Investigation of multi-systemic biomarkers and cognitive decline among long-term breast cancer survivors

Vikram Mavinkurve[1]*, Molly Crowe[2], Siok Leong[1], Sergio Corrales Guerrero[1], Katrazyna McNeal[2], Caitlin Carr[2], Heidi Tan[3], Richard Yang[3], Yuelin Li[2], Arti Hurria[3], Tim Ahles[2], and Irene Orlow[1]

[1]Departments of Epidemiology & Biostatistics and [2]Psychiatry & Behavioral Sciences, Memorial Sloan Kettering Cancer Center, New York NY; [3]Cancer and Aging Research Program, City of Hope, CA

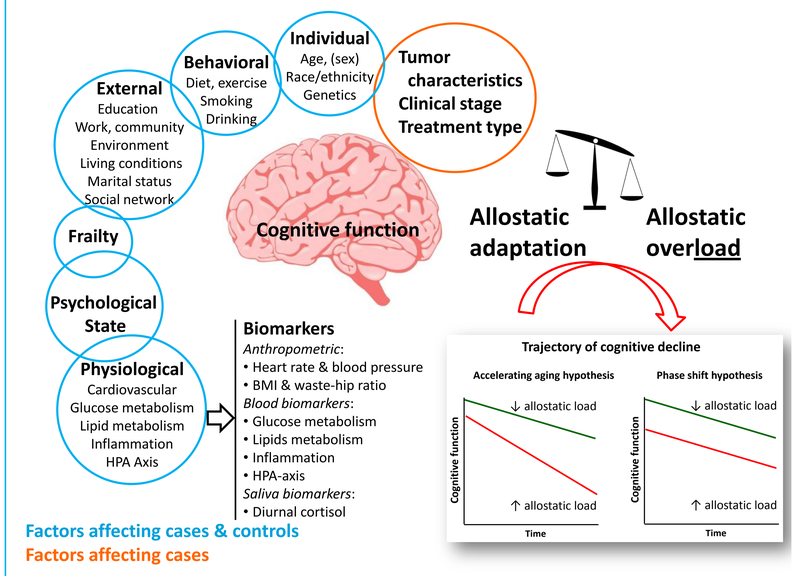
Background

Cognitive decline is among the most feared aspects of growing older. Recent evidence suggests that people diagnosed and treated for their cancers are more vulnerable to cognitive aging, in part because cancer treatments result in substantial physiological and psychological stress; however, additional risk factors are likely responsible. The cumulative physiological dysregulation associated to a lifetime of adapting to physiological and psychological stressors is referred to as 'allostatic load'. As population ages, the identification of potentially preventable risk factors that contribute to cognitive decline and affect quality of life in this vulnerable population is of paramount importance.

Hypothesis: Survivors with a high allostatic load or increase in allostatic load over time who were treated with adjuvant chemotherapy and/or endocrine therapy will have lower levels of cognitive performance (assessment 1) and over time compared to survivors with low allostatic load and healthy controls.

Overall goal: To identify the biologic basis behind cognitive decline in long term cancer survivors through the use of a multi-systemic set of biomarkers to measure the cumulative physiological wear and tear referred to as 'allostatic load'. Here we present results from our *interim* and descriptive analysis.

Figure 1: Known factors affecting allostasis, biomarkers, & proposed hypothesis



Methods

Participants:
- Females age ≥65 yr recruited at Memorial Sloan Kettering (MSK, n=35) and City of Hope (COH, n=104)
- Long-term (5yrs+) breast cancer survivors exposed to chemotherapy (n=30)
- Long term survivors not exposed to chemotherapy (n=49)
- Unaffected controls (n=60), age, race and education matched

Biospecimens:
- Fasting blood collected in tiger top (clot activator), lavender top (EDTA), grey top (fluoride/oxalate), and light blue top (citrate) collection tubes
- Saliva collected at 4 time points (awakening, 45min after, mid afternoon, bed time)

Allostatic load panel:
- 28 biomarkers to evaluate the cardiovascular (CV) system (n=3), glucose metabolism, lipids metabolism (n=8), chronic inflammation (n=15), and hypothalamic-pituitary-adrenal axis (HPA) (n=2) (Figure 1,& Table 2)
- Specimens tested at the Molecular Epidemiology Laboratory (Memorial Sloan Kettering) and at the Clinical and Translational Science Center (Weill Cornell Medical College)

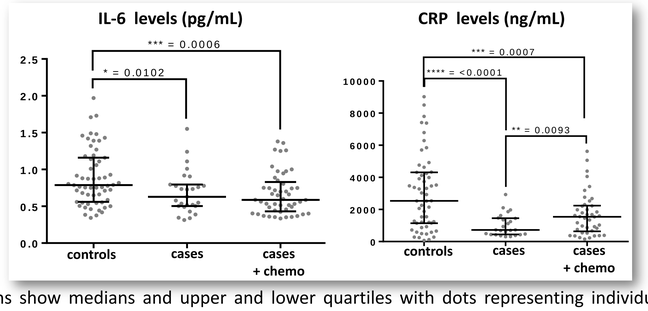
Analyses:
- Accrual and testing is ongoing.
- Preliminary analysis includes descriptive analysis of experimental data (single marker- and system-level) and quality control of sample procurement

Results

Table 1: Detectability, mean, range, and biomarkers' cut-points (N=139)

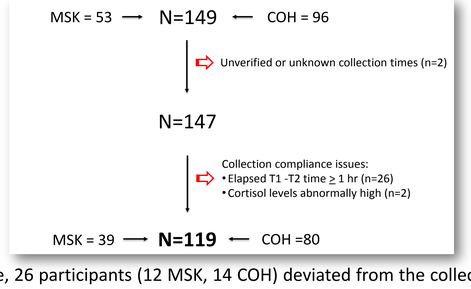
	Detectability	Mean (range)	high risk cut-off
Glucose metabolism			
Insulin	100%	13.13 (4.73 - 65.14) µIU/ml	≥14.77
Hba1c	100%	5.8 (5 - 9.1) %	≥6
Glucose	100%	97.3 (60 - 237) mg/dl	≥101
Inflammation			
IL-6	100%	1.04 (0.34 - 9.94) pg/mL	≥1.18
IFNg	100%	5.23 (1.46 - 27.3) pg/mL	≥5.47
IL-10	98.6%	0.36 (0.08 - 2.33) pg/mL	≥0.37
IL-13	100%	0.79 (0.31 -1.92) pg/mL	≥0.95
IL-8	100%	22.62 (11.9 - 63.4) pg/mL	≥24.93
TNF-alpha	100%	3.02 (0.82 - 5.2) pg/mL	≥3.34
ICAM-1	100%	398.2 (225 - 706) ng/ml	≥457.8
VCAM-1 (CD106)	100%	464.3 (299 - 732) ng/ml	≥522.5
SAA	100%	7255 (1199 - 58012) ng/ml	≥6426
IL12(p70)	87.5%	0.53 (0.1 - 21.4) pg/mL	≥0.214
IL-4	86.3%	0.04 (0.017 - 0.144) pg/ml	≥0.04
e-selectin	100%	7.62 (2.46 - 20.8) ng/ml	≥9.62
D-dimer	100%	508.1 (97 - 2588) ng/ml	≥549
Fibrinogen	100%	353.1 (166.1 - 605) mg/dL	≥421.4
CRP	100%	3829 (74.4 - 14853) ng/ml	≥4880
IL-1-beta	23%	0.33 (0.102 - 1.08) pg/mL	≥0.38
HPA axis			
Cortisol	98.4%	*see table 2 and figure 3*	
DHEAS	100%	0.36 (0.04 - 1.17) µg/mL	≤0.18
Lipid Metabolism			
Total Cholesterol	100%	189.2 (120 - 311) mg/dL	≥200.5
HDL	100%	71.92 (26 - 522) mg/dL	≤47.25
LDL	100%	104.2 (54 - 232) mg/dL	≥118
Triglycerides	100%	104.8 (24 - 288) mg/dL	≥121

Figure 2: Example of single-marker results among cases and controls



Graphs show medians and upper and lower quartiles with dots representing individual patients within the group. Outliers were removed using the Grubbs method. Asterisks denote significant differences (t – test p-values).

Figure 3. Diurnal cortisol: collection compliance & other considerations

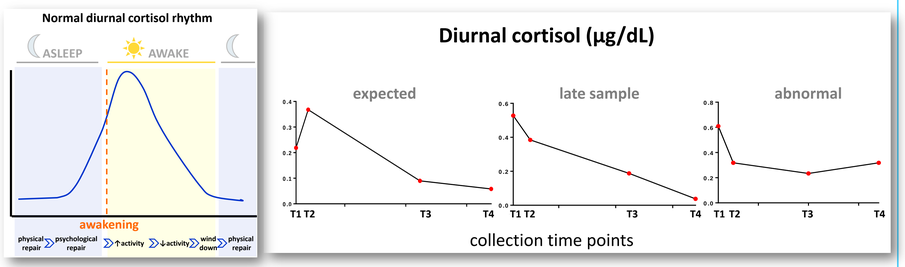


As shown above, 26 participants (12 MSK, 14 COH) deviated from the collection recommendations by collecting the second sample ≥1 hour after the first collection. 2 samples presented unexpected high levels potentially due to the unreported use of a medication. In addition, 5/147 participants were 'late raisers' (awoke ≥12 noon).

Table 2: Characteristics of 119 participants that passed QC for cortisol

	Saliva - collection time (h:m)					Cortisol levels (µg/dL)				% Change C2→C1	Slope
	T1	T2	T3	T4	T2-T1	C1	C2	C3	C4		
Overall mean	7:01	7:43	15:13	20:10	0:42	0.37	0.41	0.15	0.15	-3.4	-0.13
Min	3:45	4:20	2:05	0:00	0:15	0.01	0.05	0.01	0.01	-327	-0.55
Max	12:45	13:30	18:45	23:57	0:57	1.86	1.52	1.1	14.22	97	0.69
MSK mean	7:08	7:53	14:58	19:02	0:44	0.3	0.36	0.17	0.1	2.71	-0.13
Min	3:45	4:20	2:05	0:00	0:35	0.01	0.05	0.02	0.01	-199	-0.46
Max	12:45	13:30	18:15	23:55	0:56	0.93	0.96	0.92	0.65	97	0.3
COH mean	6:57	7:39	15:20	20:43	0:41	0.41	0.44	0.14	0.17	-6.39	-0.13
Min	4:09	4:59	3:15	0:30	0:15	0.11	0.09	0.01	0.02	-248	-0.55
Max	9:30	10:22	18:45	23:57	0:57	1.86	1.52	1.1	1.92	77	0.69

Figure 3: Examples of Diurnal Cortisol profiles in study participants



Cortisol levels follow a circadian cycle, in which lower levels are encountered during the evenings (~T4) , and rise overnight with a peak (T2) 30 to 45 minutes after awakening (T1) in the morning. Cortisol awakening response (CAR) measured by the increase in cortisol levels from T1 to T2, and slope are used to evaluate adaptation to chronic stressors

System-level average scores:

	Control	Cases	Case + chemotherapy
Inflammation	4	3	3
Glucose metabolism	0.78	0.95	1
Lipid metabolism^	1	1	1

Systems were scored by summing the marker-level scores. Marker-level scores were assigned a score of '1' if the marker was ≥ to the high risk quartile and a '0' to the rest. ^BMI and hip to waist ratio not included yet

Summary & Discussion:

- Three biomarkers had low detectability and are not suitable for inclusion in the overall allostatic load score
- Non-compliance to sample collection instructions are common (~20%) and current recommendations are being evaluated
- At the single-marker level, 2 biomarkers show significant differences between cases and controls: IL-6 and CRP; at the system-level, the average inflammation score is higher in the control group than in the cases
- Accrual is ongoing and further analyses is planned for the overall allostatic load in relation to case-control status and cognitive function at baseline and cognitive decline overtime

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