HRS Documentation Report

Flow Cytometry Results from the 2016 Venous Blood Study in the 2016 Health and Retirement Study

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Introduction

This document contains information on the flow cytometry data collected as part of the HRS 2016 Venous Blood Study (VBS).

Procedures for collection, assay, and descriptions of other blood-based variables are included in the documentation - *Venous blood collection and assay protocol in the 2016 Health and Retirement Study*. https://hrs.isr.umich.edu/publications/biblio/9065. The earlier document provided initial flow cytometry results for about 1,000 cases. This document contains additional information on our approach to flow cytometry and outlines in more detail the subsets characterized as well as descriptive information on flow cytometry for the entire 2016 VBS sample.

Additional detail on the effects of collection, processing, storage of cells and analysis of flow cytometry results are provided in:

Thyagarajan B, Barcelo H, Crimmins E, Weir D, Minnerath S, Vivek S, Faul J. Effect of delayed cell processing and cryopreservation on immunophenotyping in multicenter population studies. J Immunol Methods. 2018 Dec; 463:61-70. doi: 10.1016/j.jim.2018.09.007. Epub 2018 Sep 14. PubMed PMID: 30222961.

Barcelo H, Faul J, Crimmins E, Thyagarajan B. A Practical Cryopreservation and Staining Protocol for Immunophenotyping in Population Studies. Curr Protoc Cytom. 2018 Apr;84(1):e35. doi: 10.1002/cpcy.35. PubMed PMID: 30040214; PubMed Central PMCID: PMC6060642.

Hunter-Schlichting D, Lane J, Cole B, Flaten Z, Barcelo H, Ramasubramanian R, Cassidy E, Faul J, Crimmins E, Pankratz N, Thyagarajan B. Validation of a hybrid approach to standardize immunophenotyping analysis in large population studies: The Health and Retirement Study. Scientific Reports 2020; 10(1): 8759. PMID: 32472068; PMCID: PMC7260195.

Subsample Weights

Sample Weights for the 2016 Venous Blood Study – Full sample (PVBSWGTR)

Respondents with at least one valid venous blood result (VBS16VALID) were assigned a VBS weight. The weights were adjusted for the differential probabilities of participation by dividing the HRS 2016 sample weight by the predicted probability of having a valid venous blood result among community-dwelling 2016 HRS respondents born prior to 1960, excluding all members of the LBB cohort. The resulting interim weight was trimmed at the 1st and 99th percentiles and was then post stratified back to the entire 2016 HRS sample born prior to 1960 by age, sex, and race/ethnicity. Analysts should use *PVBSWGTR* to weight the data included with this release.

Flow Cytometry Assays

We used multiparameter flow cytometry to obtain counts and percentages for the distributions of 24 different types of T-cells, B-cells, Natural Killer (NK) cells, monocytes, and dendritic cells

(DC). Flow cytometry results are available for approximately 9300 respondents for any given cell subset.

The immune cell subsets were identified using minor modifications to the standardized protocol published by the Human Immunology Project (Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. Nat Rev Immunol. 2012 Feb 17:12(3):191-200). The modifications include addition of CD95 to more definitively identify different subsets of T cells. One vial of cryopreserved mononuclear cells containing ~4 million cells was thawed and cells were incubated at 37°C in RPMI media for 1 hour. The cells were centrifuged at 1200rpm for 10 min at room temperature. The cells were resuspended in 1X PBS and stained using the two antibody cocktails as outlined in the tables below. The cells were kept on ice until analysis. All flow cytometry measurements were performed on a LSRII or a Fortessa X20 flow cytometer (BD Biosciences, San Diego, CA). (Thyagarajan et al. 2018)

We present data as both % cells and as absolute counts.

% T-cells, % B-cells were defined as % of events in the live lymphocytes gate after removing doublets. Percentages of T and B cell subsets were defined as % of the parent population (e.g.) %CD4+ T cells was defined as a % of total T cells.

Absolute counts of T and B-cells were calculated by multiplying the % cells and the total lymphocyte count (obtained from the complete blood count (CBC)) and expressed as 10^9/L.

% monocytes, %NK cells and % DCs were defined as % peripheral blood mononuclear cells (PBMCs). Percentages of monocytes, NK cells and DC subsets were defined as % of the parent population (e.g.) %CD16+ monocytes was defined as a % of total monocytes.

Absolute counts of monocytes, NK cells and DCs were calculated by multiplying the % cells with the total PBMC count. Total PBMC count was calculated as total [white blood cell count – (neutrophil count+eosinophil count+basophil count)]. The absolute count was expressed as 10^9/L.

T cells	Percent Calculation
CD4+ T cells	CD4+ T cells/total T cells*100
CD4+ T cells: Central Memory (CM)	CD4+ CM T cells/total CD4+ T cells*100
CD4+ T cells: (TemRA)	CD4+ TemRA T cells/total CD4+T cells*100

Panel 1 (T cells and B cells)

Marker	Clone	Fluorochrome	Provider (cat #)	Volume/sample (µl)
brilliant stain buffer	NA	NA	BD (659611)	50
viability dye	NA	FVS 570 (PE)	BD (564995)	12
CD3	UCHT1	APC	BD (555335)	4
HLA-DR	G46-6	PE-CF594	BD (562331)	1
CD19	SJ25C1	PE-Cy7	BD (557835)	1
CD27	O323	FITC	Biolegend (302806)	2.5
CD8	RPA-T8	BUV395	BD (563796)	1
IgD	IA6-2	BUV737	BD (564687)	1
CCR7	G043H7	BV421	Biolegend (353208)	2.5
CD28	CD28.2	BV510	Biolegend (302936)	2.5
CD45RA	HI100	BV711	Biolegend (304138)	2.5
CD4	RPA-T4	APC-Cy7	BD (557871)	1

Panel 2 (monocytes, DC, NK)

Marker	Clone	Fluorochrome	Provider (cat #)	Volume/ sample (ul)
brilliant stain buffer	NA	NA	BD (659611)	50
viability dye	NA	FVS 570 (PE)	BD (564995)	12
CD3	UCHT1	APC	BD (555335)	4
HLA-DR	G46-6	PE-CF594	BD (562331)	1
CD19	SJ25C1	PE-Cy7	BD (557835)	1
CD11c		BB515	BD (564490)	1
CD20	2H7	BUV395	BD (563781)	1
CD16	3G8	BUV737	BD (564433)	1
CD56	NCAM16.2	BV421	BD (562751)	1
CD14	MOP9	BV510	BD (563079)	1
CD123	9F5	BV711	BD (563161)	1
CD45	2D1	APC-Cy7	BD (560178)	0.5

Table 1: Markers used to determine 24 cell subsets using flow cytometry

CELL TYPE	MARKERS	Variable Name	
		Percentages	Counts
T cells	CD3+ CD19-	PTcell_pct	PTcell_count
CD4+ T cells	CD3+ CD19- CD8- CD4+	PCD4T_pct	PCD4T_count
CD4+ T cells: Central Memory (CM)	CD3+ CD19- CD8- CD4+ CD45RA- CCR7+ CD28+	PCD4M_pct	PCD4M_count
CD4+ T cells: (TemRA)	CD3+ CD19- CD8- CD4+ CD45RA+ CCR7- CD28-	PCD4TEMRA_pct	PCD4TEMRA_count
CD4+ T cells: Effector memory (Tem)	CD3+ CD19- CD8- CD4+ CD45RA- CCR7- CD28-	PCD4TEM_pct	PCD4TEM_count
CD4+ T cells: Naïve	CD3+ CD19- CD8+ CD4+ CD45RA+ CCR7+ CD28+	PCD4N_pct	PCD4N_count
CD8+ T cells	CD3+ CD19- CD8+ CD4-	PCD8T_pct	PCD8T_count
CD8+ T cells: Central Memory (CM)	CD3+ CD19- CD8+ CD4- CD45RA- CCR7+ CD28+	PCD8M_pct	PCD8M_count
CD8+ T cells: (TemRA)	CD3+ CD19- CD8+ CD4- CD45RA+ CCR7- CD28-	PCD8TEMRA_pct	PCD8TEMRA_count
CD8+ T cells: Effector Memory (Tem)	CD3+ CD19- CD8+ CD4- CD45RA- CCR7- CD28-	PCD8TEM_pct	PCD8TEM_count
CD8+ T cells: Naïve	CD3+ CD19- CD8+ CD4- CD45RA+ CCR7+ CD28+	PCD8N_pct	PCD8N_count
B cells	CD3- CD19+	PBcell_pct	PBcell_count
IgD+ memory B cells	CD3- CD19+ IgD+ CD27+	PIgD_Plus_MemB_pct	PIgD_Plus_MemB_count
IgD- memory B cells	CD3- CD19+ IgD- CD27+	PIgD_Minus_MemB_pct	PIgD_Minus_MemB_count
Naive B cells	CD3- CD19+ IgD+ CD27-	PNaiveB_pct	PNaiveB_count
Natural Killer (NK) Cells	CD3- CD19- CD20- CD14- CD16+ CD56+	PNK_pct[1]	PNK_count
NK Cells: CD56HI	CD3- CD19- CD20- CD14- CD16+ CD56HI	PNKHI_pct	PNKHI_count
NK Cells: CD56LO	CD3- CD19- CD20- CD14- CD16+ CD56LO	PNKLO_pct	PNKLO_count
Monocytes	CD3- CD19- CD20- CD14+	PMONO_pct[2]	PMONO_count
CD16- monocytes	CD3- CD19- CD20- CD14+ CD16-	PMONO16Minus_pct	PMONO16Minus _count
CD16+ monocytes	CD3- CD19- CD20- CD14+ CD16+	PMONO16Plus_pct	PMONO16Plus_count
Dendritic cells	CD3- CD19- CD20- CD14- HLA-DR+	PDC_pct[3]	PDC_count
Myeloid Dendritic cells (DC-M)	CD3- CD19- CD20- CD14- HLA-DR+ CD11c+ CD123-	PDCm_pct	PDCm_count
Plasmacytoid Dendritic cells (DC-P)	CD3- CD19- CD20- CD14- HLA-DR+ CD11c- CD123+	PDCp_pct	PDCp_count

Table 2. Percentages - Cell Subsets: Descriptive Measures (Unweighted)

Variable	N	Mean	Std Dev	Minimum	Maximum
T cells	9359	0.699	0.130	0.020	0.971
CD4+ T cells	9359	0.677	0.138	0.000	0.966
CD4+ T cells: Central Memory (CM)	9313	0.366	0.123	0.000	0.823
CD4+ T cells: TemRA	9313	0.040	0.066	0.000	0.815
CD4+ T cells: Tem	6918	0.014	0.041	0.000	0.524
CD4+ T cells: Naïve	9313	0.430	0.178	0.000	0.947
CD8+ T cells	9359	0.248	0.119	0.014	0.929
CD8+ T cells: Central Memory (CM)	7482	0.086	0.058	0.000	0.633
CD8+ T cells: TemRA	9314	0.458	0.221	0.000	0.994
CD8+ T cells: Tem	7476	0.021	0.044	0.000	0.553
CD8+ T cells: Naïve	9314	0.228	0.163	0.000	0.905
B cells	9359	0.071	0.055	0.000	0.959
IgD+ memory B cells	9336	0.130	0.099	0.000	0.858
IgD- memory B cells	9336	0.118	0.095	0.000	0.980
Naive B cells	9336	0.645	0.174	0.000	0.978
Natural Killer (NK) Cells	9092	0.035	0.024	0.000	0.338
NK Cells: CD56HI	9309	0.009	0.017	0.000	0.420
NK Cells: CD56LO	9309	0.929	0.080	0.002	1.000
Monocytes	9092	0.030	0.022	0.000	0.280
CD16- monocytes	9306	0.951	0.087	0.049	1.000
CD16+ monocytes	9306	0.041	0.050	0.000	0.951
%Monocytes (2016 VBS file)[1]	9597	0.085	0.024	0.000	0.435
Dendritic cells	9092	0.008	0.006	0.000	0.182
Myeloid Dendritic cells (DC-M)	9308	0.716	0.125	0.021	0.986
Plasmacytoid Dendritic cells (DC-P)	9308	0.156	0.094	0.001	0.902

[1] Monocyte percent comes from the complete blood count in the 2016 VBS data release (variable name for the monocyte %: pmono).

Table 3. Absolute counts - Cell Subsets: Descriptive Measures (Unweighted)

Variable	N	Mean	Std Dev	Minimum	Maximum
T cells	9141	1.405	0.700	0.022	30.484
CD4+ T cells	9141	0.947	0.473	0.000	10.508
CD4+ T cells: Central Memory (CM)	9095	0.339	0.197	0.000	4.505
CD4+ T-cells: TemRA	9095	0.036	0.076	0.000	2.063
CD4+ T cells: Tem	6760	0.012	0.045	0.000	1.201
CD4+ T cells: Naïve	9095	0.427	0.314	0.000	3.595
CD8+ T cells	9141	0.355	0.313	0.008	16.839
CD8+ T cells: Central Memory (CM)	7309	0.028	0.029	0.000	0.644
CD8+ T cells: TemRA	9096	0.181	0.254	0.000	15.584
CD8+ T cells: Tem	7305	0.009	0.027	0.000	0.738
CD8+ T cells: Naïve	9096	0.070	0.068	0.000	1.037
B cells	9141	0.194	2.491	0.000	192.806
IgD+ memory B cells	9118	0.021	0.166	0.000	9.385
IgD- memory B cells	9118	0.047	2.095	0.000	188.988
Naive B cells	9118	0.096	0.160	0.000	8.573
Natural Killer (NK) Cells	9092	0.226	0.167	0.000	3.078
NK Cells: CD56HI	9091	0.002	0.003	0.000	0.078
NK Cells: CD56LO	9091	0.212	0.160	0.000	2.593
Monocytes Flow Data	9092	0.199	0.159	0.001	1.898
CD16- monocytes	9088	0.188	0.151	0.001	1.530
CD16+ monocytes	9088	0.008	0.020	0.000	0.805
Monocyte Count (2016 VBS file)[1]	9597	0.562	0.224	0.000	8.500
CD16- monocytes based on VBS absolute count[2]	9092	0.535	0.219	0.000	8.067
CD16+ monocytes based on VBS absolute count[2]	9092	0.023	0.031	0.000	0.653
Dendritic cells	9092	0.051	0.083	0.000	6.846
Myeloid Dendritic cells (DC-M)	9090	0.037	0.039	0.000	2.074
Plasmacytoid Dendritic cells (DC-P)	9090	0.007	0.047	0.000	4.402

[1]For monocytes, we added 3 variables to this table - monocyte count, CD16-, and CD16+ - from the complete blood count data in the 2016 VBS data release (variable name for the monocyte count: pamon).

[2]To calculate the counts of CD16+ and CD16- monocytes for the CBC data we use the following formulas:

CD16+ absolute count = % CD16+*absolute count of monocytes
CD16- absolute count = % CD16-*absolute count of monocytes

QC results

We provide results of the QC analysis done on about 200 blinded duplicates. Individual subsets do not show significant differences. We believe this indicates that our study procedures worked well and did not produce systematic bias. Large CVs between blinded duplicate samples were observed for some cell subsets that are present in very low proportions in peripheral blood. These are indicated with a *.

Percentages

		QC Pairs				
	N	Mean	Within SD (Lab)	Between SD	Reliab	CV
T cells	194	0.73	0.10	0.10	0.89	3.53
CD4+ T cells	194	0.68	0.11	0.11	0.96	2.52
CD4+ T cells: Central Memory (CM)	194	0.31	0.11	0.11	0.78	8.01
CD4+ T-cells: TemRA*	194	0.02	0.04	0.04	0.92	34.64
CD4+ T cells: Tem*	114	0.01	0.02	0.02	0.98	31.18
CD4+ T cells: Naïve	194	0.51	0.18	0.18	0.86	6.60
CD8+ T cells	194	0.25	0.09	0.09	0.97	5.41
CD8+ T cells: Central Memory (CM)	130	0.07	0.04	0.04	0.91	12.83
CD8+ T cells: TemRA	194	0.02	0.04	0.04	0.95	13.77
CD8+ T cells: Tem*	130	0.01	0.02	0.01	0.19	43.71
CD8+ T cells: Naïve	194	0.40	0.20	0.20	0.97	8.44
B cells*	194	0.07	0.04	0.04	0.88	15.89
IgD+ memory B cells*	194	0.15	0.09	0.09	0.82	16.39
IgD- memory B cells*	194	0.12	0.08	0.08	0.73	21.28
Naive B cells	194	0.63	0.16	0.16	0.90	6.50
Natural Killer (NK) Cells	202	0.08	0.04	0.04	0.90	12.98
NK Cells: CD56HI*	202	0.01	0.01	0.01	0.66	32.94
NK Cells: CD56LO	202	0.96	0.04	0.04	0.67	1.32
Monocytes*	202	0.06	0.07	0.07	0.87	29.67
CD16- monocytes	202	0.96	0.04	0.04	0.45	1.58
CD16+ monocytes*	202	0.04	0.04	0.04	0.46	32.59
Monocytes (2016 VBS file)	190	7.58	1.7	1.7	0.91	5.4
Dendritic cells*	202	0.01	0.01	0.01	0.66	23.63
Myeloid Dendritic cells (DC-M)*	202	0.64	0.12	0.12	0.86	5.16
Plasmacytoid Dendritic cells (DC-P)	202	0.25	0.12	0.12	0.91	13.18

* Large CVs (>15%) between blinded duplicate samples.

Absolute counts

	N	Mean	Within SD (Lab)	Between SD	Reliab	CV
			QC Pairs			
T cells	180	1.35	0.49	0.50	0.9695	5.02
CD4+ T cells	181	0.92	0.38	0.38	0.9676	5.86
CD4+ T cells: Central Memory (CM)	180	0.28	0.15	0.15	0.8760	8.47
CD8+ T cells: TemRA	180	0.10	0.10	0.10	0.9648	13.64
CD4+ T cells: Tem*	110	0.00	0.01	0.01	0.9634	28.13
CD4+ T cells: Naïve	180	0.48	0.29	0.30	0.9232	10.64
CD8+ T cells	180	0.34	0.17	0.17	0.9639	8.04
CD8+ T cells: Central Memory (CM)*	126	0.02	0.01	0.01	0.9098	15.73
CD8+ T cells: TemRA	180	0.10	0.10	0.10	0.9648	13.64
CD8+ T cells: Tem*	126	0.00	0.00	0.00	0.3533	44.54
CD8+ T cells: Naïve	180	0.14	0.12	0.12	0.9640	10.75
B cells*	181	0.13	0.10	0.10	0.9260	16.36
IgD+ memory B cells *	180	0.02	0.01	0.01	0.7855	20.18
IgD- memory B cells *	180	0.01	0.01	0.01	0.8163	24.29
Naïve B cells*	180	0.09	0.08	0.08	0.9376	19.14
Natural Killer (NK) Cells	188	0.18	0.10	0.10	0.9078	13.63
NK Cells: CD56HI *	188	0	0	0	0.7422	34.45
NK Cells: CD56LO	188	0.17	0.10	0.10	0.9121	13.63
Monocytes*	188	0.12	0.09	0.09	0.5933	30.28
CD16- monocytes*	188	0.12	0.09	0.09	0.6048	30.14
CD16+ monocytes *	188	0	0.01	0.01	0.3444	46.89
Monocytes (2016 VBS file)[1]	190	0.47	0.14	0.14	0.9045	6.2
CD16- monocytes based on VBS absolute count[2]	188	0.46	0.14	0.14	0.8977	7.20
CD16+ monocytes based on VBS absolute count[2]*	188	0.02	0.02	0.02	0.4637	34.30
Dendritic cells *	188	0.03	0.02	0.02	0.5684	24.16
Myeloid Dendritic cells (DC-M) *	188	0.02	0.01	0.01	0.6420	24.52
Plasmacytoid Dendritic cells (DC-P)*	188	0.01	0	0	0.6274	28.37

* Large CVs (>15%) between blinded duplicate samples were observed for some cell subsets that are present in very low proportions in peripheral blood.

[1]For monocytes, we added 3 variables to this table - monocyte count, CD16-, and CD16+ - from the complete blood count data in the 2016 VBS data release (variable name for the monocyte count: pamon).

[2]To calculate the counts of CD16+ and CD16- monocytes for the CBC data we use the following formulas:

CD16+ absolute count = % CD16+*absolute count of monocytes
CD16- absolute count = % CD16-*absolute count of monocytes

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