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Documenting Stress in Caregivers of Transplantation Patients: Initial Evidence of HPA Dysregulation

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Abstract

There is growing evidence linking caregiver stress with an increased risk for morbidity and mortality. While the emotional and practical burden experienced by caregivers is well established, the physiological changes that may affect the caregiver's health are less understood. This study sought to compare self-reported stress, anxiety, and depression along with neuroendocrine and immune markers of stress among adult caregivers of allogeneic hematopoietic stem cell transplantation patients during the acute transplant recovery period to matched non-caregivers controls. Biomarkers and self-reported data were collected at three points during the patient's HSCT: (1) before transplant, (2) after initial transplantation discharge (±7 days), and (3) six weeks after initial transplantation discharge. Mixed linear modeling was used to examine differences by group and time. Twenty-one caregivers and 20 controls completed all study procedures. The majority of caregivers were female (57.0%) and married (95.2%), with a mean age of 52±11.4 years. Caregiver perceived stress, anxiety, and depression scores were significantly higher than controls ($p<0.001$) with effect sizes (ES) ranging from 1.37 – 1.80 and they did not change over time ($p>0.05$) for either group. Caregivers had significantly lower serum cortisol levels than controls at both discharge ($p=0.013$; ES=0.81) and 6 weeks after discharge ($p=0.028$; ES=0.71) but exhibited no significant relationship between self-reported stress and serum cortisol. Additionally, caregivers showed a significant inverse relationship between stress and epinephrine levels ($r_s=-0.654$, p=0.021). These findings support the evidence of the caregiving experience being stressful. The counter-intuitive relationship between cortisol and epinephrine might suggest dysregulation of the HPA axis and central nervous system but additional research on the physiological impact of caregiving is warranted.

Declaration of Interest

The authors declare no conflicts of interest.

Keywords

Caregiver; Stress Response; Cancer; Transplant; Family; Biomarkers; Psychological; Burden

Introduction

Providing care to a family member is a common yet challenging experience (NAC, 2009). An estimated 65.7 million people in the US serve as unpaid family caregivers, affecting approximately 36.5 million households. Cancer, a diagnosis that affects 16.6 million people in the US, (ACS, 2015), is the third leading reason for needing a caregiver. Although cancer caregivers have reported positive effects from their experience (Bishop et al., 2011), the emotional and practical burden associated with caring for a person with cancer can be greater than those associated with the care of an elderly family member or one with Alzheimer's disease (NAC, 2009).

The burden of caring for a cancer patient is complex and often greatest when the patient's burden from the illness or treatment is high (Burton et al., 1997). Allogeneic hematopoietic stem cell transplantation (HSCT) is arguably the most intense of all cancer treatments, creating serious and sometimes life-threatening toxicities. Reduced intensity conditioning (RIC) regimes are often provided to patients with pre-existing conditions that preclude them from receiving a standard myeloablative HSCT. Although RIC regimens might decrease the symptom profile and impact on quality of life in patients (Andersson et al., 2009), patients are often discharged to the outpatient setting more quickly, shifting responsibility from healthcare providers to the family caregiver. As a result, a dedicated caregiver is required following all types of allogeneic transplantation. This intense support, often referred to as burden, includes caring for the transplant recipient's physical and emotional wellbeing as well as increasing household duties (Beattie & Lebel, 2011). This burden often forces a shift in roles and responsibilities for the caregiver, frequently requiring them to alter their work schedule and relocate to a temporary residence near the transplant center. Although the burden of care begins during the inpatient phase of the HSCT, the initial discharge following transplantation represents a time when the caregiver assumes responsibility for care, around the clock, for weeks if not months after transplantation. Seldom a choice, a caregiver is a critical partner in HSCT recovery and may be a factor if a patient receives and even survives the treatment (Foster et al., 2004, Frey et al., 2002).

When the burden of an experience exceeds the available resources it is perceived as stressful and physiological changes occur (Chrousos, 2009, McEwen & Seeman, 1999). These include a cascade of physiological and hormonal reactions (Koolhaas et al., 2011)that in the presence of an acute stressor, can improve physical function (Chrousos & Gold, 1992). However, in the context of a sustained stressor, the effects may be more harmful (McEwen, 2000, Sterling, 2004, Dallman, 2010, Kassel et al., 2003, Rose et al., 2008, Van Reeth et al., 2000, McReynolds et al., 2014).

While evidence exists linking the chronic stress of caregiving to an increased risk for illness, (Vitaliano et al., 2003), little research has been published documenting the stress response from cancer caregiving. The majority of published research focuses on caregivers of

individuals with dementia (Lovell & Wetherell, 2011). In caregivers of individuals with cancer, examination of the autonomic nervous system and immune system revealed that salivary cortisol (Lucini et al., 2008, Rohleder et al., 2009), and IL-6 (Rohleder et al., 2009) do not differ between caregiver and control subjects while levels of salivary alpha-amylase and hs-CRP do, suggesting increased sympathetic nervous system activity and inflammation in caregivers (Rohleder et al., 2009). Only one published study has examined the physiological changes that occur in caregivers of HSCT recipients, reporting that salivary cortisol and DHEA, as well as NK cells, IL-6, and hs-CRP, did not differ between HSCT caregivers and non-caregiver controls (Laudenslager et al., 2015).

The purpose of this study was to compare HSCT caregiver's self-reported stress, anxiety, and depression, along with neuroendocrine and immune markers of stress, to those of non-caregiver controls. It was hypothesized that transplant caregivers would report greater stress, anxiety, and depression, and display differences in their biomarkers. A second objective was to explore the relationships among the caregiver's burden, perceived stress, anxiety, and depression with their neuroendocrine and immune biomarkers. It was hypothesized that caregivers with higher levels of psychological distress and burden would have increased levels of biomarkers that suggest a risk for impaired health.

Methods

Study design and participants

This study applied a prospective repeated measure design to examine changes in perceived stress, anxiety, and depression, along with neuroendocrine and immune biomarkers of stress in allogeneic HSCT caregivers at pre-transplantation (pre-HSCT), the time of initial hospital discharge (DC), and six weeks following hospital discharge (6-week), compared to age, gender, and race/ethnicity-matched controls. This study was approved by the National Heart, Lung and Blood Institute intramural Institutional Review Board, and all participants provided written informed consent before participation. HSCT caregivers were eligible if they were an adult, English or Spanish speaking and planning to serve as an active caregiver for an individual planning to undergo their first allogeneic HSCT at the NIH Clinical Center. Caregivers were excluded from participating if they had been treated with glucocorticosteroids in the past two months, were pregnant or lactating, diagnosed with Cushing's, Addison's, or Parkinson's disease, a history of a heart transplant, pacemaker, problems with orthostatic hypotension or diagnosed with autonomic dysfunction, unwilling to refrain from smoking for 12 hours or from drinking alcohol for 24 hours prior to blood sampling, serving as a paid caregiver, serving or had previously served as a stem cell transplant donor, or were taking medicines that interfered with immune system functioning. The caregivers were approached for participation before the HSCT recipients' day of transplant.

The NIH Clinical Center Clinical Research Volunteer Program registry was used to identify age (±10 years), sex, race and ethnicity matched individuals for each caregiver. Matched controls were contacted by phone and screened for eligibility. They were excluded from participating if they were: pregnant/lactating, treated with glucocorticosteroids in the past two months, diagnosed with Cushing's, Addison's, or Parkinson's disease, had a history of a

heart transplant, pacemaker, problems with orthostatic hypotension or diagnosed with autonomic dysfunction, unwilling to refrain from smoking for 12 hours or from drinking alcohol for 24 hours prior to blood sampling, serving as an informal unpaid or were a professional paid caregiver, receiving mental health services and/or taking psychiatric medications. The non-caregiver control subject study visits were scheduled within one week of the caregiver's visit.

Procedures

After enrollment, participants were provided with instructions regarding study procedures. Each clinic visit included a history and physical, assessment of serious life stressors over the prior three months, intravenous line placement, fasting blood draw, vital signs, anthropometric measures, return of the saliva specimen, and questionnaire completion. Participants were provided with verbal and written instructions to avoid all products that included acetaminophen for five days prior to the study visit, abstain from eating, drinking and smoking for 12 hours and avoid alcohol consumption for 24 hours prior to the study visit. Participants were instructed to not eat, drink or brush their teeth for at least 15 minutes prior to saliva collection. A saliva sample was collected 30 minutes after waking the morning of the study visit. A diary was completed by the participants to document adherence to study procedures.

Clinic appointment times were consistent for each case across study time-points, typically in the early- to mid-morning. Height and weight were obtained while standing without shoes in normal clothes, using a digital height and weight scales. A peripheral intravenous catheter was placed, and blood samples were collected after a 15-minute rest period in a quiet, dark room.

Measures

Demographic factors were self-reported and included age, gender, marital status, race, ethnicity and education. The history and physical, including height and weight, were collected by a nurse practitioner and clinical research nurse.

Perceived stress was measured with the Perceived Stress Scale-14 (PSS-14). The PSS-14 is a 14-item questionnaire that captures how stressed and overwhelmed subjects felt in the last month. Items were designed to ascertain how unpredictable, uncontrollable, and overloaded respondents find their lives, as well as examine current levels of experienced stress (Cohen et al., 1983). Responses are scaled from "never" (0) to "very often" (4) and total scores can range from 0 to 54, where higher scores indicate more perceived stress. The Cronbach's alpha for the PSS-14 in this study sample ranged from .88–.91.

Caregiver burden was assessed with the Caregiving Reaction Assessment (CRA) in caregiver participants only. The CRA is designed to assess the positive and negative effects of caregiving for persons providing care to patients with chronic illnesses. The measure consists of 24 items, with responses on a 5-point Likert Scale ranging from "strongly disagree" (1) to "strongly agree" (5). The five subscales (caregiver esteem, lack of family support, impact on finances, impact on schedule, and impact on health) are scored by calculating the mean of the subscale's items after appropriate reversals. Higher subscale

scores indicate more burden, except caregiver esteem, where a higher score indicates less burden (Given et al., 1992). A total score, representing overall caregiver burden, is obtained by calculating the mean of all 24 items, after appropriate reversals; a higher total score denotes greater burden for the caregiver (Grov et al., 2006). The Cronbach's alpha for the CRA total score in this study sample ranged from .79 – .90. The alpha coefficients by subscale ranged from .60 – .73, .81 – .93, .63 – .77, .65 – .83; and .65 – .77 (esteem, support, finance, schedule, and health, respectively).

Anxiety and Depression were assessed with the Patient-Reported Outcomes Measurement Information System (PROMIS®) Anxiety Short Form-1 and Depression Short Form-8b. The PROMIS item databanks have well established validity and reliability across multiple studies (PROMIS, 2011). A total raw score for each measure (anxiety, depression) is calculated summing each item. The total raw score is converted into a T-score with a mean of 50 and standard deviation of 10; a higher score indicating more symptoms of anxiety or depression.

Assays were selected to assess HPA, autonomic nervous system (ANS), and immune system activation including salivary and serum cortisol, epinephrine, norepinephrine, catecholamine turnover, IL-6, and TNF-α. As described previously, plasma catecholamines were measured using standard high-performance liquid chromatography (HPLC) assays (Eisenhofer et al., 1986, Lenders et al., 1993). Catecholamine concentrations were determined through reverse phase liquid chromatography with electrochemical detection on alumina (Eisenhofer et al., 1986). However, concentrations of catecholamines in the blood do not fully capture the dynamic equilibrium in which these molecules live (Eisenhofer et al., 2004). Thus, catecholamine turnover rates were calculated as combined levels of dihydroxyphenylalanine (DOPA), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), norepinephrine (NE), and dihydroxyphenylglycol (DHPG) (Pacak et al., 1993). As such, these turnover rates reflect the continual process of catecholamine synthesis and metabolism, which involve the dynamic mechanisms of leakage of catecholamines from storage vesicles, sequestration of the catecholamines back into the vesicles, exocytotic release from the vesicles, and reuptake (Eisenhofer et al., 1986). Deviation of turnover rate from those of steady-state conditions can be indicative of a stress response (Shah & Donald, 1984).

The salivary cortisol awaking rise was obtained with a single specimen (30 minutes after awakening) to decrease burden for the caregiver participants. Salivary cortisol samples were collected using the Salivette container (Sarstedt Inc., Newton, NC) which required subjects to roll the cotton swabs in their mouth for approximately 2 minutes and then return them to the Salivette container. Upon receipt of the samples, the Salivette tubes were centrifuged for 10 minutes at 4000 rpm and then immediately stored in a −20°C freezer. Samples were thawed and analyzed at study completion using tandem mass spectrometry (LC-MS/MS) using an IMMULITE® 1000 autoanalyzer (Siemens, Germany). The limit of detection for this assay is 60 ng/dL and the within-run precision is 4.7%–12.2%, while the run-to-run precision is 11%–16%.

Free serum cortisol values were obtained from subjects during their clinic visits. Recognizing a single morning salivary measure of cortisol might be limiting, serum cortisol

was also obtained. Blood was centrifuged for 10 minutes at 2500 rpm, after which the serum layer was transferred out into separate vials and stored in a −20°C freezer. Serum samples were thawed and evaluated for free cortisol using a Chemiluminescence Immunoassay, run on an IMMULITE® 2000 XPI (Siemens, Germany). The limit of detection for this assay is 1 mcg/dL but quality checks were performed using mass spectrometry for all values less than 5 mcg/dL (n=5) to ensure accuracy. The within-run precision is 4.6%–8.4% and the run-to-run precision is 6.4%–13.5%. There were no significant differences between the methods. Levels of inflammatory cytokines TNF-α and IL-6 were analyzed with Quantikine® HS enzyme-linked immunosorbent assay (ELISA) kits SS600B and SSTA00D (R&D Systems, Inc. Minneapolis, MN) using a VICTOR3® 1420 Multilabel Reader (PerkinElmer, Inc. Waltham, MA).

Catecholamine, cytokine, and both salivary and serum cortisol bio-specimens were each batch-analyzed so that samples from caregivers and their matched controls were processed together. Additionally, samples from all time-points were batched for each subject to maintain analysis consistency for longitudinal evaluations.

Analysis

Initial data analysis consisted of examining the frequency distributions for all variables at each time point and computing descriptive statistics appropriate for the level of measurement (e.g., mean and standard deviation for interval level data, median for ordinal level data). Identified outliers were either removed or winsorized. Variables were natural log or square root transformed in the final model if necessary to meet normality assumption for the analyses. Relationships among perceived stress, anxiety, depression, caregiver burden (caregivers only), and neuroendocrine and immune biomarkers were examined using Spearman rho due to non-normality of most variables

Mixed model repeated measure analyses were used to determine whether there was a change in the perceived stress, anxiety, depression, neuroendocrine and immune measures of stress (salivary and serum cortisol, norepinephrine, epinephrine, CAT-turnover, IL-6, and TNF-α) over 3 study time-points between groups. The model included fixed effects of visit (time), group, and visit by group interaction. Visit was treated as a categorical variable. Since caregiver and non-caregiver subjects were matched for age and gender, these variables were not included in the model. Any other demographic variables which are significantly different between groups and are significant predictors to any of the outcomes will be entered in the model as the covariates. Restricted maximum likelihood (REML) procedure was used for model parameter estimation. Aikake information criterion (AIC) and Bayesian information criterion were used to compare and select final models. The model selection process was to compare different covariance structures. All fixed effect terms would be in the final models.

Standardized Cohen's *d* effect size (ES) was calculated using model estimated means and standard errors to characterize the magnitude of changes between groups and changes over time. All data analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC). A $p<0.05$ was considered significant.

Results

Participants

Participants were accrued from a single center between November 2011 and April 2013. Ninety-seven caregivers were screened for participation. Fifty-seven (59%) were not eligible, 12 (12%) declined participation, and two (2%) were not enrolled at the discretion of the Principal Investigator (e.g. compliance barriers). Twenty-six (27%) caregiver subjects were enrolled along with twenty-four matched non-caregiver controls (Figure 1).

Forty-one participants (21 caregivers and 20 matching normal volunteers) completed all study procedures. A total of 9 subjects were removed from study (n=5 caregiver subjects and n=4 matching normal volunteers; Figure 1). Two caregivers were removed when the care-recipient died since their responses might reflect the grief process as opposed to caregiving. When a caregiver was removed from study, the matched control subject was also removed except in the case of one caregiver who was permitted to stay on study despite the matched non-caregiver control coming off study due to sudden family crisis. This caregiver matched a control subject already enrolled who had completed all study procedures and was therefore included in the analyses. The average time from pre-HSCT time-point to the 6 week post-HSCT time point was 83.8 days (SD=3.1; range=54–170 days).

The majority of caregivers were female (57%), and married (95.2%), with a mean age of 52±11.4 years (Table 1). The caregiver sample was primarily white, non-Hispanic (57%) with eight (38%) self-reporting as Hispanic. Over half of the caregivers were spouses to the related HSCT patient (57.1%), three of whom had two active caregivers (2CG/patient) who participated in the study (n=6).

The transplant recipients' characteristics are displayed in Table 2. The majority of transplant recipients received a reduced intensity conditioning regimen (n=12; 57.1%), with peripheral blood stem cells (n=20; 95.2%). Stem cells were often from an unrelated donor (n=17; 81.0%) for an underlying life-threatening condition such as leukemia (n=7; 33.3%), lymphoma (n=7; 33.3%), or other non-malignant diseases (n=7; 33.3%).

Psychological and Biomarker Outcomes

Table 3 presents the descriptive values for burden, stress, anxiety, depression in caregiver and non-caregiver participants at all study time-points. Caregiver Reaction Assessment total scores [mean (SD)] ranged from 2.4 (±0.48) to 2.43 (±0.64) and remained stable across all time points. Caregiver PSS scores were significantly higher than non-caregiver controls ($p<0.001$), with a large ES at each time-point > 1.0). The trajectory from pre-HSCT to 6 weeks post-discharge did not change ($p>0.05$) for either group (Table 4).

The trajectory for the anxiety and depression scores from pre-HSCT to 6 weeks post-discharge also did not change ($p>0.05$) for either group. Caregiver anxiety scores (standardized) were significantly higher than non-caregiver controls ($p<0.001$). Caregiver depression scores (standardized) were significantly higher than non-caregiver controls

(p<0.001). Similar to perceived stress, the magnitude of difference (ES) between groups for anxiety and depression was large at all study time-points (Table 4).

Table 5 presents the descriptive statistics for the observed biomarkers in caregiver and non-caregiver participants at all study time-points. The mean (SD) time of collection for the morning salivary cortisol samples was 31.9 (±10.1), 28.17 (±7.08), and 30.32 (±7.45) minutes after awakening (pre-HSCT, DC, 6-week, respectively). The caregiver morning salivary cortisol levels were not significantly different from the non-caregiver controls and did not change significantly over time in either group (Table 4).

The mean time of serum cortisol collection ranged from 8:45 AM (±1:29 hours) to 8:57 AM (±1:13 hours) across the three study time points. The serum cortisol model revealed a significant group by time interaction effect (p=0.003; Figure 2). The caregiver serum cortisol levels decreased over time while the levels for non-caregiver control subjects increased (Table 5). Caregivers had significantly lower predicted mean (SE) scores than non-caregiver controls at discharge (t_{39} =−2.60, p=0.013; ES=0.81] and 6 weeks after discharge (t_{39} −2.29, p=0.028; ES=.71] (Figure 2). The serum cortisol model was computed with and without the outliers (serum drawn after 10:00 am; n=21) and the results of the model did not change.

Twenty-six (65%) cases had valid catecholamine samples at all study time-points (13 caregiver/non-caregiver dyads). Catecholamine missing data were systemic and due to processing of specimens, not subject factors. The caregiver epinephrine, norepinephrine, and CAT-turnover levels were not significantly different than the non-caregiver controls. Only the caregiver norepinephrine levels were significantly lower at time-point 2 compared to time-point 1 (Table 4).

The caregiver IL-6 model had a significant group by time interaction revealing that while the caregiver's IL-6 levels remained stable over time, IL-6 levels for non-caregiver control subjects increased. Caregiver and control IL-6 levels did not differ significantly at any study time-point. Caregiver TNF-α levels were not significantly different from those of the non-caregiver controls. Only the caregiver TNF-α levels were significantly lower at time-point 2 compared to time-point 1 (Table 4).

Correlational analyses

Caregiver burden, perceived stress, anxiety, and depression did not significantly change over time, therefore, baseline values were used to evaluate the relationship between these factors and their neuroendocrine and immune biomarkers (Table 6). Caregiver CRA scores were significantly (r_s=0.587, p<0.005) related to PSS scores with higher burden scores related to higher perceived stress scores. Higher CRA subscale scores, specifically the caregiver schedule (r_s=0.672, p=0.0008), esteem (r_s=0.664, p=0.001), and health (r_s=0.592, p=0.005) correlated with higher PSS scores.

Caregiver subjects exhibited no significant relationship between perceived stress and serum cortisol (r_s=0.095, p=0.681). However, there was a significant inverse relationship between perceived stress scores and epinephrine levels (r_s=−0.654; p=0.021), with higher caregiver stress scores related to lower levels of epinephrine.

Neither perceived stress (caregiver and non-caregiver controls) nor caregiver burden (caregiver only) was significantly related to IL-6 or TNF-α. However, inverse relationships were present in non-caregiver controls between epinephrine and IL6 (r_s=−0.711; p=0.0095). This relationship was not seen in the caregiver sample.

Discussion

The findings of this study support the literature suggesting that the burden and stress of caring for someone receiving intense cancer therapy such as an allogeneic HSCT, is substantial (Beattie & Lebel, 2011, Girgis et al., 2013). This study expands the current evidence by demonstrating that despite transitions in the treatment trajectory or intensity of conditioning regimen, caregiver burden remains high and is associated with self-reported stress, anxiety and depression during the acute phase post-HSCT. Caregivers in this study reported a level of burden, specifically around schedule and finances, comparable to caregivers of patients undergoing treatment for lung cancer (Milbury et al., 2013) and those with advanced cancers (Utne et al., 2013). Perceived stress, anxiety and depression start high and appear to be unrelenting across many months. The scores for perceived stress in caregivers was remarkable and higher than those reported in the general population (Simoneau et al., 2013), caregivers of individuals with Alzheimer's disease (Li et al., 2007) and another HSCT sample where the percentage of those receiving standard myeloablative HSCT was similar to this study (Laudenslager et al., 2015). While the anxiety and depression scores were higher than those of the general population, the anxiety scores specifically exceeded a common standard for minimally important difference (greater than 0.5 SD difference), suggesting clinical relevance.

The relationship between caregiver burden and emotional distress (perceived stress, anxiety, depression) supports previously published findings. The findings that higher scores for burden or negative impact on the caregiver's life, were positively related to anxiety and depression supports previous research (Petruzzi et al., 2013), with caregivers reporting scores not only significantly greater than the matched non-caregiver controls, but well above the scores reported in the general population and are considered clinically meaningful (>2 or 2.5 points; respectively) (Kroenke et al., 2014). The positive relationship between burden and stress seen in this sample of HSCT caregivers supports previous work in cancer caregivers (Simoneau et al., 2013, Cohen, 1988, Li et al., 2007).

Relative to physiological biomarkers, only the serum cortisol and epinephrine analyses were statistically remarkable. The serum cortisol finding reveals a different trajectory in caregivers compared to non-caregiver controls. Despite substantial self-reported burden, and perceived stress that far exceeded the controls, the caregiver levels of cortisol were decreasing over time while the levels in the control group were increasing; a descriptive increase in the non-caregivers which may be due to the experience of coming to the hospital to participate in the study noting that the change from baseline to the end of the study was not significant. In healthy systems, perceived stress and cortisol would be positively correlated, with higher stress associated with high levels of cortisol. This finding might suggest dysregulation of the HPA axis in the caregivers.

In other populations characterized by substantial stress, anxiety or depression, a blunted HPA axis response or hypocortisolemia has been reported. In women with chronic stress and premenopausal dysphoric disorder, higher stress was related to lower levels of cortisol (Girdler et al., 1998, Klatzkin et al., 2010) in individuals with depression, decreased levels of cortisol were also reported (Wu et al., 2014, Burke et al., 2005, Ahrens et al., 2008). Similarly, individuals who have reported "burn-out" in their work environments also had lower levels of cortisol (Juster et al., 2010, Pruessner et al., 1999). While some studies have reported dysregulation of the HPA axis in individuals with PTSD, the reports are not consistent (Meewisse et al., 2007). The decreasing levels of serum cortisol seen over time in this sample of caregivers, coupled with their substantial unrelenting stress, potentially could be a real-time sign that the HPA systems of these caregivers are beginning to show the wear and tear of chronic stress. However, further research is needed to validate these findings.

The second interesting finding which may supports the dysregulation of the stress system, isthe relationship between perceived stress and epinephrine, an area not previously studied in cancer caregivers. Unlike the control subjects, caregiver's perceived stress was significantly related to epinephrine levels; however, like the relationship between perceived stress and serum cortisol, epinephrine levels decreased with higher stress in the caregivers. This finding, however, should be interpreted with caution. The sample size for this analysis was small and additionally limited by processing errors and therefore, should be validated in future research.

An alternative hypothesis for the lower levels of cortisol and epinephrine in caregivers reporting higher stress is that caregivers of patients who receive a transplant will display signs of HPA adaptation or habituation to this chronic stressor. Although the evidence is still unclear how to differentiate the two responses to a chronic, primarily psychological, stressor (Rabasa et al., 2015), there is evidence that changes can occur suggesting a tolerance or de-sensitization to a repeated stressor (Grissom & Bhatnagar, 2009, Pervanidou et al., 2007, Rosmond et al., 1998). Further research with more extensive assessments including longitudinal samples, may be needed to test this hypothesis in a clinical setting.

A number of limitations exist in this study related to the physiological biomarker assessment. Documenting abnormalities of the central nervous system can be difficult and accurately interpreting the results can be challenging. The experience of caregiving for an individual undergoing HSCT is likely very individual, and a number of factors could have interfered with the physiological outcome variables that are not included as covariates in the analyses (e.g. pre-existing health conditions and outside stressors). Unhealthy caregivers may also be more vulnerable to changes in health during the chronic stress of the experience. These concurrent issues contribute to the difficulty in interpreting these study findings. In addition, with the small sample size, the study is underpowered for the biomarker effects, which are likely to be more subtle. These factors coupled with the short time frame for data collection and the possible confounding effect of the individual caregiver's health e.g. diabetes are limitations to be considered in interpreting these findings. Moreover, future research should extend the follow-up through 100 days post-HSCT, a watershed point in transplant recovery. However, having an age, gender, and ethnicity matched sample strengthens the design and allows for a unique perspective not seen in previous studies.

Conclusion

The caregivers of HSCT patients consistently describe a level of burden and emotional distress that serve as a "call to action" for health care providers. Although interventions to reduce caregiver burden, improve caregivers' ability to cope, increase their self-efficacy, and enhance their quality of life (Northouse et al., 2010, Hurley et al., 2014, Lavretsky et al., 2013) exist, identifying objective ways to document their impact on the physical health of the caregiver is likely required for the systematic uptake into policy and practice. The purpose of this study was to correlate the self-reported burden and distress with objective neuroendocrine and immune measures of the stress response to operationalize the impact on the caregiver's health. Overall, this study offers initial evidence that may help to guide future research highlighting the clinical and methodological limitations in this area. Accepting the complex nature of our neuroendocrine response is critical to the development of successful interventions for caregivers (Laudenslager, 2014). He states in *"Anatomy of Illness": Control from a Caregiver's Perspective*. "…one must be cautious in identifying any single outcome measure as presumed to reflect stress" (Laudenslager, 2014). To build on the results of this study and explore the dysregulation of the neuroendocrine stress response and impact on a caregiver's health, a broad, biopsychosocial perspective needs to be embraced.

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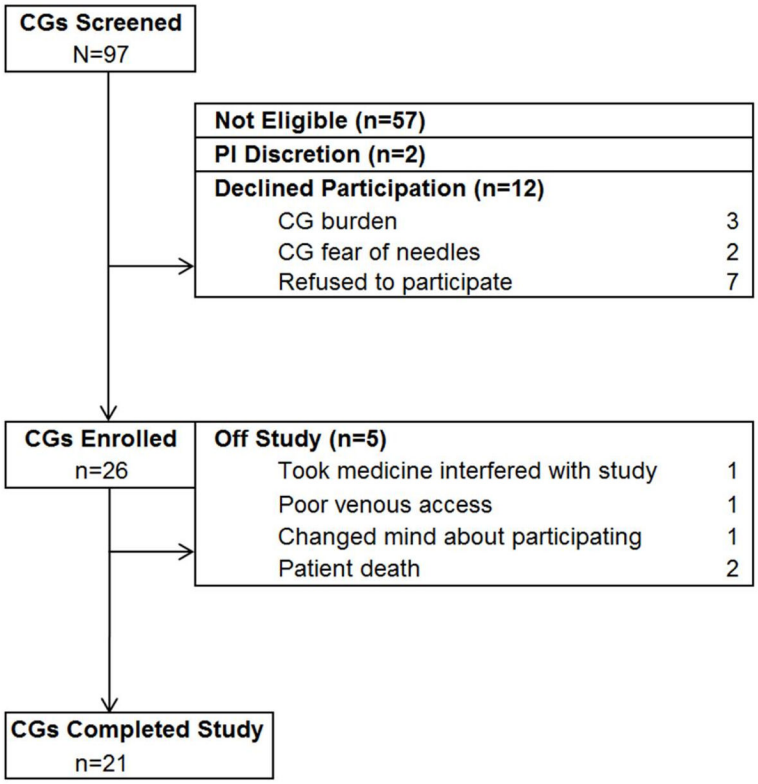

Figure 1.
Study enrollment and attrition.

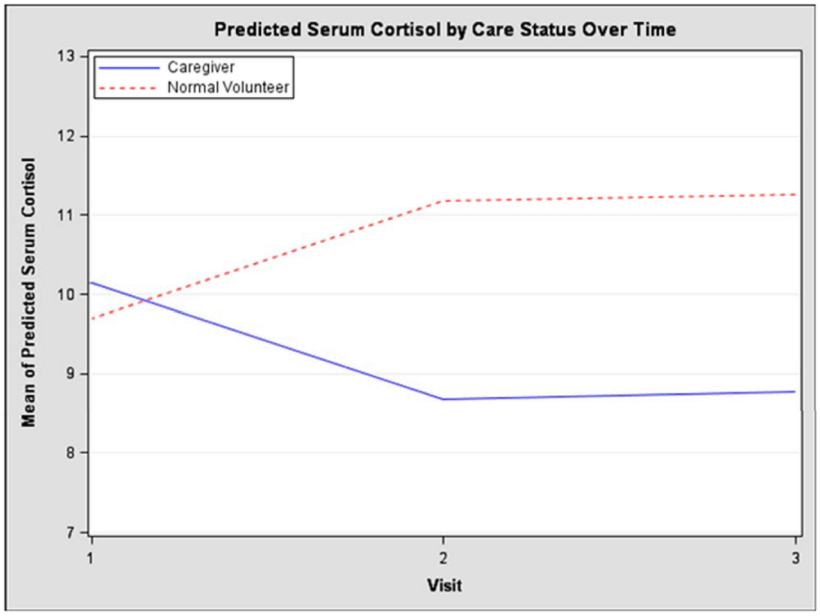

Figure 2.

Estimated means for serum cortisol from mixed model.

Note. TP, Time-point. Significant interaction (p=0.0031); Individual time point differences **p=0.013, *p=0.0275.

Table 1

Demographic characteristics (N=41)

Characteristic	CG (n=21)	Non-CG (n=20)
	n (%)	n (%)
Age, M (SD)	52.2 (11.4)	51.1 (11.0)[a]
Sex (male)	9 (43)	9 (45)
Married	20 (95.2)	16 (80.0)
Race/Ethnicity		
White, Non-Hispanic	12 (57)	12 (60)
Black, Non-Hispanic	1 (5)	1 (5)
Hispanic	8 (38)	7 (35)
Education*		
≤High School	3 (15)	0 (0)
Associate's degree/some college	9 (45)	2 (10.5)
Bachelor's degree	3 (15)	1052.6)
Graduate or professional degree	5(25)	7 (36.8)
Relationship to HSCT patient		
Spouse	12 (57.1)	
Family member, non-spouse	9 (42.9)	

Note.

[a]Age matching was ±10 years.

[b]n=20.

M, mean; SD, standard deviation; CG, caregiver, HSCT, Hematopoietic Stem Cell Transplantation.

*$p<0.012$

Table 2

Transplant information of HSCT patient

Characteristic	n (%)
Primary Disease	
Lymphoma	7 (33.3)
Leukemia	7 (33.3)
Non-Malignant Disease	7 (33.3)
Type of Transplant	
RIC	12 (57.1)
Myeloablative	9 (42.9)
Stem Cell Source	
Peripheral Blood	20 (95.2)
Cord	1 (4.8)
Stem Cell Graft Type (HLA Compatibility)	
Unrelated Donor	17 (81.0)
Related Donor	4 (19.0)
Length of Stay [Mean(SD)]	30.2 (±27.8)

Note. HSCT, Hematopoietic Stem Cell Transplantation; RIC, Reduced Intensity Conditioning; HLA, Human Leukocyte Antigen.

Table 3

Observed psychological and burden outcomes values for caregiver and non-caregiver participants

Outcome	Caregiver (n=21)			Non-Caregiver (n=20)		
	TP1 Mean (SD) Min–max	TP2 Mean (SD) Min–max	TP3 Mean (SD) Min–max	TP1 Mean (SD) Min–max	TP2 Mean (SD) Min–max	TP3 Mean (SD) Min–max
Perceived Stress Scale	25.7 (8.8)	26.1 (6.7)	26.1 (8.1)	12.9 (5.7)	13.2 (5.8)	14.4 (7.0)
	7–44	14–39	9–.46	6–25	3–28	5–33
PROMIS-Anxiety*	56.7 (8.9)	57.1 (9.3)	57.8 (7.8)	46.4 (7.8)	45.9 (7.5)	45.7 (8.5)
	36.3–70.2	36.6–72.9	36.3–67.7	36.3–62.6	36.6–58.8	36.3–61.3
PROMIS-Depression*	52.6 (7.6)	52.3 (7.9)	53 (8.2)	42.7 (5.6)	41.2 (5.4)	42.1 (7.3)
	37.1–64.4	37.1–64.4	37.1–69.3	37.1–51.2	37.1–52.3	37.1–59.7
CRA Total Score	2.4 (0.5)	2.4 (0.4)	2.4 (0.6)	-	-	-
	1.6–3.3	1.6–3.4	1.2–3.8			
Caregiver Esteem	4.4 (0.5)	4.3 (0.5)	4.3 (0.6)	-	-	-
	3.4–5	3.3–5	3–5			
Impact on Finances	2.9 (1)	2.9 (0.9)	2.8 (1.1)	-	-	-
	1–5	1.7–5	1–5			
Lack of Family Support	2.1 (0.9)	2.1 (0.9)	2.2 (1.2)	-	-	-
	1–4	1–4.8	1–4.8			
Impact on Schedule	3.7 (0.8)	3.7 (0.7)	3.7 (1)	-	-	-
	2–4.8	2.2–4.8	1–5			
Impact on Health	2.1 (0.7)	2 (0.7)	2.2 (0.8)	-	-	-
	1–3.3	1–3.5	1–3.3			

Note: M, mean; SD, standard deviation.

*Scores standardized to a mean (SD) = 50 (10) representing the average for the US general population.

Table 4

Test results and estimated mean differences from mixed model repeated measures analysis for study outcomes

	Model Effects[a]					
Outcome	**Main Effects**	**Interaction**	**Comparisons***	**Baseline**	**Discharge**	**6-Weeks Post Discharge**
					Mean difference (95% CI)	
			Physiological Markers			
Serum Cortisol, ng/dL	Time: F(2,39)=0.02, p=0.98 Group: F(1,39)=0.3.77, p=0.059	F(2,39)=0.6.71, p=0.003	TP2: t_{39}=−2.60, p=0.013 ES=0.81 TP3: t_{39}=−2.29, p=0.028 ES=0.72	0.44 (−1.45, 2.34)	−2.50 (−4.45, −0.56)	−2.50 (−4.70, −0.29)
AM Salivary Cortisol	Time: F(2,39)=0.87, p=0.426 Group: F(1,39)=0.52, p=0.47	F(2,39)=2.76, p=0.075	-----	−81.58 (−188.25, 25.09)	40.54 (−53.95, 135.04)	−37.57 (−125.92, 50.77)
Epinephrine, pg/mL[b]	Time: F(2,24)=1.35, p=0.278 Group: F(1,24)=0.76, p=0.393	F(2,24)=1.42, p=0.261	-----	1.20 (−0.22, 2.63)	−0.25 (−2.64, 2.13)	1.10 (−0.77, 2.96)
Norepinephrine, pg/mL [b]	Time: F(2,24)=3.51, p=0.046 Group: F(1,24)=0.03, p=0.86	F(2,24)=0.58, p=0.566	CG TP1 vs TP2 t_{24}=−2.55, p=0.018 ES=0.59	0.46 (−2.37, 3.28)	−0.61 (−3.04, 1.83)	−0.39 (−2.65, 1.88)
CAT-Turnover, pg/mL	Time: F(2,24)=0.47, p=0.631 Group: F(1,24)=1.22, p=0.28	F(2,24)=0.57, p=0.574	-----	−0.10 (−0.32, 0.12)	−0.02 (−0.26, 0.22)	−0.15 (−0.37, 0.06)
			Inflammation Markers			
IL-6, pg/mL [b]	Time: F(2,39)=0.17, p=0.84 Group: F(1,39)=0.15, p=0.70	F(2,39)=4.29, p=0.020	Group and Time Contrast NS	0.19 (−0.05, 0.43)	−0.08 (−0.25, 0.09)	−0.01 (−0.22, 0.19)
TNF-α, pg/mL [c]	Time: F(2,39)=6.53, p=0.0036 Group: F(1,39)=0.02, p=0.89	F(2,39)=1.68, p=0.198	CG TP1 vs TP2 t_{39}=−2.92, p=0.006 ES=0.60	0.24 (−0.22, 0.70)	−0.09 (−0.50, 0.32)	−0.23 (−0.67, 0.22)
			Psychological			
PSS [d]	Time: F(2,78)=0.85, p=0.432 Group: F(1,39)=37.26, p=0.0001	F(2,78)=0.34, p=0.713	TP1: t_{78}=5.73, p<0.0001 ES=1.79 TP2: t_{78}=5.93, p<0.0001 ES=1.80	12.77 (8.33, 17.20)	12.90 (8.46, 17.33)	11.74 (7.31, 16.18)

	Model Effects[a]					
Outcome	**Main Effects**	**Interaction**	**Comparisons***	**Baseline**	**Discharge**	**6-Weeks Post Discharge**
					Mean difference (95% CI)	
Anxiety [d]	Time: F(2,78)=0.10, p=0.909 Group: F(1,39)=29.38, p<0.0001	F(2,78)=0.14, p=0.87	TP3: t_{78}=5.27, p<0.0001 ES=1.64 TP1: t_{78}=4.40, p<0.0001 ES=1.37 TP2: t_{78}=4.55, p<0.0001 ES=1.47 TP3: t_{78}=4.86, p<0.0001 ES=1.52	6.90 (3.78, 10.02)	7.39 (4.27, 10.51)	7.62 (4.50, 10.74)
Depression [d]	Time: F(2,78)=0.99, p=0.376 Group: F(1,39)=24.12, p<0.0001	F(2,78)=0.04, p=0.961	TP1: t_{78}=4.32, p<0.0001 ES=1.35 TP2: t_{78}=4.25, p<0.0001 ES=1.41 TP3: t_{78}=4.40, p<0.0001 ES=1.38	6.26 (3.37, 9.15)	6.57 (3.68, 9.45)	6.38 (3.50, 9.27)

Note.

[a]Compound Symmetry Covariance Structure,

[b]Square root transformation;

[c]Log transformation,

[d]Unstructured Covariance Structure.

CI, Confidence Interval; AM, Morning; CAT, Catecholamine; IL-6, Interleukin-6; TNF-α, Tumor Necrosis Factor-α; PSS, Perceived Stress Scale.

*Significant comparisons only.

Table 5

Observed biomarker values for caregiver and non-caregiver participants

Biomarker	Caregiver (n=21)			Non-Caregiver (n=20)		
	TP1 Mean (SD) Min–max	TP2 Mean (SD) Min–max	TP3 Mean (SD) Min–max	TP1 Mean (SD) Min–max	TP2 Mean (SD) Min–max	TP3 Mean (SD) Min–max
Serum cortisol (mcg/dL)	10.1 (2.6)	8.7 (2.3)	[c] 8.8 (2.1)	9.7 (3.4)	11.2 (3.7)	11.3 (4.4)
	5.3–15.9	5.1–13.8	6.3–13.2	4.7–18.1	3.7–18.8	4–23.1
AM Salivary Cortisol (ng/dL)	274.6 (159.6)	[c] 309.0 (137.0)	289 (152.3)	356.2 (178)	[d] 261.5 (155.9)	[d] 324.6 (121.5)
	109–670	115–595	59–635	84–760	59–608	124–530
Epinephrine (pg/mL)	[a] 36.2 (15)	[a] 27.9 (27.5)	[b] 38.1 (15.6)	[a] 25.7 (23.3)	[a] 30 (27.9)	[b] 32.9 (34.1)
	19.6–71.2	0.3–96.8	16.8–66	4.6–87.2	0.3–84.6	0.7–126.5
Norepinephrine (pg/mL)	[a] 182.5 (105.1)	[a] 133.4 (80.3)	[b] 143.8 (67.2)	[a] 168 (80.5)	[a] 146.9 (64.4)	[b] 153.4 (73.2)
	57–372.8	31.6–286.3	68.2–259.7	92.4–351.8	78–323	68.2–291.8
CAT turnover rate (pg/mL)	[a] 3512 (852.5)	[a] 4008.9 (1492.6)	[b] 3465.1 (660.4)	[a] 3934.2 (1353)	[a] 4045.6 (1304.2)	[b] 4178.6 (1597.4)
	2278.4–5062.8	2535.2–8149.8	2530.3–4651.2	2425.7–7805.1	2969.4–7795.3	2814.2–8278.4
IL-6 (pg/mL)	[c] 0.9 (0.9)	0.7 (0.4)	[c] 0.9 (0.7)	0.6 (0.4)	[d] 0.8 (0.5)	[d] 0.8 (0.4)
	0.02–4.1	0.1–1.4	0.04–3.2	0–1.6	0.1–2.1	0.2–1.6
TNF-a (pg/mL)	[c] 1.0 (1.0)	0.6 (0.5)	[c] 0.7 (0.5)	0.8 (0.6)	0.6 (0.4)	1 (0.9)
	0.3–4.3	0.1–2.5	0.2–2.7	0.2–2.9	0.2–1.5	0.2–4.1

Note.

[a] n=12.

[b] n=13.

[c] n=20.

[d] n=19

M, mean; SD, standard deviation; CAT, Catecholamine; IL-6, Interleukin-6; TNF-α, Tumor Necrosis Factor-α.

Table 6

Baseline correlations (r_s) between biomarkers of stress and inflammation and perceived stress and burden in caregivers.

Outcome	Burden	Stress	
	CG n=21	CG n=21	Non-CG n=20
Physiological Markers			
Serum Cortisol	−0.223	−0.095	0.205
AM Salivary Cortisol	0.194	0.194	0.130
Epinephrine	0.028	−0.654*	0.442
Norepinephrine	0.049	−0.258	−0.035
CAT-Turnover	−0.385	−0.449	−0.414
Inflammation markers			
IL-6	0.196	0.196	−0.337
TNF-α	0.155	0.115	−0.156

Note. Epinephrine/Norepinephrine analysis included 13 matched caregiver/control dyads. CG, Caregiver; AM, Morning; CAT, Catecholamine; IL-6, Interleukin-6; TNF-α, Tumor Necrosis Factor-α.

* $p<0.05$;

** $p<0.01$.