: *Estimated regression coefficients (β), standard errors, t values, and p values for the effect of Socioeconomic Status (with High SES compared to Low SES as the reference) on log-transformed cytokine concentrations. All models were adjusted for age, sex, log-transformed alcohol consumption (log_Alcmp), and log-transformed smoking history (log_PackYrs). Negative coefficients indicate that High SES is associated with lower cytokine levels relative to Low SES, whereas positive coefficients indicate higher levels. Statistical significance is evaluated at the conventional 0.05 level.*

Cytokine	Coefficient	Standard Error	t value	p value
IL-1β	-0.877	0.3156	-2.7792	0.0076
IL-12p70	-1.059	0.5728	-1.8487	0.0764
IL-10	-0.4953	0.2904	-1.7058	0.094
IFN-γ	-0.5093	0.3205	-1.589	0.1181
IL-18	-0.2683	0.1699	-1.5788	0.1202
IL-17A	0.3519	0.2226	1.5806	0.1324
IL-4	-0.5797	0.4053	-1.4303	0.161
GM-CSF	0.7472	0.5259	1.421	0.1611
IP-10	-0.2137	0.2128	-1.0041	0.3198
IL-6	0.343	0.3463	0.9906	0.3267
IL-33	-0.3477	0.5094	-0.6825	0.4978
MIP-1β	-0.1515	0.2339	-0.6477	0.5199
TNF-α	-0.164	0.2603	-0.6299	0.5315
IL-5	0.2961	0.6058	0.4888	0.627
MCP-1	-0.1274	0.27	-0.4719	0.6389
IL-23	-0.4027	0.8942	-0.4503	0.6543
IL-2	-0.665	1.8233	-0.3647	0.7208
IL-13	0.0477	0.4816	0.099	0.9215
G-CSF	-0.0255	0.3658	-0.0696	0.9447

Beyond the independent effects of homelessness and SES, there were significant associations with age, sex, alcohol consumption and smoking uncovered across models. These effects, along with the effects of homelessness (Social Exclusion) and SES are presented graphically in forest plots below (fig 5.5; fig 5.6). Significant predictors are denoted by red asterisks while the strength and direction of associations are denoted by the position of black dots along the horizontal (left = negative, right = positive) – position is determined by the magnitude and sign of the relevant regression

coefficients. Whiskers on each dot indicate the 95% confidence intervals for the regression coefficients.

In the models with homelessness as the main predictor, male sex appeared negatively associated with IFN- γ , IL-2, IL-12p70 and IP-10, and positively associated with IL-17A. Age was positively associated with TNF- α alone. Pack year smoking history was positively associated with IFN- γ , IL-10 and TNF- α , while weekly alcohol consumption showed positive association with IL-18 and IP-10.

Adjusted Effects of Social Exclusion and Covariates on Cytokine Levels

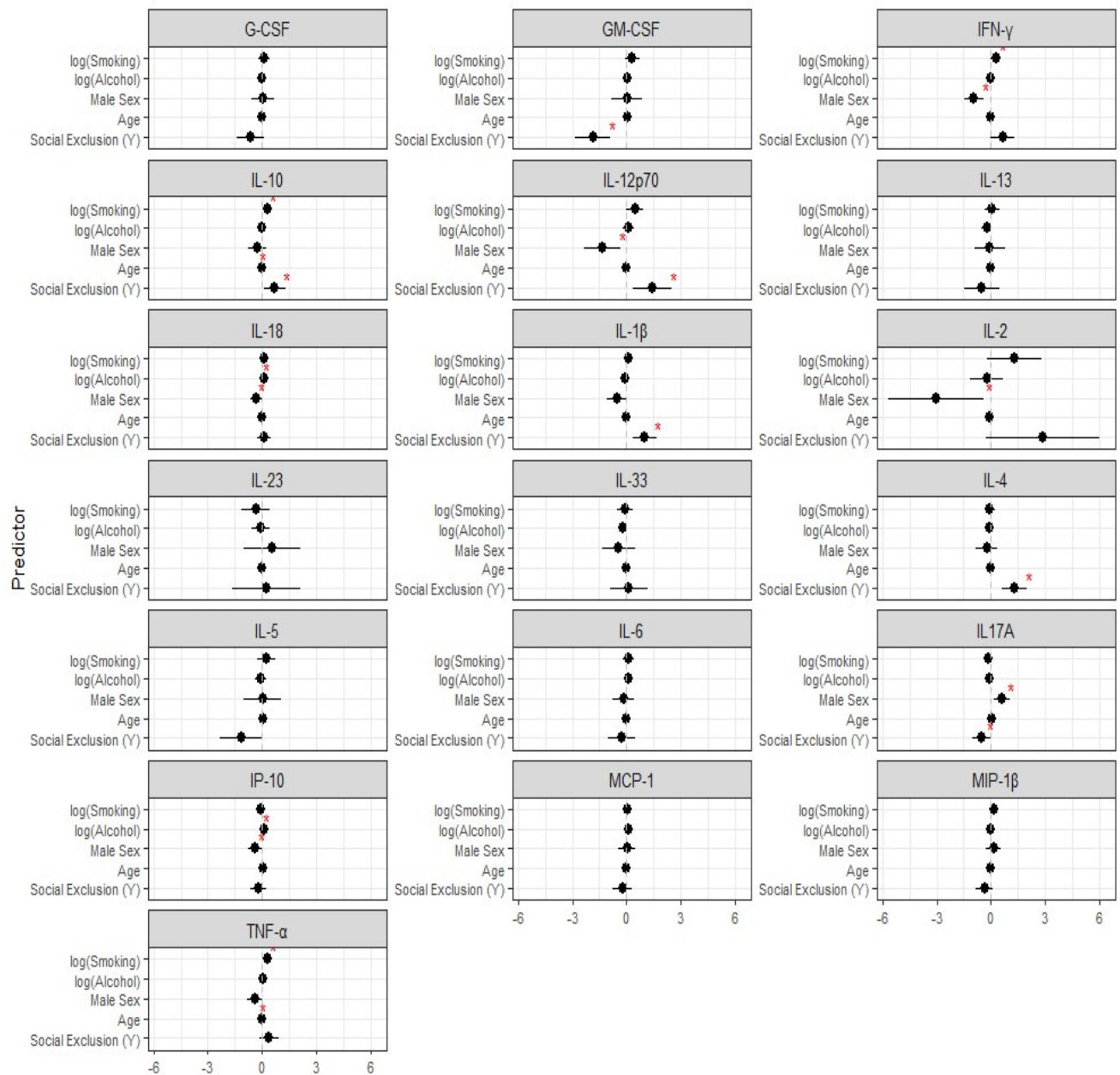


Figure 5.5 Adjusted Effects of Homelessness and Covariates on Cytokine Levels: Estimated effect sizes (black points) and 95% confidence intervals (horizontal lines) from linear regression models of log-transformed cytokine levels. The main predictor is Homelessness (Social Exclusion Y), with additional covariates including Age, Sex, log-transformed weekly units of alcohol consumed, and log-transformed pack year smoking history. Negative coefficients indicate lower log-cytokine levels relative to the reference category, whereas positive coefficients indicate higher levels. Red stars indicate coefficients that are statistically significant at $p < 0.05$. All models are adjusted for the listed covariates simultaneously.

In adjusted models of SES and the 19 cytokines/chemokines (Fig 5.6), age showed association with TNF- α and IL-10. Male sex negatively associated with IFN- γ , IL-18 and IP-10 but showed positive association with IL-17a, consistent with the models including homelessness as main predictor. Regarding the effects of the behavioural covariates, smoking positively associated with IL-10 and TNF- α , while alcohol consumption demonstrated significant relationships with IL-13 (negative) and with IL-18 and IP-10 (positive).

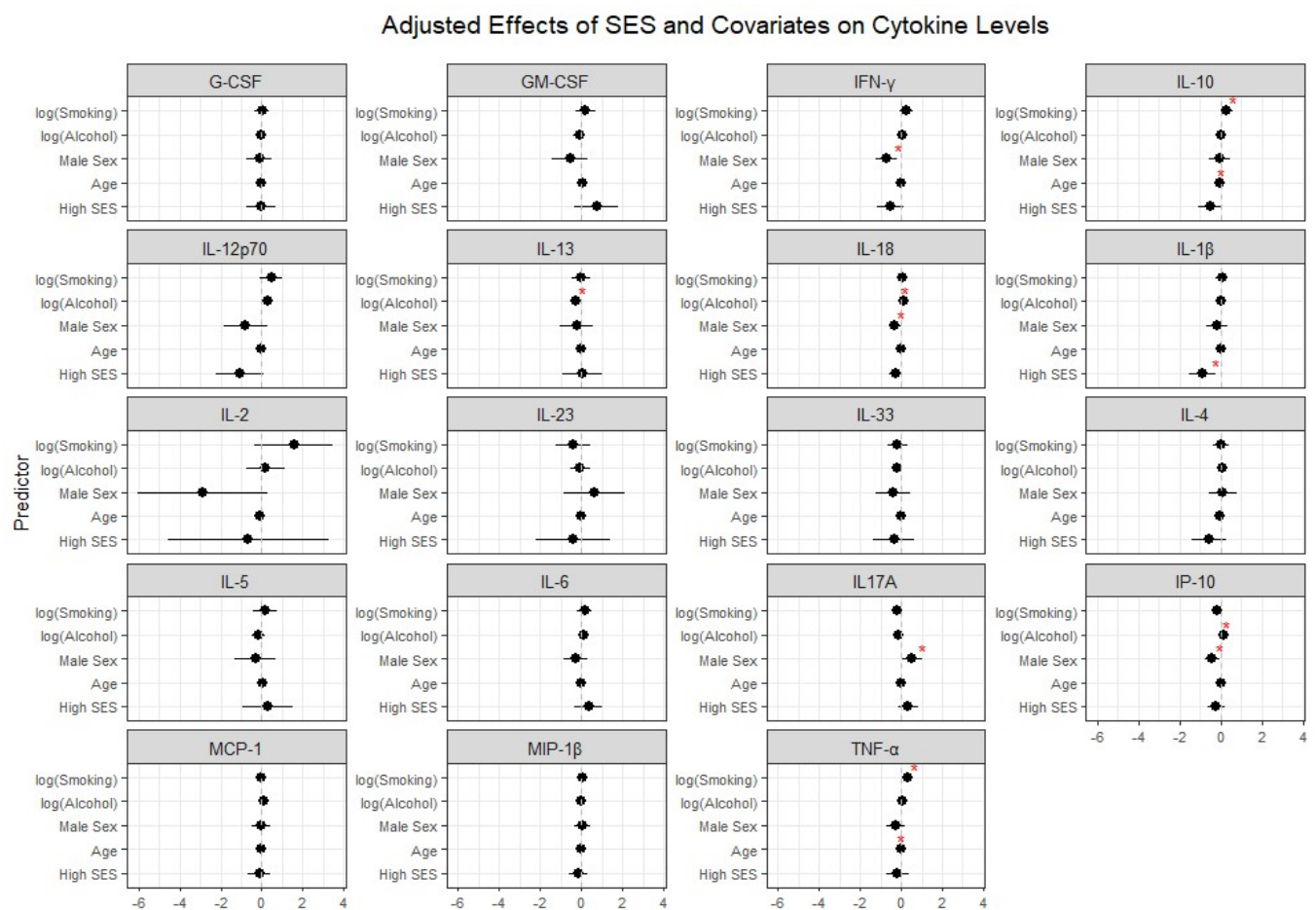


Figure 5.6 Adjusted Effects of Socioeconomic Status and Covariates on Cytokine Levels: Estimated effect sizes (black points) and 95% confidence intervals (horizontal lines) from linear regression models of log-transformed cytokine levels. The main predictor is socioeconomic status (High SES as the reference), with additional covariates including Age, Sex, log-transformed weekly units of alcohol consumed, and log-transformed pack year smoking history. Negative coefficients indicate lower log-cytokine levels for Low SES relative to High SES, whereas positive coefficients indicate higher levels. Red stars indicate coefficients that are statistically significant at $p < 0.05$. All models are adjusted for the listed covariates simultaneously.

5.4.3 Network and Hierarchical Clustering Analysis of Cytokine Profiles

To investigate patterns of coordinated immune activity, correlation- and network-based approaches were applied to log-transformed cytokine/chemokine data. The aim was to identify “modules” exhibiting shared patterns of regulation, potentially reflecting common origins or processes driving the observed immune profiles.

A heatmap of Spearman correlations across the joint cohort is shown in Figure 5.7.

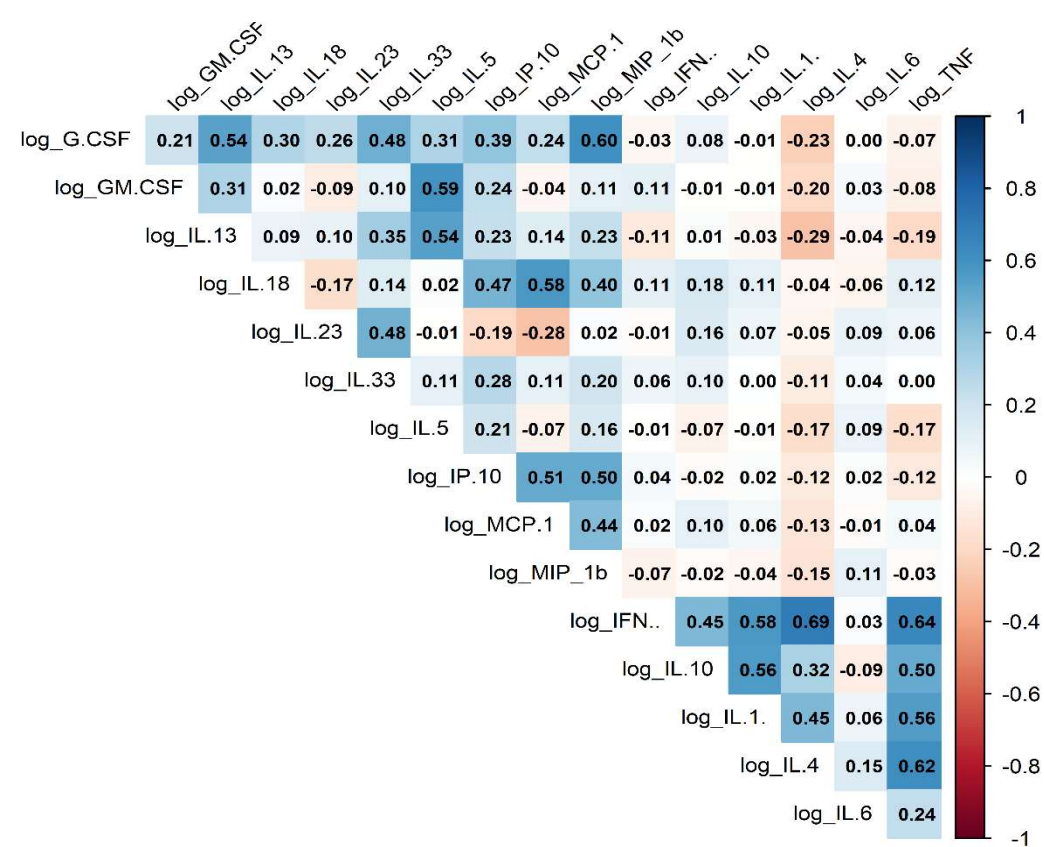


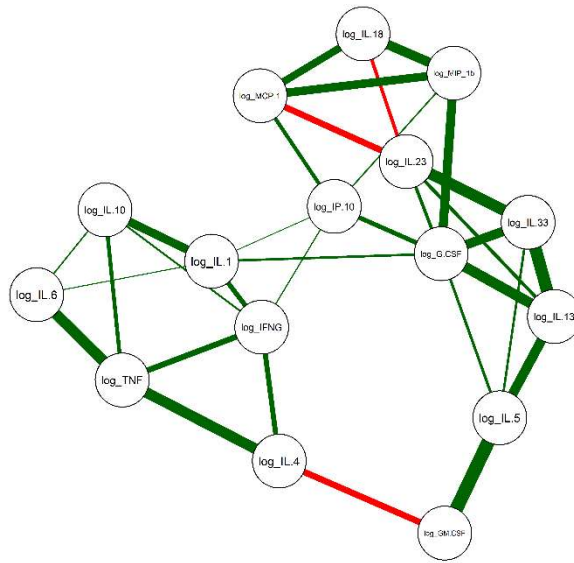
Figure 5.6 Spearman Correlation Matrix of Log-Transformed Cytokine Levels: Pairwise Spearman correlation coefficients are overlain on a heatmap. Positive correlations are indicated in blue, while negative correlations are in red. The strength of the correlation is represented by both colour intensity and numerical values within each cell. This visualisation highlights clusters of cytokines that exhibit coordinated expression patterns.

Co-regulation of cytokine/chemokine production was further explored using correlation-based network analysis. Separate Spearman correlation networks were generated for homeless and housed participants. This approach enabled the visual comparison of cytokine interconnectivity and the overall topological organisation of immune signalling across these two groups.

Notable differences were observed in the architecture of the two networks (Fig 5.8). In the case of homeless participants, a densely interconnected cluster emerged involving IL-23, G-CSF, IP-10, MCP-1, and IL-33. This cluster may reflect a coordinated axis of cytokine/chemokine activity associated with leukocyte mobilisation, chemotaxis, and inflammatory signalling. In contrast, the housed group exhibited a more fragmented architecture with reduced connectivity between these cytokines/chemokines. A shared module of IL-1 β , IFN- γ , IL-10, and TNF- α was apparent in both groups, but appeared more embedded within the wider network in excluded individuals. Red edges in the network diagrams denote negative correlations, highlighting pairs that exhibit reciprocal production patterns, an additional layer of regulation more evident in the excluded group.

These differences suggest that homelessness is associated with altered cytokine network topology, potentially indicating dysregulated or compensatory immune coordination. The emergence of distinct modules under exclusion provides a rationale for further investigation of modular patterns of immune activity.

Socially Excluded



Housed

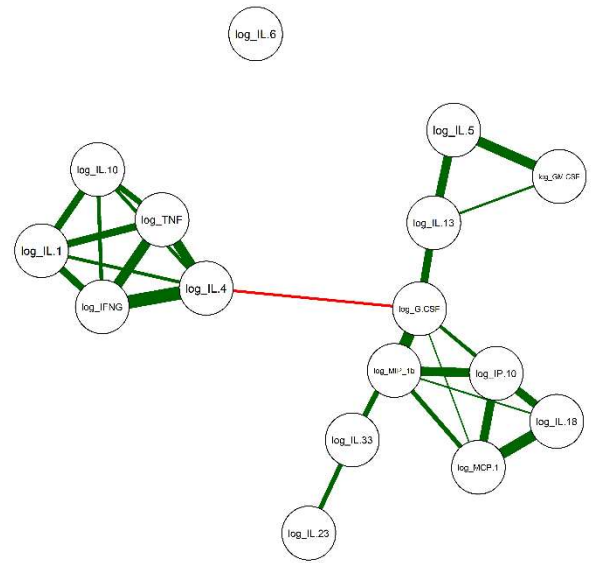
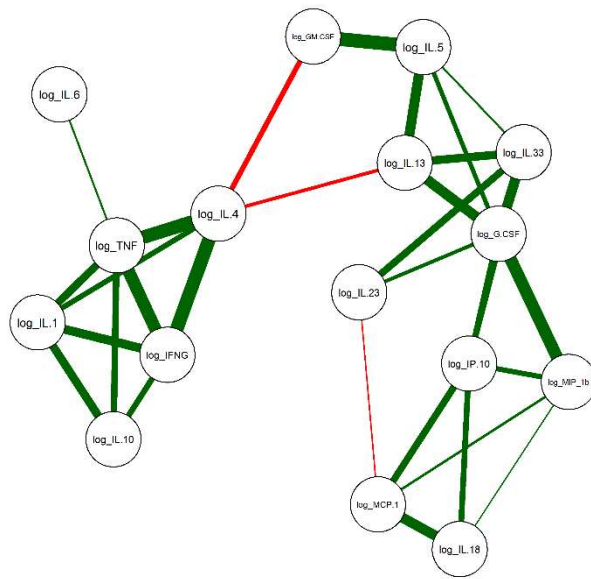


Figure 5.7 Correlation Networks by Social Exclusion Status: This panel displays Spearman correlation networks of log-transformed cytokines/chemokines among homeless (socially excluded) participants (left) versus those who were housed (right). Each node represents a cytokine/chemokine, and edges indicate pairwise Spearman correlations above a threshold ($r \geq 0.2$). The thickness and color intensity of edges correspond to the strength of the correlation – green for positive and red for negative/inverse.

Separate networks for participants classified as low SES and high SES are presented in Figure 5.9. As with the comparison by housing status, distinct topologies were observed. While both SES groups share a cluster involving IL-1 β , IFN- γ , TNF- α , and IL-10, notable differences in overall network density and connectivity patterns were evident. The high SES network appeared more modular, with defined substructures and altered co-association profiles for several cytokines, including IL-23 and IL-33. In contrast, the low SES network showed a more diffuse pattern of connectivity with additional links among IL-4, GM-CSF, and IL-5.

Low SES



High SES

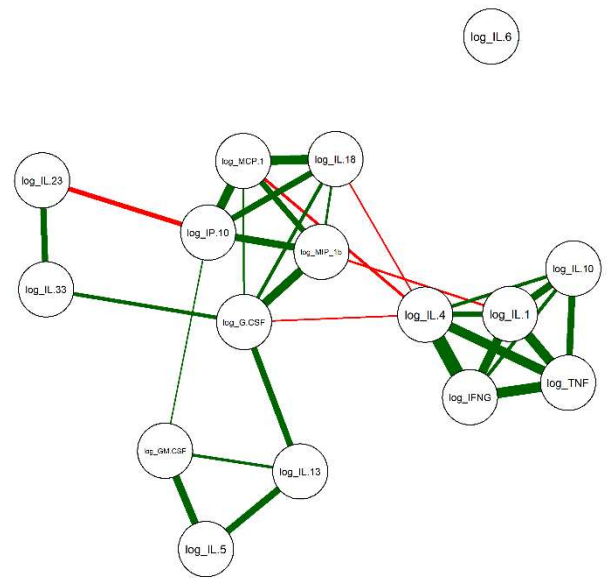


Figure 5.8 Cytokine Correlation Networks by Socioeconomic Status (SES): Spearman correlation networks are shown for low (left) and high(right) SES participants. Nodes denote cytokines, and edges represent pairwise correlations meeting the predefined threshold ($r > 0.2$). Thicker, darker green lines reflect stronger positive associations while red lines represent negative/inverse correlations.

To complement the correlation network analysis, hierarchical clustering was applied to the cytokine correlation matrix to identify broader patterns of similarity in expression profiles. Unlike network visualisations, which emphasise localised pairwise associations in specific sub-cohorts and their relative strength, hierarchical clustering provides a global view of cytokine relatedness based on overall co-expression structure. This approach revealed three major clusters of cytokines/chemokines, represented here as part of a dendrogram (Figure 5.10), with one grouping predominantly composed of pro-inflammatory mediators, another consisting of broadly immunoregulatory cytokines, and a third cluster isolating IL-6, consistent with its poor correlation with other cytokines shown in the correlation matrix plot.

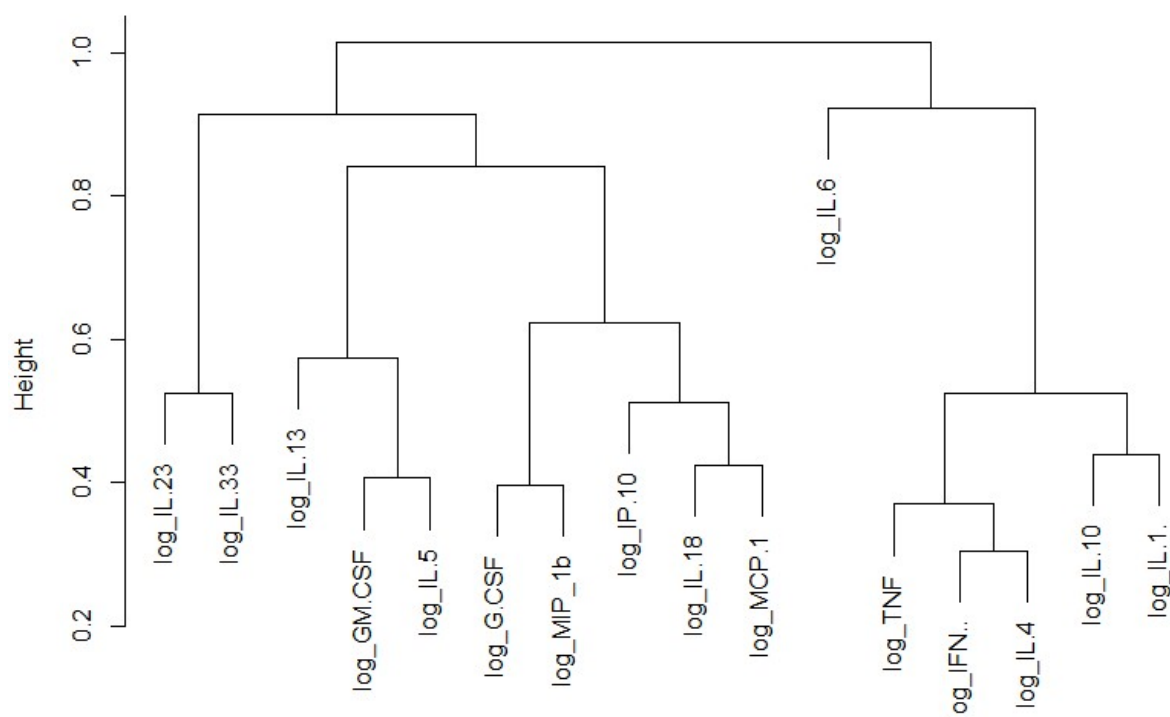


Figure 5.9 Hierarchical Clustering Dendrogram of Log-Transformed Cytokine Levels: Cytokines were clustered using an average linkage method on a distance matrix (“distance” = 1 – Spearman correlation coefficient). The vertical axis reflects the relative dissimilarity among cytokines, with shorter branch lengths indicating greater similarity.

Table 5.5 Cytokine Modules Derived from Hierarchical Clustering: modules obtained from hierarchical clustering of the Spearman correlation matrix of log-transformed cytokine levels. Cytokines were grouped based on their similarity (i.e., high correlations), with the dendrogram used to formally define the clusters. These modules represent cytokines with coordinated expression patterns.

Module	Cytokines
1	G-CSF, GM-CSF, IL-13, IL-18, IL-23, IL-33, IL-5, IP-10, MCP-1, MIP-1 β
2	IFN- γ , IL-10, IL-1 β , IL-4, TNF- α
3	IL-6

To quantify group differences in cytokine module activity, average z-scores were computed for each participant across the three modules identified by hierarchical clustering. These scores reflect relative expression within each

module. As shown in Figure 5.11 statistically significant differences were observed for Module 2, with higher scores among low SES and homeless participants. Module 2 comprised IFN- γ , IL-10, IL-1 β , IL-4, and TNF- α , cytokines broadly associated with pro-inflammatory and immune-regulatory responses, suggesting greater activity of these pathways in low SES groups. A more modest elevation in Module 1 scores was also observed in the low SES group. This module included G-CSF, GM-CSF, IL-13, IL-18, IL-23, IL-33, IL-5, IP-10, MCP-1, and MIP-1 β , encompassing chemokines and myeloid-derived cytokines often involved in immune cell recruitment and innate activation. No significant group differences were detected for Module 3, containing IL-6 on its own.

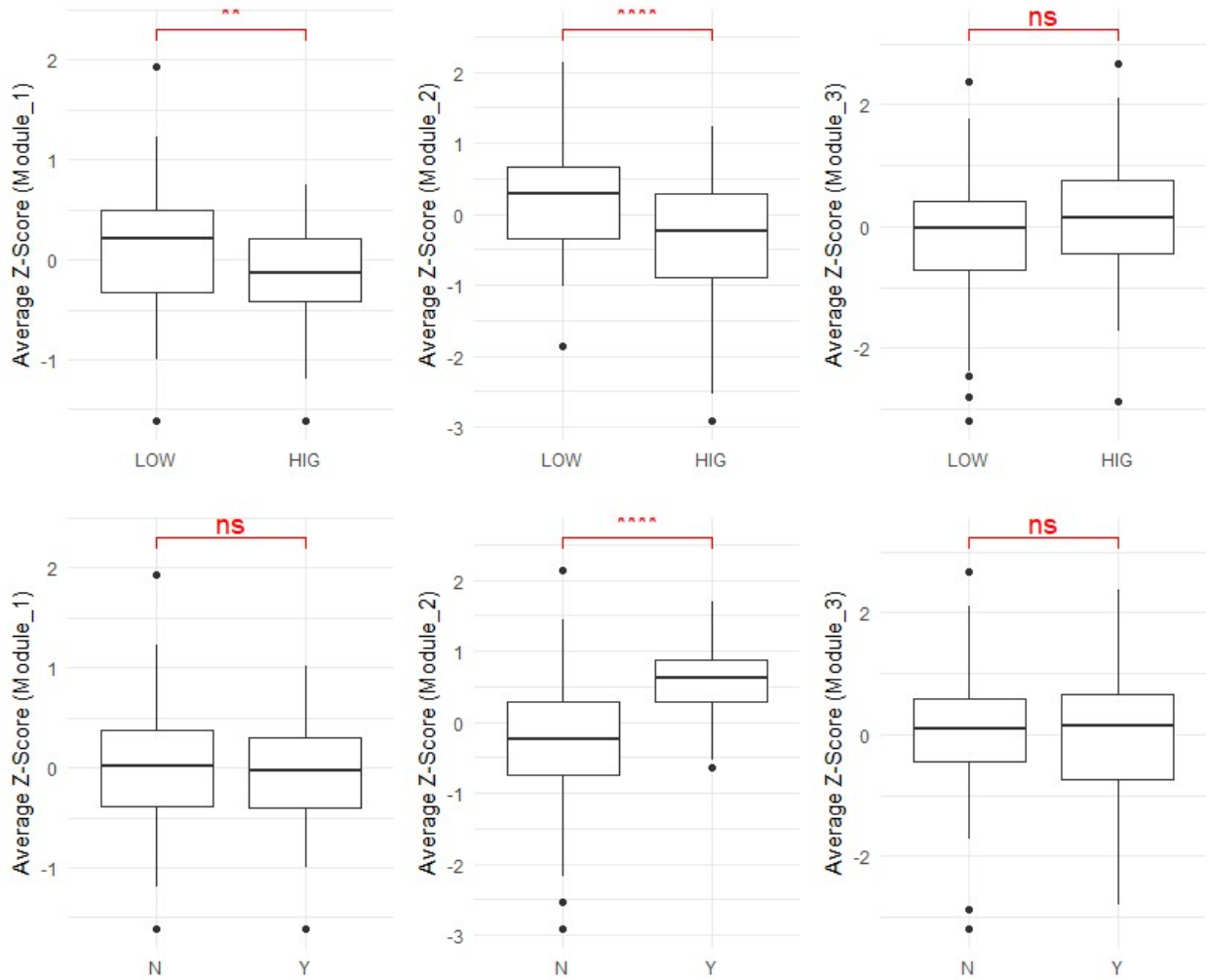


Figure 5.10 Boxplots of Module-Level Z-Scores by SES and Social Exclusion: These boxplots display each module's average z-score for participants grouped by SES (top) and homelessness (bottom). Whiskers extend to 1.5 times the interquartile range, and any points beyond these limits are shown as outliers.

5.4.4 Principal Component Analysis and PERMANOVA of Cytokine Variation

Finally, to build upon and confirm what has been shown using network- and module-based approaches, principal component analysis (PCA) was conducted to identify independent patterns of immune variation. The first five principal components (PCs) accounted for a cumulative 67.6% of the variance in cytokine expression. PC1 (21.2%) was primarily driven by G-CSF, IP-10, and MIP-1 β , while PC2 (17.5%) reflected a combination of IFN- γ , IL-1 β , IL-4, TNF- α , and IL-10, closely resembling the immune-regulatory cluster observed in

network and modular analyses. The five largest loading cytokines/chemokines and the variance explained by each principal component are set out in table 5.6 below.

Table 5.6 Summary of the First Five Principal Components in PCA of Log-Transformed Cytokines: The first five principal components (PCs), the percentage of variance explained and the top five contributing cytokines with their loadings. Loadings indicate the direction and strength of each cytokine's correlation with the principal component. All analyses are performed on log-transformed cytokine concentrations.

Principal Component	Variance Explained (%)	Top 5 Contributing Cytokines (Loadings)				
PC1	21.2	G-CSF:	IP-10:	MIP-1 β :	IL-13:	IL-18:
		0.41	0.4	0.37	0.32	0.3
PC2	17.5	IFN- γ :	IL-1 β :	IL-4:	TNF α :	IL-10:
		-0.47	-0.43	-0.42	-0.41	-0.33
PC3	12.3	IL-23:	MCP-1:	IL-5:	IL-18:	IL-13:
		0.43	-0.43	0.36	-0.35	0.34
PC4	9.1	GM-CSF:	IL-23:	IL-5:	IL-33:	G-CSF:
		-0.54	0.46	-0.45	0.36	0.29
PC5	7.5	IL-6:	IL-10:	MIP-1 β :	IL-13:	IL-23:
		-0.89	0.29	-0.24	0.23	-0.23

To visually represent separation of participants by these principal components a scatterplot of participants overlaid on the first two principal components was generated, with separate plots for housing status and SES (Figure 5.12). Despite significant overlap of the groups on these axes, this visual approach allowed for the appreciation of subtle differences and justified further analysis.

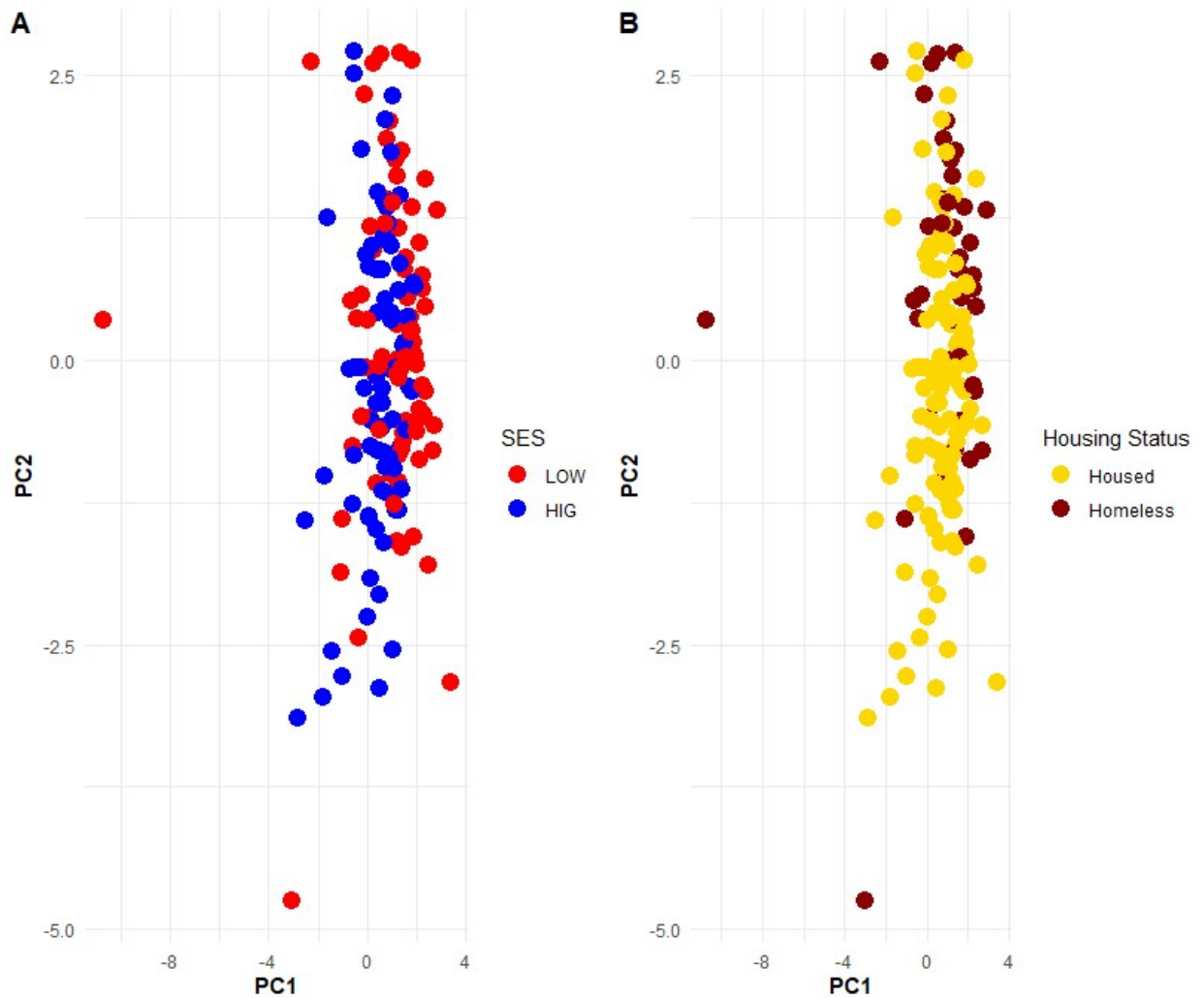


Figure 5.11 PCA Scatter Plots of Participants Coloured by SES (A) and Housing Status (B): Each point represents an individual participant's position on the first two principal components (PC1 on the x-axis, PC2 on the y-axis) derived from log-transformed cytokine data. In Panel A, participants are coloured by SES (red for Low, blue for High). In Panel B, participants are coloured by Housing Status (gold for Housed, dark red for Homeless).

To more formally test whether groups differed across the five principal components, PERMANOVA (Permutational Multivariate Analysis of Variance) was applied. The results, summarised in Table 5.7, revealed significant group-level differences for PC2 and PC5, indicating that variation in cytokine expression is structured by both SES and housing status. PC2 was most strongly influenced by IFN- γ , IL-1 β , IL-4, TNF- α , and IL-10, cytokines that collectively defined the immune-regulatory module in both network and

hierarchical clustering analyses. PC5 was dominated by IL-6, reinforcing its unique behaviour across analyses. These findings mirror earlier observations, providing convergent evidence that social adversity is associated with coordinated alterations in systemic immune activity.

Table 5.7 PERMANOVA Results for the First Five Principal Components of Log-Transformed Cytokine Levels: Degrees of freedom, sum of squares, percentage of variance explained (R^2), F statistic, and p-value for each PC when modelling the effects of SES and Housing Status (SocialExclusion). Notably, PC2 and PC5 exhibit statistically significant differences between groups, suggesting that these components capture variation related to socioeconomic and housing conditions.

Principal Component	Degrees of Freedom	Sum of Squares	% Variance Explained	F Statistic	p value
PC1	2	33.31	2.13	1.85	0.156
PC2	2	26.78	8.92	8.32	0.001***
PC3	2	1.88	1.08	0.93	0.408
PC4	2	0.73	0.55	0.47	0.649
PC5	2	5.10	4.81	4.29	0.019*

5.4.5 Low Socioeconomic Status is Associated with a More Mature, Terminally Differentiated Lymphocyte Immunophenotype

Lymphocyte immunophenotyping was carried out to capture the identity and differentiation states of a variety of circulating immune cells in participants drawn from the AESS-Vax cohort. The goal of this analysis was to explore whether the composition and phenotypic characteristics of circulating immune cells differ by socioeconomic and housing status, with a particular focus on identifying phenotypic features associated with social adversity, and in this case, low socioeconomic status.

Table 5.8 presents descriptive statistics for the major T cell, NK cell, and innate T cell subsets captured using spectral flow cytometry. Measures reported include absolute counts and relative frequencies of lymphocyte populations, as well as expression of activation and exhaustion markers within CD4+ and CD8+ compartments. The gating strategy used to identify populations is reported in Specific Methods, section 5.3.7.

Table 5.8 Descriptive Statistics for Lymphocyte Populations Identified by Immunophenotyping Spectral Cytometry Panel: Figures reported are percentages of parent populations (identified in brackets), unless stated as counts. Mean, standard deviation (SD), median and interquartile range (IQR) are reported as distributions of results for different populations vary.

Population	Mean	SD	Median	IQR	Population	Mean	SD	Median	IQR
Count Single Cells	836636.5	554611.9	776000	738132.8	NK Cells (% Live CD45 ⁺)	11.6	6.64	9.86	8.81
Count CD45⁺ Lives	343644.2	262070	259253	356863.5	NK Bright (%NK)	9.84	7.19	8.18	5.70
Count CD4⁺	109426.4	87444	85053	99795.25	NK Dim (%NK)	89.67	8.12	91.8	6.38
Count CD8⁺	41062	41292	29238	50436.5	iNKTs (% Live CD45 ⁺)	0.59	0.45	0.40	0.59
CD4⁺ Naïve (%CD4 ⁺)	30.1	18.4	29.5	29.15	MAITs (% Live CD45 ⁺)	3.01	2.04	2.62	1.77
CD4⁺ Effector memory (%CD4 ⁺)	38.1	21.7	37.15	31.1	CD4⁺ CD57⁺ (%CD4 ⁺)	5.47	3.94	4.32	3.30
CD4⁺ Central Memory (%CD4 ⁺)	19	13.5	16.4	11.5	CD4⁺ KLRG1⁺ (%CD4 ⁺)	5.11	5.65	3.82	4.11
CD4⁺ TEMRA (%CD4 ⁺)	12.8	10	10.65	8.34	CD4⁺ PD1⁺ (%CD4 ⁺)	1.26	0.89	1.08	0.95
CD8⁺ Naïve (%CD8 ⁺)	40.1	19.8	40	24.18	CD4⁺ TIGIT⁺ (%CD4 ⁺)	5.72	3.72	5.23	3.76
CD8⁺ Effector Memory (%CD8 ⁺)	25.1	13.8	22.05	19.78	CD4⁺ NKG2D⁺ (%CD4 ⁺)	3.42	3.89	1.91	2.83

CD8⁺ Central Memory (%CD8 ⁺)	12.7	9.48	10.9	8.59	CD4⁺ Ki67 (%CD4 ⁺)	3.02	1.52	2.55	1.59
CD8⁺ TEMRA (%CD8 ⁺)	22.1	13.8	19.15	12.6	CD4⁺ CD69⁺ (%CD4 ⁺)	3.9	3.43	3.22	2.89
CD4⁺ Early EM (%CD4 ⁺ EM)	77.7	10.9	79.7	12.08	CD4⁺ CD137⁺ (%CD4 ⁺)	0.99	0.77	0.72	0.89
CD4⁺ Early-Like EM (%CD4 ⁺ EM)	13.2	7.6	12.35	8.92	CD4⁺ CD154⁺ (%CD4 ⁺)	1.18	0.77	0.92	0.74
CD4⁺ Intermediate EM (%CD4 ⁺ EM)	4.4	5.84	1.55	4.08	CD4⁺ CD161⁺ (%CD4 ⁺)	14.1	6.91	12.9	7.66
CD4⁺ Terminal EM (%CD4 ⁺ EM)	4.68	2.47	4.19	2.77	CD8⁺ CD57⁺ (%CD8 ⁺)	5.47	3.94	25.55	22.75
CD4⁺ RA Terminal Effector (% CD4 ⁺ TEMRA)	11.1	12.4	6.43	11.38	CD8⁺ KLRG1⁺ (%CD8 ⁺)	17.6	16.4	12.55	21.13
CD8⁺ Early EM (%CD8 ⁺ EM)	50.9	16.4	54.05	21.33	CD8⁺ PD1⁺ (%CD8 ⁺)	4.04	3.24	3.19	2.13
CD8⁺ Early-Like EM (%CD8 ⁺ EM)	10.4	6.38	9.69	8.105	CD8⁺ TIGIT⁺ (%CD8 ⁺)	6.3	7.33	5.18	4.17
CD8⁺ Intermediate EM (%CD8 ⁺ EM)	18.7	16.1	11.8	17.87	CD8⁺ NKG2D⁺ (%CD8 ⁺)	7.56	6.67	5.65	7
CD8⁺ Terminal EM (%CD8 ⁺ EM)	20	12.1	18.95	13.45	CD8⁺ Ki67⁺ (%CD8 ⁺)	2.22	1.13	2.04	1.25
CD8⁺ RA TEMRAs (%CD8 ⁺ TEMRA)	28.1	23.2	18.5	33.75	CD8⁺ CD69⁺ (%CD8 ⁺)	3.66	2.6	3.25	2.02
Tregs (%CD4⁺)	5.49	2.54	4.96	2.86	CD8⁺ CD137⁺ (%CD8 ⁺)	6.72	10.4	3.42	5.47
Vδ1 (% Live CD45⁺)	0.59	0.53	0.41	0.64	CD8⁺ CD154⁺ (%CD8 ⁺)	1.42	0.75	1.28	0.48

Vδ2 (% Live CD45⁺)	2.6	3.28	1.64	2.23	CD8⁺ CD161⁺ (%CD8⁺)	9.74	7.99	7.43	5.68

The first lymphocyte populations examined were innate-like and unconventional T cells, including mucosal-associated invariant T (MAIT) cells, invariant natural killer T (iNKT) cells, and $\gamma\delta$ T cells (Vδ1 and Vδ2 subsets). These populations were identified early in the gating strategy and analysed as a proportion of total live CD45+ lymphocytes.

While there was no specific hypothesis related to these populations at the outset MAIT and Vδ2 cell frequencies appear significantly reduced in low SES participants, whereas iNKT and Vδ1 frequencies showed no significant differences between groups (Fig 5.13). The decreased abundance of circulating MAIT and Vδ2 cells in the low SES group may reflect the impact of chronic antigenic stimulation associated with low SES. Repeated stimulation at mucosal and barrier sites can promote terminal differentiation, apoptosis, or tissue egress, depleting these cells from the circulation. This is consistent with reports showing that MAIT cells are especially sensitive to chronic microbial challenge¹⁸⁶ and that circulating Vδ2 cells are more prone to depletion under persistent antigen exposure than the more tissue-resident Vδ1 subsets¹⁸⁷.

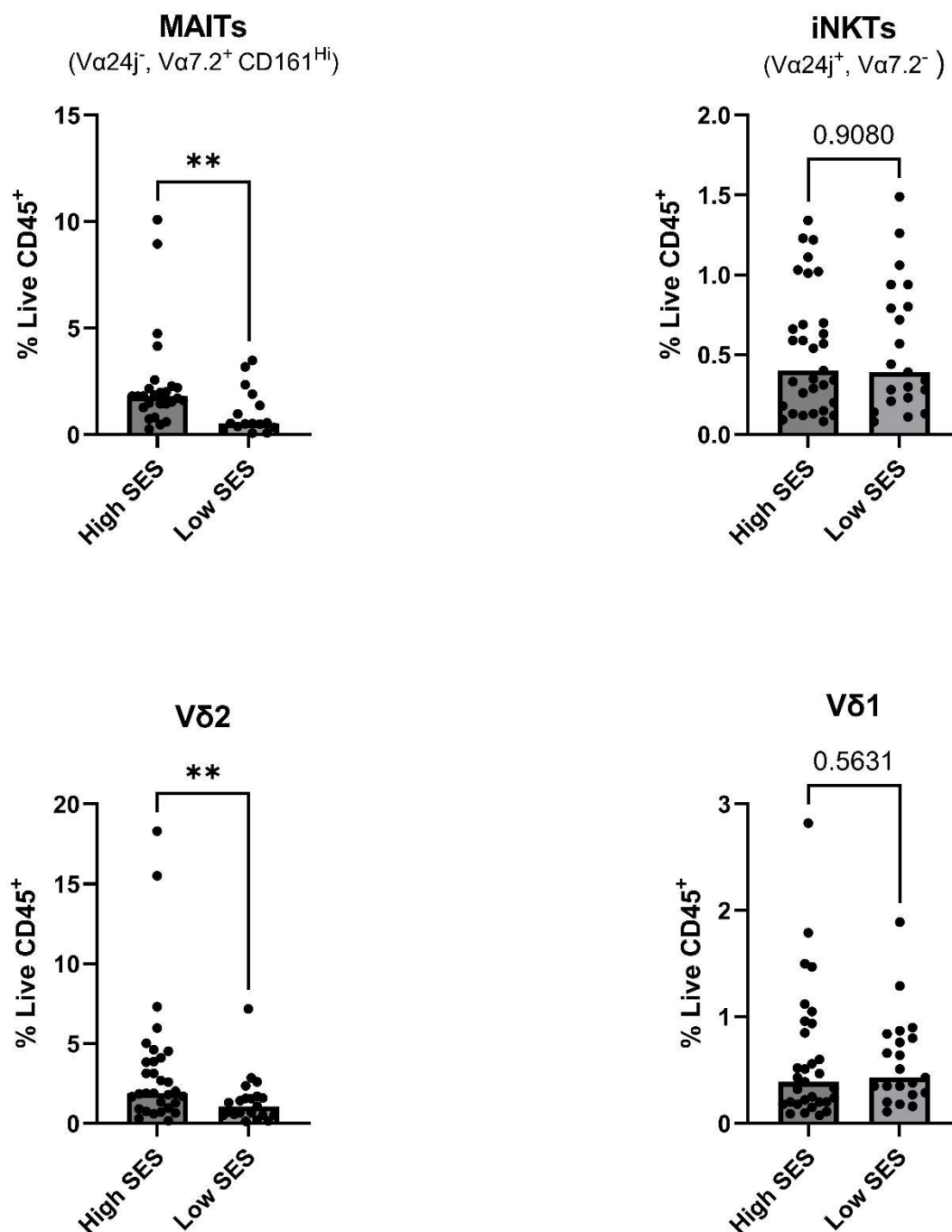


Figure 5.12 Innate-Like Lymphocytes as Proportion of Live CD45⁺ Cells in High and Low Socioeconomic Status Participants: Statistical tests shown are Mann-Whitney U-tests on high (n=31) and low (n=21) participants.

Next, T lymphocytes were identified as conventional CD3⁺CD56⁻ T cells that lacked markers of innate-like or unconventional T cell lineages (i.e., Vα7.2⁺,

V α 24J⁺, V δ 1⁺, and V δ 2⁺). Differences in the frequencies of major T cell subsets across SES groups are illustrated in Figure 5.14. A modest but statistically significant reduction in the proportion of CD4⁺ T cells was observed in low SES participants, while no significant differences were noted in CD8⁺ T cells.

Notably, the frequency of regulatory T cells (T_{regs}; CD4⁺CD25⁺CD127⁻) was significantly increased in the low SES group. This observation aligns with previous findings showing that psychosocial stress can promote T_{reg} expansion, potentially through sustained glucocorticoid signalling and prolonged sympathetic nervous system activation, both of which are known to enhance regulatory T cell induction and stability^{188–190}. Additionally, in settings where chronic antigen exposure and immune activation are more likely, as may be the case for low SES^{191,192} and excluded individuals^{10,193,194}, due to increased occupational and domestic exposure to pathogens, T_{regs} may proliferate to limit immune-mediated damage and maintain homeostasis. Together, these mechanisms could reflect a shift toward a more immunosuppressive phenotype in adversity-exposed individuals, which may impair immune responsiveness and surveillance.

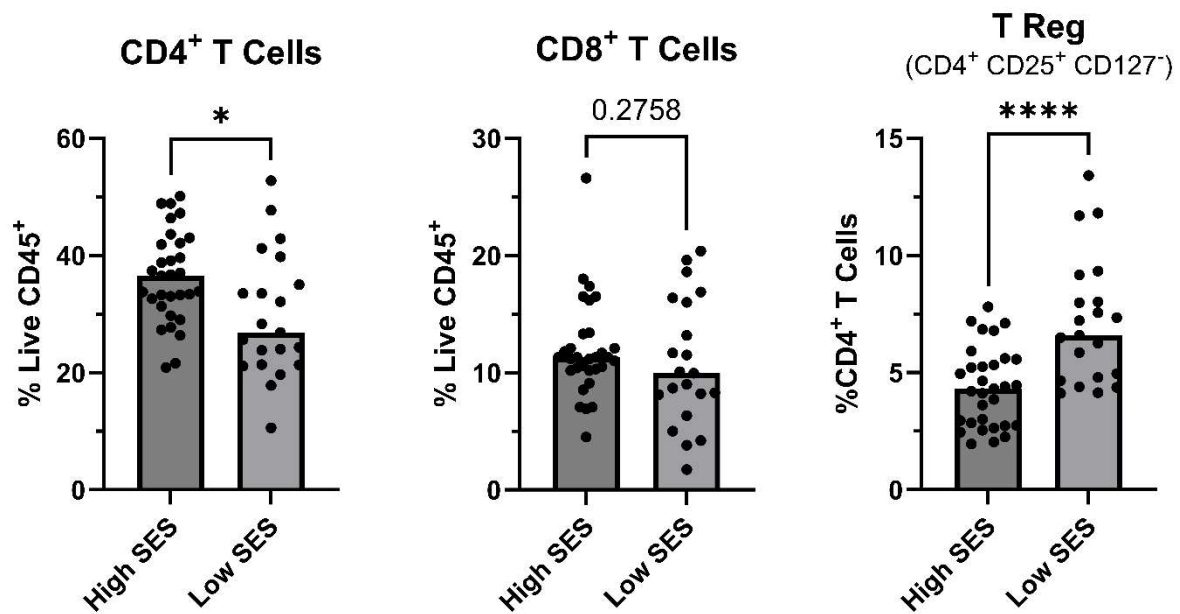


Figure 5.13 CD4⁺, CD8⁺ and T Regulatory Lymphocytes by Socioeconomic Status: Statistical tests shown are Mann-Whitney U-tests on high (n=31) and low (n=21) participants.

To build on basic observations of T lymphocyte populations, CD4⁺ and CD8⁺ subsets were examined based on the expression of CCR7 and CD45RA, markers that delineate naïve, central memory, effector memory, and terminally differentiated (TEMRA) populations. As shown in Figure 5.15 clear differences were observed in T cell subset distribution by SES group.

Low SES participants exhibited a significantly reduced frequency of naïve CD4⁺ and CD8⁺ T cells, alongside an increased representation of CD4⁺ and CD8⁺ TEMRAs, a highly differentiated, often senescent population associated with repeated antigenic stimulation and chronic stress. Although differences in central and effector memory populations were less pronounced, the overall pattern is consistent with a shift toward a more antigen-experienced and differentiated T cell phenotype in individuals from lower socioeconomic backgrounds.

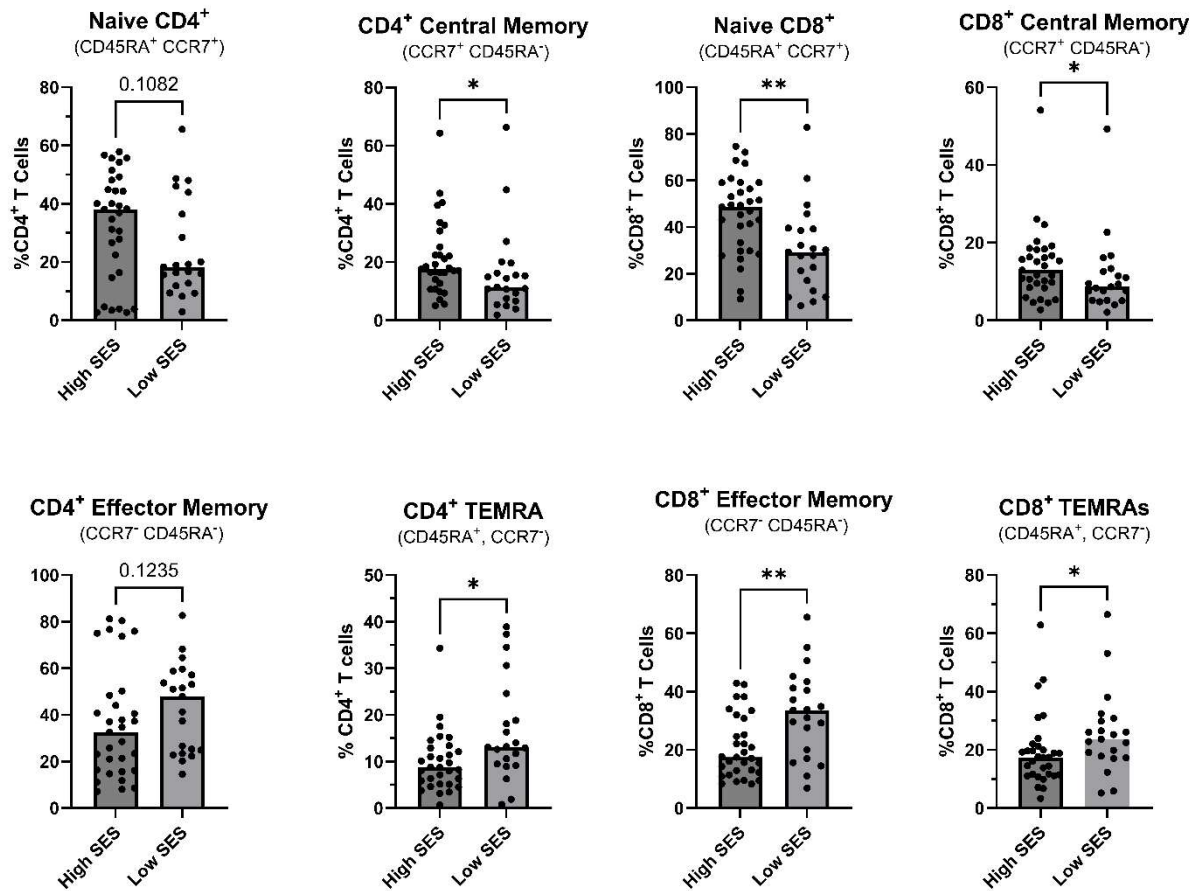


Figure 5.14 CD4⁺ and CD8⁺ Lymphocyte Subsets Defined by Expression of CCR7 and CD45RA Shown by Socioeconomic Status: Statistical tests shown are Mann-Whitney U-tests on high (n=31) and low (n=21) participants. Shown here are Naive, Central Memory, Effector Memory and T Effector Memory Re-expressing CD45RA (TEMRA) subsets.

To complement analyses of T cell subsets, Effector memory T cells (T_{EM}) were further characterised by stratifying CD4⁺ and CD8⁺ subsets into early, intermediate, and terminal effector phenotypes based on the expression of co-stimulatory receptors, CD27 and CD28. These stages reflect cumulative antigen experience, with repeated stimulation driving progressive loss of CD27 and CD28. As shown in Figure 5.16, participants from low SES backgrounds exhibited significantly higher frequencies of CD8⁺ terminal

effector T cells (CD27⁻CD28⁻), along with an increase in early effectors. No significant differences were observed among CD4⁺ subsets.

In parallel, RA TEMRA subsets, defined by re-expression of CD45RA and loss of CD27 and CD28, were also examined to identify the most terminally differentiated and senescent cells. Although group differences in RA TEMRAs did not reach statistical significance, both CD4⁺ and CD8⁺ compartments showed a clear trend toward higher frequencies in low SES participants.

The increased proportion of terminal effectors within the CD8⁺ T_{EM} pool and trend observed in RA TEMRAs supports the notion of an adversity-associated shift toward more differentiated, senescent T cell phenotypes, consistent with the broader patterns identified across the chapter.

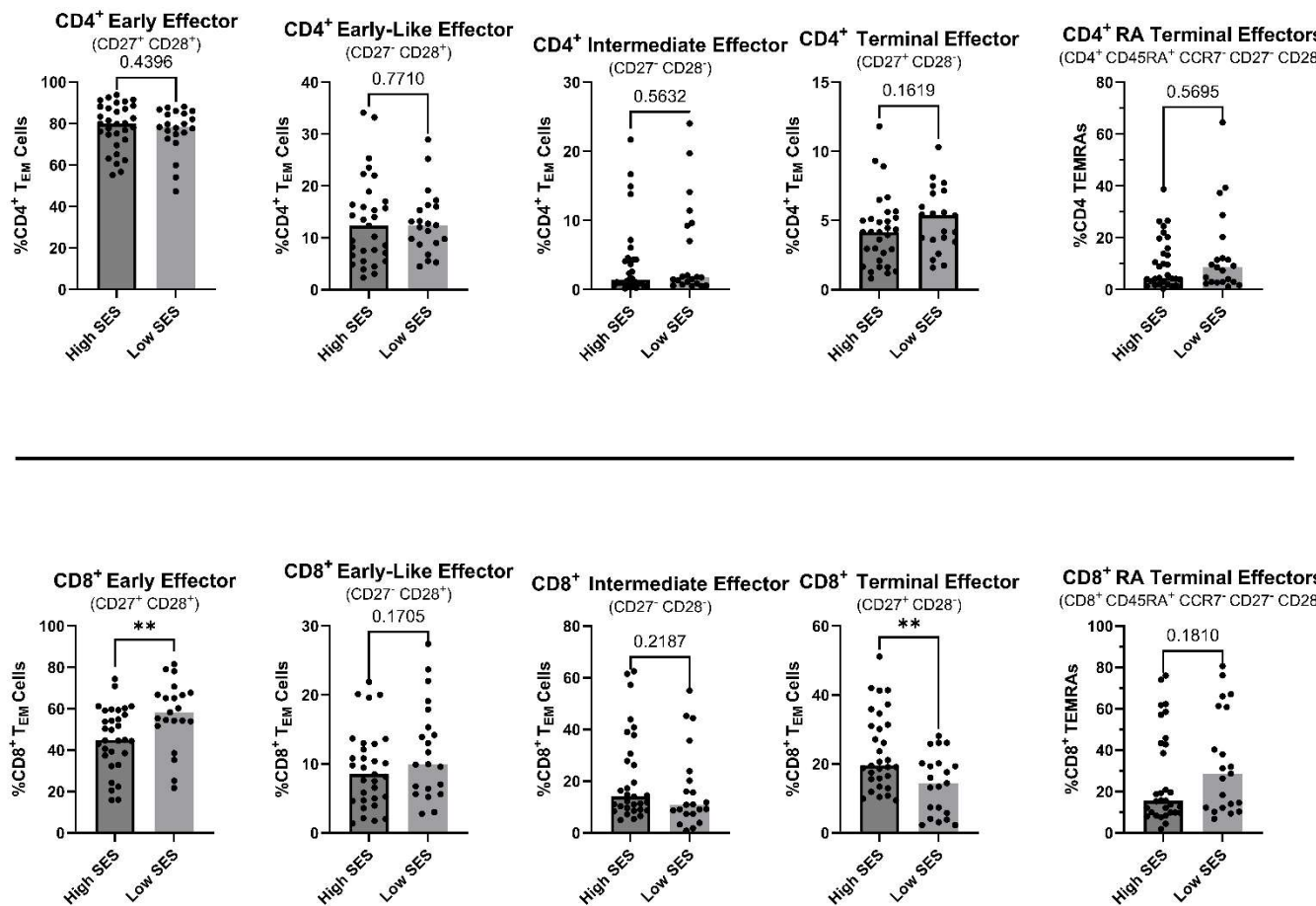


Figure 5.16 CD4⁺ and CD8⁺ Effector Memory Subsets as Proportion of T_{EM}s and RA-TEMRA as a Proportion of TEMRA; Defined by CD27 and CD28 Expression, Shown by Socioeconomic Status: Statistical tests shown are Mann-Whitney U-tests on high (n=31) and low (n=21) participants.

To further assess the differentiation and functional state of CD4⁺ and CD8⁺ cells, the expression of common senescence and exhaustion markers was analysed (Figure 5.17). Across both compartments, a clear pattern emerged, with individuals from low SES backgrounds displaying significantly higher frequencies of cells expressing markers of senescence (CD57 and KLRG1) and exhaustion (PD-1 and TIGIT). These phenotypic changes are consistent with an immunological profile characterised by accumulated terminal differentiation and regulatory exhaustion. Although the functional capacity of these cells was not directly assessed, the increased expression of CD57,

KLRG1, PD-1, and TIGIT suggests a shift toward a state of reduced proliferative potential and immune responsiveness. While such phenotypes have been widely linked to chronic immune activation, it is also plausible that sustained psychosocial stress contributes to these outcomes through neuroendocrine signalling and altered immune regulation.

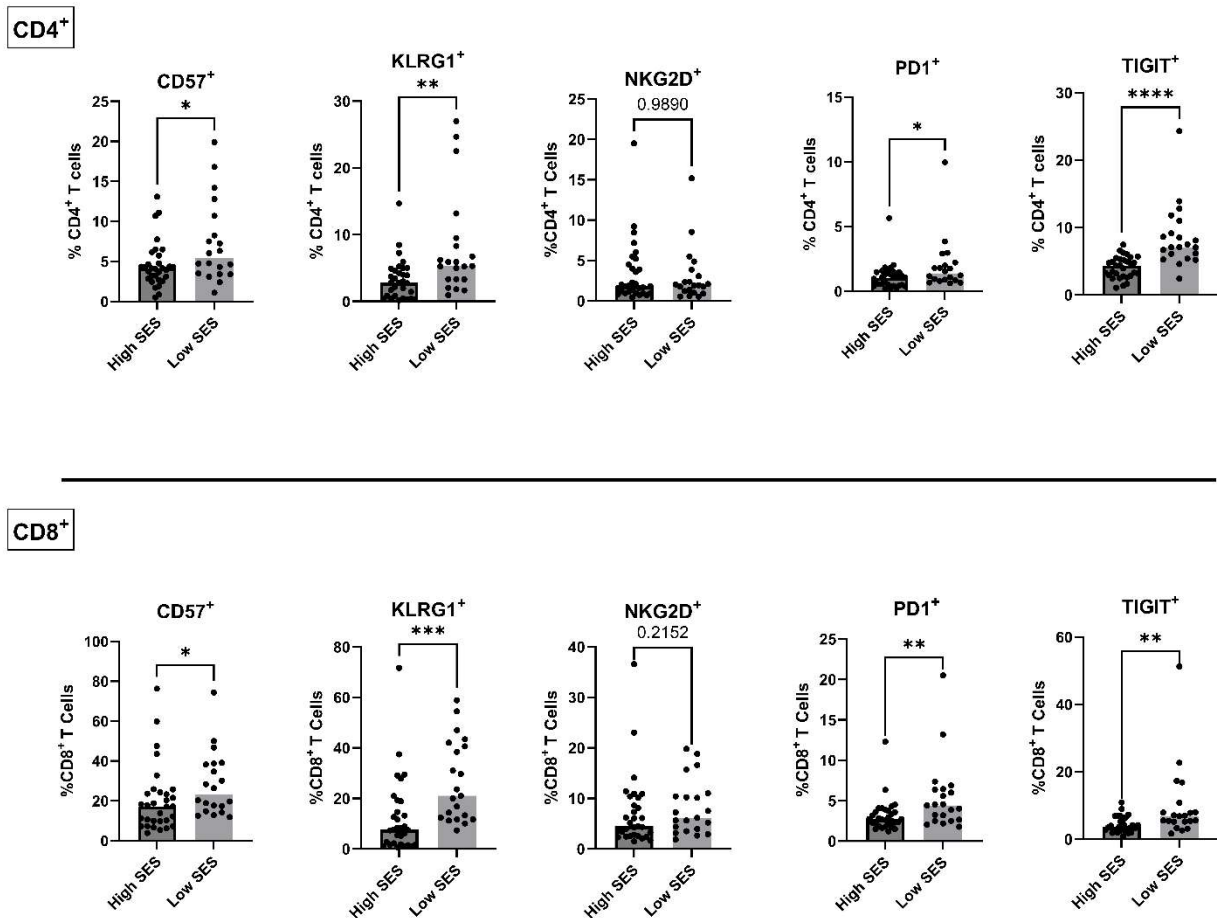
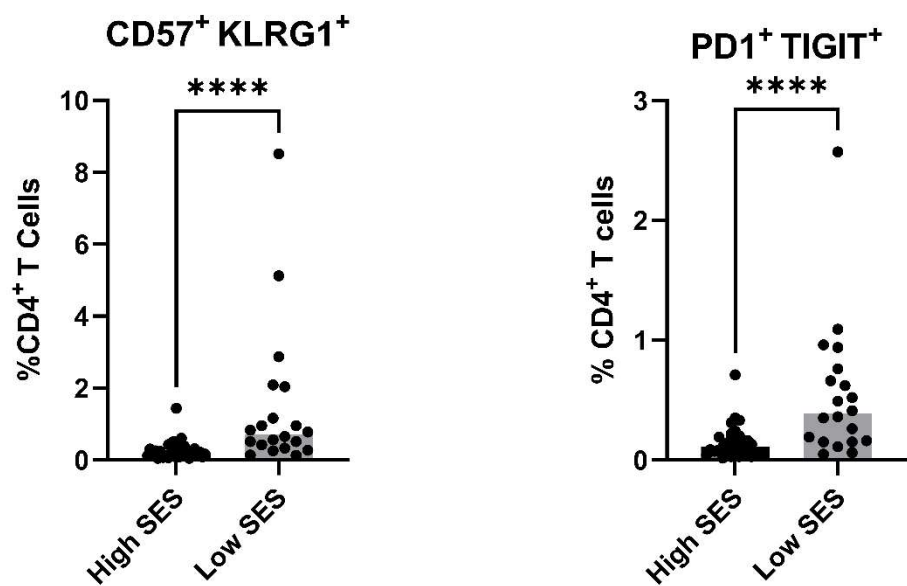


Figure 5.17 Expression of Common Senescence and Exhaustion Markers on CD4⁺ and CD8⁺ T Lymphocytes by Socioeconomic Status: Shown here are senescence markers (CD57, KLRG1, NKG2D) and exhaustion markers (PD1 and TIGIT). Statistical tests shown are Mann-Whitney U-tests on high (n=31) and low (n=21) participants.

To validate and extend findings from single-marker analyses, co-expression of senescence (CD57⁺KLRG1⁺) and exhaustion (PD-1⁺TIGIT⁺) markers was assessed in both CD4⁺ and CD8⁺ T cells (Figure 5.18). Strikingly, the proportion of T cells co-expressing these marker pairs was significantly higher in participants from low SES backgrounds. Co-expression provides a more robust indication of terminal differentiation and functional impairment than individual marker analysis alone. This provides stronger evidence for an expansion of senescent and exhausted T cell populations in both CD4⁺ and CD8⁺ compartments. Co-expression of these markers is often associated with diminished proliferative capacity and impaired effector function, supporting the notion that chronic social stress and environmental exposures in low SES contexts contribute to the emergence of a more immunosenescent and potentially less responsive T cell pool, which may be relevant to observations from the AESS-Vax study.

CD4⁺



CD8⁺

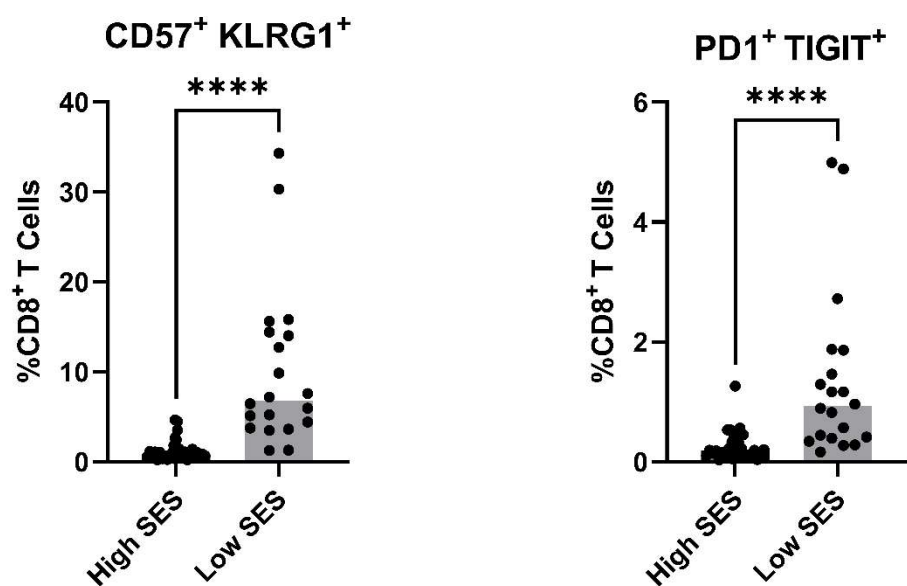


Figure 5.18 Co-expression of Senescence (CD57⁺ KLRG1⁺) and Exhaustion Markers (PD1⁺ TIGIT⁺) on CD4⁺ and CD8⁺ T Cells by Socioeconomic Status: Statistical tests shown are Mann-Whitney U-tests on high (n=31) and low (n=21) participants.

5.4.6 Adjusted Models Confirm Differences in Lymphocyte Immunophenotype by SES Group Persist After Controlling for CMV Status, Age and Sex

To examine whether the observed differences in immune phenotypes by SES group persisted after accounting for relevant covariates, a series of linear models were constructed with immune parameters as outcomes and SES as the primary predictor. All models were adjusted for age, sex, and CMV serostatus, given their known associations with immune ageing and lymphocyte differentiation.

As shown in Table 5.9, low SES remained significantly associated with a range of features previously identified as differing between groups. These included higher frequencies of CD57+KLRG1+ and PD1+TIGIT+ CD4+ and CD8+ T cells, consistent with a more terminally differentiated or exhausted phenotype. Low SES was also associated with lower proportions of total CD4+ T cells and a significant expansion of CD4+ TEMRA cells and Tregs. Frequencies of CD4+ and CD8+ naive T cells were lower in the low SES group, while effector memory CD8+ T cells were significantly increased.

These associations suggest that SES-related differences in immune phenotype cannot be attributed solely to demographic or latent viral reactivation factors and instead may reflect broader processes of immune dysregulation linked to chronic psychosocial adversity.

Table 5.9 Associations Between Socioeconomic Status and Immune Cell Phenotypes After Covariate Adjustment: Linear regression models examining the effect of low (vs high) socioeconomic status (SES) on immune phenotypes related to T cell differentiation, activation, and exhaustion. All models were adjusted for age, sex, and CMV serostatus (NEG as reference group). Estimates reflect the direction and magnitude of SESLOW effects relative to the HIG reference group.

Population	Estimate (LOW vs HIG)	Std. Error	t-value	p-value
PD1 ⁺ TIGIT ⁺ CD8 ⁺	1.19	0.340	3.49	0.00138
CD8 ⁺ Effector Memory	13.4	3.96	3.37	0.00192
CD57 ⁺ KLRG1 ⁺ CD8 ⁺	6.58	2.10	3.14	0.00358
Tregs (% CD4 ⁺)	2.10	0.715	2.93	0.00605
CD4 ⁺ TEMRA	7.94	2.79	2.85	0.00748
CD57 ⁺ KLRG1 ⁺ CD4 ⁺	1.28	0.507	2.52	0.0166
PD1 ⁺ TIGIT ⁺ CD4 ⁺	0.354	0.149	2.38	0.0233
CD4 ⁺ Naïve	-14.3	5.85	-2.44	0.0200
Total CD4 ⁺ (% live)	-6.70	3.06	-2.19	0.0355
CD8 ⁺ Naïve	-10.1	5.23	-1.92	0.0631
CD4 ⁺ Central Memory	-8.69	5.11	-1.70	0.0989
CD8 ⁺ Early Effector	8.25	5.06	1.63	0.112
CD57 ⁺ CD4 ⁺	1.93	1.23	1.56	0.127
CD8 ⁺ Terminal Effector	-5.33	3.59	-1.49	0.147
PD1 ⁺ CD4 ⁺	0.818	0.612	1.34	0.190
CD8 ⁺ TEMRA	-4.04	4.48	-0.900	0.374
Vα7.2 ⁺ (Possible MAITs)	-0.582	0.625	-0.931	0.359
Vδ2 ⁺ T cells	-1.06	1.10	-0.965	0.342
CD8 ⁺ Central Memory	0.712	2.95	0.241	0.811
CD57 ⁺ CD8 ⁺	0.0943	5.48	0.0172	0.986

5.5 Discussion:

5.5.1 Soluble Signatures of Social Adversity – Cytokine and Chemokine Analyses

Chronic psychosocial adversity has been repeatedly and robustly linked to dysregulation of fundamental aspects of human biology, particularly in the immune system, where it has been associated with elevated inflammation and general dysregulation. Drawing on the merged AESS-Vax and PATH datasets, a consistent pattern of immune dysregulation in both homeless

individuals and low SES participants was observed, with marked changes in circulating cytokine/chemokine profiles and T cell differentiation states that may have functional consequences for health and ageing.

Homeless individuals exhibited a pronounced immune shift, with cytokines and chemokines including IL-1 β , TNF- α , IFN- γ , IL-4, IL-12p70, IL-10, IL-2, and IL-18, elevated relative to housed peers. After adjusting for the effects of age, sex, alcohol consumption, and smoking history, six markers (IL-1 β , IL-4, IL-10, IL-12p70, IL-17A (reduced in homeless participants), and GM-CSF (also reduced in homeless participants) remained independently associated with homelessness. These data suggest that there is a simultaneous upregulation of pro-inflammatory and regulatory signalling pathways, with some evidence of suppressed T_H17 and myeloid-lineage cytokine expression.

Low SES participants also showed elevated levels of multiple cytokines, including IL-1 β , TNF- α , IFN- γ , IL-10, MIP-1 β , IP-10, G-CSF, and IL-18. However, only IL-1 β remained statistically significant in adjusted regression models, suggesting that while immune alterations exist across the social gradient, the most extreme adversity (i.e., homelessness) exerts the strongest biological impact. This is consistent with a dose-response model in which cumulative stress and exclusion drive progressive immune dysregulation.

Strikingly, IL-6 was not significantly altered in either group, even prior to covariate adjustment. This contrasts with an extensive body of literature linking IL-6 to chronic stress, low socioeconomic status, and age-related inflammation, particularly in older or comorbid cohorts. While our study cohort was somewhat younger than those typically used in ageing research, low SES and homeless participants still presented with age and comorbidity

profiles indicative of elevated biological risk. As discussed in Chapter 3, this was especially true for PATH participants, who exhibited pronounced frailty and multimorbidity. Therefore, it is unlikely that the absence of a significant IL-6 signal reflects a universally low-risk population. It is plausible that differences in IL-6 levels were present but not detectable due to low absolute concentrations that were further reduced by serum dilutions. Regardless, these findings support the view that IL-6 is not a universally reliable biomarker of social adversity.

The broader cytokine profile in adversity-exposed individuals reflected a combination of heightened inflammation, compensatory immune regulation, and chemotaxis. IL-10 elevation in particular, may signify a compensatory regulatory mechanism in response to ongoing immune activation, consistent with observations in chronic psychosocial stress and inflammaging literature.

Network and module analyses revealed distinct patterns in cytokine co-expression. Homeless and low SES participants showed denser, more interconnected cytokine networks, especially involving chemokines and myeloid-associated mediators such as IL-23, IP-10, and MCP-1. A core module comprising IL-1 β , TNF- α , IFN- γ , and IL-10 was present across network, but composite scores derived from concentrations of module components were significantly higher in homeless and low SES groups. These patterns, visually apparent in the networks suggest systems-level reorganisation of immune signalling, with tighter coupling between pro- and anti-inflammatory pathways in the context of social adversity.

5.5.2 T Cell Immunophenotypes and Immune Ageing in Excluded Populations

Turning to results from immunophenotyping, at the broadest level, individuals from low SES and homeless groups exhibited statistically significant reductions in total CD4⁺ T cell numbers; resulting in lower CD4:CD8 ratios in low SES and homeless participants on average. Within both compartments, naïve T cell subsets were significantly reduced, while terminally differentiated effector memory cells re-expressing CD45RA (TEMRA) were increased, particularly among the CD8⁺ T cells. These alterations were not explained by chronological age or sex differences, suggesting that the shift toward more differentiated T cell phenotypes is linked to adversity exposure. This is consistent with what has been shown in the past in the context of chronic stress^{195,196}, low socioeconomic status^{197,198} and immune ageing¹⁹⁹, where higher TEMRA:Naïve and TEMRA:effector ratios have emerged as key biological indicators of accelerated ageing.

Increased frequencies of regulatory T cells were observed in low SES participants too. This expansion may reflect stress-adaptive responses driven by glucocorticoid or sympathetic signalling, functioning to limit immune-mediated tissue damage under conditions of sustained immune activation. Similar expansions have been documented in chronically stressed populations, such as long-term caregivers, and are thought to reflect prolonged HPA axis activation and glucocorticoid exposure^{45,62}.

Unconventional T cell populations also showed evidence of adversity-associated depletion. Frequencies of mucosal-associated invariant T (MAIT) cells and Vδ2 T cells were lower in low SES participants. These populations are known to be highly sensitive to chronic antigenic stimulation¹⁸⁷. Their reduced representation in circulation may reflect homing to peripheral tissues

following sustained activation, functional exhaustion, or apoptosis. This pattern aligns with previous studies reporting unconventional T cell depletion in chronically stressed populations²⁰⁰, potentially driven by persistent antigen exposure and immune activation at epithelial and mucosal barriers.

To explore shifts in conventional T cell populations in more detail, we examined effector memory and TEMRA subset composition in CD4⁺ and CD8⁺ T cells. Both compartments showed increased proportions of terminal effector phenotypes, including CD27⁻ CD28⁻ cells, terminal effectors (CCR7⁻ CD45RA⁻ CD27⁻ CD28⁻) and RA TEMRAs (CCR7⁻ CD45RA⁺ CD27⁻ CD28⁻), the most differentiated and senescent subsets, though the trend in RA TEMRAs did not reach statistical significance for CD4⁺ or CD8⁺ cells. Expression of senescence (CD57, KLRG1) and exhaustion markers (PD-1, TIGIT) was elevated, with greater co-expression in adversity-exposed individuals. Co-expression of these markers is a stronger indicator of functional impairment than individual markers alone, reflecting both reduced proliferative capacity and effector dysfunction.

Finally, to assess whether these immunophenotypic differences could be explained by differences in demographic or health relevant covariates of SES and social exclusion, linear regression models were deployed. In a model with SES as primary predictor and age, sex and CMV serostatus as covariates, differences in CD4⁺ and CD8⁺ TEMRA cells, T_{Regs}, and senescence and exhaustion markers (CD57, KLRG1, PD-1, TIGIT) all remained statistically significant. A similar pattern was seen for social exclusion (included as an appendix), which remained associated with greater expression of senescence markers and higher proportions of terminal effector phenotypes. Previous studies reporting SES-related differences in T cell phenotype have frequently

identified cytomegalovirus (CMV) infection as a major explanatory factor^{197,201}. However, the results presented here show that adversity-related changes in T cell phenotype persisted even after adjusting for CMV serostatus, along with age and sex. These findings suggest that CMV alone does not account for the observed immunophenotypic pattern in social adversity.

5.5.3 Novelty, Limitations, and Future Directions

To our knowledge, this study is among the first to use high-dimensional cytometry to characterise immune phenotypes in cohorts experiencing homelessness and low SES, with a specific focus on T cell subset distributions and phenotype. The integration of spectral cytometry with cytokine network analysis has also enabled a more systems-level view of immune reorganisation under adversity, a methodological shift that is beginning to happen at speed at the cutting edge of psychoneuroimmunology.

Despite its novelty, the AESS-Vax and PATH studies have notable limitations. A key strength of the dataset, its breadth, also introduced analytical challenges, and several planned functional immunology analyses could not be completed due to time constraints. In particular, an activation panel was designed to assess responses to SARS-CoV-2 spike peptide stimulation in PBMCs, examining activation marker expression and cytokine production, tying analysis in this chapter to the aims of Chapter 4 and the AESS-Vax study. However, the panel was not fully optimised in time for inclusion in this thesis. Similarly, dedicated myeloid and B cell-focused cytometry panels were developed but remained unrefined by the study's endpoint. Data captured to date will be retained, and follow-up experiments using biobanked samples will be completed for inclusion in publication(s).

The PATH study was designed as a pilot to explore the feasibility of recruiting and sampling individuals experiencing homelessness for the study of accelerated-ageing and to assess key methodological and logistical aspects of this task. The immunological data generated from PATH (and AESS-Vax) provide valuable preliminary insights into adversity-associated immune changes and will inform future study designs. Having now established effect sizes, we are better positioned to power prospective studies that explore the biology of social adversity. Future work will also incorporate multi-omic approaches, including profiling conserved transcriptional response to adversity (CTRA), assessing chromatin accessibility, and characterising the landscape of pathogen exposure using serological and metagenomic tools. The latter will be particularly important for testing hypotheses around latent infections (e.g. CMV) and their role in driving phenotypic shifts observed here. Longitudinal follow-up represents another critical next step. Time-series sampling would allow for assessment of the stability or progression of the observed phenotypes and to link these to clinically meaningful outcomes such as vaccine responsiveness, infection rates, or frailty. In doing so, we can move beyond cross-sectional associations to a more dynamic understanding of how adversity becomes biologically embedded over time.

5.6 Conclusion:

This chapter has provided evidence that the immune system is a key interface through which social adversity may influence health. By integrating cytokine, chemokine, and high-dimensional immunophenotyping data, it demonstrates that social exclusion and low socioeconomic status are associated with quantifiable, biologically meaningful alterations in immune dynamics. These

include shifts in circulating immune markers, depletion of naïve and unconventional lymphocytes, and expansion of exhausted and senescent T cell subsets, together suggesting an adversity-associated immunophenotype. Importantly, these changes are observable even in relatively young adults (the average age of all subgroups is under fifty years). Taken together with findings in Chapters 3 and 4, this chapter strengthens the case that (psycho)social adversity is biologically embodied through the immune system, and underscores the need for socially inclusive research, policy, and clinical practice. The findings presented here lay the groundwork for future mechanistic and longitudinal studies aimed at better understanding, and ultimately interrupting, the pathways that allow poverty, inequality, social exclusion and chronic stress to negatively affect long term health.

Chapter 6: Discussion

Over the past century and a half, humanity has achieved dramatic improvements in life expectancy and overall health, particularly in countries of the Global North. These gains are often seen as triumphs of medicine, and in many respects they are. The advent of antibiotics, the development of vaccines, and the discovery of life-saving treatments for previously untreatable infectious and genetic diseases have played an enormous role in extending lives and reducing suffering. However, many of the most substantial improvements in population health have come about independent of these biomedical breakthroughs. The steepest declines in mortality track historically with the introduction of clean municipal water supplies, sanitation systems, improvements in housing quality and reductions in housing density. These were structural interventions that drastically reduced exposure to infection and pathological deprivation. In this historical sense, biomedicine followed where material reform had already begun to lead.

If the nineteenth and early twentieth centuries were defined by the epidemiological transition from infectious to chronic disease, then our present moment is defined by a new, more insidious threat; a rising burden of diseases shaped by inequality, chronic stress, and environmental disruption. As physician and activist Rupa Marya writes, *“our bodies are inflamed because our world is inflamed”*²⁰². Political, social, and ecological harm is metabolised in the body as persistent biological damage. People living in conditions of precarity and exclusion; housing instability, material deprivation, exclusion from meaningful employment, racialisation, and chronic adversity, experience that harm not as abstract disadvantage, but as real, cumulative injury to the body’s regulatory systems. These injuries show up not in sudden outbreaks,

but in slow-moving epidemics of diabetes, depression, cancer, cardiovascular disease, autoimmune dysfunction and early-onset frailty.

This is not a new idea. Over 150 years ago, Rudolf Virchow wrote that *“medicine is a social science, and politics nothing but medicine on a grand scale.”*⁷ In our time, this idea has been expanded upon by researchers and practitioners like Gabor Maté, who argues that illness is not an aberration but a consequence; an adaptation to environments that are themselves pathological. In *The Myth of Normal*²⁰³, Maté reframes chronic disease as a socially and culturally patterned biological response to the demands, disconnection, and distortions of modern life. These conditions, he argues, do not simply reflect individual vulnerability, they reflect systemic failures of care.

This is not to deny the immense value of modern science and medicine. Biomedical science not only provides breakthrough treatments for diseases, it has unlocked a deeper, more integrative and actionable understanding of human health and disease. Furthermore, developments in immunology, genomics, and systems biology have given us unprecedented tools to understand how inequality becomes biologically embedded; how lived experiences of exclusion, instability, and exposure become inscribed in the body’s cells, circuits, and signalling networks. What’s needed now is a synthesis: a science that treats health as both biological and social, recognising that every social exposure; every stressor, every unmet need leaves a molecular residue.

The return of the old killers, cholera, typhus and tuberculosis may be highly unlikely but the progress of the last two centuries is by no means guaranteed. Inequality is soaring throughout the world, between and within societies. The

fine ecological and climatological balances that have allowed populations to grow and spread and flourish are being pushed to breaking point. According to a 2023 update to the Planetary Boundaries framework, six of nine thresholds critical to life on Earth have already been breached²⁰⁴. We are living through a period of multiple, entangled crises; ecological, social, economic, and political. All of these crises have the potential to massively disrupt global health. Some of these disruptions are already visible in the form of emergent and accelerating disease patterns. The new diseases of our time; early immune ageing, chronic inflammation, metabolic dysfunction, and widespread mental ill-health, are not caused by single pathogens. They are shaped by the social architecture that we live and age within. To understand these new pathologies, and to intervene meaningfully, we need to ask how the world around us is making us sick. This thesis begins from that question.

6.1 The Biology of Exclusion: Immune Function Across the Social Divide

The idea that health is socially patterned is well-established but our understanding of how social adversity affects health lacks depth. The data presented in this thesis suggest that immune function is socially patterned too. Across two independent cohorts (AESS-Vax and PATH), we observed consistent immunological differences between housed and homeless participants, as well as between people from low and high socioeconomic backgrounds. These differences included not only diminished vaccine-induced immune responses (Chapter 4), but alterations in cytokine and chemokine profiles and changes to the phenotype of circulating immune cells (Chapter 5). These biological differences mirrored and potentially

mediated clear gradients in health status; measured by number of diagnosed conditions, self-rated health, and medication burden (Chapter 3).

Experiences of psychosocial stress across the lifecourse were also shown to be unevenly distributed across the social gradient, however direct evidence of psychosocial stress (as captured using the scales in this study) mediating immune changes was not found.

Importantly, these patterns did not appear randomly distributed. They followed a clear social logic. In many cases, immune and health-related measures showed dose-response relationship, with housed high-SES participants at one end, low-SES housed participants in the middle, and individuals experiencing homelessness at the most extreme end of the spectrum. However, the transition across this gradient was not always linear. For many measures, people experiencing homelessness did not simply occupy the lowest position on a gradient; they exhibited biologically distinct patterns that point toward qualitative differences in risk. This group, often multiply excluded, not only faces a higher burden but a qualitatively different form of exposure and risk; where overlapping experiences of exclusion, deprivation, shame, stigma, violence and environmental stress interact and compound one another to exert stronger biological effects. The result is not just an intensification of existing gradients, but the emergence of distinctive immune patterns; chronic low-grade inflammation, accelerated immune ageing, and dysregulation of regulatory and effector function

These findings suggest that extreme exclusion may cross biological thresholds not typically reached by more moderate forms of disadvantage. In doing so, the health of people experiencing homelessness can serve as a magnifying lens; revealing the mechanisms that underpin the broader social gradient in

health. Understanding how risk becomes biologically embedded in this context can help illuminate the pathways that allow inequality to shape disease more generally. This approach reflects the idea, introduced in Chapter 1, that studying the extremes of exclusion provides crucial insight into the full spectrum of social determination.

6.2 The Other Vaccine Inequality: Vaccine-Induced Immunity is Socially Patterned:

Discussions of vaccine inequality in public health often centre on access: who receives a vaccine, when, and under what conditions. During the COVID-19 pandemic, this framing dominated global discourse, as high-income countries hoarded the vast majority of early vaccine supplies while many low- and middle-income countries faced prolonged delays. Yet a less recognised but equally significant form of vaccine inequality lies in the variability of immune response among those who are vaccinated.

Vaccine-induced immunity reflects the functional state of the immune system at the time of antigen exposure, a state shaped not only by age and comorbidities, but by cumulative psychosocial and material conditions. Because vaccination introduces the same antigen at a standardised dose, it offers a natural experiment for comparing immune function across social groups. In this sense, differences in vaccine response may reflect deeper biological consequences of structural inequality.

In Chapter 4, we showed that both anti-Spike antibody titres and IFN- γ responses to SARS-CoV-2 antigens were lower among participants from lower socioeconomic backgrounds and lower again in those experiencing homelessness. These associations remained robust after adjustment for key

covariates including age, sex, alcohol use, and smoking. While causality cannot be inferred, the consistency of these results aligns with prior research in social immunology and psychoneuroimmunology, where chronic stress has been shown to suppress vaccine responses (see Table 1.2, chapter 1 for review).

Although mediation by psychosocial stress was not supported statistically, the distribution of stress exposure in our sample suggests biological plausibility. It is possible that the scales developed to date do not capture the degree of difference in intensity of adversity experienced by people experiencing homelessness – e.g. the ACES questionnaire will give an equal score to participants abused once as to those who experience severe, recurrent abuse. Participants experiencing homelessness scored highest across all three stress indices. Mediation analysis in the AESS-Vax dataset returned null results; however, post-hoc power calculations indicated the study was underpowered to detect effects of the magnitude observed. This study provides foundational effect size estimates for future work and, to our knowledge, is the first to examine stress as a potential mediator of mRNA vaccine-induced immunity.

These findings highlight the need for more robust, harmonised, and context-sensitive measures of psychosocial experience. Metrics and constructs like loneliness, perceived safety, social connectedness, and eudaimonic well-being, each shown to influence immune regulation in prior research, including work by Steve Cole and colleagues^{205–207}, may offer greater explanatory power than the indices used in AESS-Vax.

The COVID-19 vaccination programme offered a rare, if challenging, opportunity to investigate immune responses across socially stratified groups. National vaccine policies evolved in real-time, with changes according to

vaccine availability and political pressure. A significant proportion of participants experiencing homelessness received the single-dose Johnson & Johnson vaccine²⁰⁸, while others received mRNA formulations. This heterogeneity, combined with a volatile national rollout, introduced barriers to follow-up and consistency. Nonetheless, our findings demonstrate a clear social gradient in immune response, reinforcing the utility of vaccine studies as a means of uncovering the biological imprint of inequality.

Future research may benefit from leveraging routine immunisation against influenza, pneumococcal infections, or hepatitis B as more stable bases for examining social patterning in immunity. These vaccines are widely administered, immunologically well-characterised, and less affected by the volatility of emergency public health responses. They also permit more reliable prospective follow-up and improved comparability across cohorts.

The AESS-Vax study was designed to include deeper immunological profiling than is presented here. Specifically, we planned to stimulate cryopreserved PBMCs with SARS-CoV-2 antigens to assess activation-induced markers (AIMs)^{209,210} and cytokine production. These assays would have enabled characterisation of antigen-specific T cell responses and, with further optimisation, B cell phenotypes. We also trialled hair sample collection for cortisol analysis early in the study, but uptake was low, as many participants declined or were unable to provide sufficient sample. While these assays were not completed in time, biobanked samples remain available and present an opportunity for future analyses that integrate immunological and neuroendocrine data.

Finally, future studies should incorporate clinical endpoints to assess the real-world implications of differential vaccine responsiveness. Although unrealised in AESS-Vax for practical and logistical reasons, the pandemic presented an opportunity to link immunogenicity with breakthrough infection. Longitudinal monitoring of infection, hospitalisation, and disease severity would offer critical insight into how social conditions shape clinically relevant variation in immune responses. As biomedical scientists grow more adept at working with large, complex datasets, new opportunities are emerging to investigate the biological underpinnings of unequal vaccine responses and the broader mechanisms that allow social inequality and adversity *under the skin*.

6.3 Immune Composition and Function Across the Social Gradient

The AESS-Vax study examined immune function in response to a standardised antigenic challenge. However, much of how a person responds to immune challenge is dictated by the immune status of the individual at the time of encounter with threat or need to activate wound healing responses. Therefore, this thesis also explored the immune system at rest: its cellular composition, the phenotype of its cells, and the circulating cytokine and chemokine environment. These data, presented in Chapter 5, offer a complementary perspective on immune vulnerability and dynamics under conditions of social adversity.

Here, participants from low SES backgrounds exhibited skewed cytokine and chemokine profiles, characterised by elevations in inflammatory mediators (TNF- α , IL-1 β , MCP-1, IFN- γ) and reductions in regulatory cytokines like IL-10. Both principal component and modular analyses pointed to a shared

underlying pattern: a shift towards chronic low-grade, systemic inflammation. This circulating cytokine/chemokine pattern is consistent with what has been described in contexts of chronic stress and adversity^{60,100,211,212}. Immune phenotyping further revealed higher frequencies of late-differentiated and terminally differentiated T cell subsets, particularly CD8+ TEMRA cells and CD27-CD28- phenotypes. These subsets are typically associated with immune ageing, chronic antigenic stimulation, and reduced proliferative capacity^{197,213,214}.

The profiles we observed align closely with the literature on "inflammaging", a state of persistent, low-grade inflammation associated with Immunosenescence (itself a canonical "hallmark of ageing")^{215,216} and increased disease risk in older adults²¹⁷⁻²¹⁹. The convergence of elevated inflammatory signalling and an aged T cell phenotype suggests that chronic exposure to social disadvantage may drive premature immunological ageing, even in relatively young populations. These findings support the growing evidence that structural inequality accelerates biological wear-and-tear, reshaping not only health risk but the architecture of immune defence itself. The immune profile observed here may help explain why age-associated comorbidities such as cardiovascular disease, chronic obstructive pulmonary disease, and dementia frequently emerge earlier among socially excluded groups^{9,71,220,221}, particularly individuals experiencing homelessness. The pathogenesis of these conditions often involves chronic inflammation, altered wound healing, and dysregulated adaptive immune responses, all of which have been observed here.

6.4 Future Directions

Methodological and logistical constraints encountered throughout the course of AESS-Vax and PATH have been mentioned. These limitations necessarily shape the strength of inference and the kinds of claims that can be made. Yet taken together, the patterns observed; spanning vaccine responses, cytokine signatures, and immune cell phenotypes, point toward deep and wide-ranging alterations to the immune system. These may represent an adversity-associated immunophenotype: a distinctive biological signature that emerges in low socioeconomic status and chronic adversity.

To extend this analysis beyond the circulating immune proteome and T cell phenotypes future work should include detailed analyses of B cell subsets and myeloid populations, including classical, intermediate, and non-classical monocytes. While not a focus of this thesis, prior research has shown that chronic psychosocial stress can alter myeloid cell distributions, increasing the prevalence of inflammatory monocytes such as Ly6C^{hi} subsets in murine models of social defeat^{63,88}. Similar shifts have been reported in human studies of acute, experimentally-induced stress²²², alongside stress-associated changes in neutrophil-to-lymphocyte ratios²²³, a marker increasingly recognised for its prognostic value in inflammatory and psychiatric conditions^{224,225}.

Biobanked PBMCs from the AESS-Vax study remain available and represent a valuable opportunity to build a more comprehensive map of immune cell architecture in the context of psychosocial and economic adversity. With sufficient phenotypic resolution and sample integration, future analyses may

help elucidate how exclusion and adversity affect both innate and adaptive immune processes.

Beyond extending the scope of immune analyses, future work must explore whether observed effects are reversible. Investigating the extent and timing of biological changes in response to improved social conditions remains a crucial next step. To paraphrase Karl Marx, while science provides an interpretation of the world, *“the point is to change it”*²²⁶.

There may be particular merit to integrating biological analyses into the evaluation of social interventions that aim to improve health and welfare. For example, longitudinal studies could be conducted alongside Housing First programmes, community-based family support services, or cash transfer schemes targeting socially excluded populations. Previous work by Steve Cole and colleagues has shown that contemplative practices like mindfulness, meditation and tai chi can downregulate the Conserved Transcriptional Response to Adversity in stressed individuals^{79,81,227,228,229}. These findings prove the plasticity of immune profiles like the CTRA, justifying further research into interventions to mitigate their pathological impacts. However, unlike the individual-level interventions previously studied, the types of social programmes proposed here would address the root causes of adversity through structural change; perhaps offering more enduring reversal of underlying biological vulnerability by treating causes rather than symptoms.

Finally, as the field moves forward, it will be important to integrate the biology of exclusion into the architecture of population cohort studies. Large-scale studies of health and ageing like The Irish Longitudinal Study on Ageing (TILDA), the US Health and Retirement Study and the Midlife in US (MIDUS)

study already incorporate measures of SES but often exclude individuals experiencing the most extreme forms of disadvantage. One way forward may be to develop adjunct studies that mirror the structure of existing cohorts while focusing specifically on multiply excluded groups. IDS-TILDA²³⁰, which incorporates adults with intellectual disabilities into the broader TILDA framework, offers a useful precedent. Doing so would not only increase representativeness but would allow researchers to trace how exclusion interacts with ageing, comorbidity, and immune function over time.

6.5 Conclusion:

This thesis has argued that inequality leaves a biological trace, that social exclusion and material deprivation are not just social facts, but immunological forces. Across two independent studies, using diverse methodologies and outcome measures, we have shown that individuals exposed to structural disadvantage exhibit altered immune function, reduced vaccine responsiveness, and evidence of accelerated immune ageing. These effects are not marginal. They are consistent, patterned, and fit an emerging model of “Social Immunology” that aims to understand the immune system in its native context, as a system that maintains organismal homeostasis in response to threats that are real and perceived, internal and environmental, immediate and chronic.

The implications are clear. Addressing health inequality cannot rely solely on downstream interventions or individualised care. It requires grappling with the social conditions that shape immune development, resilience, and decline. This is not only a moral imperative. It is a scientific one. If immune function

reflects social experience, then social policy becomes a tool of public health, and reducing inequality becomes a form of preventative medicine.

This realisation invites a broader rethinking of what health science is for, and how it should be done. Humans are social animals; our biology emerges not in isolation, but in relationship; to one another, to institutions, to history and our environments. To understand human health, we must look beyond genes and cells, to the social and political structures in which those genes and cells are embedded. A science that ignores the social is not just incomplete, it is inaccurate. As Rudolf Virchow observed: *“If disease is an expression of individual life under unfavourable conditions, then epidemics must be indicative of mass disturbances of mass life.”*²³¹

And as Lancet Editor-in-Chief, Richard Horton has written:

*“A healthy society is much more than a community in which the causes of disease are minimised. It is one where, at the very least, human creativity is free to flourish, individuals have the liberty to be who they wish to be and the spirit of life (all life and not merely human life) prospers”.*²³²

References

1. Marmot, M. Inclusion health: addressing the causes of the causes. *The Lancet* **391**, 186–188 (2018).
2. Xu, J. *et al.* Products - Data Briefs - Number 456 - December 2022.
<https://www.cdc.gov/nchs/products/databriefs/db456.htm> (2022)
doi:10.15620/cdc:122516.
3. The Association Between Income and Life Expectancy in the United States, 2001-2014 | Equity, Diversity, and Inclusion | JAMA | JAMA Network.
<https://jamanetwork.com/journals/jama/fullarticle/2513561>.
4. *The Growing Gap in Life Expectancy by Income: Implications for Federal Programs and Policy Responses*. (National Academies Press, Washington, D.C., 2015). doi:10.17226/19015.
5. Hiam, L., Harrison, D., McKee, M. & Dorling, D. Why is life expectancy in England and Wales ‘stalling’? *J Epidemiol Community Health* **72**, 404–408 (2018).

6. Arceo-Gomez, E. O. *et al.* The income gradient in COVID-19 mortality and hospitalisation: An observational study with social security administrative records in Mexico. *The Lancet Regional Health - Americas* **6**, 100115 (2022).
7. Virchow, R. C. Report on the Typhus Epidemic in Upper Silesia. *Am J Public Health* **96**, 2102–2105 (2006).
8. Marmot, M., Friel, S., Bell, R., Houweling, T. A. & Taylor, S. Closing the gap in a generation: Health equity through action on the social determinants of health. *The Lancet* **372**, 1661–1669 (2008).
9. Ivers, J.-H. & Barry, J. *MORTALITY AMONGST THE HOMELESS POPULATION IN DUBLIN*. (2018).
10. Ingram, C., Buggy, C., Elabbasy, D. & Perrotta, C. Homelessness and health-related outcomes in the Republic of Ireland: a systematic review, meta-analysis and evidence map. *J Public Health (Berl.)* **32**, 1855–1876 (2024).
11. Mantell, R. *et al.* Accelerated aging in people experiencing homelessness: A rapid review of frailty prevalence and determinants. *Frontiers in Public Health* **11**, (2023).
12. Five-year standardised mortality ratios in a cohort of homeless people in Dublin | BMJ Open. <https://bmjopen.bmj.com/content/9/1/e023010>.
13. England, E., Thomas, I., Mackie, P. & Browne-Gott, H. A typology of multiple exclusion homelessness. *Housing Studies* **39**, 695–719 (2024).
14. D'Sa, S. *et al.* The psychological impact of childhood homelessness—a literature review. *Ir J Med Sci* **190**, 411–417 (2021).

15. McEwen, B. S. Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York academy of sciences* **840**, 33–44 (1998).
16. Spruill, T. M. Chronic psychosocial stress and hypertension. *Curr Hypertens Rep* **12**, 10–6 (2010).
17. McCrory, C. *et al.* Towards a consensus definition of allostatic load: a multi-cohort, multi-system, multi-biomarker individual participant data (IPD) meta-analysis. *Psychoneuroendocrinology* **153**, 106117 (2023).
18. McEwen, B. S. & Stellar, E. Stress and the individual: Mechanisms leading to disease. *Archives of internal medicine* **153**, 2093–2101 (1993).
19. McCrory, C. *et al.* How does socio-economic position (SEP) get biologically embedded? A comparison of allostatic load and the epigenetic clock(s). *Psychoneuroendocrinology* **104**, 64–73 (2019).
20. Parker, H. W., Abreu, A. M., Sullivan, M. C. & Vadiveloo, M. K. Allostatic Load and Mortality: A Systematic Review and Meta-Analysis. *Am J Prev Med* **63**, 131–140 (2022).
21. Gruenewald, T. L., Seeman, T. E., Karlamangla, A. S. & Sarkisian, C. A. Allostatic load and frailty in older adults. *Journal of the American Geriatrics Society* **57**, 1525–1531 (2009).
22. Danese, A. & McEwen, B. S. Adverse childhood experiences, allostasis, allostatic load, and age-related disease. *Physiology & behavior* **106**, 29–39 (2012).
23. Gruenewald, T. L. *et al.* History of socioeconomic disadvantage and allostatic load in later life. *Social science & medicine* **74**, 75–83 (2012).

24. Alotiby, A. Immunology of Stress: A Review Article. *J Clin Med* **13**, 6394 (2024).
25. Godoy, L. D., Rossignoli, M. T., Delfino-Pereira, P., Garcia-Cairasco, N. & de Lima Umeoka, E. H. A Comprehensive Overview on Stress Neurobiology: Basic Concepts and Clinical Implications. *Front Behav Neurosci* **12**, 127 (2018).
26. Lazarus, R. S. & Folkman, S. *Stress, Appraisal, and Coping*. (Springer publishing company, 1984).
27. Cole, S. W. *et al.* Social regulation of gene expression in human leukocytes. *Genome Biol* **8**, R189 (2007).
28. Lee, M. J. *et al.* Transcriptomic analyses of black women in neighborhoods with high levels of violence. *Psychoneuroendocrinology* **127**, 105174 (2021).
29. Kitayama, S., Akutsu, S., Uchida, Y. & Cole, S. W. Work, meaning, and gene regulation: Findings from a Japanese information technology firm. *Psychoneuroendocrinology* **72**, 175–181 (2016).
30. Renna, M. E. A review and novel theoretical model of how negative emotions influence inflammation: The critical role of emotion regulation. *Brain, Behavior, & Immunity - Health* **18**, 100397 (2021).
31. Brosschot, J. F., Verkuil, B. & Thayer, J. F. Generalized Unsafety Theory of Stress: Unsafe Environments and Conditions, and the Default Stress Response. *Int J Environ Res Public Health* **15**, 464 (2018).
32. Gjerstad, J. K., Lightman, S. L. & Spiga, F. Role of glucocorticoid negative feedback in the regulation of HPA axis pulsatility. *Stress* **21**, 403–416 (2018).
33. Neural regulation of endocrine and autonomic stress responses | Nature Reviews Neuroscience. <https://www.nature.com/articles/nrn2647>.

34. Mueller, B., Figueroa, A. & Robinson-Papp, J. Structural and functional connections between the autonomic nervous system, hypothalamic–pituitary–adrenal axis, and the immune system: a context and time dependent stress response network. *Neurological Sciences* **43**, 951–960 (2022).
35. Lohse, M. J. The ins and outs of adrenergic signaling. *J Mol Med (Berl)* **93**, 955–962 (2015).
36. Perez, D. M. *The Adrenergic Receptors: In the 21st Century*. (Springer Science & Business Media, 2006).
37. Grisanti, L. A., Perez, D. M. & Porter, J. E. Modulation of Immune Cell Function by α 1-Adrenergic Receptor Activation. *Curr Top Membr* **67**, 113–138 (2011).
38. Hering, L., Rahman, M., Potthoff, S. A., Rump, L. C. & Stegbauer, J. Role of α 2-Adrenoceptors in Hypertension: Focus on Renal Sympathetic Neurotransmitter Release, Inflammation, and Sodium Homeostasis. *Front Physiol* **11**, 566871 (2020).
39. Sharma, D. & Farrar, J. D. Adrenergic regulation of immune cell function and inflammation. *Seminars in Immunopathology* **42**, 709–717 (2020).
40. Scanzano, A. & Cosentino, M. Adrenergic regulation of innate immunity: a review. *Front Pharmacol* **6**, 171 (2015).
41. Kadmiel, M. & Cidlowski, J. A. Glucocorticoid receptor signaling in health and disease. *Trends Pharmacol Sci* **34**, 518–530 (2013).
42. Ayroldi, E. *et al.* Mechanisms of the anti-inflammatory effects of glucocorticoids: genomic and nongenomic interference with MAPK signaling pathways. *FASEB J* **26**, 4805–4820 (2012).

43. Oakley, R. H. & Cidlowski, J. A. The biology of the glucocorticoid receptor: new signaling mechanisms in health and disease. *J Allergy Clin Immunol* **132**, 1033–1044 (2013).
44. Dhabhar, F. S., Miller, A. H., McEwen, B. S. & Spencer, R. L. Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms. *The Journal of Immunology* **154**, 5511–5527 (1995).
45. Dhabhar, F. S. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. *Neuroimmunomodulation* **16**, 300–317 (2009).
46. Felten, D. L., Ackerman, K. D., Wiegand, S. J. & Felten, S. Y. Noradrenergic sympathetic innervation of the spleen: I. Nerve fibers associate with lymphocytes and macrophages in specific compartments of the splenic white pulp. *J Neurosci Res* **18**, 28–36, 118–21 (1987).
47. Williams, J. M. & Felten, D. L. Sympathetic innervation of murine thymus and spleen: A comparative histofluorescence study. *The Anatomical Record* **199**, 531–542 (1981).
48. Nance, D. M. & Sanders, V. M. Autonomic innervation and regulation of the immune system (1987–2007). *Brain, Behavior, and Immunity* **21**, 736–745 (2007).
49. Panuncio, A. L., De La Peña, S., Gualco, G. & Reissenweber, N. Adrenergic innervation in reactive human lymph nodes. *J Anat* **194 (Pt 1)**, 143–6 (1999).
50. Spengler, R. N., Chensue, S. W., Giacherio, D. A., Blenk, N. & Kunkel, S. L. Endogenous norepinephrine regulates tumor necrosis factor- α production from macrophages in vitro. *The Journal of Immunology* **152**, 3024–3031 (1994).

51. Grailer, J. J., Haggadone, M. D., Sarma, J. V., Zetoune, F. S. & Ward, P. A. Induction of M2 Regulatory Macrophages through the β 2-Adrenergic Receptor with Protection during Endotoxemia and Acute Lung Injury. *Journal of Innate Immunity* **6**, 607–618 (2014).
52. Ağaç, D., Estrada, L. D., Maples, R., Hooper, L. V. & Farrar, J. D. The β 2-adrenergic receptor controls inflammation by driving rapid IL-10 secretion. *Brain, Behavior, and Immunity* **74**, 176–185 (2018).
53. Seiffert, K. *et al.* Catecholamines Inhibit the Antigen-Presenting Capability of Epidermal Langerhans Cells. *The Journal of Immunology* **168**, 6128–6135 (2002).
54. Ramer-Quinn, D. S., Baker, R. A. & Sanders, V. M. Activated T helper 1 and T helper 2 cells differentially express the beta-2-adrenergic receptor: a mechanism for selective modulation of T helper 1 cell cytokine production. *J Immunol* **159**, 4857–67 (1997).
55. Frohman, E. M., Vayuvegula, B., Gupta, S. & van den Noort, S. Norepinephrine inhibits gamma-interferon-induced major histocompatibility class II (Ia) antigen expression on cultured astrocytes via beta-2-adrenergic signal transduction mechanisms. *Proceedings of the National Academy of Sciences* **85**, 1292–1296 (1988).
56. Estrada, L. D., Ağaç, D. & Farrar, J. D. Sympathetic neural signaling via the β 2-adrenergic receptor suppresses T-cell receptor-mediated human and mouse CD8⁺ T-cell effector function. *European Journal of Immunology* **46**, 1948–1958 (2016).

57. Nakai, A., Hayano, Y., Furuta, F., Noda, M. & Suzuki, K. Control of lymphocyte egress from lymph nodes through β 2-adrenergic receptors. *Journal of Experimental Medicine* **211**, 2583–2598 (2014).
58. Miller, L. E., Jüsten, H. P., Schölmerich, J. & Straub, R. H. The loss of sympathetic nerve fibers in the synovial tissue of patients with rheumatoid arthritis is accompanied by increased norepinephrine release from synovial macrophages. *Faseb j* **14**, 2097–107 (2000).
59. Levick, S. P., Murray, D. B., Janicki, J. S. & Brower, G. L. Sympathetic nervous system modulation of inflammation and remodeling in the hypertensive heart. *Hypertension* **55**, 270–276 (2010).
60. Miller, G. E., Cohen, S. & Ritchey, A. K. Chronic psychological stress and the regulation of pro-inflammatory cytokines: a glucocorticoid-resistance model. *Health Psychol* **21**, 531–41 (2002).
61. O'Connor, M.-F., Schultze-Florey, C. R., Irwin, M. R., Arevalo, J. M. G. & Cole, S. W. Divergent gene expression responses to Complicated Grief and Non-complicated Grief. *Brain, Behavior, and Immunity* **37**, 78–83 (2014).
62. Miller, G. E. *et al.* A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF- κ B signaling. *Biological psychiatry* **64**, 266–272 (2008).
63. Miller, G. E. *et al.* Greater inflammatory activity and blunted glucocorticoid signaling in monocytes of chronically stressed caregivers. *Brain, behavior, and immunity* **41**, 191–199 (2014).

64. Christian, L. M. *et al.* Understanding the health effects of caregiving stress: New directions in molecular aging. *Ageing Research Reviews* **92**, 102096 (2023).
65. Kinsey, S. G., Bailey, M. T., Sheridan, J. F. & Padgett, D. A. The inflammatory response to social defeat is increased in older mice. *Physiology & behavior* **93**, 628–636 (2008).
66. Egan, R. J. *et al.* Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behavioural brain research* **205**, 38–44 (2009).
67. Simons, N. D. & Tung, J. Social status and gene regulation: conservation and context dependence in primates. *Trends in cognitive sciences* **23**, 722–725 (2019).
68. Cole, S. W. The Conserved Transcriptional Response to Adversity. *Curr Opin Behav Sci* **28**, 31–37 (2019).
69. Cole, S. W. *et al.* Myeloid differentiation architecture of leukocyte transcriptome dynamics in perceived social isolation. *Proceedings of the National Academy of Sciences* **112**, 15142 (2015).
70. Friedman, E. M. & Herd, P. Income, education, and inflammation: differential associations in a national probability sample (the MIDUS study). *Psychosomatic medicine* **72**, 290 (2010).
71. Wirtz, P. H. & von Känel, R. Psychological stress, inflammation, and coronary heart disease. *Current cardiology reports* **19**, 1–10 (2017).
72. Sloan, E. K. *et al.* The sympathetic nervous system induces a metastatic switch in primary breast cancer. *Cancer Research* **70**, 7042–7052 (2010).

73. Antoni, M. H. *et al.* Stress management, leukocyte transcriptional changes and breast cancer recurrence in a randomized trial: An exploratory analysis. *Psychoneuroendocrinology* **74**, 269–277 (2016).
74. Knight, J. M. *et al.* Low socioeconomic status, adverse gene expression profiles, and clinical outcomes in hematopoietic stem cell transplant recipients. *Clinical Cancer Research* **22**, 69–78 (2016).
75. Shaashua, L. *et al.* Perioperative COX-2 and β -adrenergic blockade improves metastatic biomarkers in breast cancer patients in a phase-II randomized trial. *Clinical Cancer Research* **23**, 4651–4661 (2017).
76. Haldar, R. *et al.* Perioperative COX2 and β -adrenergic blockade improves biomarkers of tumor metastasis, immunity, and inflammation in colorectal cancer: A randomized controlled trial. *Cancer* **126**, 3991–4001 (2020).
77. Knight, J. M. *et al.* Propranolol inhibits molecular risk markers in HCT recipients: a phase 2 randomized controlled biomarker trial. *Blood Advances* **4**, 467–476 (2020).
78. Hiller, J. G. *et al.* Preoperative β -Blockade with Propranolol Reduces Biomarkers of Metastasis in Breast Cancer: A Phase II Randomized Trial. *Clinical Cancer Research* **26**, 1803–1811 (2020).
79. Black, D. S. *et al.* Yogic meditation reverses NF- κ B and IRF-related transcriptome dynamics in leukocytes of family dementia caregivers in a randomized controlled trial. *Psychoneuroendocrinology* **38**, 348–355 (2013).

80. Bower, J. E. *et al.* Yoga reduces inflammatory signaling in fatigued breast cancer survivors: A randomized controlled trial. *Psychoneuroendocrinology* **43**, 20–29 (2014).
81. Irwin, M. R. *et al.* Tai Chi compared with cognitive behavioral therapy and the reversal of systemic, cellular and genomic markers of inflammation in breast cancer survivors with insomnia: A randomized clinical trial. *Brain, Behavior, and Immunity* **120**, 159–166 (2024).
82. Nelson-Coffey, S. K., Fritz, M. M., Lyubomirsky, S. & Cole, S. W. Kindness in the blood: A randomized controlled trial of the gene regulatory impact of prosocial behavior. *Psychoneuroendocrinology* **81**, 8–13 (2017).
83. Lee, S.-H., Cole, S. W., Park, J. & Choi, I. Loneliness and immune gene expression in Korean adults: The moderating effect of social orientation. *Health Psychology* **40**, 686 (2021).
84. Dorian, B. *et al.* Aberrations in lymphocyte subpopulations and function during psychological stress. *Clinical and Experimental Immunology* **50**, 132 (1982).
85. Herbert, T. B. & Cohen, S. Stress and immunity in humans: a meta-analytic review. *Psychosom Med* **55**, 364–379 (1993).
86. McKim, D. B. *et al.* Social Stress Mobilizes Hematopoietic Stem Cells to Establish Persistent Splenic Myelopoiesis. *Cell Reports* **25**, 2552–2562.e3 (2018).
87. Heidt, T. *et al.* Chronic variable stress activates hematopoietic stem cells. *Nature medicine* **20**, 754–758 (2014).

88. Powell, N. D. *et al.* Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via β -adrenergic induction of myelopoiesis. *Proceedings of the National Academy of Sciences* **110**, 16574–16579 (2013).
89. Snyder-Mackler, N. *et al.* Social status alters immune regulation and response to infection in macaques. *Science* **354**, 1041–1045 (2016).
90. Lea, A. J. *et al.* Dominance rank-associated gene expression is widespread, sex-specific, and a precursor to high social status in wild male baboons. *Proceedings of the National Academy of Sciences* **115**, E12163–E12171 (2018).
91. Surjit, M. *et al.* Widespread negative response elements mediate direct repression by agonist-liganded glucocorticoid receptor. *Cell* **145**, 224–241 (2011).
92. Vandevyver, S., Dejager, L. & Libert, C. Comprehensive Overview of the Structure and Regulation of the Glucocorticoid Receptor. *Endocrine Reviews* **35**, 671–693 (2014).
93. Lovell, B., Moss, M. & Wetherell, M. A. Perceived stress, common health complaints and diurnal patterns of cortisol secretion in young, otherwise healthy individuals. *Hormones and behavior* **60**, 301–305 (2011).
94. Maniam, J., Antoniadis, C. & Morris, M. J. Early-Life Stress, HPA Axis Adaptation, and Mechanisms Contributing to Later Health Outcomes. *Frontiers in Endocrinology* **5**, (2014).
95. Berens, A. E., Jensen, S. K. G. & Nelson, C. A. Biological embedding of childhood adversity: from physiological mechanisms to clinical implications. *BMC Medicine* **15**, 135 (2017).

96. Cohen, S., Tyrrell, D. A. & Smith, A. P. Negative life events, perceived stress, negative affect, and susceptibility to the common cold. *Journal of personality and social psychology* **64**, 131 (1993).
97. Cohen, S. *et al.* Types of stressors that increase susceptibility to the common cold in healthy adults. *Health psychology* **17**, 214 (1998).
98. Cohen, S., Doyle, W. J. & Skoner, D. P. Psychological stress, cytokine production, and severity of upper respiratory illness. *Psychosomatic medicine* **61**, 175–180 (1999).
99. Cohen, S. *et al.* Reactivity and vulnerability to stress-associated risk for upper respiratory illness. *Psychosomatic medicine* **64**, 302–310 (2002).
100. Cohen, S. *et al.* Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. *Proceedings of the National Academy of Sciences* **109**, 5995–5999 (2012).
101. Janicki-Deverts, D., Cohen, S., Turner, R. B. & Doyle, W. J. Basal salivary cortisol secretion and susceptibility to upper respiratory infection. *Brain, behavior, and immunity* **53**, 255–261 (2016).
102. Stoeger, T. & Adler, H. “Novel” Triggers of Herpesvirus Reactivation and Their Potential Health Relevance. *Frontiers in Microbiology* **9**, (2019).
103. Liu, T., Khanna, K. M., Chen, X., Fink, D. J. & Hendricks, R. L. Cd8+ T Cells Can Block Herpes Simplex Virus Type 1 (HSV-1) Reactivation from Latency in Sensory Neurons. *Journal of Experimental Medicine* **191**, 1459–1466 (2000).
104. St. Leger, A. J. & Hendricks, R. L. CD8+ T cells patrol HSV-1-infected trigeminal ganglia and prevent viral reactivation. *J. Neurovirol.* **17**, 528–534 (2011).

105. Desimio, M. G., Covino, D. A., Cancrini, C. & Doria, M. Entry into the lytic cycle exposes EBV-infected cells to NK cell killing via upregulation of the MICB ligand for NKG2D and activation of the CD56^{bright} and NKG2A⁺KIR⁺CD56^{dim} subsets. *Front Immunol* **15**, 1467304 (2024).
106. Moustaki, A., Argyropoulos, K. V., Baxevanis, C. N., Papamichail, M. & Perez, S. A. Effect of the simultaneous administration of glucocorticoids and IL-15 on human NK cell phenotype, proliferation and function. *Cancer Immunol Immunother* **60**, 1683–1695 (2011).
107. Chimote, A. A. *et al.* Immune and ionic mechanisms mediating the effect of dexamethasone in severe COVID-19. *Front. Immunol.* **14**, (2023).
108. Liberman, A. C. *et al.* Regulatory and Mechanistic Actions of Glucocorticoids on T and Inflammatory Cells. *Front. Endocrinol.* **9**, (2018).
109. Ives, A. M. & Bertke, A. S. Stress Hormones Epinephrine and Corticosterone Selectively Modulate Herpes Simplex Virus 1 (HSV-1) and HSV-2 Productive Infections in Adult Sympathetic, but Not Sensory, Neurons. *Journal of Virology* **91**, 10.1128/jvi.00582-17 (2017).
110. Workman, A. *et al.* Cellular Transcription Factors Induced in Trigeminal Ganglia during Dexamethasone-Induced Reactivation from Latency Stimulate Bovine Herpesvirus 1 Productive Infection and Certain Viral Promoters. *Journal of Virology* **86**, 2459–2473 (2012).
111. Chang, M. *et al.* β -Adrenoreceptors Reactivate Kaposi's Sarcoma-Associated Herpesvirus Lytic Replication via PKA-Dependent Control of Viral RTA. *Journal of Virology* **79**, 13538–13547 (2005).

112. Hayward, S. E. *et al.* A systematic review of the impact of psychosocial factors on immunity: implications for enhancing BCG response against tuberculosis. *SSM-population health* **10**, 100522 (2020).
113. Shirtcliff Elizabeth, A., Coe Christopher, L. & Pollak Seth, D. Early childhood stress is associated with elevated antibody levels to herpes simplex virus type 1. *Proceedings of the National Academy of Sciences* **106**, 2963–2967 (2009).
114. Brook, M. J., Christian, L. M., Hade, E. M. & Ruffin, M. T. The Effect of Perceived Stress on Epstein-Barr Virus Antibody Titers in Appalachian Ohio Women. *Neuroimmunomodulation* **24**, 67–73 (2017).
115. Phillips, A. C., Carroll, D., Khan, N. & Moss, P. Cytomegalovirus is associated with depression and anxiety in older adults. *Brain, Behavior, and Immunity* **22**, 52–55 (2008).
116. Pawelec, G. & Derhovanessian, E. Role of CMV in immune senescence. *Virus research* **157**, 175–179 (2011).
117. Bjornevik, K. *et al.* Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. *Science* (2022).
118. Frasca, D., Diaz, A., Romero, M., Landin, A. M. & Blomberg, B. B. Cytomegalovirus (CMV) seropositivity decreases B cell responses to the influenza vaccine. *Vaccine* **33**, 1433–1439 (2015).
119. Bowyer, G. *et al.* Reduced Ebola vaccine responses in CMV+ young adults is associated with expansion of CD57+ KLRG1+ T cells. *Journal of Experimental Medicine* **217**, (2020).

120. Petry, L. J., Weems, L. B. & Livingstone, J. N. Relationship of stress, distress, and the immunologic response to a recombinant hepatitis B vaccine. *J Fam Pract* **32**, 481–486 (1991).
121. Jabaaij, L. *et al.* Influence of perceived psychological stress and distress on antibody response to low dose rDNA hepatitis B vaccine. *Journal of Psychosomatic Research* **37**, 361–369 (1993).
122. Kohut, M. L., Cooper, M. M., Nickolaus, M. S., Russell, D. R. & Cunnick, J. E. Exercise and psychosocial factors modulate immunity to influenza vaccine in elderly individuals. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* **57**, M557–M562 (2002).
123. Burns, V. E., Carroll, D., Ring, C., Harrison, L. K. & Drayson, M. Stress, Coping, and Hepatitis B Antibody Status. *Psychosomatic Medicine* **64**, (2002).
124. Burns, V. E., Drayson, M., Ring, C. & Carroll, D. Perceived stress and psychological well-being are associated with antibody status after meningitis C conjugate vaccination. *Psychosomatic Medicine* **64**, 963–970 (2002).
125. Burns, V. E., Carroll, D., Drayson, M., Whitham, M. & Ring, C. Life events, perceived stress and antibody response to influenza vaccination in young, healthy adults. *Journal of Psychosomatic Research* **55**, 569–572 (2003).
126. Hayney, M. S. *et al.* The association between psychosocial factors and vaccine-induced cytokine production. *Vaccine* **21**, 2428–2432 (2003).
127. Cohen, S., Kamarck, T. & Mermelstein, R. Perceived stress scale. *Measuring stress: A guide for health and social scientists* **10**, 1–2 (1994).

128. Noone, P. A. The holmes–rahe stress inventory. *Occupational Medicine* **67**, 581–582 (2017).
129. Galvan, M. J., Payne, B. K., Hannay, J., Georgeson, A. R. & Muscatell, K. A. What Does the MacArthur Scale of Subjective Social Status Measure? Separating Economic Circumstances and Social Status to Predict Health. *Annals of Behavioral Medicine* **57**, 929–941 (2023).
130. Bastianon, C. D. et al. Perceived Stress Scale (PSS-10) psychometric properties in migrants and native Germans. *BMC Psychiatry* **20**, 450 (2020).
131. Komaroff, A. L., Masuda, M. & Holmes, T. H. The social readjustment rating scale: a comparative study of Negro, Mexican and white Americans. *Journal of Psychosomatic Research* (1968).
132. McLennan, J. D., MacMillan, H. L. & Afifi, T. O. Questioning the use of adverse childhood experiences (ACEs) questionnaires. *Child Abuse & Neglect* **101**, 104331 (2020).
133. Kovács-Tóth, B., Oláh, B., Kuritárné Szabó, I. & Fekete, Z. Psychometric properties of the Adverse Childhood Experiences Questionnaire 10 item version (ACE-10) among Hungarian adolescents. *Front. Psychol.* **14**, (2023).
134. Giatti, L., do Valle Camelo, L., de Castro Rodrigues, J. F. & Barreto, S. M. Reliability of the MacArthur scale of subjective social status-Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). *BMC public health* **12**, 1–7 (2012).
135. Church, S., Rogers, E., Rockwood, K. & Theou, O. A scoping review of the Clinical Frailty Scale. *BMC Geriatr* **20**, 393 (2020).

136. OECD. *Health at a Glance 2019: OECD Indicators*. (Organisation for Economic Co-operation and Development, Paris, 2019).
137. Ni Cheallaigh, C. *et al.* HOMELESSNESS IS ASSOCIATED WITH PREMATURE ONSET OF MULTIMORBIDITY AND INCREASED USAGE OF ACUTE HOSPITAL SERVICES. *Age and Ageing* **45**, 54 (2016).
138. Kennedy, B. *et al.* Low stress resilience in late adolescence and risk of smoking, high alcohol consumption and drug use later in life. *J Epidemiol Community Health* **73**, 496–501 (2019).
139. Hawn, S. E., Cusack, S. E. & Amstadter, A. B. A Systematic Review of the Self-Medication Hypothesis in the Context of Posttraumatic Stress Disorder and Comorbid Problematic Alcohol Use. *Journal of Traumatic Stress* **33**, 699–708 (2020).
140. Khantzian, E. J. The Self-Medication Hypothesis of Addictive Disorders: Focus on Heroin and Cocaine Dependence. in *The Cocaine Crisis* (ed. Allen, D. F.) 65–74 (Springer US, Boston, MA, 1987). doi:10.1007/978-1-4613-1837-8_7.
141. Lembke, A. Time to Abandon the Self-Medication Hypothesis in Patients with Psychiatric Disorders. *The American Journal of Drug and Alcohol Abuse* <https://www.tandfonline.com/doi/abs/10.3109/00952990.2012.694532> (2012).
142. Biltz, R. G., Sawicki, C. M., Sheridan, J. F. & Godbout, J. P. The neuroimmunology of social-stress-induced sensitization. *Nature Immunology* **23**, 1527–1535 (2022).
143. Furman, D. *et al.* Chronic inflammation in the etiology of disease across the life span. *Nat Med* **25**, 1822–1832 (2019).

144. Etzel, L., Apsley, A. T., Hastings, W. J., Ye, Q. & Shalev, I. Early life adversity is associated with differential gene expression in immune cells: A cluster-based analysis across an acute psychosocial stressor. *Brain, Behavior, and Immunity* **119**, 724–733 (2024).
145. Maphosa, P. Homelessness and the Assessment of Housing Needs 2014: Report for Dublin. (2017).
146. June 2024 Monthly Homeless Report and Homeless Quarterly Progress Report Quarter 2 2024 published. <https://www.gov.ie/en/press-release/b2a49-june-2024-monthly-homeless-report-and-homeless-quarterly-progress-report-quarter-2-2024-published/> (2024).
147. Family homelessness has risen almost six-fold since 2014, Focus Ireland says. *The Irish Times* <https://www.irishtimes.com/ireland/social-affairs/2024/06/14/family-homelessness-has-risen-almost-six-fold-since-2014-focus-ireland-says/>.
148. Hwang, S. W., Wilkins, R., Tjepkema, M., O'Campo, P. J. & Dunn, J. R. Mortality among residents of shelters, rooming houses, and hotels in Canada: 11 year follow-up study. *BMJ* **339**, b4036 (2009).
149. Henwood, B. F., Katz, M. L. & Gilmer, T. P. Aging in place within permanent supportive housing. *Int J Geriatr Psychiatry* **30**, 80–87 (2015).
150. Liu, M. & Hwang, S. Health care for homeless people. *Nature Reviews Disease Primers* **7**, (2021).

151. McCutchen, C., Hyland, P., Shevlin, M. & Cloitre, M. The occurrence and co-occurrence of ACEs and their relationship to mental health in the United States and Ireland. *Child Abuse & Neglect* **129**, 1–9 (2022).
152. ACEs at Cork Simon: Exploring the Connection between Early Trauma and Later Negative Life Events among Cork Simon Service Users.
<https://www.corksimon.ie/what-we-do/campaigning/cork-simon-research/>
(2017).
153. Hyland, P. *et al.* Trauma, PTSD, and complex PTSD in the Republic of Ireland: prevalence, service use, comorbidity, and risk factors. *Soc Psychiatry Psychiatr Epidemiol* **56**, 649–658 (2021).
154. Jackson, C. Social Exclusion and Gender: Does One Size Fit All? *Eur J Dev Res* **11**, 125–146 (1999).
155. Zhang, M. F. & Gordon, D. Understanding Gender Inequality in Poverty and Social Exclusion through a Psychological Lens: Scarcities, Stereotypes, and Suggestions. in *The Cambridge Handbook of the International Psychology of Women* (eds. Cheung, F. M. & Halpern, D. F.) 390–401 (Cambridge University Press, 2020). doi:10.1017/9781108561716.033.
156. Adler, N. E., Epel, E. S., Castellazzo, G. & Ickovics, J. R. Relationship of subjective and objective social status with psychological and physiological functioning: Preliminary data in healthy, White women. *Health psychology* **19**, 586 (2000).
157. Thoits, P. A. Stress and health: major findings and policy implications. *J Health Soc Behav* **51 Suppl**, S41-53 (2010).

158. Stringhini, S. *et al.* Socioeconomic status and the 25 × 25 risk factors as determinants of premature mortality: a multicohort study and meta-analysis of 1·7 million men and women. *Lancet* **389**, 1229–1237 (2017).
159. Shonkoff, J. P., Garner, A. S., Committee on Psychosocial Aspects of Child and Family Health, Committee on Early Childhood, Adoption, and Dependent Care, & Section on Developmental and Behavioral Pediatrics. The lifelong effects of early childhood adversity and toxic stress. *Pediatrics* **129**, e232-246 (2012).
160. Marmot, M. Social determinants of health inequalities. *Lancet* **365**, 1099–1104 (2005).
161. Weekly low-risk alcohol guidelines. *HSE.ie* <https://www2.hse.ie/living-well/alcohol/health/improve-your-health/weekly-low-risk-alcohol-guidelines/>.
162. Jin, Q. *et al.* Association of Sleep Duration With All-Cause and Cardiovascular Mortality: A Prospective Cohort Study. *Front. Public Health* **10**, (2022).
163. Cappuccio, F. P., Cooper, D., D’Elia, L., Strazzullo, P. & Miller, M. A. Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. *European Heart Journal* **32**, 1484–1492 (2011).
164. Emmel, N., Hughes, K., Greenhalgh, J. & Sales, A. Accessing Socially Excluded People — Trust and the Gatekeeper in the Researcher-Participant Relationship. *Sociological Research Online* **12**, 43–55 (2007).
165. Holborow, M. Homes in Crisis Capitalism : Gender, Work and Revolution. 1–224 (2024).

166. Spratt, T., Devaney, J. & Frederick, J. Adverse Childhood Experiences: Beyond Signs of Safety; Reimagining the Organisation and Practice of Social Work with Children and Families. *The British Journal of Social Work* **49**, 2042–2058 (2019).
167. Kong, J., Liu, Y., Goldberg, J. & Almeida, D. M. Adverse childhood experiences amplify the longitudinal associations of adult daily stress and health. *Child Abuse Negl* **122**, 105337 (2021).
168. Taylor, K. S., Steptoe, A. & Iob, E. The relationship of adverse childhood experiences, hair cortisol, C-reactive protein, and polygenic susceptibility with older adults' psychological distress during the COVID-19 pandemic. *Mol Psychiatry* **27**, 5038–5048 (2022).
169. Mosley-Johnson, E., Campbell, J. A., Garacci, E., Walker, R. J. & Egede, L. E. Stress that Endures: Influence of Adverse Childhood Experiences on Daily Life Stress and Physical Health in Adulthood. *J Affect Disord* **284**, 38–43 (2021).
170. Xie, H., Shih, C.-H., Aldooan, S. D., Wall, J. T. & Wang, X. Hypothalamus volume mediates the association between adverse childhood experience and PTSD development after adulthood trauma. *Transl Psychiatry* **13**, 1–8 (2023).
171. Richard, Georgia, Reddy, Conor, Ní Cheallaigh, Clíona. Recruitment and Engagement of People Experiencing Homelessness in Observational Research: Pearls from a Social Inclusion Research Group. *In Preparation*.
172. Riester, E. *et al.* Performance evaluation of the Roche Elecsys Anti-SARS-CoV-2 S immunoassay. *Journal of Virological Methods* **297**, 114271 (2021).

173. Perkmann, T. *et al.* The Comparability of Anti-Spike SARS-CoV-2 Antibody Tests is Time-Dependent: a Prospective Observational Study. *Microbiol Spectr* **10**, e01402-21.
174. O'Connor, T. G. *et al.* Prenatal maternal anxiety predicts reduced adaptive immunity in infants. *Brain, Behavior, and Immunity* **32**, 21–28 (2013).
175. Tatar, M., Shoorekchali, J. M., Faraji, M. R. & Wilson, F. A. International COVID-19 vaccine inequality amid the pandemic: Perpetuating a global crisis? *J Glob Health* **11**, 03086.
176. Khoury, D. S. *et al.* Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* **27**, 1205–1211 (2021).
177. Cromer, D. *et al.* Neutralising antibody titres as predictors of protection against SARS-CoV-2 variants and the impact of boosting: a meta-analysis. *Lancet Microbe* **3**, e52–e61 (2022).
178. Sette, A. & Crotty, S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell* **184**, 861–880 (2021).
179. van den Boogaard, J., Rots, N. Y., van der Klis, F., de Melker, H. E. & Knol, M. J. Is there an association between socioeconomic status and immune response to infant and childhood vaccination in the Netherlands? *Vaccine* **38**, 3480–3488 (2020).
180. Hoes, J. *et al.* Socioeconomic Status Is Associated With Antibody Levels Against Vaccine Preventable Diseases in the Netherlands. *Front. Public Health* **6**, (2018).

181. Eskenazi, B. *et al.* Undernutrition and antibody response to measles, tetanus and Haemophilus Influenzae type b (Hib) vaccination in pre-school south African children: The VHEMBE birth cohort study. *Vaccine* **46**, 126564 (2025).
182. Lopman, B. A. *et al.* Understanding Reduced Rotavirus Vaccine Efficacy in Low Socio-Economic Settings. *PLOS ONE* **7**, e41720 (2012).
183. Friedman, E. M. & Herd, P. Income, education, and inflammation: differential associations in a national probability sample (the MIDUS study). *Psychosomatic medicine* **72**, 290 (2010).
184. Lustig, A. *et al.* Telomere Shortening, Inflammatory Cytokines, and Anti-Cytomegalovirus Antibody Follow Distinct Age-Associated Trajectories in Humans. *Front. Immunol.* **8**, (2017).
185. Almanan, M. *et al.* IL-10–producing Tfh cells accumulate with age and link inflammation with age-related immune suppression. *Science Advances* **6**, eabb0806 (2020).
186. Amini, A., Pang, D., Hackstein, C.-P. & Klenerman, P. MAIT Cells in Barrier Tissues: Lessons from Immediate Neighbors. *Front Immunol* **11**, 584521 (2020).
187. Kurioka, A. & Klenerman, P. Aging unconventionally: $\gamma\delta$ T cells, iNKT cells, and MAIT cells in aging. *Seminars in Immunology* **69**, 101816 (2023).
188. Tian, W. *et al.* Chronic Stress: Impacts on Tumor Microenvironment and Implications for Anti-Cancer Treatments. *Frontiers in Cell and Developmental Biology* **9**, (2021).
189. Ronaldson, A. *et al.* Increased percentages of regulatory T cells are associated with inflammatory and neuroendocrine responses to acute psychological stress

- and poorer health status in older men and women. *Psychopharmacology (Berl)* **233**, 1661–1668 (2016).
190. Lei, Y. *et al.* Investigating the crosstalk between chronic stress and immune cells: implications for enhanced cancer therapy. *Front Neurosci* **17**, 1321176 (2023).
 191. Stebbins, R. C. *et al.* Persistent socioeconomic and racial and ethnic disparities in pathogen burden in the United States, 1999–2014. *Epidemiol Infect* **147**, e301 (2019).
 192. Semenza, J. C. & Giesecke, J. Intervening to reduce inequalities in infections in Europe. *Am J Public Health* **98**, 787–792 (2008).
 193. Aldridge, R. W. *et al.* Causes of death among homeless people: a population-based cross-sectional study of linked hospitalisation and mortality data in England. *Wellcome Open Res* **4**, 49 (2019).
 194. Beijer, U., Wolf, A. & Fazel, S. Prevalence of tuberculosis, hepatitis C virus, and HIV in homeless people: a systematic review and meta-analysis. *Lancet Infect Dis* **12**, 859–870 (2012).
 195. Prather, A. A. *et al.* Associations between chronic caregiving stress and T cell markers implicated in immunosenescence. *Brain, Behavior, and Immunity* **73**, 546–549 (2018).
 196. Social stressors associated with age-related T lymphocyte percentages in older US adults: Evidence from the US Health and Retirement Study | PNAS.
<https://www.pnas.org/doi/10.1073/pnas.2202780119>.

197. Aiello, A. E. *et al.* Income and markers of immunological cellular aging. *Psychosom Med* **78**, 657–666 (2016).
198. Reid, B. M. *et al.* Persistent skewing of the T-cell profile in adolescents adopted internationally from institutional care. *Brain Behav Immun* **77**, 168–177 (2019).
199. Mogilenko, D. A., Shchukina, I. & Artyomov, M. N. Immune ageing at single-cell resolution. *Nat Rev Immunol* **22**, 484–498 (2022).
200. Rudak, P. T. *et al.* Chronic stress physically spares but functionally impairs innate-like invariant T cells. *Cell Rep* **35**, 108979 (2021).
201. Klopach, E. T. *et al.* Socioeconomic status and immune aging in older US Adults in the Health and Retirement Study. *Biodemography Soc Biol* **67**, 187–202 (2022).
202. Marya, R. & Patel, R. *Inflamed: Deep Medicine and the Anatomy of Injustice*. (Penguin UK, 2021).
203. Maté, G. & Maté, D. *The Myth of Normal: Trauma, Illness & Healing in a Toxic Culture*. (Random House, 2022).
204. Earth beyond six of nine planetary boundaries | Science Advances.
<https://www.science.org/doi/10.1126/sciadv.adh2458>.
205. Cole, S. W. *et al.* Loneliness, eudaimonia, and the human conserved transcriptional response to adversity. *Psychoneuroendocrinology* **62**, 11–17 (2015).
206. Fredrickson, B. L. *et al.* A functional genomic perspective on human well-being. *Proceedings of the National Academy of Sciences* **110**, 13684–13689 (2013).

207. Lori, A. *et al.* Dynamic Patterns of Threat-Associated Gene Expression in the Amygdala and Blood. *Front. Psychiatry* **9**, (2019).
208. J&J vaccine administered through homeless services.
<https://www.rte.ie/news/coronavirus/2021/0506/1214053-covid-19-ireland/>
(2021).
209. Feng, S. *et al.* Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nature Medicine* **27**, 2032–2040 (2021).
210. Lozano-Ojalvo, D. *et al.* Differential effects of the second SARS-CoV-2 mRNA vaccine dose on T cell immunity in naive and COVID-19 recovered individuals. *Cell Reports* **36**, 109570 (2021).
211. Powell, N. D., Tarr, A. J. & Sheridan, J. F. Psychosocial stress and inflammation in cancer. *Brain, Behavior, and Immunity* **30**, S41–S47 (2013).
212. Miller, G. E. *et al.* A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-kappaB signaling. *Biol Psychiatry* **64**, 266–72 (2008).
213. Henson, S. M., Riddell, N. E. & Akbar, A. N. Properties of end-stage human T cells defined by CD45RA re-expression. *Curr Opin Immunol* **24**, 476–481 (2012).
214. Pawelec, G. Hallmarks of human “immunosenescence”: adaptation or dysregulation? *Immunity & Ageing* **9**, 15 (2012).
215. López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. Hallmarks of aging: An expanding universe. *Cell* **186**, 243–278 (2023).
216. The Hallmarks of Aging - PMC.
<https://pmc.ncbi.nlm.nih.gov/articles/PMC3836174/>.

217. Franceschi, C. *et al.* Immunobiography and the Heterogeneity of Immune Responses in the Elderly: A Focus on Inflammaging and Trained Immunity. *Front Immunol* **8**, 982 (2017).
218. Inflamm-aging: An Evolutionary Perspective on Immunosenescence - FRANCESCHI - 2000 - Annals of the New York Academy of Sciences - Wiley Online Library.
<https://nyaspubs.onlinelibrary.wiley.com/doi/abs/10.1111/j.1749-6632.2000.tb06651.x>.
219. Rodrigues, L. P. *et al.* Hallmarks of aging and immunosenescence: Connecting the dots. *Cytokine & Growth Factor Reviews* **59**, 9–21 (2021).
220. Associations between Homelessness and Alzheimer’s Disease and Related Dementia: A Systematic Review - Ganesh M. Babulal, Rohan Rani, Paris Adkins-Jackson, Adam C. Pearson, Monique M. Williams, 2022.
https://journals.sagepub.com/doi/full/10.1177/07334648221109747?casa_token=QPdkveenVYcAAAAA%3ARVDUb3n9w9rRIjV0sicMhaOBL1Mw64weD3lpRWknW6sUWskNDI5Qi5frMTCE8Rj9t1ZH9gLQmyofQ.
221. Burki, T. K. Homelessness and respiratory disease. *The Lancet Respiratory Medicine* **1**, 767–768 (2013).
222. van de Wouw, M. *et al.* Acute stress increases monocyte levels and modulates receptor expression in healthy females. *Brain, Behavior, and Immunity* **94**, 463–468 (2021).

223. Howard, R., Scheiner, A., Kanetsky, P. A. & Egan, K. M. Sociodemographic and lifestyle factors associated with the neutrophil-to-lymphocyte ratio. *Annals of Epidemiology* **38**, 11-21.e6 (2019).
224. Shan, M. *et al.* Association between neutrophil to lymphocyte ratio and depression among US adults: From a large population-based cross-sectional study. *Journal of Psychosomatic Research* **162**, 111041 (2022).
225. Ghafari, S. S., Yousefi, Z., Bakhtiari, E., mohammadi mahdiabadi hasani, mohammad hossein & Hassanzadeh, G. Neutrophil-to-lymphocyte ratio as a predictive biomarker for early diagnosis of depression: A narrative review. *Brain Behav Immun Health* **36**, 100734 (2024).
226. Theses On Feuerbach by Karl Marx.
<https://www.marxists.org/archive/marx/works/1845/theses/>.
227. Dutcher, J. M., Cole, S. W., Williams, A. C. & Creswell, J. D. Smartphone mindfulness meditation training reduces Pro-inflammatory gene expression in stressed adults: A randomized controlled trial. *Brain, Behavior, and Immunity* **103**, 171–177 (2022).
228. Bower, J. E. *et al.* Mindfulness meditation for younger breast cancer survivors: A randomized controlled trial. *Cancer* **121**, 1231–1240 (2015).
229. Hemond, C. C. *et al.* A single-arm, open-label pilot study of neuroimaging, behavioral, and peripheral inflammatory correlates of mindfulness-based stress reduction in multiple sclerosis. *Sci Rep* **14**, 14044 (2024).
230. McCarron, M. *et al.* Patterns of multimorbidity in an older population of persons with an intellectual disability: Results from the intellectual disability supplement

- to the Irish longitudinal study on aging (IDS-TILDA). *Research in Developmental Disabilities* **34**, 521–527 (2013).
231. Lange, K. W. Rudolf Virchow, poverty and global health: from “politics as medicine on a grand scale” to “health in all policies”. *Global Health Journal* **5**, 149–154 (2021).
232. Horton, R. Offline: The secrets of a healthy society. *The Lancet* **387**, 325 (2016).

Appendix 1 – AESS Vax Participant Questionnaire:

N.B. All data collected will be pseudonymised (study number), collected and stored on an encrypted server based in the EU. Clinical researchers on this project will not have access to survey data, and laboratory-based researchers will not have access to any personally identifiable information.

Study Number:

Part I: Basic Participant Information:

N.B. For multiple choice questions please circle the appropriate option

1) **Age:**

- A. 25-29
- B. 30-34
- C. 35-39
- D. 40-44
- E. 45-49
- F. 50-54
- G. 55-59
- H. 60-64
- I. 65-69
- J. 70-74
- K. 75-79
- L. 80+

2) **Ethnicity (Circle appropriate answer):**

- A. White
- B. Black
- C. East Asian
- D. South Asian
- E. West Asian
- F. Arab
- G. Latin American
- H. Aboriginal or First Nations
- I. Roma
- J. Minceir/Traveller
- K. Other _____

3) **Height (cm):** _____

4) **Weight (kg):** _____

5) **What is the highest level of education you have achieved?**

- A. Primary Education
- B. Some Secondary
- C. Junior Certificate
- D. Leaving Certificate
- E. FETAC Level 5 or 6
- F. Bachelors Degree (Level 7 or 8)
- G. Masters Degree or Higher Diploma
- H. PhD or other Level 10
- I. Other _____

6) **Relationship Status:**

- A. Married
- B. Single
- C. Co-habiting/Long-Term Relationship
- D. Separated
- E. Divorced
- F. Other _____

7) **Which category best describes your current housing status (tick all that apply)?**

- A. Homeowner
- B. Council, Approved Housing Body, or other social tenancy
- C. Private Rental
- D. Private Rental (more than 2yrs at same address)
- E. Short-term rental or subletting
- F. Homeless

- G. Living with family/friends
- H. Formerly homeless (*)
- I. Other _____

7.1) How many people are usually resident at your address?

- A. 0-2 others
- B. 3-4 others
- C. 4-5 others
- 6. 6+ others

7.2) If you answered 7 with (F) Homeless, which best describes your situation as a homeless person?

- A. Own door/Supported Accommodation
- B. Own room in hub/hotel/hostel
- C. Night by night Emergency Accommodation
- D. Rough-sleeper
- E. Staying with friends/family ("couch-surfing")
- F. Other _____

7.3) If you answered 7 with H, how many years were you homeless for?

8) Have you ever been incarcerated (including child/adolescent detention, "borstal" etc)?

- (A) Never
- (B) Once (less than 5yrs)
- (C) Once (more than 5yrs)
- (D) Twice
- (E) More than twice

Details:

8.1) If you answered D or E, could you provide more detail on your history of incarceration? (ages when incarcerated, time served etc.)

9) Have you been diagnosed with any long-term or chronic health condition(s)? If so, list them:

10) Are you currently taking any medications? If so, list them:

11) Are you, or have you ever been a smoker?

- A. Yes, currently
- B. Yes, previously
- C. No

11.1) If you answered A or B, how many cigarettes do/did you smoke on average per day? How many years have you/did you smoke(d)?

___ cigarettes per day

___ years smoking

12) Do you consume alcohol?

- A. Yes
- B. No
- C. In the past, but not now

12.1) If you answered A, how many units of alcohol do you consume per week on average?

1 unit = 1/3 glass wine, 1/3 pint of standard beer or cider or 1 measure of spirits

- A. 1-5 units
- B. 6-10 units
- C. 11-15 units
- D. 16-20 units
- E. 20-30 units
- F. Over 30 units per week – estimate ___

13) On the average night, how many hours sleep do you get?

- A. Less than 4
- B. 4-5 hours
- C. 6-7 hours
- D. 8-9 hours
- E. More than 9 hours

14) Do you or have you used illegal drugs?

- A. Yes, currently
- B. Yes, occasionally
- C. Yes, previously
- D. No

14.1) If you answered A or B, which drugs do you use (tick all that apply)?

- A. Cannabis

- B. Cocaine (Powder)**
- C. Crack Cocaine**
- D. Heroin**
- E. Methamphetamines**
- F. Amphetamines**
- G. Ketamine and/or other dissociatives (e.g. PCP, DXM, NOS)**
- H. Benzodiazepines**
- I. Opioid substitutes (methadone etc.)**
- J. Other _____**

Part II: McArthur Ladder – Perceived Social Status:

At the **top** of the ladder are the people who are the best off – those who have the most money, the most education and the most respected jobs. At the **bottom** are the people who are the worst off – who have the least money, least education, and the least respected jobs or no job. The higher up you are on this ladder, the closer you are to the people at the very top; the lower you are, the closer you are to the people at the very bottom.

Where would you place yourself on this ladder?

Please place a large “X” on the rung where you think you stand at this time in your life, relative to other people.



Part III: Perceived Stress Scale:

Circle the appropriate response in each case:

- 1) In the last 6-8 weeks, how often have you been upset because of something that happened unexpectedly?
 - (1) Never
 - (2) Almost Never
 - (3) Sometimes
 - (4) Fairly often
 - (5) Very often

- 2) In the last 6-8 weeks, how often have you felt that you were unable to control the important things in your life?
 - (1) Never
 - (2) Almost Never
 - (3) Sometimes
 - (4) Fairly often
 - (5) Very often

- 3) In the last 6-8 weeks, how often have you felt nervous and stressed?
 - (1) Never
 - (2) Almost Never
 - (3) Sometimes
 - (4) Fairly often
 - (5) Very often

- 4) In the last 6-8 weeks, how often have you felt confident about your ability to handle your personal problems?
 - (1) Never
 - (2) Almost Never
 - (3) Sometimes
 - (4) Fairly often
 - (5) Very often

- 5) In the last 6-8 weeks, how often have you felt that things were going your way?
 - (1) Never
 - (2) Almost Never
 - (3) Sometimes
 - (4) Fairly often
 - (5) Very often

- 6) In the last 6-8 weeks, how often have you found that you could not cope with all the things that you had to do?
 - (1) Never
 - (2) Almost Never

- (3) Sometimes
- (4) Fairly often
- (5) Very often

7) In the last 6-8 weeks, how often have you been able to control irritations in your life?

- (1) Never
- (2) Almost Never
- (3) Sometimes
- (4) Fairly often
- (5) Very often

8) In the last 6-8 weeks, how often have you felt that you were on top of things?

- (1) Never
- (2) Almost Never
- (3) Sometimes
- (4) Fairly often
- (5) Very often

9) In the last 6-8 weeks, how often have you been angered because of things that happened that were outside of your control?

- (1) Never
- (2) Almost Never
- (3) Sometimes
- (4) Fairly often
- (5) Very often

10) In the last 6-8 weeks, how often have you felt difficulties were piling up so high that you could not overcome them?

- (1) Never
- (2) Almost Never
- (3) Sometimes
- (4) Fairly often
- (5) Very often

Part IV: Holmes-Rahe Scale (Life Event Stress):

From the following list, **circle** all those you have experienced in the **past 12 months**:

1. Death of a spouse
2. Divorce
3. Marital Separation from mate
4. Detention in jail or other institution
5. Death of a close family member
6. Major personal injury or illness
7. Marriage
8. Being fired at work
9. Marital reconciliation with mate
10. Retirement from work
11. Major change in the health or behaviour of a family member
12. Pregnancy
13. Sexual Difficulties
14. Gaining a new family member (i.e. Birth , adoption, older adult moving in, etc.)
15. Major business readjustment
16. Major change in financial state (i.e. a lot worse or better off than usual)
17. Death of a close friend
18. Changing to a different line of work
19. Major change in the number of arguments w/spouse (i.e. either a lot more or a lot less than usual)
20. Taking on a mortgage (for home, business, etc.)
21. Foreclosure on a mortgage or loan
22. Major change in responsibilities at work (i.e. promotion, demotion, etc. .)
23. Son or daughter leaving home (marriage, attending college, joined mil.)
24. In-law troubles
25. Outstanding personal achievement
26. Spouse beginning or ceasing work outside the home
27. Beginning or ceasing formal schooling
28. Major change in living condition (new home, remodelling, deterioration of neighbourhood or home etc.)
29. Revision of personal habits (dress manners, associations, quitting smoking)
30. Troubles with the boss
31. Major changes in working hours or conditions
32. Changes in residence
33. Changing to a new school

34. Major change in usual type and/or amount of recreation
35. Major change in church activity (i.e. a lot more or less than usual)
36. Major change in social activities (clubs, movies, visiting, etc. .)
37. Taking on a loan (car, t v, freezer, etc.)
38. Major change in sleeping habits (a lot more or a lot less than usual)
39. Major change in number of family get-togethers
40. Major change in eating habits (a lot food intake, or very different meal hours or surroundings?)
41. Vacation
42. Major holidays
43. Minor violations of the law (traffic tickets, jaywalking, disturbing the peace. etc.)

Part V: Childhood Trauma Questionnaire:

Circle the appropriate response in each case:

A) Emotional Abuse:

- 1) Did a parent or other adult in the household swear at you, insult you or put you down?
 - (1) Never
 - (2) Once or Twice
 - (3) Sometimes
 - (4) Often
 - (5) Very often

- 2) Did a parent or other adult in the household act in a way that made you feel that you might be physically hurt?
 - (1) Never
 - (2) Once or Twice
 - (3) Sometimes
 - (4) Often
 - (5) Very often

B) Physical Abuse:

- 1) Did a parent or other adult in the household push, grab, slap or throw something at you?
 - (1) Never
 - (2) Once or Twice
 - (3) Sometimes
 - (4) Often
 - (5) Very often

- 2) Did a parent or other adult in the household hit you so hard you had marks or were injured?
- (1) Never
 - (2) Once or Twice
 - (3) Sometimes
 - (4) Often
 - (5) Very often

C) Sexual Abuse:

- 1) Did an adult or person at least 5 years older than you touch or fondle you in a sexual way?
- (1) Never
 - (2) Once or Twice
 - (3) Sometimes
 - (4) Often
 - (5) Very often
- 2) Did an adult or person at least 5 years older than you have you touch their body in a sexual way?
- (1) Never
 - (2) Once or Twice
 - (3) Sometimes
 - (4) Often
 - (5) Very often
- 3) Did an adult or person at least 5 years older than you attempt oral, anal, or vaginal intercourse with you?
- (1) Never
 - (2) Once or Twice
 - (3) Sometimes
 - (4) Often
 - (5) Very often
- 4) Did an adult or person at least 5 years older than you actually have oral, anal or vaginal intercourse with you?
- (1) Never
 - (2) Once or Twice
 - (3) Sometimes
 - (4) Often
 - (5) Very often

D) Substance Abuse in the Household:

1) Did you ever live with anyone who was a problem drinker or alcoholic?

- (1) Never
- (2) Once or Twice
- (3) Sometimes
- (4) Often
- (5) Very often

2) Did you ever live with anyone who used street drugs?

- (1) Never
- (2) Once or Twice
- (3) Sometimes
- (4) Often
- (5) Very often

E) Mental Illness in the Household:

1) Was a household member depressed or mentally ill

- (1) Never
- (2) Once or Twice
- (3) Sometimes
- (4) Often
- (5) Very often

2) Did a household member attempt suicide or commit suicide?

- (1) Never
- (2) Once or Twice
- (3) Sometimes
- (4) Often
- (5) Very often

F) Domestic Violence in Household

1) Was your mother/stepmother pushed, grabbed, slapped or had something thrown at her?

- (1) Never
- (2) Once or Twice
- (3) Sometimes
- (4) Often
- (5) Very often

2) Was your mother/stepmother kicked, bitten, hit with a fist, or hit with something hard?

- (1) Never
- (2) Once or Twice
- (3) Sometimes

- (4) Often
- (5) Very often

3) Was your mother/stepmother repeatedly hit over at least a few minutes?

- (1) Never
- (2) Once or Twice
- (3) Sometimes
- (4) Often
- (5) Very often

4) Was your mother/stepmother threatened or hurt by a knife or gun?

- (1) Never
- (2) Once or Twice
- (3) Sometimes
- (4) Often
- (5) Very often

G) History of Incarceration in Household:

1) Did a household member go to prison or jail?

- (1) Never
- (2) Once or Twice
- (3) Sometimes
- (4) Often
- (5) Very often

H) Parental Separation or Divorce:

1) Were your parents/guardians separated or divorced?

- (1) Never
- (2) Once or Twice
- (3) Sometimes
- (4) Often
- (5) Very often

Appendix 2: PATH-I Participant Questionnaire:

1. Clinical Frailty Score:

Clinical Frailty Scale*



1 Very Fit – People who are robust, active, energetic and motivated. These people commonly exercise regularly. They are among the fittest for their age.



2 Well – People who have **no active disease symptoms** but are less fit than category 1. Often, they exercise or are very **active occasionally**, e.g. seasonally.



3 Managing Well – People whose **medical problems are well controlled**, but are **not regularly active** beyond routine walking.



4 Vulnerable – While **not dependent** on others for daily help, often **symptoms limit activities**. A common complaint is being “slowed up”, and/or being tired during the day.



5 Mildly Frail – These people often have **more evident slowing**, and need help in **high order IADLs** (finances, transportation, heavy housework, medications). Typically, mild frailty progressively impairs shopping and walking outside alone, meal preparation and housework.



6 Moderately Frail – People need help with **all outside activities** and with **keeping house**. Inside, they often have problems with stairs and need **help with bathing** and might need minimal assistance (cuing, standby) with dressing.



7 Severely Frail – **Completely dependent for personal care**, from whatever cause (physical or cognitive). Even so, they seem stable and not at high risk of dying (within ~ 6 months).



8 Very Severely Frail – Completely dependent, approaching the end of life. Typically, they could not recover even from a minor illness.



9 Terminally Ill - Approaching the end of life. This category applies to people with a **life expectancy <6 months**, who are **not otherwise evidently frail**.

Scoring frailty in people with dementia

The degree of frailty corresponds to the degree of dementia. Common **symptoms in mild dementia** include forgetting the details of a recent event, though still remembering the event itself, repeating the same question/story and social withdrawal.

In **moderate dementia**, recent memory is very impaired, even though they seemingly can remember their past life events well. They can do personal care with prompting.

In **severe dementia**, they cannot do personal care without help.

* 1. Canadian Study on Health & Aging, Revised 2008.

2. K. Rockwood et al. A global clinical measure of fitness and frailty in elderly people. CMAJ 2005;173:489-495.

Score: _____

2. Grip Strength:

Left: _____

Right: _____

3. Height and Weight:

Height: _____

Weight: _____

4. Timed Up and Go (If space permits):

Time taken to get up from standing, walk 3 metres and sit back down

Time: _____ seconds

5. Employment Status:

A. What is your current employment status? – Circle most applicable:

- i. Retired
- ii. Employed
- iii. Self-Employed
- iv. Unemployed
- v. Permanent Illness or disability
- vi. Full time carer
- vii. Full time education or training

B. If you are employed what is your current job?

C. If you are unemployed, when did you become unemployed?

6. Parental Occupation in Childhood:

What occupation did your parent(s) or caregivers have in childhood:

7. Care and Incarceration:

- i. Were you in care or foster care as a child? If yes, please give details:

- ii. Have you ever been to prison or juvenile detention? If yes, please give details:

8. Do you drink?

A. How often do you have a drink containing alcohol?

—

B. On a typical day when you are drinking, how many standard drinks/units do you consume?

—

C. How often do you consume 6 or more drinks?

—

9. Smoking:

A. How many cigarettes do you smoke per day?

—

B. At what age did you start smoking?

—

10. Drugs:

Do you currently use drugs? Y/N

A. What types of drugs do you use/did you use?

—

B. How often do you use drugs/did you use drugs?

—

C. On a day you use/used drugs, how many times per day do you use/did you use them?

—

D. Do you/did you use more than one type of drug on the same occasion?

—

11. Sleep:

Approx How Many Hours Do You Sleep at Night? _____

12. Falls:

Have you fallen in the last year? Y/N circle as appropriate and answer following:

A. How many times have you fallen in the past year?

B. Were any of the falls non-accidental/have no cause?

C. Did you injure yourself seriously enough to need medical treatment?

D. Are you afraid of falling?

E. Have you ever fractured bones in your body?

13. Traumatic Brain Injury:

A. Have You Ever Hit Your Head or Been Hit on the Head? Y/N

B. Have You Ever Been Seen In A&E Due to A Head Injury? Y/N

C. Did You Ever Lose Consciousness or have a dazed or confused experience Due to A Head Injury? Y/N

D. Do You Experience Any of these Problems Since the Head Injury? Y/N

E. Did You Ever Have Any Significant Sickness After Hitting Your Head? Y/N

F. How Would You Rate Your Day to Day Memory?

14. Oral Health:

A. What is you current dental situation?

B. Does your dental situation cause you pain and/or discomfort daily?

C. Do you have access to dental services when you need them?

15. Health Supports:

A. Do you have GP Supports?

B. Do you have a keyworker? Y/N

C. Do you have a case manager? Y/N

D. What hospital do you attend?

E. Over the last 12 months which of the following services have you availed of? **Circle all that apply**

- i. Methadone/Opioid Replacement
- ii. Needle Exchange
- iii. Other Addiction Service
- iv. Keyworker
- v. Alcohol Detox Rehab
- vi. Drug Detox Rehab
- vii. Home Help
- viii. Personal Care Attendant
- ix. Meals
- x. Food Parcels

16. Family and Relationships:

A. Do you have children? Y/N

B. How many children do you have? _____

C. How often do you feel you lack companionship?

- i. Hardly ever/Never

- ii. Some of the time
- iii. Often
- D. How often do you feel left out?
 - i. Hardly ever/Never
 - ii. Some of the time
 - iii. Often
- E. How often do you feel isolated from others?
 - i. Hardly ever/Never
 - ii. Some of the time
 - iii. Often
- F. How often do you feel lonely?
 - i. Often/Always
 - j. Some of the time
 - k. Occasionally
 - l. Hardly ever
 - m. Never

17. Mental Health and Wellbeing – MHC-SF:

Please answer the following questions are about how you have been feeling during the past month. Place a check mark in the box that best represents how often you have experienced or felt the following:

During the past month, how often did you feel ...	NEVER	ONCE OR TWICE	ABOUT ONCE A WEEK	ABOUT 2 OR 3 TIMES A WEEK	ALMOST EVERY DAY	EVERY DAY
1. happy						
2. interested in life						
3. satisfied with life						
4. that you had something important to contribute to society						
5. that you belonged to a community (like a social group, or your neighborhood)						
6. that our society is a good place, or is becoming a better place, for all people						

7. that people are basically good						
8. that the way our society works makes sense to you						
9. that you liked most parts of your personality						
10. good at managing the responsibilities of your daily life						
11. that you had warm and trusting relationships with others						
12. that you had experiences that challenged you to grow and become a better person						
13. confident to think or express your own ideas and opinions						
14. that your life has a sense of direction or meaning to it						

18. **Life Satisfaction:**

A. Cantril Ladder: Please imagine a ladder with steps numbered from zero at the bottom to ten at the top. Suppose we say that the top of the ladder represents the best possible life for you and the bottom of the ladder represents the worst possible life for you. If the top step is 10 and the bottom step is 0, on which step of the ladder do you feel you personally stand at the present time?

B. I am satisfied with my life? Y/N

C. What brings satisfaction? e.g Family, friends, pets, exercise, substances/alcohol, religion, helping others, eating, arts etc.

D. To what extent do you help others?

19. **Holmes-Rahe Life Event Stress Inventory:**

From the following list, **circle** all those you have experienced in the **past 12 months**:

44. Death of a spouse
45. Divorce
46. Marital Separation from mate
47. Detention in jail or other institution
48. Death of a close family member
49. Major personal injury or illness
50. Marriage
51. Being fired at work
52. Marital reconciliation with mate
53. Retirement from work
54. Major change in the health or behaviour of a family member
55. Pregnancy
56. Sexual Difficulties
57. Gaining a new family member (i.e. Birth , adoption, older adult moving in, etc.)
58. Major business readjustment
59. Major change in financial state (i.e. a lot worse or better off than usual)
60. Death of a close friend
61. Changing to a different line of work
62. Major change in the number of arguments w/spouse (i.e. either a lot more or a lot less than usual)
63. Taking on a mortgage (for home, business, etc.)
64. Foreclosure on a mortgage or loan
65. Major change in responsibilities at work (i.e. promotion, demotion, etc. .)
66. Son or daughter leaving home (marriage, attending college, joined mil.)
67. In-law troubles
68. Outstanding personal achievement
69. Spouse beginning or ceasing work outside the home
70. Beginning or ceasing formal schooling
71. Major change in living condition (new home, remodelling, deterioration of neighbourhood or home etc.)
72. Revision of personal habits (dress manners, associations, quitting smoking)
73. Troubles with the boss
74. Major changes in working hours or conditions
75. Changes in residence
76. Changing to a new school
77. Major change in usual type and/or amount of recreation
78. Major change in church activity (i.e. a lot more or less than usual)

79.	Major change in social activities (clubs, movies, visiting, etc. .)
80.	Taking on a loan (car, t v, freezer, etc.)
81.	Major change in sleeping habits (a lot more or a lot less than usual)
82.	Major change in number of family get-togethers
83.	Major change in eating habits (a lot food intake, or very different meal hours or surroundings?
84.	Vacation
85.	Major holidays
86.	Minor violations of the law (traffic ticket s, jaywalking, disturbing the peace. etc.)

20. Adverse Childhood Experiences:

Prior to your 18th birthday:

1. Did a parent or other adult in the household often or very often... Swear at you, insult you, put you down, or humiliate you?
or Act in a way that made you afraid that you might be physically hurt?

If yes enter 1 _____

2. Did a parent or other adult in the household often or very often... Push, grab, slap, or throw something at you?
or Ever hit you so hard that you had marks or were injured?

If yes enter 1 _____

3. Did an adult or person at least 5 years older than you ever... Touch or fondle you or have you touch their body in a sexual way?
or Attempt or actually have oral, anal, or vaginal intercourse with you?

If yes enter 1 _____

3. Did you often or very often feel that ... No one in your family loved you or thought you were important or special?
or Your family didn't look out for each other, feel close to each other, or support each other?

If yes enter 1 _____

5. Did you often or very often feel that ... You didn't have enough to eat, had to wear dirty clothes, and had no one to protect you?

or Your parents were too drunk or high to take care of you or take you to the doctor if you needed it?

If yes enter 1 _____

6. Was a biological parent ever lost to you through divorce, abandonment, or other reason?

If yes enter 1 _____

7. Was your mother or stepmother: Often or very often pushed, grabbed, slapped, or had something thrown at her?

or Sometimes, often, or very often kicked, bitten, hit with a fist, or hit with something hard? or Ever repeatedly hit over at least a few minutes or threatened with a gun or knife?

If yes enter 1 _____

8. Did you live with anyone who was a problem drinker or alcoholic or who used street drugs?

If yes enter 1 _____

9. Was a household member depressed or mentally ill or did a household member attempt suicide?

If yes enter 1 _____

10. Did a household member go to prison?

If yes enter 1 _____

21. Berkman-Syme Social Network Index:

A. How many *close friends* do you have, people that you feel at ease with and can talk to about private matters?

- i. None
- ii. 1 or 2
- iii. 3 to 5

- iv. 6 to 9
 - v. 10 or more
 - vi. Unknown
- B. How many close friends do you see at least once per month?
- i. None
 - ii. 1 or 2
 - iii. 3 to 5
 - iv. 6 to 9
 - v. 10 or more
 - vi. Unknown
- C. How many *relatives* do you have, people that you feel at ease with and can talk to about private matters?
- i. None
 - ii. 1 or 2
 - iii. 3 to 5
 - iv. 6 to 9
 - v. 10 or more
 - vi. Unknown
- D. How many relatives do you see at least once per month?
- i. None
 - ii. 1 or 2
 - iii. 3 to 5
 - iv. 6 to 9
 - v. 10 or more
 - vi. Unknown
- E. Do you participate in any groups, such as a social or work group, self-help group, drop in centre, community group, religious congregation or public service?
- i. No
 - ii. Yes
 - iii. Unknown
- F. About how often do you go to religious meetings or services?
- i. Never or almost never
 - ii. Once or twice a year
 - iii. Every few months
 - iv. Once or twice a month
 - v. Once a week
 - vi. More than once a week
 - vii. Unknown
- G. Is there someone available to you whom you can count on to listen to you when you need to talk?

- i. None
- ii. 1 or 2
- iii. 3 to 5
- iv. 6 to 9
- v. 10 or more
- vi. Unknown

H. Is there someone available to give you good advice about a problem?

- i. None
- ii. 1 or 2
- iii. 3 to 5
- iv. 6 to 9
- v. 10 or more
- vi. Unknown

I. Is there someone available to you who shows you love and affection?

- i. None
- ii. 1 or 2
- iii. 3 to 5
- iv. 6 to 9
- v. 10 or more
- vi. Unknown

J. Can you count on anyone to provide you with emotional support – talking over problems or helping you make a difficult decision?

- i. None
- ii. 1 or 2
- iii. 3 to 5
- iv. 6 to 9
- v. 10 or more
- vi. Unknown

K. Do you have as much contact as you would like with someone you feel close to, someone in who you can trust and confide?

- i. None
- ii. 1 or 2
- iii. 3 to 5
- iv. 6 to 9
- v. 10 or more
- vi. Unknown

22. External Shame - OAS-2:

- A. I think others look down on me
 - a. Never
 - b. Seldom
 - c. Sometimes
 - d. Frequently
 - e. Almost Always

- B. People distance themselves from me when I make mistakes
 - a. Never
 - b. Seldom
 - c. Sometimes
 - d. Frequently
 - e. Almost Always

- C. Others think there is something missing in me
 - a. Never
 - b. Seldom
 - c. Sometimes
 - d. Frequently
 - e. Almost Always

- D. People see me as unimportant compared to others
 - a. Never
 - b. Seldom
 - c. Sometimes
 - d. Frequently
 - e. Almost Always

- E. Other people see me as not measuring up to them
 - a. Never
 - b. Seldom
 - c. Sometimes
 - d. Frequently
 - e. Almost Always

- F. Other people see me as somehow defective as a person
 - a. Never
 - b. Seldom
 - c. Sometimes
 - d. Frequently
 - e. Almost Always

G. Others think there is something missing in me

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

H. Others see me as empty and unfulfilled

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

23. Internal Shame – ISS-S and ISS-SE

1. I feel like I am never quite good enough

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

2. I feel somewhat left out

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

3. I think people look down on me

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

4. All in all I am inclined to feel like a success

- a. Never
- b. Seldom
- c. Sometimes

- d. Frequently
 - e. Almost Always
5. I scold myself and put myself down
- a. Never
 - b. Seldom
 - c. Sometimes
 - d. Frequently
 - e. Almost Always
6. I feel insecure about others opinions of me
- a. Never
 - b. Seldom
 - c. Sometimes
 - d. Frequently
 - e. Almost Always
7. Compared to other people I feel like I somehow never measure up
- a. Never
 - b. Seldom
 - c. Sometimes
 - d. Frequently
 - e. Almost Always
8. I see myself as being very small and insignificant
- a. Never
 - b. Seldom
 - c. Sometimes
 - d. Frequently
 - e. Almost Always
9. I feel I have much to be proud of
- a. Never
 - b. Seldom
 - c. Sometimes
 - d. Frequently
 - e. Almost Always
10. I feel intensely inadequate and full of self doubt
- a. Never
 - b. Seldom
 - c. Sometimes

- d. Frequently
- e. Almost Always

11. I feel as if I am somehow defective as a person like there is something wrong with me

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

12. When I compare myself to others I am just not as important

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

13. I have an overpowering dread that my faults will be revealed in front of others

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

14. I feel I have a number of good qualities

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

15. I see myself striving for perfection only to continually fall short

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

16. I think others are able to see my defects

- a. Never

- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

17. I could beat myself over the head club when I make a mistake

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

18. On the whole I am satisfied with myself

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

19. I would like to shrink away when I make a mistake

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

20. I replay painful events over and over in my mind until I am overwhelmed

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

21. I feel I am a person of worth at least on an equal plane with others

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

22. At times I feel like I will break into a thousand pieces

- a. Never

- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

23. I feel as if I have lost control over my bodily functions and my feelings

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

24. Sometimes I feel no bigger than a pea

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

25. At times I feel so exposed that I wish the earth would open up and swallow me

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

26. I have this painful gap within me that I have not been able to fill

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

27. I feel empty and unfulfilled

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

28. I take a positive attitude towards myself

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

29. My loneliness is more like emptiness

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always

30. I feel like there is something missing

- a. Never
- b. Seldom
- c. Sometimes
- d. Frequently
- e. Almost Always