



## Grief, depressive symptoms, and inflammation in the spousally bereaved

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### ABSTRACT

Grief is conceptualized by strong negative emotions, which include longing, sadness, and preoccupations with thoughts, recollections, and images of the spouse. In the initial months after the loss of a spouse, those who are widowed are at risk for cardiovascular problems and premature mortality. In the general population, depression is characterized by chronic low-grade inflammation, a key predictor of cardiovascular problems, morbidity, and mortality. Although depression and grief share similarities, they are distinct constructs. We aimed to identify if grief was related to inflammation among those who had a spouse recently die. We also sought to determine if those who are widowed and already experience elevated levels of depressive symptoms compared with the general population had higher levels of inflammation compared with those who are widowed who report fewer depressive symptoms. Ninety-nine recently bereaved individuals ( $M = 84.74$  days since passing,  $SD = 18.17$ ) completed a blood draw and psychological assessments. Proinflammatory T cell-derived cytokines were assessed, which included interferon gamma (IFN- $\gamma$ ), interleukin (IL)-6, tumor necrosis factor alpha (TNF- $\alpha$ ), IL17-A, and IL-2. Bereaved individuals with a higher grief severity (using an established cut-score) had higher levels of the proinflammatory cytokines IFN- $\gamma$ , IL-6, and TNF- $\alpha$  than those with less grief severity. Those who experienced higher levels of depression exhibited elevated levels of proinflammatory cytokines compared with those who had lower levels of depression (using a continuous measure of depressive symptoms, as well as an established cut score). This is the first study to demonstrate that inflammatory markers can distinguish those who are widowed based on grief severity such that those who are higher on grief severity have higher levels of inflammation compared with those who are lower on grief severity. These findings also add to the broader literature on depression and inflammation by showing that even in a population with high levels of depressive symptoms, there is a positive relationship between depression and inflammation.

### 1. Introduction

The loss of a spouse is an extremely stressful life event that puts people at risk for mental and physical health problems (Stahl et al., 2016). Bereavement is linked with an increased risk of cardiovascular disease (CVD), and premature mortality (Moon et al., 2013). Even after adjusting for established risk factors, the death of a spouse is associated with an increased rate of mortality from cardiovascular events (Hart et al., 2007). The first 3 months after the loss of a spouse puts people at the greatest risk for cardiovascular events (Shor et al., 2012). In a matched cohort study using a primary care database, sixteen percent of the bereaved group experienced a myocardial infarction (MI) or a

stroke within 30 days after the loss compared with 0.08% of the non-bereaved controls during the same period (Carey et al., 2014). This association existed separately for MI, pulmonary embolisms, and acute coronary syndrome. Furthermore, at 90 days after the loss, bereaved individuals were still at considerable risk for non-MI acute coronary syndrome and a pulmonary embolism compared with non-bereaved comparisons (Carey et al., 2014).

Stressful life events, and the negative emotions they generate, can enhance inflammation through several neuroendocrine and autonomic nervous system pathways (Slavich and Irwin, 2014). Grief is conceptualized by strong negative emotions, which include shock and even denial that one's spouse is gone (Shear, 2015). Grief consists of intense

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separation distress, longing, and sadness. It is common for those who are grieving to become preoccupied with thoughts, recollections, and images of the spouse (Shear, 2015). Grief is also focused on excessive avoidance of reminders of the loss (Shear, 2015). We do not know if grief severity is associated with elevated levels of inflammation.

Higher grief severity is associated with elevated levels of depressive symptoms; however, depression and grief are distinct constructs (Milic et al., 2017). Inflammation plays an important part in depression's pathogenesis for a subcategory of depressed individuals; depression also primes or sensitizes larger cytokine responses to stress (Fagundes et al., 2013; Kiecolt-Glaser et al., 2015; Miller and Blackwell, 2006). However, the positive association between depression and inflammation has not been demonstrated in a population of bereaved adults; those who are widowed score above the clinical cut-off for depression much more often than in the general population, especially during the first few months after the loss (Zisook and Shuchter, 1991). There is a possibility that a ceiling effect exists such that depression is already so elevated that there is no variance to detect a positive relationship between the “most depressed (those who have more depressive symptoms)” bereaved adults and bereaved adults who are “less depressed (those who have fewer depressive symptoms).”

In this study, we aimed to identify if grief was related to inflammation among those who had a spouse recently die. We also evaluated if depressive symptoms were associated with inflammation among bereaved adults. Given that bereaved individuals generally have high levels of depressive symptoms, it is unclear if there is still a gradient increase in inflammation as depressive symptoms rise in this population. Spousally bereaved individuals completed a blood draw and psychological assessments very shortly after the loss of their spouse. We hypothesized that those who had a higher degree of grief symptomatology would have greater levels of inflammation, as well as more depressive symptoms, relative to those with lower grief symptoms post-loss. We also hypothesized that bereaved individuals with more depressive symptoms would have higher levels of inflammation compared with bereaved individuals with fewer depressive symptoms.

## 2. Methods

### 2.1. Study sample

Individuals who recently experienced the loss of their spouse were contacted and recruited from obituaries, support groups, flyer distribution, online postings, and community events. All recruitment strategies were approved by the local Institutional Review Board (IRB). Exclusion criteria included significant visual or auditory impairment, being pregnant or nursing (women), autoimmune and inflammatory diseases, having experienced bereavement due to loss of another loved one in the last year, and getting divorced within the past year. All participants were English-speakers to ensure understanding of the questionnaires. We reached out to 1302 bereaved individuals (via mailers). Of those, 168 bereaved individuals agreed to be screened. A total of 118 bereaved individuals were eligible to participate. Of those, 99 participated. Bereaved participants must have recently lost their spouse no later than 14 weeks before the visit. Participants must have been married to their partner for at least 3 years before the loss because at this point in the relationship, bereaved individuals are “fully attached” according to the adult attachment literature (Fagundes and Schindler, 2012). We assumed a medium effect size (using Cohen's *d*) based on previous work linking stimulated cytokine production to psychosocial outcomes (Collado-Hidalgo et al., 2006). When choosing group size for the a priori power analysis, we could not find specific data regarding prevalence rates for the grief cut point immediately after the loss. However, the prevalence rate for major depression in the first month after the loss is 41% (Zisook and Shuchter, 1991). Accordingly, we utilized a conservative estimate by assuming that at least 35% of our sample would be in the smaller group for the case-categorical cut-off of

grief and depression. Using this percentage, we had 0.81 power to detect an effect.

Research assistants administered assessments at the participant's home or in the Bioscience Research Collaborative Community Research Center in the Texas Medical Center. During these visits, participants completed a questionnaire packet, which included self-report demographic questionnaires and clinical questionnaires. Anthropometric measurements including weight, height, and waist circumference and non-fasting blood samples were collected during the early hours of the morning. All samples were collected between 7:30 and 11:00 AM to control for diurnal variation.

The day before the visit, a research assistant called the participants and reminded them of the next day visit. Given that inflammatory markers may be elevated during acute illnesses (e.g., upper respiratory infections), we asked participants if they were experiencing any illness symptoms (e.g., fever, congestion, sore throat, or acute infections due to injury). Also, participants were asked to avoid any strenuous physical activity 48 h before all visits and were asked whether they followed these directions during the reminder phone call. Participants were re-scheduled for a different time if they were ill or did not follow the exercise restriction.

### 2.2. Measures

#### 2.2.1. Grief severity & depressive symptoms

The Inventory of Complicated Grief (ICG) measure was developed to assess symptoms of grief known to predict long-term functional impairments. The ICG measures 19 different grief-related symptoms. It takes approximately 5 min to complete and provides important information regarding a set of symptoms that comprise complicated grief. The internal consistency of the 19-item ICG was high (Cronbach's  $\alpha = 0.94$ ). We used a case categorical approach as our primary index and a continuous approach as a secondary index. Individuals that were considered to have a high degree of grief severity were those with ICG scores  $\geq 25$  (Prigerson et al., 1995). This cut-score is established for identifying syndromal levels of grief (a case-categorical approach); individuals reporting ICG total scores at this cut-point or above had significantly worse mental and physical health, social functioning, and pain (Prigerson et al., 1995). We utilized the cut-off as a case-categorical variable. There is ongoing debate about how many months need to pass after death to be diagnosed with a clinical diagnosis of complicated grief; however, the vast majority of researchers and clinicians would consider 3 months after the loss too early for a formal diagnosis (Shear, 2015). Accordingly, it should not be interpreted as a proxy for complicated grief (Prigerson et al., 1995) in the present investigation.

The Center for Epidemiologic Studies Depression Scale (CES-D) was used as a measure to assess for the prevalence of depression and was also included in regression models as a control variable, due to its close association with inflammation ( $\alpha = .91$ ) (Radloff, 1977). The CES-D is a widely utilized measure of depression. Higher scores on this scale indicate greater depressive symptomatology. The cut-score for clinical depression is 16. We used both a case categorical approach with the 16 cut-score and a continuous approach in the current investigation. Importantly, none of the items in the ICG are identical to those in the CES-D. Every item in the ICG references the deceased. There is one question that is conceptually similar (“I feel lonely” and “I feel lonely a great deal of time ever since s/he died”).

#### 2.2.2. Inflammation

To measure the reactivity of T cells to mitogenic stimulation, we used whole blood cell cultures to induce cytokine production. The proinflammatory T cell-derived cytokines included IL-6, TNF- $\alpha$ , IFN- $\gamma$ , IL17-A, and IL-2. We adopted this approach rather than analyzing serum level cytokines because circulating (serum or plasma) levels are often close to, and more often below, the limit of detection of the assay, and they exhibit extreme variability as a result of a number of factors

including diurnal variation, changes in plasma volume, and enlargement of the cell pool. We are able to obtain a more complete signature by measuring the capacity of immune cells to produce inflammatory mediators after *ex vivo* stimulation (Marsland et al., 2017). This method more likely represents the *in vivo* situation where cytokines are produced by the immune system in response to stress or infection. Whole blood, diluted 1:10 with RPMI-1640 (Gibco, Grand Island, NY), 100 U/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine was stimulated with anti-CD2/CD28 monoclonal antibodies final concentration anti-CD2.1/anti-CD2.2 0.33 µg/ml and anti-CD28 1.33 µg/ml at 37 °C/5% CO<sub>2</sub> in 96-well round-bottomed plates. Supernatants were collected after 72 h of culture and stored at –80 °C until they were analyzed using multiplex assays according to the manufacturer's instructions (R&D Biosystems) (Korenromp et al., 2011). T cell mitogen-induced secretion of IL-6, TNF-α, IFN-γ, IL17-A, and IL-2 were assessed (Korenromp et al., 2011).

### 2.2.3. Sleep disturbance

The Pittsburgh Sleep Quality Index (PSQI) was used as a measure of sleep disturbance. The PSQI is a widely used instrument for the evaluation of sleep disturbances which consists of seven component scores that are aggregated in a global score with a range of 0–21 (Buysse et al., 1989). Individual subscales included Subjective Sleep Quality, Sleep Latency, Sleep Duration, Sleep Efficiency, Sleep Disturbances, Use of Sleep Medication and Daytime Dysfunction. Higher scores on each subscale of the global score are indicative of greater sleep disturbance.

### 2.2.4. Comorbid conditions

The Charlson Comorbidity Index (CCI) was used to assess for comorbid conditions. This measure is the most widely used comorbidity index for predicting mortality (D'Hoore et al., 1993). The measure assigns weights to 19 physical health comorbid conditions based on their potential influence on one-year mortality. This was used as a covariate in the analysis as is common in the field of psychoneuroimmunology (Fagundes et al., 2014).

### 2.2.5. Physical activity

Physical activity was measured by the International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003). The IPAQ contains items that measure the frequency and duration of vigorous-intensity activities, moderate-intensity activities, and walking during a 7-day period. The respective frequency and duration values for vigorous activities, moderate activities, and walking were first multiplied together. The resulting volumes (minutes/week) of vigorous activities, moderate activities, and walking were then multiplied by their respective metabolic equivalents (MET). Finally, the resulting individual MET values were summed to form a continuous measure of physical activity in units of total MET-minutes/week. This was a covariate in the analysis.

### 2.2.6. Other covariates

Demographic factors, health behaviors, and body mass index (BMI) were also included in models as covariates. Specifically, participants provided self-reports of their age, gender, education, days since death of spouse, smoking status, and nicotine use. BMI was computed as weight in kilograms divided by height in meters squared. Total family income was also assessed using an ordinal scale starting at 0 if the family did not have any income in the prior year. On this scale, 1 indicates a total family income between \$5000 and \$11,999 and 8 represents an income of \$100,000 or greater. We also documented all medications.

## 2.3. Statistical analysis

Preliminary statistical analyses included descriptive statistics and assessment of normality of distributions. After careful inspection, we had no need to remove outliers. The inflammatory markers were

skewed as would be expected (Shields et al., 2016). Accordingly, (base 10) log transformed values were calculated for each marker. After running each analysis, we examined the residuals to ensure they were normally distributed.

Multiple imputation was employed to impute missing data (which was fewer than 5% of the sample and did not include any immune data nor data on bereavement status). Multiple imputation produces unbiased parameter estimates that appropriately reflect the true variability of the missing data and has been shown through simulation studies to be a more valid and less biased analytical approach than listwise deletion. Multiple imputation has been shown to perform well when data are missing at random and is even acceptable under some cases of nonrandom missingness (Enders, 2017). It is robust to departures from normality assumptions and performs well even with low sample size. Following standard practice, the imputation procedure was repeated five times in order to approximate the true measurement variance represented in real data. All analyses were completed with each of the full imputed data sets, and the coefficients generated by each separate data set were averaged to produce final estimates (Enders, 2017).

Given that most assays in the field of psychoneuroimmunology produce multiple immune markers representing a similar construct, there is generally risk of type I error. Thus, before analyzing each cytokine individually, we created a composite index of all proinflammatory markers to determine statistical significance. This is an established way to evaluate multiple immune markers in order to protect type I error; if the composite index is statistically significant, then the markers are analyzed individually (Fagundes et al., 2012). Other possible solutions include a Bonferroni adjustment; however, this approach has increasingly been dismissed given it is too conservative in modest size samples (Nakagawa, 2004; Perneger, 1998). The proinflammatory composite had a Cronbach α of 0.92. For each cytokine, the z scores derived from the (base 10) log transformed values were calculated and averaged to produce the summary construct for each participant. We first ran two general linear models (one adjusted and one unadjusted) to assess the association between grief scores and the composite cytokine index. We then ran individual general linear models to assess the association between grief severity and each individual inflammatory marker. In the unadjusted models, grief severity was the independent variable and each individual cytokine (i.e., IL-6, TNF-α, IFN-γ, IL17-A, and IL-2) was used as a separate dependent variable. In the adjusted models, we included physical activity, age, sex, use of antidepressants, BMI, statins, days since death, total family income, smoking, education, sleep, and comorbidities in the analyses. In the field of psychoneuroimmunology, it is standard to select a priori covariates; the aforementioned covariates were selected a priori decision based on previous work in the field (Fagundes et al., 2013; Kiecolt-Glaser et al., 2015; Miller and Blackwell, 2006). We ran the grief analyses described above twice. In the first set of “grief severity models” we used a case categorical approach using the cut-score previously described. We then used the same procedures evaluating grief as a continuous variable.

When examining the association between depressive symptoms and inflammation, we first ran two general linear models (one adjusted and one unadjusted) to assess the association between depressive symptoms and the composite cytokine index. We then ran ten general linear models to evaluate the association between depressive symptoms and each inflammatory marker. In the first set of “depression models”, which were unadjusted, depressive symptoms was the independent variable and each individual cytokine (i.e., IL-6, TNF-α, IFN-γ, IL17-A, and IL-2) was used as a separate dependent variable. In the second set of models, we adjusted for physical activity, age, sex, use of antidepressants, BMI, statins, days since death, total family income, smoking, education, sleep, and comorbidities. We ran the depression analyses described using a case categorical approach using the cut-score for major depression disorder (MDD). We then used the same procedure evaluating depression as a continuous variable.

**Table 1**  
Descriptive statistics for variables included in the models based on the case categorical cut-off for grief and depression.

Characteristic	Overall (n = 99) Mean (SD) %	≥ grief cut point (n = 35) Mean (SD) %	< grief cut point (n = 64) Mean (SD) %	$\eta^2$ or Cramer's $V^*$	P-Value	Overall (n = 99) Mean (SD) %	≥ depress cut point (n = 46) Mean (SD) %	< depress cut point (n = 53) Mean (SD) %	$\eta^2$ or Cramer's $V^*$	P-Value
Total Physical Activity										
Mean (SD)	2853.08 (3872.88)	3122.30 (4761.63)	2705.87 (3301.45)	.004	0.60	2853 (3872.8)	3122.30 (2872.63)	2622.22 (4417.35)	.006	0.79
Age, years										
Mean (SD)	68.61 (10.70)	65.33 (11.93)	70.42 (9.56)	.01	0.024	68.61 (10.70)	64.67 (11.93)	72.00(9.56)	.10	0.010
Sex										
Females	72	69	73	.05*	0.65	72	38.6	32.4	.26*	0.007
Education – Highest Grade										
Mean (SD)	16.10 (2.80)	16.92 (2.59)	15.64 (2.83)	.05	0.029	16.10 (2.80)	16.18 (2.59)	16.03 (2.83)	.001	0.98
Antidepressants										
Currently Taking	25	11	14	.10*	0.23	25	15.6	9.40	.01*	0.08
BMI										
Mean (SD)	26.80 (4.71)	27.13 (4.31)	26.61 (4.95)	.002	0.60	26.79 (4.71)	27.59 (4.31)	26.12(4.95)	.025	0.60
Nicotine Use										
Currently Using	0.05	0.03	0.02	.34*	0.24	0.05	0.03	0.06	.004	0.65
Statins										
Currently Taking	0.41	0.13	0.28	0.05*	0.60	0.41	0.25	0.16	.013*	0.26
Days Since Passing										
Mean (SD)	84.79 (18.26)	83.69 (17.07)	85.39 (18.98)	.002	0.67	84.79 (18.26)	84.60 (17.07)	85.00(18.98)	.00	0.90
Total Family Income										
Mean (SD)	6.81 (1.56)	6.76 (1.80)	6.84 (1.42)	.00	0.82	6.81 (1.56)	6.90 (1.80)	6.74 (1.42)	.003	0.61
Pittsburgh Sleep Quality Index - Total										
Mean (SD)	7.30 (6.08)	8.53 (6.52)	6.62 (5.66)	.04	0.19	7.30 (6.08)	8.89 (6.52)	5.94 (5.66)	.073	0.05
Charlson Comorbidity Index										
Mean (SD)	0.24 (0.94)	0.19 (0.86)	0.27 (0.94)	.004	0.74	0.24 (0.94)	0.40 (0.86)	0.06 (0.94)	.033	0.06
IL-6										
Mean (SD)	4.54 (1.37)	5.00 (1.35)	4.28 (1.31)	.07	0.013	4.53 (1.36)	4.83 (1.35)	4.28 (1.31)	.04	0.047
Adjusted Mean	4.65	5.04	4.26	.07	0.016	4.65	4.83	4.28	.03	0.15
IL-17A										
Mean (SD)	5.80 (0.92)	5.98 (1.04)	5.70 (0.85)	.02	0.16	5.80 (0.92)	6.01 (1.04)	5.61 (0.85)	.05	0.034
Adjusted Mean	5.85	6.04	5.67	.01	0.10	5.85	6.01	5.60	.09	0.03
IL-2										
Mean (SD)	7.11 (0.62)	7.16 (0.53)	7.08 (0.67)	.00	0.55	7.11(0.62)	7.14 (0.53)	7.08 (0.67)	.004	0.55
Adjusted Mean	7.13	7.20	7.07	.01	0.39	7.13	7.14	7.08	.001	0.57
TNF- $\alpha$										
Mean (SD)	6.50 (1.34)	6.94 (1.22)	6.26 (1.36)	.06	0.016	6.50 (1.34)	6.98 (1.22)	6.17(1.36)	.07	0.001
Adjusted Mean	6.59	6.92	6.27	.05	0.042	6.59	6.88	6.12	.02	0.051
IFN- $\gamma$										
Mean (SD)	8.10 (1.64)	8.67 (1.49)	7.78 (1.64)	.07	0.009	8.08(1.63)	8.58(1.49)	7.69(1.64)	.08	0.006
Adjusted Mean	8.22	8.65	7.79	.04	0.026	8.22	8.58	7.69	.08	0.028

### 3. Results

First, we evaluated the association between inflammatory markers and grief using the case categorical approach. Using the composite index of each cytokine (IL-6, TNF- $\alpha$ , and IFN- $\gamma$ , IL17-A, and IL-2), bereaved individuals who met or exceeded the grief cut-point had higher levels of the cytokine composite in the unadjusted ( $p = .029$ ;  $\eta^2 = 0.05$ ) and adjusted models ( $p = .035$ ;  $\eta^2 = 0.05$ ) relative to those who were below the cut-point (means are not meaningful and thus not reported because they are calculated with cytokine z scores). When we ran each cytokine as an individual dependent variable, bereaved individuals who met or exceeded the grief cut-point had higher levels of the proinflammatory cytokines IL-6, TNF- $\alpha$ , and IFN- $\gamma$  in both adjusted and unadjusted models compared with those below the cut-point (see Tables 1 and 2). Grief was not associated with IL17-A or IL-2. Adjusted and unadjusted means, standard deviations, and  $p$ -values are presented in Table 1. As presented, those who met or exceeded the grief cut-point were younger and had more education. We then examined grief as a continuous variable. Using this approach, grief symptoms were not significantly associated with elevations in the composite index of

inflammation ( $r = .12$ ,  $p = .23$ ) for the unadjusted model or the adjusted model ( $b = 0.01$ ,  $p = .18$ , semi-partial  $r = 0.14$ ). Thus, we did not probe the individual cytokines.

In the depression analyses, we examined the association between depressive symptoms and proinflammatory cytokines using the case categorical approach (Table 1). Using the composite index of each cytokine, compared with those that did not meet the categorical cut-off for possible MDD, those who met the cut-off for possible MDD exhibited greater elevations of inflammation in the composite index in the unadjusted model ( $p = .020$ ,  $\eta^2 = 0.05$ ) and the adjusted model ( $p = .020$ ,  $\eta^2 = 0.05$ ). In the unadjusted regression models examining each individual cytokine individually, compared with those who did not meet the categorical cut-off for possible MDD, those who did meet the cut-off exhibited greater elevations in the proinflammatory cytokines IL-6, TNF- $\alpha$ , IFN- $\gamma$ , and IL17-A. However, this was not the case for IL-2. In the adjustment models, compared with those that did not meet the categorical cut-off for MDD among bereaved individuals, those who met the categorical cut-off for MDD among bereaved individuals had higher levels of the proinflammatory cytokines TNF- $\alpha$ , IFN- $\gamma$ , and IL17-A. This was not the case for IL-2 or IL-6.

**Table 2**  
Correlation table for variables included in the model based on the continuous scores for grief and depression.

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Days Since Passing														
2. Comorbidity Index	-.11*													
3. Sleep Quality Index	.00	-.01												
4. Income	.08	.08	-.01											
5. Age	.11**	.14**	-.09*	-.03										
6. BMI	-.13**	.04	.13**	.07	-.04									
7. Physical Activity	-.11*	.13**	-.49**	.04	.07	-.10*								
8. IFN- $\gamma$	.03	-.08*	-.07	.08	-.18**	.12**	.04							
9. IL-17A	-.10*	.01	-.08	.09*	-.08	.07	.09*	.55**						
10. IL-2	-.06	-.11**	-.10*	.01*	-.03	.14**	.04	.62**	.55**					
11. TNF- $\alpha$	.04	-.09*	.01	.08*	-.11**	.13**	.02	.95**	.65**	.71**				
12. IL-6	.02	-.18**	-.07	.06	-.15**	.11**	.01	.79**	.60**	.70**	.84**			
13. Grades Completed	.06	-.03	.23**	.20**	-.06	.18**	-.11*	.02	-.15**	-.11**	.001	-.06		
14. Grief (continuous)	-.17	.05	.30	-.07	-.27**	-.01	-.00	.16	.10	-.07	.14	.11	.06	
15. Depressive symptoms (continuous)	-.08	-.10	.43*	-.00	-.36**	.15	-.02	.26**	.19	.04	.26**	.24*	.09	.66**

Note: \* Correlation is significant at the 0.05 level (2-tailed). \*\* Correlation is significant at the 0.01 level (2-tailed).

We then modeled depression as a continuous variable. Using the composite index of each cytokine, bereaved individuals who had more depressive symptoms had higher levels of the cytokine composite in the unadjusted ( $r = .23$ ,  $p = .024$ ) model and the adjusted model ( $p = .049$ ; semi-partial  $r = .27$ ). The average depression score was 1.26 points above the clinical cut-off ( $M = 17.26$ ). In the unadjusted regression model (i.e. zero-order correlation), those with greater depressive symptoms had elevated levels of the proinflammatory cytokines IL-6 ( $r = .24$ ,  $p < .001$ ), TNF- $\alpha$  ( $r = .26$ ,  $p < .001$ ), IFN- $\gamma$  ( $r = 0.26$ ,  $p < .001$ ). However, depressive symptoms were not associated with IL17-A ( $r = 0.19$ ,  $p = 0.07$ ) or IL-2 ( $r = 0.04$ ,  $p = .92$ ).

Adjusted regression models showed that those with greater depressive symptoms had elevated levels of the proinflammatory cytokines IL-6 ( $b = 0.045$ ,  $p = .017$ , semi-partial  $r = .28$ ), TNF- $\alpha$  ( $b = 0.042$ ,  $p = .039$ , semi-partial  $r = .25$ ), IFN- $\gamma$  ( $b = 0.06$ ,  $p = .001$  semi-partial  $r = .29$ ), IL17-A ( $b = 0.027$ ,  $p = .044$  semi-partial  $r = .24$ ). Depressive symptoms were not associated with IL-2 ( $b = 0.01$ ,  $p = .45$  semi-partial  $r = 0.10$ ).

Given the association between grief (cut-score) and inflammation, and the association between depressive symptoms and inflammation, we sought to examine if the association between grief and inflammation was mediated by a continuous measure of depressive symptoms. We tested mediation via examining the path coefficient of the indirect effect by estimating and bootstrapping a confidence interval (percentile, bias-corrected, and accelerated), which was generated for the total and indirect effects. Specifically, we tested indirect effects with 5000 bootstrap resamples (Preacher and Hayes, 2008). We examined if the association between grief (A) and the inflammatory composite (Y) was mediated by depressive symptoms (M). Mediation is indicated when zero is not included in the bootstrap confidence intervals. Bootstrapping results were the determinant of mediation in these analyses. In the unadjusted model, bootstrap results indicated mediation from grief to inflammation through depressive symptoms did not exist because zero was in the confidence interval ( $a*b =$  indirect effect (X on Y through M) Effect size ( $r^2$ ) = .12, SE = .12, 95% CI, ((-.09)–(.76)). In the adjusted model, bootstrap results indicated mediation from grief to inflammation through depressive symptoms did not exist because zero was in the confidence interval ( $a*b =$  indirect effect (X on Y through M) Effect size ( $r^2$ ) = .15, SE = .16, 95% CI, ((-.10)–(.51)). Thus, in both the adjusted and unadjusted model, there was no evidence of mediation.

#### 4. Discussion

Bereaved individuals with higher grief severity, using a case categorical approach, had higher levels of proinflammatory T cell-mediated

cytokine production (an important CVD risk factor) than those who did not. Those who experienced more depressive symptoms showed elevated levels of proinflammatory cytokines compared with those who had fewer depressive symptoms. Those who met the clinical cut-off for MDD also had higher levels of proinflammatory cytokine production than those who did not. However, depression did not mediate the relationships with grief and proinflammatory cytokines. Importantly, these findings remained after adjusting for established confounding factors.

Researchers in the field of psychosomatic medicine have long discussed that grief should be conceptualized as a “disease” given the overlap between grief and sickness behaviors such as fatigue and depression, although Engel (1961) disagreed with this notion. To our knowledge, this is the first study to demonstrate that spousally bereaved individuals with greater grief symptomatology had higher levels of inflammation than those with lower levels of grief. Neuroinflammation is a biological mechanism involved in the initiation of “sickness behaviors” across a variety of populations (Dantzer et al., 2008). It is possible that increased inflammation among those who experience considerable grief actually promotes complicated grief, an important direction for future work.

The average depression score for bereaved individuals was slightly more than one point above the cut-off score (Radloff, 1977). Although a formal diagnosis of major depressive disorder (MDD) can only occur after a clinical interview, this score is important. This is the first study to demonstrate that in a bereaved population, there is a relationship between inflammation and depression (Fagundes et al., 2013; Kiecolt-Glaser et al., 2015; Miller and Blackwell, 2006). Furthermore, higher grief severity was associated with elevated levels of depressive symptoms.

Research psychologists have argued that grief and depression are conceptually distinguishable; this claim has been supported by empirical data (Boelen et al., 2010; Prigerson et al., 1996). One-third of the variance between grief and depression in the current investigation overlapped. A mediation analysis indicated that depression did not explain the association between grief and inflammation; however, caution should be warranted given the sample size (MacKinnon, 2008). Future work that evaluates grief, depression, and inflammation in a considerably larger sample will be crucial to understand these complex, albeit intertwined, relationships. In the current investigation, the sample size precludes us from stating with confidence whether the similar or dissimilar variance between depression and inflammation is involved in the relationship between grief and inflammation. This will be an interesting direction for future research. Importantly, bereavement was recently removed as an exclusion when diagnosing depression in the Diagnostic and Statistical Manual of Mental Disorders 5

(DSM–5). This decision was made under the premise that there is no scientific basis to avoid making a major depression diagnosis after the loss of a loved one, while freely making a diagnosis of major depression after other major life stressors that precipitate poor mental health (Florence et al., 2015). The answer to the question of if the same or different variance between inflammation and depression is responsible for the association between grief and inflammation could be informative for future editions of the DSM.

Depression and inflammation are intertwined through a bidirectional feedback loop such that depression primes the inflammatory response, while inflammation is involved in the pathogenesis of depression. (Fagundes et al., 2013; Kiecolt-Glaser et al., 2015; Miller and Blackwell, 2006). However, a positive association between depression and inflammation has not been demonstrated in a population of bereaved adults. A high proportion of bereaved individuals exceed self-reported clinical cut-offs for possible major depressive disorder in comparison to those who are not bereaved (Zisook and Shuchter, 1991). Thus, there was a high probability that depressive symptoms were consistently elevated to the point that there was no variance to detect a positive relationship between the “most depressed (those who had more depressive symptoms)” bereaved adults and bereaved adults who had elevated depressive symptoms but were “less depressed (those who had fewer depressive symptoms).” Accordingly, similar to the general population, inflammation is an important marker of elevated levels of depression among bereaved adults, which could have clinical implications.

This study adds evidence to the proposition, recently put forth, that determining subtypes of depression and inflammatory levels when making diagnoses and treatment plans would be useful. The proponents of this approach, who subscribed to an evolutionary psychiatry perspective, argued that depression is not a unitary disease but a rather a constellation of 12 subtypes (Rantala et al., 2017). They proposed grief to be one of these subtypes. The authors suggested that inflammation can augment short-term changes in mood after exposure to some of these 12 subtypes (e.g., grief), thus thwarting recovery.

After evaluating the cytokines as a composite, we analyzed each cytokine individually. Importantly, IL-6, TNF- $\alpha$ , and IFN- $\gamma$  emerged as the cytokines related to higher levels of grief in both adjusted and unadjusted models, but not IL17-A and IL-2. A recent meta-analysis revealed a moderate increase in both stimulated IL-6 and TNF- $\alpha$  production that peaked between 10 and 30 min after acute stress and IFN- $\gamma$  10 min post-stress. Yet, there were no effects of stress on IL17-A and IL-2. Thus, it is possible that these three markers are more sensitive to the stress associated with grief than the other markers measured in the current study (Marsland et al., 2017).

Bereaved individuals are clearly vulnerable for cardiovascular events during the early months after the death of a spouse. Yet we know little about which bereaved individuals are most at risk and why. Researchers have called for a better understanding of the factors associated with these events (Carey et al., 2014). Inflammation is central to all phases of cardiovascular disease (CVD) from initial lesion to end-stage thrombotic complications, and has been hypothesized to be a key mechanism underlying bereavement-related CVD risk (Libby et al., 2009; Pearson et al., 2003). Although most of this work has evaluated the link between serum levels of cytokines and CVD, there is work linking stimulated cytokines to CVD risk. The advantage of this approach is that the range of measuring cytokines is much larger than is possible with the very low values in serum or plasma and the capacity of the immune system to perform under challenging conditions is better reflected by the production capacity of the immune system than under rest conditions (van Zuiden et al., 2011).

Hong et al. (2015) showed that relationships between inflammation dysregulation mediated by sympathoadrenal activation and blood pressure (BP) is evident among individuals with normal to mildly elevated BP. They argued that bAR-mediated inflammation control (BARIC) responses of blood monocytes to isoproterenol (Iso) is an

important indicator of BP and CVD risk factor, especially given the critical role of monocytes in atherogenic processes. Bellingrath et al. (2013) found that LPS-stimulated cytokine production in association with an effort-reward-imbalance, which is a known risk factor of CVD due to a lower sensitivity to regulation by glucocorticoids.

Our manuscript focuses on inflammation and the intensity of grief and depression. There are several papers underlining a relation between depression and stimulated inflammation. Gaspersz et al. (2017) showed that within a large MDD sample, the anxious distress specifier was associated with increased innate cytokine production capacity but not with basal inflammation. Majd et al. (2018) demonstrated higher stimulated TNF- $\alpha$  production with higher depressive symptoms in men and women. In a prospective longitudinal study in a large cohort of military personnel, stimulated cytokine production was associated with depressive symptoms, whereas there was no relation with serum cytokines (van Zuiden et al., 2011).

An important strength of our study is its rigorous methodology, including careful consideration of time of day of blood draw and homogeneity in type of loss. The sample is predominately white, a limitation that should be addressed in future work. Future studies should investigate inflammation over time to examine the time course of grief-related changes in inflammation. During the initial days to months after a loss, acute grief can vary in intensity, nature, and time course; however, for most people, grief diminishes over time (Prigerson et al., 1995). Yet there are cases in which grief actually increases. Bonanno et al., (2002) identified five unique trajectories of affective changes after the loss of a loved one: common grief, chronic grief, chronic depression, depression followed by improvement, and resilience. It would be interesting for researchers to examine these trajectories over time as they relate to inflammatory biomarkers.

Previous work (approximately 3 months after the death of a spouse) demonstrated that those who exceeded the threshold of the case categorical cut-off we utilized, experienced greater impairments in global functioning, mood, sleep, and self-esteem at 18 months after the loss (Prigerson et al., 1995). Based on work suggesting elevated levels of inflammation predict future depression, it will be interesting to examine if baseline levels of inflammation at approximately 3 months after the loss predict impairment in global functioning, mood, sleep, and self-esteem at 18 months after the loss.

Although the case-categorical cut-off is sometimes used to assess complicated grief, it is important to note that participants in this investigation would not qualify for a complicated grief diagnosis given that six months had not passed since the loss occurred. The diagnosis of complicated grief has gone through many iterations. There is a lack of consensus regarding the criteria and even formal name for complicated grief. Two suggestions for criteria that were presented to the working group for the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) led to an the inclusion in the DSM-5 of an entity called, “persistent complex bereavement disorder” (incorporating some of the criteria from each proposal) as a condition requiring further research (Shear, 2015). According to the provisional proposed guidelines for the diagnosis of prolonged grief disorder in the International Classification of Diseases, 11th Revision, one must have a significant grief response for at least six months (Shear, 2015). It will be interesting for future work to examine the relationship between inflammation and grief after the six-month mark (Shear, 2015).

It is noteworthy that grief was associated with inflammatory markers when assessed using the categorical cut-off, but not when assessed continuously. In the grief literature, there is an active discussion regarding whether researchers should use a continuous or categorical approach (Holland et al., 2009). Some argue that there is a categorical difference between those suffering from extreme grief and those who are not (Newson et al., 2011). Yet others believe that it should be evaluated across the spectrum (Holland et al., 2009). The current study demonstrates that, in the case of people’s inflammatory biology, there is a categorical difference. This is similar to other populations in the field

of psychoneuroimmunology where people differ in inflammatory levels using a case-categorical approach, but not when the same construct is measured continuously. Most notably, in the cancer-related fatigue literature, inflammation is consistently associated with fatigue when assessed as a distinct category (Collado-Hidalgo et al., 2006). However, there is generally no relationship when evaluated continuously, even though statistical power is generally higher when a continuous measure is used.

Up until now, the vast majority of studies examining grief and biological markers of physical health have focused on comparing those who are bereaved with those who are not bereaved to demonstrate risk differences (Schultze-Florey et al., 2012). However, there has been almost no work examining individual differences in the biological markers of physical health within bereaved individuals. Clearly, not everyone who is widowed is at risk for poor physical health outcomes. Yet we know almost nothing about who is most at risk for elevated biological markers of disease risk. Showing differences between grief severity is a critical first step; however, future work paying careful attention to individual differences within bereaved individuals is critically needed to inform targeted interventions. It is problematic to assume that the same risk factors for psychological health will map on to biological risk (Segerstrom and Miller, 2004).

## 5. Conclusion

In conclusion, this study adds to our growing understanding of the mechanisms that underlie CVD among those who are bereaved. Specifically, it is (a) the first study to demonstrate that inflammatory markers can distinguish those with higher grief severity compared with those with lower grief severity, and (b) a relationship exists between depression and inflammation among bereaved individuals. Future longitudinal studies that examine links between grief and inflammation are an important next step. These findings add to the growing literature about the biological processes that underlie grief.

## Conflicts of interest

The authors have no conflicts of interest.

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