Supplement

Supplementary Methods

Persistent Organic Pollutant Measurement
Plasma samples were analyzed using methods representing a modification of methods presented by CDC, 2007.[9] For this method each 2 mL plasma sample was spiked with internal standards using mixtures of labeled PCBs (EC-5325, CIL, Andover, MA, USA), OCPs (ES-5465, CIL, Andover, MA, USA), and PBDEs (EO-5277, CIL, Andover, MA, USA). Recovery standards used $^{13}C_6$-TCDD, $^{13}C_{12}$-CB-208, and $^{13}C_{12}$-BDE-139 (CIL, Andover, MA, USA). Native standards (PCBs, EC-5433; OCPs, ES-5467; PBDEs, EO-5103; CIL, Andover, MA, USA) were used for calibration and method validation. Liquid-liquid extractions used a hexane/methyl tert-butyl ether mixture (1:1 v/v, Fisher Scientific, Fair Lawn, NJ, USA), and the extract was concentrated and prepared for cleanup using solid-phase extraction silica (Sep-Pak® Vac 1 cc/50 mg, Waters Corporation, Milford, MA, USA) and Florisil (Sep-Pak® Plus, Waters Corporation, Milford, MA, USA) cartridges, both under vacuum, with a hexane/dichloromethane mixture (1:1 v/v, Fisher Scientific, Fair Lawn, NJ, USA) mixture added to elute the desired compounds. These cartridges, selected to improve consistency between samples and to minimize background levels, were pre-cleaned with a hexane/dichloromethane mixture, and then conditioned with hexane. The eluate was concentrated to 100 µL under nitrogen gas blowdown. After transfer to an insert and a GC vial, 2 µL samples were injected into the GC-MS/MS (Agilent 7890B/7010B, Agilent Technologies, Santa Clara, CA, USA) equipped with a DB-5MS column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA) for separation. Helium was used as the carrier gas (0.7 mL/min) and methane as the MS reagent gas. The mass-selective detector operated in electron impact mode set for selected ion using an ionization energy of 38 eV and the dynamic multiple reaction monitoring (MRM) mode to enhance sensitivity. Each run used multiple time windows to scan only a few m/z ions at a given retention time. The method was developed and verified for a suite of 60 PCBs, 20 OCPs and 12 PBDEs.

Quality Assurance Procedures
Quality assurance activities included using authentic standards (certified surrogate and calibration standard solutions), blanks, matrix spikes, duplicates, and analyses of standard reference materials. To minimize blank contamination, all glassware, consumables, and evaporator attachments were rinsed with hexane and baked overnight before use. Target compounds were detected at mostly trace levels, except for the more volatile PCBs, which were found at intermediate levels. Internal and calibration standard solution preparation and spiking used only designated micro-syringes. Solvent blends were made on the day of use from identified and tracked reagents. We utilized multiple blanks, National Institute of Standards and Technology (NIST) reference standard materials (RSMs), and calibration check samples in each sequence (20-40 samples) and checked calibration within each sequence. Background and matrix effects were determined using fetal bovine serum blanks. Six- and seven-point calibration curves were developed using certified standards. Linear calibration curves, based on standards with concentrations ranging from 0.5 to 1000 pg/mL, were generated using the ratio of the peak area of the analyte to the labeled internal standard. The R^2 values of the curve were equal or greater than 0.99.

Quality assurance metrics included accuracy measured as the percent deviation from NIST reference standard materials (RSMs 1957 and 1958, NIST, USA); recovery as the percent of spike recovery; and precision as the percentage coefficient of variation of multiple sample

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analyses, calculated for all chemicals in the RSM. Both methods achieved acceptance criteria of ±25% for most analytes, e.g., Method B attained an accuracy of 12 ± 8% for OCPs and PBDEs and 16 ± 15% for PCBs; and spike recoveries averaged 92 ± 6% for the OCPs and PBDEs and 82 ± 5% for PCBs. Accuracy was slightly lower but still acceptable for the RSM 1957, which is a low concentration and unspiked material; this RSM 1957 is rarely used or reported for this purpose but served as an independent check. The limit of detection (LOD) was estimated as the instrumental detection limit (amount of analyte detected with a signal-to-noise ratio of three) and the preconcentration factor. LODs for OCPs and PBDEs averaged 0.006 ± 0.007 ng/L and 0.003 ± 0.002 ng/mL for PCBs. Nondetections were set to LOD/$\sqrt{2}$ (**Supplementary Table S1** provides LODs).

Supplementary Tables

Supplementary Table S1. Persistent organic pollutants (ng/mL)

Abbreviations: POP, persistent organic pollutant; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; HCB, hexachlorobenzene; PeCB, pentachlorobenzene; DDE, dichlorodiphenyldichloroethylene; LOD, limit of detection.

		Cases								Controls									
POP	**% < LOD**	**N**	**Mean**	**SD**	**Min**	**Q25**	**Q50**	**Q75**	**Max**	**N**	**Mean**	**SD**	**Min**	**Q25**	**Q50**	**Q75**	**Max**	**P-Value**	**Q-Value (BH)**
alpha-HCH	34.9	164	0.009	0.054	0.001	0.001	0.004	0.007	0.697	105	0.003	0.003	0.001	0.001	0.002	0.005	0.017	0.005	0.010
c/t-heptachlorepoxide	2.2	164	0.020	0.017	0.002	0.009	0.014	0.027	0.102	105	0.013	0.009	0.002	0.006	0.011	0.017	0.051	0.000	0.001
cis-Nonachlor	29.6	164	0.020	0.050	0.003	0.003	0.009	0.017	0.559	105	0.008	0.008	0.003	0.003	0.006	0.010	0.056	0.001	0.003
gamma-HCH	33.8	164	0.050	0.199	0.001	0.001	0.011	0.042	1.909	105	0.016	0.024	0.001	0.001	0.005	0.022	0.125	0.013	0.021
HCB	2.2	164	0.141	0.111	0.001	0.059	0.110	0.190	0.563	105	0.106	0.096	0.001	0.047	0.088	0.138	0.743	0.009	0.015
Mirex	1.9	164	0.156	1.427	0.002	0.015	0.024	0.043	18.287	105	0.382	3.669	0.002	0.012	0.018	0.025	37.615	0.000	0.001
Oxychlordane	27.4	164	0.062	0.056	0.007	0.007	0.050	0.089	0.270	105	0.037	0.035	0.007	0.007	0.021	0.058	0.185	0.000	0.002
PBDE-100	17.7	164	0.020	0.042	0.002	0.004	0.008	0.014	0.360	105	0.012	0.018	0.002	0.003	0.007	0.013	0.129	0.084	0.112
PBDE-153	8.3	164	0.094	0.259	0.004	0.016	0.030	0.064	2.903	105	0.060	0.184	0.004	0.010	0.023	0.043	1.747	0.008	0.014
PBDE-47	6.9	164	0.090	0.203	0.004	0.019	0.037	0.070	2.114	105	0.067	0.136	0.004	0.014	0.032	0.068	1.116	0.158	0.183
PBDE-99	36.3	164	0.019	0.048	0.003	0.003	0.007	0.012	0.448	105	0.012	0.024	0.003	0.003	0.006	0.010	0.197	0.112	0.140
PeCB	48.5	164	0.013	0.013	0.006	0.006	0.006	0.016	0.083	105	0.012	0.010	0.006	0.006	0.006	0.013	0.063	0.534	0.550
PCB-105	26.1	164	0.011	0.018	0.001	0.002	0.006	0.013	0.179	105	0.009	0.011	0.001	0.001	0.005	0.011	0.076	0.261	0.294
PCB-11	34.6	164	0.012	0.014	0.002	0.002	0.008	0.016	0.089	105	0.011	0.011	0.002	0.002	0.007	0.016	0.052	0.470	0.497
PCB-118	11.3	164	0.059	0.098	0.001	0.018	0.036	0.062	0.930	105	0.040	0.043	0.001	0.012	0.032	0.049	0.241	0.096	0.123
PCB-138	1.6	164	0.186	0.163	0.002	0.078	0.135	0.254	0.965	105	0.127	0.124	0.002	0.051	0.100	0.157	1.033	0.002	0.005
PCB-153	2.5	164	0.237	0.208	0.001	0.094	0.170	0.329	1.168	105	0.166	0.157	0.001	0.065	0.139	0.233	1.339	0.007	0.012
PCB-156	1.6	164	0.050	0.046	0.002	0.020	0.037	0.066	0.312	105	0.037	0.053	0.002	0.014	0.029	0.045	0.512	0.005	0.009
PCB-157	6.0	164	0.014	0.014	0.001	0.005	0.009	0.018	0.078	105	0.009	0.012	0.001	0.003	0.006	0.011	0.108	0.001	0.003
PCB-167	22.8	164	0.009	0.010	0.002	0.003	0.006	0.012	0.070	105	0.006	0.006	0.002	0.002	0.004	0.007	0.041	0.014	0.021
PCB-170	1.6	164	0.093	0.073	0.002	0.042	0.073	0.127	0.421	105	0.069	0.066	0.002	0.030	0.053	0.080	0.430	0.001	0.003
PCB-178	14.6	164	0.013	0.013	0.001	0.005	0.009	0.019	0.089	105	0.011	0.014	0.001	0.004	0.008	0.011	0.090	0.023	0.033
PCB-180	0.8	164	0.213	0.173	0.003	0.101	0.165	0.271	1.015	105	0.147	0.123	0.003	0.068	0.121	0.184	0.842	0.000	0.002
PCB-187	14.3	164	0.041	0.044	0.005	0.011	0.026	0.055	0.304	105	0.032	0.035	0.005	0.010	0.022	0.040	0.217	0.132	0.158
PCB-189	41.5	164	0.004	0.003	0.002	0.002	0.004	0.006	0.019	105	0.003	0.003	0.002	0.002	0.002	0.004	0.018	0.003	0.006
PCB-194	6.0	164	0.056	0.048	0.005	0.025	0.042	0.067	0.280	105	0.038	0.035	0.005	0.016	0.030	0.043	0.206	0.000	0.001
PCB-199	5.2	164	0.046	0.040	0.005	0.023	0.034	0.054	0.236	105	0.034	0.035	0.005	0.016	0.027	0.039	0.244	0.002	0.005

PCB-202	13.7	164	0.011	0.011	0.002	0.005	0.008	0.014	0.071	105	0.008	0.009	0.002	0.003	0.006	0.009	0.056	0.002	0.005
PCB-206	8.5	164	0.027	0.035	0.001	0.010	0.018	0.031	0.327	105	0.017	0.019	0.001	0.008	0.013	0.019	0.159	0.000	0.001
PCB-208	15.4	164	0.010	0.011	0.001	0.003	0.006	0.011	0.086	105	0.006	0.006	0.001	0.003	0.004	0.007	0.044	0.002	0.005
PCB-209	13.2	164	0.016	0.019	0.001	0.005	0.010	0.017	0.139	105	0.008	0.008	0.001	0.003	0.006	0.010	0.048	0.000	0.001
PCB-28/31	16.2	164	0.005	0.006	0.001	0.002	0.003	0.007	0.055	105	0.006	0.008	0.001	0.001	0.004	0.006	0.065	0.774	0.774
PCB-74	5.2	164	0.046	0.052	0.002	0.015	0.031	0.053	0.355	105	0.032	0.027	0.002	0.011	0.025	0.046	0.124	0.042	0.058
PCB-8	22.0	164	0.007	0.008	0.001	0.002	0.005	0.009	0.047	105	0.006	0.007	0.001	0.001	0.004	0.009	0.033	0.399	0.435
p,p'-DDE	0.0	164	1.358	1.743	0.044	0.462	0.754	1.471	13.747	105	0.777	0.711	0.118	0.305	0.582	0.903	4.615	0.000	0.002
trans-Nonachlor	0.0	164	0.117	0.109	0.006	0.051	0.083	0.138	0.851	105	0.065	0.045	0.007	0.034	0.052	0.082	0.264	0.000	0.000

Supplementary Table S2. Matched ALS and Control Participant Demographics

Covariate	Cases (N = 76)	Controls (N = 41)
Age at sample collection (years)	63.1 (58.8-68.2)	63.3 (59.2-67.5)
Body Mass Index (kg/m^2)*	26.0 (23.0-30.1)	26.2 (23.4-29.6)
Sex		
Female	39 (51.3)	26 (63.4)
Male	37 (48.7)	15 (36.6)
Military Service		
No	70 (92.1)	33 (80.5)
Yes	4 (5.3)	4 (9.8)
Missing	2 (2.6)	4 (9.8)
Education		
High School or Less	23 (30.3)	4 (9.8)
Some Postsecondary	27 (35.5)	9 (22.0)
Bachelor's Degree	15 (19.7)	11 (26.8)
Graduate Degree	9 (11.8)	13 (31.7)
Missing	2 (2.6)	4 (9.8)
Race		
American Indian / Alaska Native	0 (0.0)	0 (0.0)
Asian	1 (1.3)	0 (0.0)
Black or African American	1 (1.3)	1 (2.4)
White	74 (97.4)	40 (97.6)
Unreported	0 (0.0)	0 (0.0)
ALSFRS-R	36 (30-40)	
Time Between Symptom Onset and Diagnosis (Years)	1.04 (0.71-1.70)	
Time Between Diagnosis and Sample Acquisition (Years)	0.48 (0.31-0.95)	
Observed Death		
Yes	67 (88.2)	
No	9 (11.8)	
El Escorial Criteria		
Possible / Suspected	12 (15.8)	
Probable, Lab Supported	15 (19.7)	
Probable	23 (30.3)	
Definite	26 (34.2)	
Onset Segment		
Bulbar	29 (38.2)	
Cervical	24 (31.6)	
Lumbar	23 (30.3)	
Cannot be Determined	0 (0.0)	

Table of descriptive statistics for the study population. For continuous variables, median (25th – 75th percentile), and for categorical variables, N (%). *Body Mass Index is observed for 73 cases and 36 controls. ALSFRS-R, revised amyotrophic lateral sclerosis functional rating scale.

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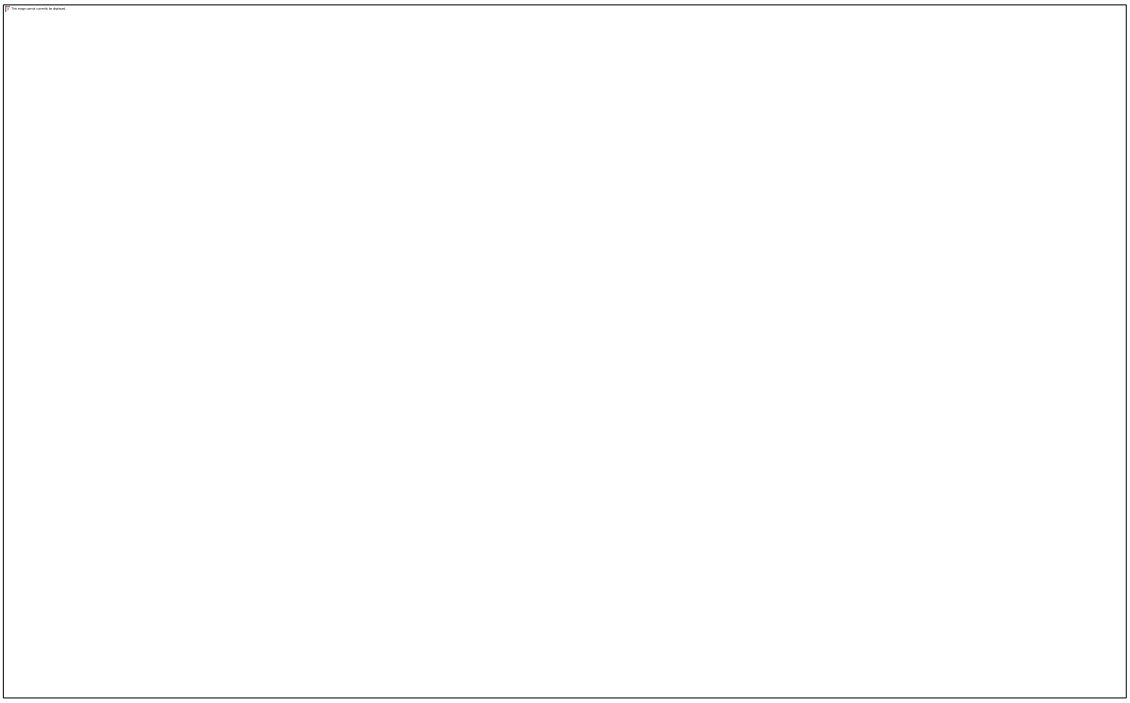
Supplementary Figures

Supplementary Figure S1. Distribution of ERS^{risk} by ALS and control
Distribution of the ERS^{risk} by ALS and control group. ERS, environmental risk score.



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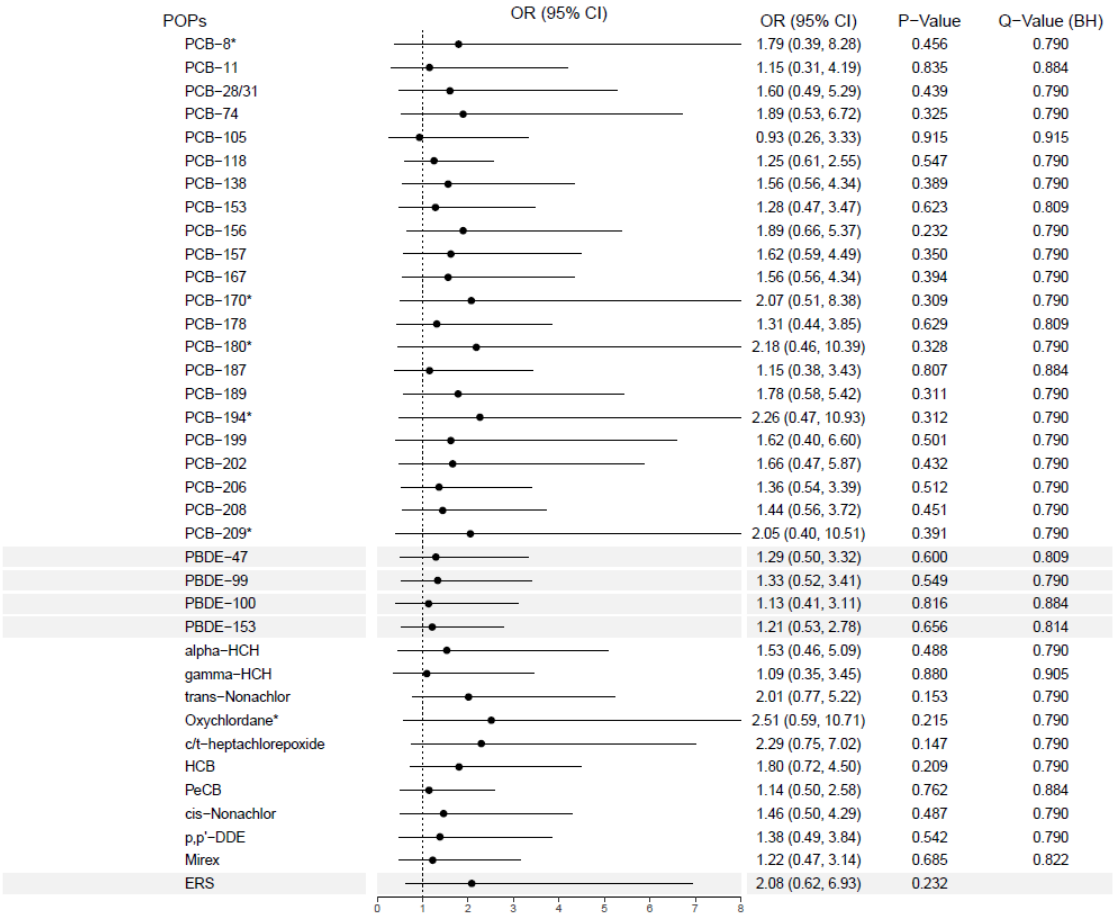
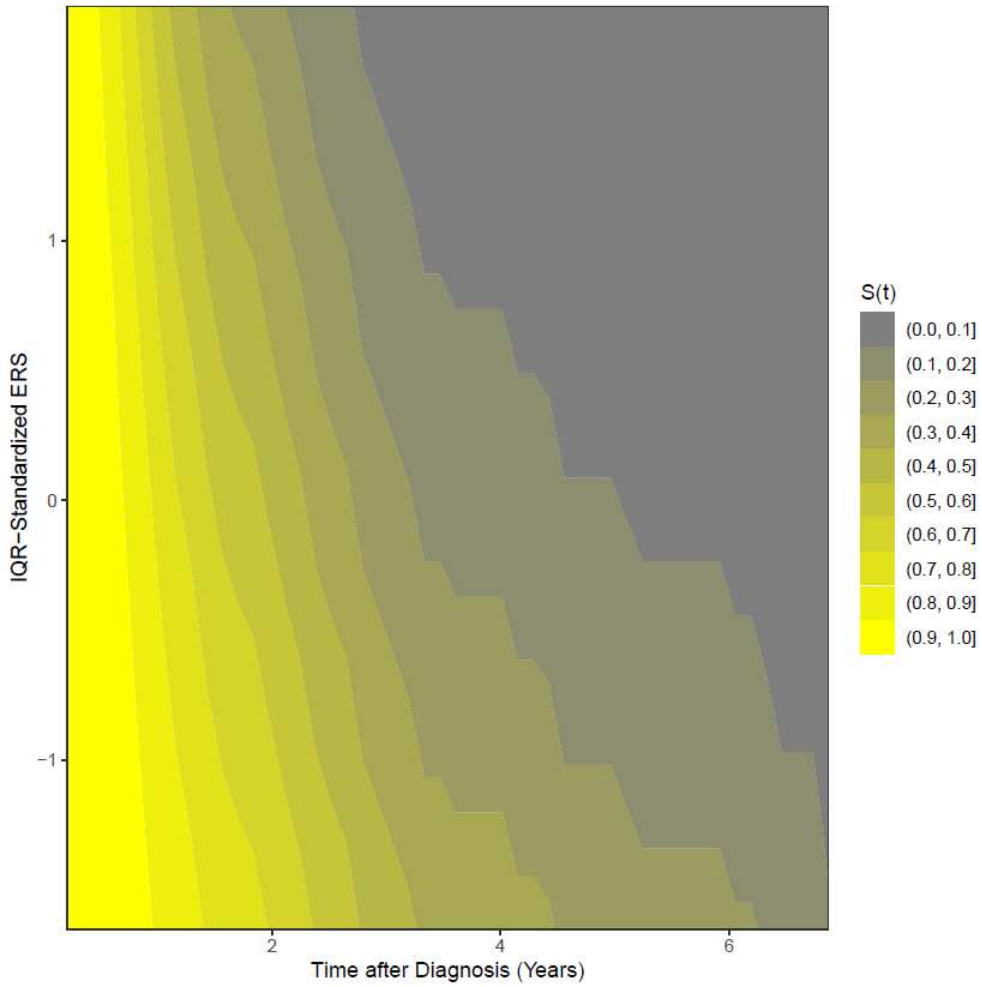
Supplementary Figure S2. Matched cohort adjusted single pollutant and mixture associations between POPs and case/control status

Single POP logistic regression models where the outcome is case/control status using matching on age and sex. The variables of interest are log-transformed standardized POP concentrations, and the covariates are education, continuous BMI, and continuous BMI slope. Abbreviations: BH, Benjamini-Hochberg; BMI, body mass index; CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; ERS, environmental risk score; HCB, hexachlorobenzene; HR, hazard ratio; OCP, organochlorine pesticide; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; PeCB, pentachlorobenzene; POP, persistent organic pollutant.



Supplementary Figure S3. Cohort contour survival plot
Contour survival plot indicating the survival time from diagnosis based on environmental risk score, visualized and grouped by survival probability (S(t)).
Abbreviations: ERS, environmental risk score; IQR, interquartile range; S(t), survival probability.



Supplementary Figure S4. Adjusted survival curves by ERS quartile
Dashed lines indicate the median survival in each environmental risk score (ERS) strata. The estimated adjusted median survival time is 2.26 years for Quartile 1, 2.24 years for Quartile 2, 1.68 years for Quartile 3, and 1.38 years for Quartile 4. With Quartile 1 as the reference, hazard ratios and 95% confidence intervals by quartile are as follows: Quartile 2 (HR = 1.01, 95% CI 0.59-1.75, p = 0.960); Quartile 3 (HR = 1.65, 95% CI 0.95-2.86, p = 0.075); Quartile 4 (HR = 2.31, 95% CI 1.30-4.13, p = 0.005).