1. **Title Page**

Clinical Associations of Leukocyte Telomere Length in a Cohort of Repatriated Prisoners of War

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14. ABSTRACT
A cohort consisting of 127 American repatriated prisoners of war (RPWs) was studied in order to ascertain whether there were statistically significant correlations between Leukocyte Telomere Length (LTL) obtained from a commercially available LTL test and 47 clinical indicators of health, environmental stress and aging. Methods: Data was obtained from an annual examination of the RPW cohort. LTLs were determined by a commercial laboratory and compared to the results from common blood and urine tests, historical data related to incarceration length and solitary confinement, and qualitative health assessment tools. Analysis of descriptive statistical data, the construction of stepwise linear and logistic regression models and Fisher exact calculations were performed. Results: There is no apparent difference between the demographically homogenous RPW cohort?s mean LTL (6.48 ? 1.18 Kb) and the commercial laboratory?s mean LTL [6.49 Kb (no SD available)] for its sample population of undisclosed demographics. Statistical analysis did not yield any significant correlations between LTL and the clinical data studied. Discussion: The negative statistical results of this study, coupled with the questionable quality of the commercial LTL data utilized, does not support our program's use of the publicly available LTL test studied in this work for monitoring repatriate health.

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2. Abstract Page:

Introduction: A cohort consisting of 127 American repatriated prisoners of war (RPWs) was studied in order to ascertain whether there were statistically significant correlations between Leukocyte Telomere Length (LTL) obtained from a commercially available LTL test and 47 clinical indicators of health, environmental stress and aging. **Methods:** Data was obtained from an annual examination of the RPW cohort. LTLs were determined by a commercial laboratory and compared to the results from common blood and urine tests, historical data related to incarceration length and solitary confinement, and qualitative health assessment tools. Analysis of descriptive statistical data, the construction of stepwise linear and logistic regression models and Fisher exact calculations were performed. **Results:** There is no apparent difference between the demographically homogenous RPW cohort's mean LTL (6.48 ± 1.18 Kb) and the commercial laboratory's mean LTL [6.49 Kb (no SD available)] for its sample population of undisclosed demographics. Statistical analysis did not yield any significant correlations between LTL and the clinical data studied. **Discussion:** The negative statistical results of this study, coupled with the questionable quality of the commercial LTL data utilized, does not support our program's use of the publicly available LTL test studied in this work for monitoring repatriate health.

Key Words: RPW, biomarker, resiliency, aging

3. **Main Text:**

INTRODUCTION

The Robert E. Mitchell Center for Prisoner of War Studies (REMC) manages the Repatriated Prisoner of War (RPW) program to care for American repatriates with the primary goal of identifying the health related risks of posttraumatic stress and prisoner of war (POW) internment for future members of the armed forces of the United States of America. A major thrust of current studies at the REMC is to determine the impact of captivity on the development of physical and/or psychological pathology and to ascertain health trends in aging populations (6).

Telomeres are disposable, protective regions of repetitive DNA sequences that prevent the DNA replication process or environmental damage from degrading the ends of chromosomes. However, these protected ends are truncated with each cell division such that they may eventually be exhausted with a decrease in telomere length being correlated with increasing age at the population level. In addition, telomere lengths from individual tissues have been correlated with certain diseases in those tissues (3). With the promise of a new method to screen for health concerns, analysis of telomere length has been promoted as a commercial biomarker of aging and disease (4). However, these commercially available blood tests utilize the readily accessible leukocyte telomere length (LTL) that has not been definitively established as a marker of overall health or sufficiently correlated with results from tissue sample telomere lengths (9).

In an attempt to determine the possible utility of LTL testing as a health screening instrument for the aging cohort of repatriates, the REMC added LTL measurements to its laboratory panel in 2011. This preliminary study was undertaken to determine if a commercial

LTL test could be used as a surrogate marker of health, environmental stress and aging by determining if significant correlations exist between the LTLs of the RPW cohort and 47 established clinical indicators of health regularly obtained during their periodic examinations (see Table I). This study had both a cross-sectional component and a retrospective cohort component. LTL's and the other laboratory tests used in this study were collected concurrently at one physical examination. While components of the qualitative measures of disease burden (see Table I) and the historical data related to incarceration are retrospective.

METHODS

150 REMC repatriates received LTL testing as part of their annual physical in 2011. Blood samples were collected according to instructions provided by the commercial laboratory. These instructions required no patient preparation. Blood samples were collected in one tube containing sodium citrate and were neither frozen nor centrifuged as instructed. The samples were shipped overnight to the commercial laboratory in the provided kit that contained a frozen cold pack.

One RPW was excluded from this study because of the presence of leukemia that greatly inflated the subject's LTL. 22 other subjects were excluded from this study due to administrative difficulties associated with obtaining proper consent. The remaining 127 subjects were not screened for acute infections or other health states that could briefly skew LTL values (2).

Commercial LTL data was received as a "score" [i.e. LTL in kilobases (kb)] and as a "percentile" calculated with reference to the commercial laboratory's subject database. The age range of the 127 subjects was from 61 to 85 years with a mean age of 71.9 ± 5.62 years. The group was fairly homogenous in their demographics: 126 were male, 124 were aviation officers,

125 were Caucasian. Demographics of subjects used by the commercial laboratory to establish a reference group were not disclosed by the commercial laboratory. Descriptive statistical analysis of the LTL data was performed to include mean LTL with standard deviation and the identification of outliers. All statistical analyses were performed with SAS 9.2.

Forty-nine clinically-related variables (see Table I) were analyzed for correlation with LTL. These data were collected as part of the routine annual examination by the staff of the REMC. Laboratory testing of blood and urine samples was performed by the military hospital affiliated with the REMC. Of the 47 variables, five sets of data were incomplete to include the following: "torture duration" and the urine tests for cortisol, norepinephrine, epinephrine and dopamine. The large numbers of variables were screened for possible correlations by building stepwise linear regression models and logistic regression models with an n=69 subset (all 47 variables present and no missing data points in any variables) and with an n=127 subset (the five incomplete variable sets removed and no missing data in any of the remaining 42 variables).

One of the main goals of this study was to use stepwise linear regression to determine a minimum set of variables chosen from all available variable sets in order to build a statistical model that would successfully explain our cohort's LTL. In addition, linear regression was performed on groups of clinically related variables such as those associated with the risk of cardiovascular disease or "POW Related" data (see Table I). The desired features of a successful linear regression model for our study included an F statistic from analysis of variance (ANOVA) calculations that was less than a standard p-value of 0.05, no collinear variables, no more than 10 total variables (to limit over-fitting) and a total linear regression coefficient (i.e. R^2 or coefficient of determination) of 0.70 or greater. Furthermore, an ideal linear model would seek to minimize mean square error (MSE) and Mallow's C(p) (8).

The complete data set (n=69 with 47 variables) and subset of complete variable categories (n=127 with 42 variables) were evaluated by logistic regression for LTLs divided into tertiles. The acceptance criteria for the odds ratios obtained from logistic regression was a χ^2 probability < 0.05. Fisher Exact testing was also carried out in contingency table fashion for the "POW Related" data. Here, upper and lower LTL tertiles were compared to upper and lower values of these four data types. For example, Fisher Exact calculations were carried out to determine if there was a significant difference between the number of repatriates with shorter (lowest tertile) LTLs or those with longer (highest tertile) LTLs and whether they were considered resilient or not by psychological evaluation (see Table II). Using only upper and lower tertiles limited the number of subjects to 79 in the Fisher Exact calculations.

RESULTS

Analysis of the RPW data revealed a mean LTL of 6.48 ± 1.18 kb. No significant LTL outliers were present in the 127 subjects analyzed. A scatter plot of LTL (y) in kb versus Age (x) in years demonstrated an inversely proportional relationship with a least squares linear trend line of $y = -0.0655x + 11.19$. This indicates a loss of 65.5 bases per year. However, the R^2 of 0.0976 for this trend line reveals a poor fit for the data.

No linear regression calculations were able to meet the requirements of an acceptable model of LTL behavior. That is, no combination of clinical variables, without collinearity, added sequentially or selected as a group of known risk factors for disease produced a statistically significant model without over fitting the data or producing an unacceptable coefficient of determination. Likewise, logistic regression modeling failed to produce statistically significant odds ratios. Lastly, Fisher Exact calculations did not show a statistically significant

correlation between LTL and length of incarceration, solitary confinement, resiliency or torture severity.

DISCUSSION

This is a negative statistical results study. Despite extensive statistical testing that included multiple linear regression models, logistic regression models and Fisher Exact testing, the authors did not find statistically significant correlations between the LTLs of our cohort and the results of clinical tests regularly used at the REMC as measures of overall health. Limitations of this work are those inherent in a cross-sectional study (i.e. collecting data at one point in time) and the retrospective nature of using surveys and historical data. Also, the power to detect statistical differences is limited by the fixed number of subjects in the cohort, the use of LTL upper and lower tertiles, and the varying levels of data completeness for five of the variables. Furthermore, not screening our cohort for acute infections that may transiently increase LTL has the potential to bias results (2). However, incorporating white blood cell counts into the statistical analysis did mitigate some of this concern.

It should be noted that some of the aforementioned shortcomings are consistent with how commercial laboratories market the use of LTL tests. The commercial laboratory used in this study gave no instructions for preparation of patients prior to sample collection or indications for follow up or longitudinal testing. Furthermore, the test reports provided by the commercial laboratory gave only LTL and its percentile calculated from the database of its own clients of undisclosed description. Though the mean LTL of 6.48 ± 1.18 kb in our cohort is comparable to the mean of 6.49 kb obtained from the commercial laboratory database, without statistical measures of dispersion and demographic information that influence LTL, like ethnicity and gender (1), it is difficult to draw conclusions about its significance. Also information was not

provided as to how the clinician should interpret the results. This is not unexpected as there is currently no consensus on how the clinician should interpret telomere lengths or what could be done for the patient based on their LTL test results (9).

As it is debatable as to whether various LTL measurement protocols give consistent and comparable LTL values (9) and the laboratory we used was not forthcoming with their protocol, we were wary of drawing conclusions from comparisons of our data with the literature. That having been said, our analysis provided no statistically significant correlations between LTL and cardiovascular related variables listed in Table I and is not in congruence with the association of decreased telomere length with cardiovascular disease (5). However, as in many studies found in the literature that associate telomere length with morbidities, these studies obtained telomere lengths from the organ tissue affected by the disease of interest and not LTL. Furthermore, a comparison of our RPW cohort to a similar cohort of 436 male, Finnish, business executives was made using an unpaired, two tailed, *t*-test. The Finnish cohort had a mean age of 75.9 ± 4.0 and a mean telomere length of 8.2 ± 0.4 kb (7) as compared to a mean RPW age of 71.9 ± 5.62 years and a mean LTL of 6.48 ± 1.18 kb. This is a highly statistically significant difference between the mean LTLs of the Finnish cohort and our repatriate cohort at a p-value of 0.001. Though it is intriguing to speculate about this length discrepancy, without knowledge of the commercial laboratory's protocols, we cannot rule out that laboratory protocol differences alone could explain these findings.

The negative statistical results of this study coupled with the nascent state of telomere testing in clinical practice and the scant information provided by the commercial laboratory used in this study does not support the use of LTL measurements as a health monitoring or research tool in our RPW cohort at this time. For future consideration, having established an initial LTL

for members of our cohort, there may be value in additional LTL testing for longitudinal monitoring. Yet, we caution the reader that LTL is not necessarily the same as telomere length in other tissues and may not always be used interchangeably. Moreover, various competing laboratory procedures for determining LTL may differ in their results and commercial laboratories may not provide enough information to make LTL tests meaningful for your clinical and research needs. It is suggested that the use of a laboratory with validated and published laboratory protocols, such as a university or reference laboratory, be used in LTL studies.

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5. References:

1. Diez Roux AV, Ranjit N, Jenny NS, Shea S, Cushman M, Fitzpatrick A, et al. Race/ethnicity and telomere length in the Multi-Ethnic Study of Atherosclerosis: Race/ethnicity and telomere length. Aging Cell. 2009 May 26;8(3):251–7.

2. Dolcetti R, De Rossi A. Telomere/telomerase interplay in virus-driven and virus-independent lymphomagenesis: pathogenic and clinical implications: TELOMERE/TELOMERASE INTERPLAY. Medicinal Research Reviews. 2012 Mar;32(2):233–53.

3. Fitzpatrick AL, Kronmal RA, Kimura M, Gardner JP, Psaty BM, Jenny NS, et al. Leukocyte Telomere Length and Mortality in the Cardiovascular Health Study. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2011 Apr 1;66A(4):421–9.

4. Mather KA, Jorm AF, Parslow RA, Christensen H. Is Telomere Length a Biomarker of Aging? A Review. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2011 Feb 1;66A(2):202–13.

5. Moslehi J, DePinho RA, Sahin E. Telomeres and Mitochondria in the Aging Heart. Circulation Research. 2012 Apr 27;110(9):1226–37.

6. NMOTC. Robert E. Mitchell Foundation [Internet]. 2014 [cited 2014 May 25]. Available from: http://www.remcf.org/index.html

7. Strandberg TE, Saijonmaa O, Tilvis RS, Pitkälä KH, Strandberg AY, Salomaa V, et al. Telomere Length in Old Age and Cholesterol Across the Life Course. Journal of the American Geriatrics Society. 2011 Oct;59(10):1979–81.

8. Weiner IB, editor. Handbook of Psychology. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2003.

9. Wolinsky H. Testing time for telomeres. EMBO reports. 2011 Sep 1;12(9):897–900.

TABLES

Table I: Clinical data analyzed in this study.

Non-invasive:

- Age
- Height
- Weight
- Body Mass Index (BMI)*
- Systolic Blood Pressure (SBP)*
- Diastolic Blood Pressure (DBP)*
- Mean Arterial Pressure (MAP)*
- Dominant Grip Strength
- Walking Speed
- Forced Expiratory Volume in one second (FEV1)
- Pack Years of Tobacco smoking*

POW Related:

- Capture duration
- Solitary duration
- Percent weight loss
- Torture

Qualitative Markers of Disease Burden:

Cumulative Illness Rating Scale (CIRS) Severity Index

- CIRS Heart*
- CIRS Vascular*

Short Form Health Survey Physical Component Summary (SF12)

- Quality of Life Inventory (QOLI) T-score
- Sleep Efficiency
- Physical Disease Burden

Urine Tests:

- Cortisol*
- Norepinephrine
- Epinephrine
- Dopamine

Blood Tests:

- Hematocrit (HCT)
- Hemoglobin (HGB)
- White Blood Cell Count (WBC)
- Mean Corpuscular Volume (MCV)
- Mean Corpuscular Hemoglobin (MCH)
- Mean Corpuscular Hemoglobin Concentration (MCHC)
- LDL/HDL Ratio*
- Hemoglobin A1C*
- AM Cortisol
- Dehydroepiandrosterone sulfate (DHEAS)
- Blood Urea Nitrogen (BUN)
- Creatinine
- Calcium
- Total Bilirubin
- Fasting Blood Sugar (FBS)
- Albumen
- Alkaline Phosphatase
- Aspartate aminotransferase (AST or SGOT)
- Alanine aminotransferase (ALT or SGPT)
- Potassium
- Platelets
- C-Reactive Protein Ultra-sensitive (CRP)*

* Markers of cardiovascular risk

Table II. Fisher exact test for presence of resiliency versus upper/lower LTL tertile.

	Lower LTL Tertile	Upper LTL Tertile	Totals
Resilient	12	13	25
Not Resilient	32	22	54
Totals	44	35	79
Two-tailed P value			0.4657