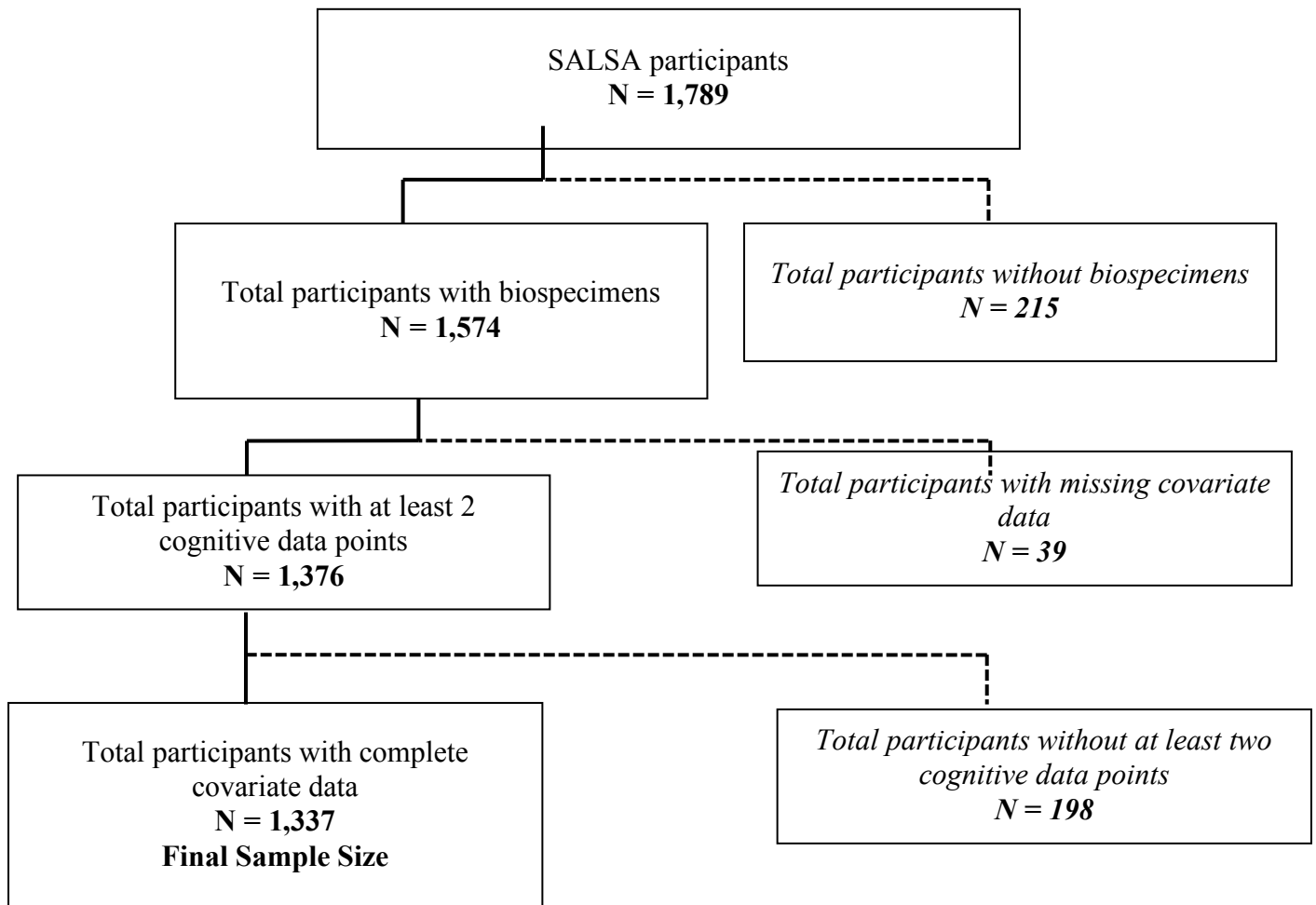


eFigure 1. Sample flow chart for cognitive decline analyses showing how the study sample was derived. Final study sample, N = 1,337.



Laboratory methods.

HSCRP The Genzyme Diagnostics (formerly Equal Diagnostics) High sensitivity CRP kit was used for testing the samples. This is a wide range CRP assay which measures 0 - 160 mg/L. This kit measures low as well as high samples. The CRP Ultra Wide Range Reagent Kit is a latex-enhanced turbidimetric in vitro immunoassay. CRP in the sample binds to the specific anti-CRP antibody, which has been absorbed to latex particles, and agglutinates. The agglutination is detected as an absorbance change when read at 570nm on the automated Cobas Mira Chemistry Analyzer from Roche Diagnostics Corporation. The magnitude of change is proportional to the quantity of CRP in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known concentrations.

A summary report of guidelines resulting from a scientific conference sponsored by Centers for Disease Control and Prevention and the American Heart Association (Circulation 2004;110:e545-549.) recommended the following categories for interpretation of hs-CRP test results:

< 1.0 mg/L = Low risk of developing cardiovascular disease.

1.0 - 3.0 mg/L = Average risk of developing cardiovascular disease.

> 3.0 mg/L = High risk of developing cardiovascular disease.

IL-6

The Human IL-6 Chemiluminescent Immunoassay (R&D Systems, Q60000B) is a plate assay which employs the quantitative sandwich enzyme immunoassay technique using a monoclonal antibody specific for IL-6 which has been pre-coated onto a microplate. Standards, QC's and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody during a 2 hr incubation on an orbital shaker at RT. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-6 is added to the wells and incubated for 3 hrs on an orbital shaker at RT. Following a wash to remove any unbound antibody-enzyme reagent, an enhanced luminol/peroxide substrate solution is added to the wells, incubated for 5-20 min and then read in a microplate luminometer (ICN Titertek Luminoscan set to read for 2.0 seconds) which measures the intensity of the light emitted. Light is produced in proportion to the amount of IL-6 bound in the initial step. The counts are transferred to a computer program (StatLia) for analysis with a range of 0.5 – 200 pg/mL. We report the following coefficients of variation. Inter-assay CV: 15.8% at 24.2 pg/mL, 19.3% at 67.4 pg/mL, 14.8 at 6.1 pg/mL, 13.3 at 144.2 pg/mL. Intra-assay 2.6% at 24.2 pg/mL, 3.0% at 67.4 pg/mL, 3.5 at 6.1 pg/mL, 2.8 at 144.2 pg/mL.

TNF- α

The Human TNF- α Chemiluminescent Immunoassay (R&D Systems, QTA00B) is a plate assay which employs the quantitative sandwich enzyme immunoassay technique using a monoclonal antibody specific for TNF- α which has been pre-coated onto a microplate. Standards, QC's and samples are pipetted into the wells and any TNF- α present is bound by the immobilized antibody during a 3 hr incubation on an orbital shaker at RT. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for TNF- α is added to the wells and incubated for 2 hrs on an orbital shaker at RT. Following a wash to remove any unbound antibody-enzyme reagent, an enhanced luminol/peroxide substrate solution is added to the wells, incubated for 5-20 min and then read in a microplate luminometer (ICN Titertek Luminoscan set to read for 2.0 seconds) which measures the intensity of the light emitted. Light is produced in proportion to the amount of TNF- α bound in the initial step. The counts are transferred to a computer program (StatLia) for analysis with a range of 1.1 - 560 pg/mL. We report the following coefficients of variation. Inter-assay CV: 8.3% at 116 pg/mL, 8.8% at 35.6 pg/mL, 9.6% at 58.3 pg/mL, 11.3% at 162 pg/mL. Intra-assay 2.6% at 116 pg/mL, 2.1% at 35.6 pg/mL, 3.5% at 58.3 pg/mL, 2.6% at 162 pg/mL.

Viral IgG antibodies

ELISA testing for CMV, HSV1, and HSV2 IgG antibodies were done using the Wampole Laboratories (Princeton, NJ) Diagnostic ELISA test kits. The test was performed following manufacturer's instructions and each kit contained internal calibrations and controls. The antibody level was expressed in terms of mean optical density ratio. The sensitivity and specificity of each kit is 96.4 and 93.3 for CMV; 97.1 and 96.8 for HSV-1; 100% and 98.8% for HSV-2.

Helicobacter pylori IgG quantitative test. Samples will be tested at the CLASS laboratory to test the serum for IgG antibodies to *H.Pylori* using an enzyme linked immunoassay that has been approved by the U.S. Food and Drug Administration. Briefly, immobilized partially purified *H. pylori* antigens on the wells of a microwell plate are used to bind antibody from diluted subject serum. Excess non-specific IgG antibodies in sera are washed away with buffer and an anti-human IgG enzyme conjugate is added to the wells. The conjugate forms a sandwich by binding with antigen-antibody complex. An enzyme substrate is added that provides a colormetric signal for the presence of bound antibody. The intensity of the color corresponds directly with the amount of serum *H.pylori* antibody bound to the well, indicating the level of antibody found in serum.

Cortisol, salivary

The salivary cortisol testing was collected with Salivettes (Sarstedt, Newton, NC) and assayed unextracted with DPC Coat a Count cortisol kits (DPC, Los Angeles, CA). Interassay variability was 6.5%, intraassay variation was 5% and assay sensitivity was 0.1ng/mL.

eTable 1. Fixed Effects Estimates from Linear Mixed Effects Models of Cytokines and Cognitive Decline in the SALSA, n=1,337

Variable		Crude Models		IP Weighted Models		Cortisol Interaction Models		
		Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	
Intercept		1.9740	(1.941, 2.007)	2.0076	(1.973, 2.042)	2.1481	(1.925, 2.372)	
IL-6	1	0.1651	(0.116, 0.215)	0.1210	(0.070, 0.172)	-0.6759	-(0.993, -0.359)	
IL6	0	ref	. .	ref	. .	ref	. .	
Age		0.0124	(0.003, 0.022)	0.0145	(0.004, 0.025)	-0.0563	-(0.123, 0.011)	
Agesq		0.0021	(0.002, 0.003)					
Interleukin - 6	Age*IL-6	1	0.0010	-(0.013, 0.015)	0.0107	-(0.004, 0.025)	0.0518	-(0.042, 0.146)
	Age*IL-6	0	ref	. .	ref	. .	ref	. .
	Cortisol					-0.0554	-(0.142, 0.031)	
	Cortisol*IL-6					0.3135	(0.191, 0.436)	
	Cortisol*IL-6					ref	. .	
	Age*Cortisol*IL-6	1				0.0108	-(0.014, 0.036)	
	Age*Cortisol*IL-6	0				0.0278	(0.002, 0.054)	
	Intercept		2.0085	(1.975, 2.042)	2.0524	(2.019, 2.086)	1.7776	(1.583, 1.972)
	TNF-alpha	1	0.0967	(0.045, 0.148)	0.0355	-(0.018, 0.089)	0.2363	-(0.119, 0.592)
	TNF-alpha	0	ref	. .	ref	. .	ref	. .
	Age		0.0132	(0.003, 0.023)	0.0172	(0.007, 0.027)	-0.0237	-(0.081, 0.033)
	Agesq		0.0022	(0.002, 0.003)				
Tumor Necrosis Factor - alpha	Age*TNF-alpha	1	0.0004	-(0.014, 0.014)	0.0076	-(0.007, 0.022)	-0.0547	-(0.158, 0.048)
	Age*TNF-alpha	0	ref	. .	ref	. .	ref	. .
	Cortisol					0.1084	(0.033, 0.184)	
	Cortisol*TNF-alpha					-0.0804	-(0.217, 0.057)	
	Cortisol*TNF-alpha					ref	. .	
	Age*Corisol*TNF-alpha	1				0.0396	(0.007, 0.072)	
	Age*Corisol*TNF-alpha	0				0.0159	-(0.006, 0.038)	

	Intercept		1.9995	(1.965, 2.034)	2.0370	(2.001, 2.073)	1.9147	(1.688, 2.142)
	CRP	1	0.0983	(0.049, 0.148)	0.0473	-(0.004, 0.098)	-0.2284	-(0.553, 0.096)
	CRP	0	ref	. .	ref	. .	ref	. .
	Age		0.0178	(0.008, 0.028)	0.0233	(0.013, 0.033)	-0.0500	-(0.115, 0.015)
	Agesq		0.0021	(0.001, 0.003)				
C-reactive Protein	Age*CRP	1	-0.0066	-(0.020, 0.007)	-0.0039	-(0.018, 0.011)	0.0097	-(0.085, 0.105)
	Age*CRP	0	ref	. .	ref	. .	ref	. .
	Cortisol						0.0478	-(0.041, 0.136)
	Cortisol*CRP						0.1066	-(0.019, 0.232)
	Cortisol*CRP						ref	. .
	Age*Cortisol*CRP						0.0226	-(0.004, 0.049)
	Age*Cortisol*CRP						0.0287	(0.003, 0.054)

eTable 2. Fixed Effects Estimates from Linear Mixed Effects Models of Pathogen Seropositivity and Cognitive Decline in the SALSA, n=1,303

	Variable		Crude Models		IP Weighted Models		Cortisol Interaction Models	
			Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
HSV-1	Intercept		2.0162	(1.943, 2.090)	2.0662	(1.990, 2.142)	1.7016	(1.162, 2.241)
	HSV-1	1	-0.0047	-(0.083, 0.074)	-0.0314	-(0.113, 0.050)	0.0949	-(0.474, 0.664)
	HSV-1	0	ref	. .	ref	. .	0.0000	. .
	Age		0.0099	-(0.011, 0.031)	0.0190	-(0.002, 0.041)	-0.1007	-(0.262, 0.061)
	Age2		0.0025	(0.002, 0.003)				
	Age*HSV-1	1	0.0049	-(0.017, 0.027)	0.0037	-(0.019, 0.027)	0.0703	-(0.100, 0.241)
	Age*HSV-1	0	ref	. .	ref	. .	0.0000	. .
	Cortisol						0.1416	-(0.067, 0.350)
	Cortisol*HSV-1						-0.0488	-(0.269, 0.171)
	Cortisol*HSV-1						0.0000	. .
	Age*Cortisol*HSV-1	1					0.0205	-(0.001, 0.042)
	Age*Cortisol*HSV-1	0					0.0453	-(0.016, 0.107)
CMV	Intercept		1.8069	(1.745, 1.869)	1.8793	(1.814, 1.945)	1.3580	(0.935, 1.781)
	CMV	1	0.2508	(0.183, 0.319)	0.1853	(0.113, 0.257)	0.4555	-(0.007, 0.918)
	CMV	0	ref	. .	ref	. .	ref	. .
	Age		-0.0010	-(0.020, 0.018)	0.0042	-(0.016, 0.025)	0.0592	-(0.067, 0.185)
	Age2		0.0025	(0.002, 0.003)				
	Age*CMV	1	0.0163	-(0.005, 0.037)	0.0202	-(0.002, 0.042)	-0.1198	-(0.258, 0.018)
	Age*CMV	0	ref	. .	ref	. .	0.0000	. .
	Cortisol						0.1991	(0.040, 0.359)
	Cortisol*CMV						-0.1013	-(0.277, 0.074)
	Cortisol*CMV						0.0000	. .
	Age*Cortisol*CMV	1					0.0327	(0.011, 0.054)
	Age*Cortisol*CMV	0					-0.0219	-(0.070, 0.026)

	Intercept		2.0308	(1.999, 2.063)	2.0415	(2.009, 2.074)	1.7147	(1.512, 1.918)
	VZV	1	-0.0670	-(0.126, -0.008)	-0.0014	-(0.062, 0.060)	0.3682	-(0.026, 0.762)
	VZV	0	ref	. .	ref	. .	ref	. .
	Age		0.0133	(0.004, 0.023)	0.0194	(0.010, 0.029)	-0.0428	-(0.105, 0.019)
	Age2		0.0025	(0.002, 0.003)				
VZV	Age*VZV	1	0.0040	-(0.012, 0.020)	0.0073	-(0.010, 0.025)	0.0081	-(0.111, 0.127)
	Age*VZV	0	ref	. .	ref	. .	0.0000	. .
	Cortisol						0.1268	(0.049, 0.205)
	Cortisol*VZV						-0.1453	-(0.299, 0.009)
	Cortisol*VZV						0.0000	. .
	Age*Cortisol*VZV	1					0.0240	-(0.015, 0.063)
	Age*Cortisol*VZV	0					0.0237	(0.000, 0.047)
	Intercept		1.6898	(1.601, 1.779)	1.7943	(1.701, 1.887)	1.2220	(0.474, 1.970)
	H. pylori	1	0.3530	(0.260, 0.446)	0.2711	(0.174, 0.368)	0.5881	-(0.180, 1.356)
	H. pylori	0	ref	. .	ref	. .	ref	. .
	Age		0.0157	-(0.009, 0.041)	0.0238	-(0.002, 0.050)	-0.0878	-(0.315, 0.139)
	Age2		0.0025	(0.002, 0.003)				
H. pylori IgG	Age*H. pylori	1	-0.0024	-(0.028, 0.024)	-0.0024	-(0.029, 0.025)	0.0540	-(0.179, 0.287)
	Age*H. pylori	0	ref	. .	ref	. .	0.0000	. .
	Cortisol						0.2191	-(0.066, 0.505)
	Cortisol*H. pylori						-0.1196	-(0.413, 0.174)
	Cortisol*H. pylori						0.0000	. .
	Age*Cortisol*H. pylori	1					0.0211	(0.001, 0.042)
	Age*Cortisol*H. pylori	0					0.0422	-(0.043, 0.128)
	Intercept		1.9984	(1.966, 2.031)	2.0582	(2.025, 2.092)	1.7541	(1.547, 1.961)
	T. gondii	1	0.0404	-(0.015, 0.096)	-0.0591	-(0.117, -0.001)	0.1736	-(0.205, 0.552)

T. gondii IgG	T. gondii	0	ref	. .	ref	. .	ref	. .
	Age		0.0159	(0.006, 0.026)	0.0230	(0.013, 0.033)	-0.0246	-(0.086, 0.037)
	Age2		0.0025	(0.002, 0.003)				
	Age*T. gondii	1	-0.0056	-(0.022, 0.010)	-0.0032	-(0.020, 0.014)	-0.0554	-(0.174, 0.063)
	Age*T. gondii	0	ref	. .	ref	. .	0.0000	. .
	Cortisol						0.1183	(0.039, 0.198)
	Cortisol*T.gondii						-0.0919	-(0.239, 0.055)
	Cortisol*T.gondii						0.0000	. .
	Age*Cortisol*T.gondii	1					0.0388	(0.000, 0.078)
	Age*Cortisol*T.gondii	0					0.0181	-(0.005, 0.042)
