Received: 25 September 2019 | Revised: 30 January 2020 | Accepted: 20 February 2020

DOI: 10.1002/eat.23278

ORIGINAL ARTICLE

International Journal of EATING DISORDERS WILEY

Coping strategies and stress-induced natural killer cell redistribution in women with eating disorders

Francisco J. Vaz-Leal[1,2] | María Isabel Ramos-Fuentes[2] | Juan Antonio Guisado-Macías[1,2] | Guadalupe Espárrago-Llorca[3] | Celia Redondo-Rodríguez[4] | Beatriz Bajo-Cabello[5] | Laura Rodríguez-Santos[2]

[1]University Hospital/University Hospital Network, Extremadura Health Service, Badajoz, Spain

[2]Faculty of Medicine, University of Extremadura, Badajoz, Spain

[3]Eating Disorders Unit, University Hospital Network, Extremadura Health Service, Cáceres, Spain

[4]Faculty of Education, University of Extremadura, Badajoz, Spain

[5]College of Nursing and Occupational Therapy, University of Extremadura, Cáceres, Spain

Correspondence
Francisco J. Vaz-Leal, Facultad de Medicina. Campus Universitario. Avda. Elvas, s/n. 06006 Badajoz. Spain.
Email: fjvaz@unex.es

Funding information
European Regional Development Fund and Junta deExtremadura, Grant/Award Number: GR18136

Action Editor: Guido Frank

Abstract

Background: Patients with eating disorders (ED) are very sensitive and responsive to psychosocial stress. Stress response includes changes in immune cell distribution and may be modulated by the capability to cope with stressors. Thus, the present study sought to analyze the association between coping strategies and immune response (natural killer [NK] cell redistribution following psychosocial stress) in patients with anorexia nervosa (AN) and bulimia nervosa (BN) and healthy controls (HC).

Method: Twenty-four AN patients, 29 BN patients, and 58 HC were studied. A multidimensional assessment tool, the COPE Inventory, was used to assess coping strategies. The number of NK cells was quantified in peripheral blood before and after the application of the Trier Social Stress Test (TSST). Potentially mediating variables, such as weight status, severity of eating pathology, depression, anxiety, and impulsivity were controlled.

Results: The three groups differed in intensity and direction of cell redistribution: The TSST was followed in BN patients by a significant decrease in the number of NK cells, whereas HC displayed a moderate decrease and AN a clear increase. Specific correlations between coping strategies and NK cell mobilization were found, especially in BN patients (positive for "planning" and negative for "substance abuse").

Conclusion: Recognition and subsequent modification of the dysfunctional coping strategies used by patients with ED could contribute to improving their immune status, strengthening their resilience and increasing their ability to overcome the disease.

KEYWORDS

anorexia nervosa, bulimia nervosa, leukocyte, natural killer cells, stress, Trier Social Stress Test

1 | INTRODUCTION

Patients with eating disorders (ED) are very sensitive and responsive to psychosocial stress. When faced with stress-inducing situations, patients with anorexia nervosa (AN) and bulimia nervosa (BN) display strong neuroendocrine, psychological, and behavioral reactions (Het et al., 2015; P. Monteleone et al., 2011, 2012; P. Monteleone, Scognamiglio, Monteleone, Perillo, & Maj, 2014; Paszynska, Dmitrzak-Weglarz, Tyszkiewicz-Nwafor, & Slopien, 2016; Pirke, Platte, Laessle, Seidl, & Fichter, 1992; Vaz-Leal, Rodríguez-Santos, García-Herráiz, Monge-Bautista, & López-Vinuesa, 2011; Vaz-Leal et al., 2018).

Psychosocial stress can be understood as the result of demands from the interpersonal environment and the individual capacity to cope with them. In line with this approach, some animal studies have shown that coping styles can have a significant modulatory effect on

stress responses at the neurobiological level (Azpiroz, De Miguel, Fano, & Vegas, 2008; De Miguel et al., 2011; Koolhaas, 2008; Koolhaas, de Boer, Coppens, & Buwalda, 2010; Reimert, Rodenburg, Ursinus, Kemp, & Bolhuis, 2014; Vegas, Fano, Brain, Alonso, & Azpiroz, 2006). However, as the coping styles identified in animals are elementary in many ways, the results of these studies cannot be directly transferred to human research, for which more complex models are necessary (Folkman & Lazarus, 1985; Lazarus, DeLongis, Folkman, & Gruen, 1985). To our knowledge, studies of this type have not yet been published.

Patients with ED are prone to use dysfunctional and ineffective coping methods (Brytek-Matera & Schiltz, 2013; Ghaderi & Scott, 2000; Hernando et al., 2019; Jaúregui-Lobera, Estébanez, Santiago-Fernández, Álvarez Bautista, & Garrido, 2009; Nagata, Matsuyama, Kiriike, Iketani, & Oshima, 2000; Tobin & Griffing, 1995; Troop, Holbrey, & Treasure, 1998). This seems to correlate with higher levels of depression, anxiety, and hostility (Dennard & Richards, 2013; Tobin & Griffing, 1995; Vaz-Leal et al., 2007; Vaz-Leal, Rodríguez-Santos, Melero-Ruiz, Ramos-Fuentes, & García-Herráiz, 2010), such that coping style seems to be able to identify and discriminate ED patients more effectively than the actual characteristics of the ED (Villa et al., 2009).

One of the neurobiological changes induced by stress is mobilization/redistribution of immune cells, a necessary step in immune response (Mandal & Viswanathan, 2015). Activation of the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic adrenal medulary (SAM) axis causes the release of glucocorticoids and catecholamines, which stimulate cell receptors and induce immune cell mobilization (Bartolomucci, 2007; Dhabhar, Malarkey, Neri, & McEwen, 2012; Ince, Weber, & Scheiermann, 2018).

Natural killer (NK) cells ($CD56^{+}CD3^{-}$) are a subpopulation of lymphocytes distributed throughout the body. Because of their cytotoxicity and their capacity to produce cytokines, they are usually the primary effectors against potential dangers, such as virally infected or neoplastic cells (Mandal & Viswanathan, 2015; Peng & Tian, 2014). As they play an early and crucial role in the immune response and are easily quantified in peripheral blood, the assessment of NK cells can be a reliable indicator of immune status and immune system activity.

In light of the foregoing considerations, we assumed that coping strategies would correlate with the intensity of NK cell redistribution following a situation of experimentally induced psychosocial stress. We expected ED patients and healthy controls (HC) to display differentiated patterns of immune response and specific correlations between coping strategies and NK cell redistribution.

2 | METHODS

2.1 | Participants

The study was performed on three groups: (a) women with restrictive AN (ANR) and no history of BN (AN group); (b) normal weight women with BN and no history of AN (BN group); and (c) normal-weight healthy women (HC group).

Fifty-three female Caucasian patients sequentially seeking treatment during a calendar year at our university network ED unit (January to December 2017) were recruited using the selection criteria described below. Twenty-four patients met the inclusion criteria for the AN group and 29 patients met the criteria for the BN group. For the HC group, 58 Caucasian women were recruited from the students attending a university course on ED and young professionals undergoing training in the ED. All participants (patients and controls) were free of psychotropic medication for at least 15 days before assessment and were not taking hormonal contraceptives.

Selection criteria for the patients in the AN group were as follows: (a) meet DSM-5 diagnostic criteria for AN, restricting type; (b) no history of BN; (c) age below 30; and (d) consent to enter the study.

Selection criteria for the patients in the BN group were as follows: (a) meet DSM-5 diagnostic criteria for BN; (b) no history of AN; (c) age below 30; (d) to have a body mass index (BMI) within the normal range of 20.0–24.9 kg/m^2; and (e) consent to participate in the study.

Inclusion criteria in the HC group were as follows: (a) no personal history of ED or other psychiatric illnesses; (b) BMI within the normal range 20–24.9 kg/m^2; (c) age below 30; and (d) consent to participate in the study.

Exclusion criteria for all candidates were: (a) medication or drug use in the previous 2 weeks; (b) use of contraceptive pills in the last 8 weeks; (c) history of head injury, neurological disease, or immune system-related disease; and (d) history of childhood trauma.

A female clinical psychologist, a member of the ED unit, discussed the study with potential participants, and those who met the screening criteria were approached to participate in the assessment phase through face-to-face interviews. All clinical scales used in the study had Spanish validated versions.

Past and/or present ED were confirmed using the interview for the diagnosis of ED (Kutlesic et al., 1998), with their criteria adapted to DSM-5. The childhood trauma questionnaire-short form (CTQ-SF; Bernstein et al., 2003) was used to assess childhood trauma history. The Spanish CTQ-SF consists of five factors: emotional abuse, physical abuse, sexual abuse, physical neglect, and emotional neglect.

The University Hospital Institutional Review Board and the University of Extremadura Ethics Committee approved all research procedures. The study was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants signed informed consent forms. Participants did not receive financial compensation to take part in the study. Seven patients were excluded from the study (two AN and five BN). In five of these cases, suspicion/evidence of childhood trauma was the reason. Two patients (one with AN and one with BN) refused to participate in the study. Differences in age and eating pathology of excluded individuals by comparison with the subjects included in the study were not found.

Information about age and BMI of the participants is shown in Table 1. As can be observed, significant differences between groups with regard to age did not exist. As a direct consequence of the design of the study, significant differences in BMI between groups did exist.

TABLE 1 Characteristics of the sample

	AN patients* (*N* = 24)	BN patients* (*N* = 29)	Controls* (*N* = 58)	*F***	*p****
Age (years)	22.8 (3.9)	21.8 (3.3)	21.0 (3.2)	2.58	.08
Body mass index (kg/m^2)	16.8 (1.0)	22.8 (1.4)	21.7 (1.3)	168.98	<.001[a,b,c]
EAT-40 score	59.3 (24.1)	51.4 (14.9)	8.0 (2.9)	122.03	<.001[b,c]
BITE total score	18.3 (10.2)	36.4 (9.6)	2.9 (2.4)	159.99	<.001[a,b,c]
BDI score	27.1 (8.5)	23.1 (9.3)	3.3 (4.1)	140.01	<.001[b,c]
IBS score	38.5 (12.0)	44.5 (18.7)	30.3 (4.8)	11.45	<.001[b,c]
STAI-S score	26.8 (12.3)	28.1 (9.0)	14.0 (6.0)	30.00	<.001[b,c]
STAI-T score	36.1 (12.0)	37.7 (10.5)	16.2 (5.9)	76.01	<.001[b,c]

[a]AN versus BN.
[b]AN versus controls.
[c]BN versus controls.
Abbreviations: BDI, beck depression inventory; BITE, bulimia investigatory test Edinburgh; EAT-40, eating disorders inventory-40 items; IBS, impulsive behavior scale; STAI-S, state-trait anxiety scale-state score; STAI-T, state-trait anxiety scale-trait score.
*Mean (*SD*); **$F_{2,108}$ in all cases; ***Post hoc (Scheffe).

2.2 | Psychopathological assessment

To assess the severity of eating pathology, the Eating Attitudes Test-40 items (EAT-40) (Garner & Garfinkel, 1979), and the Bulimia Investigation Test Edinburgh (BITE) (Henderson & Freeman, 1987), were used. Depressive symptom severity was assessed using the Beck Depression Inventory (BDI; Beck & Steer, 1993). Impulsivity assessment was performed using the Impulsive Behavior Scale-Revised (IBS-R) (Rossotto, Yager, & Rorty, 1998). In the time immediately preceding the Trier Social Stress Test (TSST) (Section 2.5), participants completed the State–Trait Anxiety Inventory (STAI) (Spielberger, Gorssuch, Lushene, Vagg, & Jacobs, 1983).

2.3 | Assessment of coping strategies

For identification of coping strategies, the 60-item COPE Inventory (Carver, Scheier, & Weintraub, 1989) was used. The COPE Inventory provides 15 dimensions that identify the ways subjects deal with stressors. The dimensions are clearly defined and can be regarded as "positive" or "negative," "problem-focused" or "emotion-focused," "confrontational" or "avoidant," "social-supported" or "self-sufficient," and so forth. The Spanish version of the COPE Inventory by Crespo and Cruzado (1997) was selected for the present study.

2.4 | Collection of biological samples and laboratory methods

After psychopathological assessment, biological samples were obtained from each subject. The process for obtaining and analyzing the samples was as follows.

On the first day, the participants came to the blood drawing room for venepuncture at 8:00 a.m., after 12 hr of fasting. After placement of a winged steel needle into the vein, the participants rested for 30 min. A 7-ml blood sample was then obtained. Quantification of lymphocyte subpopulations was performed by immunophenotyping and flow cytometric analysis. Immunofluorescence staining was performed using a standardized, lysed, no-wash whole-blood staining technique. The expression of NK-cell antigens ($CD56^+CD16^+CD3^-$) was determined. Flow-count fluorospheres were used to directly determine absolute counts. The same process was repeated at 8:00 a.m. on the third day of the study, 21 hr after the TSST. A member of the research team monitored the potential interference of recent undesirable events by means of a telephone interview with the subjects on the days of the first and the second blood collection. No serious negative events were reported by any of the participants.

2.5 | Social stress induction

On the second day of the study, at 10 a.m., the participants were exposed to the TSST in its traditional form of public speaking and mental arithmetic. The procedure has been described in detail elsewhere (Allen et al., 2017; Birkett, 2011; Kirschbaum, Pirke, & Hellhammer, 1993). In all the subjects with menstrual activity, the assessment was scheduled to be held during the follicular phase. The professionals who participated in the TSST were blind to the group to whom the participants belonged. As stated above, the interference of unwanted stressful experiences during the 3-days of the study was controlled.

2.6 | Statistical analysis

The three groups were compared for age and BMI using one-way analysis of variance (ANOVA), with Scheffé post hoc comparisons. The same procedure was used to compare the scores from the clinical scales (EAT-40, BITE, BDI, IBS, and STAI) as well as the COPE

dimensions. Spearman's correlation coefficients were used to analyze the association between the scores from the clinical scales, the baseline counts of lymphocyte and NK cells, and the percentage change of lymphocyte and NK cells after the TSST. Bonferroni's correction was applied to compensate for errors due to multiple statistical comparisons. As a result, the significance level was set at $p < .007$ when the clinical items were analyzed and at $p < .0034$ in the case of COPE dimensions.

Repeated-measures ANOVA with Greenhouse–Geisser correction was used to analyze the changes over time in total lymphocyte and NK cell counts. One intra-subjects factor (Time) comprising two variables (cell counts before and after the TSST) was considered as well as one between-subjects factor (Group) comprising three categories: (a) AN group, (b) BN group, and (c) Control group.

In order to correlate the coping strategies with the changes in NK cell distribution, a multiple regression study (stepwise, forward) was performed in each group, using as the dependent variable the percentage change in NK cell counts [([Post TSST−Basal]/Basal) × 100] and as independent variables the dimensions of the COPE Inventory. To control for the potential influence of weight status, depression, anxiety, and impulsivity, these variables were included as covariates in the equations. The IBM SPSS 22.0 statistical package was used for statistical analysis in all cases.

3 | RESULTS

Table 1 displays the clinical characteristics of the sample. As can be observed, there were significant differences between groups for all items (excepting age). Except for the BITE score, there were no differences between AN and BN patients at this level.

When the association between the scores from the clinical scales and the number and percentage of lymphocytes and NK cells before and after the TSST were analyzed, weak results above the corrected level of statistical significance were obtained.

Table 2 contains the data related to the COPE dimensions. Significant differences, basically between patients and HC, were found for 9 of the 15 items.

The counts of lymphocyte and NK cells before and after TSST are shown in Table 3. It can be observed that after TSST, the counts of total circulating lymphocytes were below the basal values in the three groups. The drop in the counts was greater in BN patients (−21.3%) than in AN patients and HC, which showed similar values (−8 and −8.6%, respectively). The ANOVA for the repeated-measures factorial study revealed a significant effect of Time ($F_{1,108} = 50.30$; $p < .001$; $\eta^2_p = .32$) and Time × Group interaction ($F_{2,108} = 5.24$; $p < .007$; $\eta^2_p = .09$).

Counts of NK cells in peripheral blood 1 day after the TSST showed discordant results for the three groups. In the AN group, the number of NK cells was elevated by 18.6%, whereas in BN patients and the controls the counts dropped by 34 and 9%, respectively. The ANOVA for the repeated-measures study revealed a significant effect of Group ($F_{1,108} = 4.32$; $p < .016$; $\eta^2_p = .07$) and a significant Time × Group interaction ($F_{2,108} = 4.1$; $p < .02$; $\eta^2_p = .07$).

Table 4 shows the results of the multiple regression study. As can be observed, analysis of the BN group yielded a strong regression model ($F_{1,27} = 13.93$; $p < .001$) based on two predictors: "Substance use" and "Planning." For the AN group, analysis provided a model with one significant predictor, "Behavioral disengagement," though the

TABLE 2 COPE dimensions

	AN patients* (*N* = 24)	BN patients* (*N* = 29)	Controls* (*N* = 58)	*F***	*p*
Active coping	9.0 (3.4)	8.5 (1.2)	10.4 (1.4)	10.46	<.001[b,c]
Planning	8.9 (3.0)	9.8 (2.3)	11.1 (1.5)	2.27	0.11
Suppression of competing activities	9.5 (2.3)	10.3 (1.2)	10.1 (1.0)	10.92	<.001[b,c]
Restraint-coping	9.0 (2.9)	8.4 (1.7)	9.4 (1.1)	3.30	.04 [b,c]
Use of instrumental social support	10.7 (3.7)	9.4 (2.3)	12.1 (2.1)	11.65	<.001[c]
Positive reinterpretation and growth	10.5 (3.4)	10.1 (2.4)	12.3 (2.1)	9.34	<.001[b,c]
Acceptance	9.3 (3.2)	9.8 (2.1)	11.4 (1.5)	9.73	<.001[b,c]
Denial	6.9 (2.4)	7.7 (3.0)	6.2 (1.8)	4.18	.02[c]
Religious coping	6.3 (2.9)	5.3 (1.9)	7.9 (3.1)	9.64	<.001[b,c]
Use of emotional social support	10.3 (3.7)	9.6 (2.5)	13.5 (1.6)	31.38	<.001[b,c]
Focus on and venting of emotions	9.7 (3.1)	10.9 (2.1)	10.8 (2.5)	1.77	.18
Behavioral disengagement	8.3 (2.5)	8.6 (2.8)	6.7 (1.5)	9.09	<.001[b,c]
Mental disengagement	9.2 (2.2)	9.6 (1.6)	8.5 (1.5)	4.23	.02[c]
Substance use	4.0 (0.3)	5.4 (2.8)	4.7 (1.0)	5.36	<.01[a]
Humor	6.5 (2.9)	6.3 (1.8)	8.9 (2.7)	13.81	<.001[b,c]

[a]Post hoc (Scheffe): AN versus BN.
[b]Post hoc (Scheffe): AN versus controls.
[c]Post hoc (Scheffe): BN versus controls.
*Mean (*SD*); **$F_{2,108}$ in all cases.

TABLE 3 Immune cell counts before and after the TSST

	AN patients* (N=24)		BN patients* (N=29)		Controls* (N=58)	
	Count*	Range	Count*	Range	Count*	Range
Lymphocytes						
Pre TSST	2264 (553)	1296–3519	2388 (546)	1597–3563	2446 (650)	1379–4505
Post TSST	2084 (539)	1197–3103	1879 (485)	1214–2839	2235 (612)	1236–4273
NK cells						
Pre TSST	167 (61)	61–265	206 (87)	78–370	247 (122)	52–669
Post TSST	216 (145)	58–653	162 (103)	48–386	238 (127)	69–573

*Mean (*SD*).

TABLE 4 Predictors of change in NK cell count following the TSST

Group	Predictor	B	IC 95%	*p*
AN patients	Behavioral disengagement	−7.1	−13.4 to −0.9	.027
BN patients	Substance use	−18.6	−26.3 to −11.0	<.001
	Planning	16.4	7.1–25.7	.001
Controls	Use of emotional social support	9.2	0.7–17.7	.034

statistical significance was weak ($F_{1,22}$ = 5.61; p < .027). For HC, a model based on one variable ("Use of emotional social support") with modest significance was found ($F_{1,56}$ = 4.71; p = .03). Inclusion of BMI and the scores on the clinical scales as covariates did not induce variations in the achieved results in any case.

4 | DISCUSSION

Coping strategies seem to be able to modulate the impact of psychosocial stress on immune system activity (Koolhaas et al., 1999; Koolhaas, de Boer, Buwalda, & van Reenen, 2007). To our knowledge, this is the first study performed on humans (who were also ED patients) that analyzed the influence of specific and complex coping strategies on immune cell mobilization following psychosocial stress. Our study essentially confirms the results of animal research, revealing the existence of significant correlations between coping strategies and immune cell trafficking. It also shows some specific characteristics of immune cell responses to psychosocial stress in ED patients.

Hormones released by the HPA and SAM axes in response to acute stress influence blood vessel stiffness and adhesion, allowing the trafficking of immune cells from the vasculature to specific tissues and vice versa. Cell migration explains the changes observed in cell counts over time. In the case of lymphocytes, the passage of the cells from the tissues into the blood in the first phase of stress response results in an initial increase of the peripheral counts, which is followed by a decrease that indicates trafficking of the cells out of the blood and back into the tissues (Dhabhar et al., 2012). In accordance with this pattern of response, a moderate decrease in the number of lymphocytes was detected in our HC group. Our ED patients also responded in a similar way, but with specific patterns of response: While in AN patients the decrease in the number of lymphocytes was similar to that in the HC, the process was three to four times more intense in the BN patients. We consider this discordance perhaps to be due to the existence of differences in the reactivity to stress of the HPA and SAM axes in AN and BN (Garaci et al., 2019; A. M. Monteleone et al., 2015, 2017; P. Monteleone et al., 2011, 2012, 2014; Nagata, Yamada, Iketani, & Kiriike, 2006; Paszynska et al., 2016; Pirke et al., 1992; Vaz-Leal, Rodríguez-Santos, García-Herráiz, & Ramos-Fuentes, 2011; Vaz-Leal et al., 2007, 2010; Vaz-Leal, Rodríguez-Santos, García-Herráiz, Monge-Bautista, & López-Vinuesa, 2011). In a recent study, we reported that AN-restricting type patients display a pattern of stress-induced cortisol release similar to that of healthy individuals, whereas a blunted cortisol response is observed in BN patients (Vaz-Leal et al., 2018). As cell migration is triggered by cortisol and other stress-related hormones, these results could support the idea that bulimic symptoms can induce specific disturbances in neurobiological stress response.

In relation to the pattern of stress-mediated redistribution of NK cells, our three groups differed from all others: HC showed a decrease in relation to the above baseline situation of about 9%; BN patients displayed a strong decrease in counts (more than 30%); and AN patients had a no less intense pattern of cell mobilization, although in the opposite direction, with an increase of almost 20%. We consider that the apparent "proliferation" of NK cells observed in our AN patients should be addressed in the context of other specific "paradoxical" responses to immune challenges observed in AN, which can result in symptoms sometimes difficult to explain, such as asymptomatic or paucisymptomatic responses to infections described some years ago (Birmingham et al., 2003; Garaci et al., 2019; Gupta & Sivakumar, 1994). These manifestations could be mediated by malnutrition or single nutritional deficiencies and, again, by specific

disturbances in the activity of the HPA and SAM axes (Lambert et al., 1997; Slotwinska & Slotwinski, 2017).

With regard to the main topic of our study, the results reveal the existence of significant correlations between coping style and cell mobilization, both positive and negative. As the results from the AN group and the HC group, albeit significant, have little statistical power and need further confirmation, we will focus on the results for the BN group. As already stated, we found in our BN group a strong negative correlation between cell mobilization and substance use, as well as a positive correlation with planning. This means that those of our patients who used alcohol/substances for dealing with interpersonal stress had a weaker immune response than those who did not. This is in agreement with previous studies performed by our group, which examined the clinical and neurobiological impact of alcohol and other substances on BN (Vaz-Leal et al., 2007, 2015). In contrast with this feature, the capability of the patients to think about ways and strategies to solve problems (planning) seems to be associated with a better immune response. Overall, these results agree with those of many experimental studies developed in the context of animal research, which have shown that animals with passive-reactive coping styles have lower and weaker immune reactivity than those with a proactive style (Azpiroz et al., 2008; Koolhaas et al., 2010; Vargas et al., 2018; Zozulya, Gabaeva, Sokolov, Surkina, & Kost, 2008).

We note that our study has several limitations. First, our study does not provide a mechanistic explanation of how the immune system is involved in specific behaviors. It should be borne in mind that the aim of our study was not to address the issue of the relationship between ED and immune changes, which according to what we currently know are bidirectional (Gibson & Mehler, 2019; Hedman et al., 2019) and probably based on shared mechanisms (Duncan et al., 2017; Raevuori et al., 2014; Wotton, James, & Goldacre, 2016; Zerwas et al., 2017). It is clear that starvation and nutritional deficiencies may result in immune deficiencies, but it is equally evident that the role of inflammation in the etiopathogenesis of ED has received increasing confirmation every day (Gibson & Mehler, 2019). In this context, our study aimed only to analyze some of the modifications that psychosocial stress would induce in the functioning of the immune system of our probands in order to verify whether something that had been described in animals (i.e., how stress is handled can influence final response) occurred in humans. We have replicated in some ways the methodology of the animal studies, adapting it to the requirements of clinical human research. Second, the size of our sample might seem small, but it is similar to and even higher than those of analogous studies and reflects the characteristics of the context where the study was developed and the need to control seasonality and other temporal factors. Finally, the choice of dimensional psychopathological assessment tools may be considered a further limitation, but was conditioned by the methodology of the study.

Contrariwise, we consider our study to have a number of strengths. In many neurobiological studies of ED, patients with AN and BN are classified as a single group or are assigned to different groups but with no consideration of their clinical antecedents. We know that patients very often "move" along the spectrum of ED over time, retaining traits of their previous conditions, such that an uncontrolled history of AN or BN can be considered a distorting factor (Vaz-Leal, Rodríguez-Santos, García-Herráiz, Monge-Bautista, & López-Vinuesa, 2011). Thus, one of the strengths of our study is that three well-defined and clinically homogeneous groups of young women were used, and this strict selection probably contributed to controlling some conflicting biases, as some neuroendocrinological mechanisms can depend on age and sex. Finally, we excluded from the study those individuals who had a history of childhood trauma, as we know that people exposed to traumatic childhood experiences tend to present a distorted neurobiological reactivity to stress in adulthood (A. M. Monteleone et al., 2019; Smiarowska et al., 2014; Steiger et al., 2001).

5 | CONCLUSION

ED is a life-threatening clinical condition. Patients are frequently exposed to serious clinical complications, many of which are affected by stress. It is known that stress has a negative influence on health: Unwanted events are related to changes in immune activity, which may increase susceptibility to immune diseases or other disorders as well as tumor progression. Immune cell redistribution is a mechanism that promotes immuno-enhancement of specific compartments and is related to the effectivity of the immune response. As suggested by the data, the response to social stress could be modulated by the coping strategies used by the subjects, so that modification of these strategies could strengthen patients' resilience, maintaining the functionality of their immune status and their ability to confront the disease. In our opinion, this is a powerful reason to continue research in this field.

ACKNOWLEDGMENTS

Supported by the European Regional Development Fund—FEDER—and the regional Government of Extremadura—Junta de Extremadura—(GR18136).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Francisco J. Vaz-Leal https://orcid.org/0000-0002-4872-3924

REFERENCES

Allen, A. P., Kennedy, P. J., Dockray, S., Cryan, J. F., Dinan, T. G., & Clarke, G. (2017). The Trier Social Stress Test: Principles and practice. *Neurobiology of Stress*, *6*, 113–126. https://doi.org/10.1016/j.ynstr.2016.11.001

Azpiroz, A., De Miguel, Z., Fano, E., & Vegas, O. (2008). Relations between different coping strategies for social stress, tumor development and neuroendocrine and immune activity in male mice. *Brain, Behavior, and Immunity*, *22*, 690–698. https://doi.org/10.1016/j.bbi.2007.10.007

Bartolomucci, A. (2007). Social stress, immune functions and disease in rodents. *Frontiers in Neuroendocrinology*, *28*, 28–49. https://doi.org/10.1016/j.yfrne.2007.02.001

Beck, A. T., & Steer, R. A. (1993). *Beck depression inventory. Manual*. San Antonio, TX: The Psychological Corporation.

Bernstein, D. P., Stein, J. A., Newcomb, M. D., Walker, E., Pogge, D., Ahluvalia, T., … Zule, W. (2003). Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse & Neglect*, *27*, 169–190. https://doi.org/10.1016/s0145-2134(02)00541-0

Birkett, M. A. (2011). The Trier social stress test protocol for inducing psychological stress. *Journal of Visualized Experiments*, *56*, 3228. https://doi.org/10.3791/3238

Birmingham, C. L., Hodgson, D. M., Fung, J., Brown, R., Wakefield, A., Bartrop, R., & Beumont, P. (2003). Reduced febrile response to bacterial infection in anorexia nervosa patients. *The International Journal of Eating Disorders*, *34*, 269–272. https://doi.org/10.1002/eat.10189

Brytek-Matera, A., & Schiltz, L. (2013). Comparative structural study of the configuration of coping strategies among female patients with eating disorders and a non-clinical control group. *Psychiatria Danubina*, *25*, 359–365.

Carver, C. S., Scheier, M. F., & Weintraub, J. K. (1989). Assessing coping strategies: A theoretically based approach. *Journal of Personality and Social Psychology*, *56*, 267–283.

Crespo, M., & Cruzado, J. (1997). La evaluación del afrontamiento: adaptación española del cuestionario COPE con una muestra de estudiantes universitarios. *Anal Modif Conducta*, *23*, 797–830.

De Miguel, Z., Vegas, O., Garmendia, L., Arregi, A., Beitia, G., & Azpiroz, A. (2011). Behavioral coping strategies in response to social stress are associated with distinct neuroendocrine, monoaminergic and immune response profiles in mice. *Behavioural Brain Research*, *225*, 554–561. https://doi.org/10.1016/j.bbr.2011.08.011

Dennard, E. E., & Richards, C. S. (2013). Depression and coping in subthreshold eating disorders. *Eating Behaviors*, *14*, 325–329. https://doi.org/10.1016/j.eatbeh.2013.05.011

Dhabhar, F. S., Malarkey, W. B., Neri, E., & McEwen, B. S. (2012). Stress-induced redistribution of immune cells–From barracks to boulevards to battlefields: A tale of three hormones. *Psychoneuroendocrinology*, *37*, 1345–1368. https://doi.org/10.1016/j.psyneuen.2012.05.008

Duncan, L., Yilmaz, Z., Gaspar, H., Walters, R., Goldstein, J., Anttila, V., … Bulik, C. M. (2017). Significant locus and metabolic genetic correlations revealed in genome-wide association study of anorexia nervosa. *The American Journal of Psychiatry*, *174*, 850–858. https://doi.org/10.1176/appi.ajp.2017.16121402

Folkman, S., & Lazarus, R. S. (1985). If it changes it must be a process: Study of emotion and coping during three stages of a college examination. *Journal of Personality and Social Psychology*, *48*, 150–170.

Garaci, F. G., Vasili, E., Bocchinfuso, F., Lacche, A., Nardone, V., Di Donna, C., … Floris, R. (2019). Paucisymptomatic pulmonary and right ear tuberculosis in young woman suffering from anorexia and bulimia nervosa. *Radiology Case Reports*, *14*, 423–426. https://doi.org/10.1016/j.radcr.2018.10.019

Garner, D. M., & Garfinkel, P. E. (1979). The Eating Attitudes Test: An index of the symptoms of anorexia nervosa. *Psychological Medicine*, *9*, 273–279.

Ghaderi, A., & Scott, B. (2000). Coping in dieting and eating disorders: A population-based study. *The Journal of Nervous and Mental Disease*, *188*, 273–279. https://doi.org/10.1097/00005053-200005000-00004

Gibson, D., & Mehler, P. S. (2019). Anorexia nervosa and the immune system: A narrative review. *Journal of Clinical Medicine*, *8*(11), 1915. https://doi.org/10.3390/jcm8111915

Gupta, K., & Sivakumar, K. (1994). Anorexia nervosa and tuberculosis: Case reports and a review of immune mechanisms. *The International Journal of Eating Disorders*, *15*, 301–304.

Hedman, A., Breithaupt, L., Hubel, C., Thornton, L. M., Tillander, A., Norring, C., … Bulik, C. M. (2019). Bidirectional relationship between eating disorders and autoimmune diseases. *Journal of Child Psychology and Psychiatry*, *60*, 803–812. https://doi.org/10.1111/jcpp.12958

Henderson, M., & Freeman, C. P. L. (1987). A self-rating scale for bulimia: The "BITE". *The British Journal of Psychiatry*, *150*, 18–24.

Hernando, A., Pallas, R., Cebolla, A., García-Campayo, J., Hoogendoorn, C. J., & Roy, J. F. (2019). Mindfulness, rumination, and coping skills in young women with eating disorders: A comparative study with healthy controls. *PLoS One*, *14*, e0213985. https://doi.org/10.1371/journal.pone.0213985

Het, S., Vocks, S., Wolf, J. M., Hammelstein, P., Herpertz, S., & Wolf, O. T. (2015). Blunted neuroendocrine stress reactivity in young women with eating disorders. *Journal of Psychosomatic Research*, *78*, 260–267. https://doi.org/10.1016/j.jpsychores.2014.11.001

Ince, L. M., Weber, J., & Scheiermann, C. (2018). Control of leukocyte trafficking by stress-associated hormones. *Frontiers in Immunology*, *9*, 3143. https://doi.org/10.3389/fimmu.2018.03143

Jaúregui-Lobera, I., Estébanez, S., Santiago-Fernández, M. J., Álvarez Bautista, E., & Garrido, O. (2009). Coping strategies in eating disorders. *European Eating Disorders Review*, *17*, 220–226. https://doi.org/10.1002/erv.920

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The "Trier Social Stress Test." A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*, 76–81. https://doi.org/10.1159/000119004

Koolhaas, J. M. (2008). Coping style and immunity in animals: Making sense of individual variation. *Brain, Behavior, and Immunity*, *22*, 662–667. https://doi.org/10.1016/j.bbi.2007.11.006

Koolhaas, J. M., de Boer, S. F., Buwalda, B., & van Reenen, K. (2007). Individual variation in coping with stress: A multidimensional approach of ultimate and proximate mechanisms. *Brain, Behavior and Evolution*, *70*, 218–226. https://doi.org/10.1159/000105485

Koolhaas, J. M., de Boer, S. F., Coppens, C. M., & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology*, *31*, 307–321. https://doi.org/10.1016/j.yfrne.2010.04.001

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., … Blokhuis, H. J. (1999). Coping styles in animals: Current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Reviews*, *23*, 925–935.

Kutlesic, V., Williamson, D. A., Donald, A., Gleaves, D. H., Barbin, J. M., & Murphy-Eberenz, K. P. (1998). The Interview for the Diagnosis of Eating Disorders-IV: Application to DSM-IV diagnostic criteria. *Psychological Assessment*, *10*, 41–48.

Lambert, M., Hubert, C., Depresseux, G., Vande Berg, B., Thissen, J. P., Nagant de Deuxchaisnes, C., & Devogelaer, J. P. (1997). Hematological changes in anorexia nervosa are correlated with total body fat mass depletion. *The International Journal of Eating Disorders*, *21*, 329–334.

Lazarus, R. S., DeLongis, A., Folkman, S., & Gruen, R. (1985). Stress and adaptational outcomes: The problem of confounded measures. *The American Psychologist*, *40*, 770–785.

Mandal, A., & Viswanathan, C. (2015). Natural killer cells: In health and disease. *Hematology/Oncology and Stem Cell Therapy*, *8*, 47–55. https://doi.org/10.1016/j.hemonc.2014.11.006

Monteleone, A. M., Monteleone, P., Marciello, F., Pellegrino, F., Castellini, G., & Maj, M. (2017). Differences in cortisol awakening response between binge-purging and restrictive patients with anorexia nervosa. *European Eating Disorders Review*, *25*, 13–18. https://doi.org/10.1002/erv.2485

Monteleone, A. M., Monteleone, P., Serino, I., Scognamiglio, P., Di Genio, M., & Maj, M. (2015). Childhood trauma and cortisol awakening response in symptomatic patients with anorexia nervosa and bulimia nervosa. *The International Journal of Eating Disorders*, *48*, 615–621. https://doi.org/10.1002/eat.22375

Monteleone, A. M., Ruzzi, V., Pellegrino, F., Patriciello, G., Cascino, G., Del Giorno, C., … Maj, M. (2019). The vulnerability to interpersonal stress in eating disorders: The role of insecure attachment in the emotional and cortisol responses to the Trier social stress test. *Psychoneuroendocrinology*, *101*, 278–285. https://doi.org/10.1016/j.psyneuen.2018.12.232

Monteleone, P., Scognamiglio, P., Canestrelli, B., Serino, I., Monteleone, A. M., & Maj, M. (2011). Asymmetry of salivary cortisol and alpha-amylase responses to psychosocial stress in anorexia nervosa but not in bulimia nervosa. *Psychological Medicine*, *41*, 1963–1969. https://doi.org/10.1017/S0033291711000092

Monteleone, P., Scognamiglio, P., Monteleone, A. M., Perillo, D., & Maj, M. (2014). Cortisol awakening response in patients with anorexia nervosa or bulimia nervosa: Relationships to sensitivity to reward and sensitivity to punishment. *Psychological Medicine*, *44*, 2653–2660. https://doi.org/10.1017/S0033291714000270

Monteleone, P., Tortorella, A., Scognamiglio, P., Serino, I., Monteleone, A. M., & Maj, M. (2012). The acute salivary ghrelin response to a psychosocial stress is enhanced in symptomatic patients with bulimia nervosa: A pilot study. *Neuropsychobiology*, *66*, 230–236. https://doi.org/10.1159/000341877

Nagata, T., Matsuyama, M., Kiriike, N., Iketani, T., & Oshima, J. (2000). Stress coping strategy in Japanese patients with eating disorders: Relationship with bulimic and impulsive behaviors. *The Journal of Nervous and Mental Disease*, *188*, 280–286. https://doi.org/10.1097/00005053-200005000-00005

Nagata, T., Yamada, H., Iketani, T., & Kiriike, N. (2006). Relationship between plasma concentrations of cytokines, ratio of CD4 and CD8, lymphocyte proliferative responses, and depressive and anxiety state in bulimia nervosa. *Journal of Psychosomatic Research*, *60*, 99–103. https://doi.org/10.1016/j.jpsychores.2005.06.058

Paszynska, E., Dmitrzak-Weglarz, M., Tyszkiewicz-Nwafor, M., & Slopien, A. (2016). Salivary alpha-amylase, secretory IgA and free cortisol as neurobiological components of the stress response in the acute phase of anorexia nervosa. *The World Journal of Biological Psychiatry*, *17*, 266–273. https://doi.org/10.3109/15622975.2016.1163419

Peng, H., & Tian, Z. (2014). NK cell trafficking in health and autoimmunity: A comprehensive review. *Clinical Reviews in Allergy and Immunology*, *47*, 119–127. https://doi.org/10.1007/s12016-013-8400-0

Pirke, K. M., Platte, P., Laessle, R., Seidl, M., & Fichter, M. M. (1992). The effect of a mental challenge test of plasma norepinephrine and cortisol in bulimia nervosa and in controls. *Biological Psychiatry*, *32*, 202–206.

Raevuori, A., Haukka, J., Vaarala, O., Suvisaari, J. M., Gissler, M., Grainger, M., … Suokas, J. T. (2014). The increased risk for autoimmune diseases in patients with eating disorders. *PLoS One*, *9*, e104845. https://doi.org/10.1371/journal.pone.0104845

Reimert, I., Rodenburg, T. B., Ursinus, W. W., Kemp, B., & Bolhuis, J. E. (2014). Selection based on indirect genetic effects for growth, environmental enrichment and coping style affect the immune status of pigs. *PLoS One*, *9*, e108700. https://doi.org/10.1371/journal.pone.0108700

Rossotto, E., Yager, J., & Rorty, M. (1998). The impulsive behavior scale. In J. Vanderlinden & W. Vandereycken (Eds.), *Trauma, dissociation, and impulse dyscontrol in eating disorders*. Philadelphia, PA: Brunner/Mazel.

Slotwinska, S. M., & Slotwinski, R. (2017). Immune disorders in anorexia. *Central European Journal of Immunology*, *42*, 294–300. https://doi.org/10.5114/ceji.2017.70973

Smiarowska, M., Safranow, K., Dziedziejko, V., Bialecka, M., Koziolek, M., & Samochowiec, J. (2014). Association of plasma hormones, nutritional status, and stressful life events in anorexia nervosa patients. *Postępy Higieny i Medycyny Doświadczalnej (Online)*, *68*, 162–171.

Spielberger, C. D., Gorssuch, R. L., Lushene, P. R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Steiger, H., Gauvin, L., Israel, M., Koerner, N., Ng Ying Kin, N. M., Paris, J., & Young, S. N. (2001). Association of serotonin and cortisol indices with childhood abuse in bulimia nervosa. *Archives of General Psychiatry*, *58*, 837–843.

Tobin, D. L., & Griffing, A. S. (1995). Coping and depression in bulimia nervosa. *The International Journal of Eating Disorders*, *18*, 359–363.

Troop, N. A., Holbrey, A., & Treasure, J. L. (1998). Stress, coping, and crisis support in eating disorders. *The International Journal of Eating Disorders*, *24*, 157–166.

Vargas, R., Balasch, J. C., Brandts, I., Reyes-Lopez, F., Tort, L., & Teles, M. (2018). Variations in the immune and metabolic response of proactive and reactive *Sparus aurata* under stimulation with vibrio anguillarum vaccine. *Scientific Reports*, *8*, 17352. https://doi.org/10.1038/s41598-018-35863-w

Vaz-Leal, F. J., Ramos-Fuentes, M. I., Rodríguez-Santos, L., Chimpén-López, C., Fernández-Sánchez, N., Zamora-Rodríguez, F. J., … Guisado-Macías, J. A. (2018). Blunted cortisol response to stress in patients with eating disorders: Its association to bulimic features. *European Eating Disorders Review*, *26*, 207–216. https://doi.org/10.1002/erv.2581

Vaz-Leal, F. J., Ramos-Fuentes, M. I., Rodríguez-Santos, L., Flores-Mateos, I. S., Franco-Zambrano, A., Rojo-Moreno, L., & Beato-Fernández, L. (2015). Neurobiological and clinical variables associated with alcohol abuse in bulimia nervosa. *European Eating Disorders Review*, *23*, 185–192. https://doi.org/10.1002/erv.2352

Vaz-Leal, F. J., Rodríguez-Santos, L., García-Herráiz, M. A., Monge-Bautista, M., & López-Vinuesa, B. (2011). Bulimia nervosa with history of anorexia nervosa: Could the clinical subtype of anorexia have implications for clinical status and treatment response? *The International Journal of Eating Disorders*, *44*, 212–219. https://doi.org/10.1002/eat.20805

Vaz-Leal, F. J., Rodríguez-Santos, L., García-Herráiz, M. A., & Ramos-Fuentes, M. I. (2011). Neurobiological and psychopathological variables related to emotional instability: A study of their capability to discriminate patients with bulimia nervosa from healthy controls. *Neuropsychobiology*, *63*, 242–251. https://doi.org/10.1159/000323445

Vaz-Leal, F. J., Rodríguez-Santos, L., Melero, M. J., Ramos, M. I., Monge, M., & López-Vinuesa, B. (2007). Hostility and helper T-cells in patients with bulimia nervosa. *Eating and Weight Disorders*, *12*, 83–90.

Vaz-Leal, F. J., Rodríguez-Santos, L., Melero-Ruiz, M. J., Ramos-Fuentes, M. I., & García-Herráiz, M. A. (2010). Psychopathology and lymphocyte subsets in patients with bulimia nervosa. *Nutritional Neuroscience*, *13*, 109-115. https://doi.org/10.1179/147683010X12611460764129.

Vegas, O., Fano, E., Brain, P. F., Alonso, A., & Azpiroz, A. (2006). Social stress, coping strategies and tumor development in male mice: Behavioral, neuroendocrine and immunological implications. *Psychoneuroendocrinology*, *31*, 69–79. https://doi.org/10.1016/j.psyneuen.2005.05.013

Villa, V., Manzoni, G. M., Pagnini, F., Castelnuovo, G., Cesa, G. L., & Molinari, E. (2009). Do coping strategies discriminate eating disordered individuals better than eating disorder features? An explorative study on female inpatients with anorexia and bulimia nervosa. *Journal of Clinical Psychology in Medical Settings*, *16*, 297–303. https://doi.org/10.1007/s10880-009-9172-9

Wotton, C. J., James, A., & Goldacre, M. J. (2016). Coexistence of eating disorders and autoimmune diseases: Record linkage cohort study, UK. *The International Journal of Eating Disorders*, *49*, 663–672. https://doi.org/10.1002/eat.22544

Zerwas, S., Larsen, J. T., Petersen, L., Thornton, L. M., Quaranta, M., Koch, S. V., … Bulik, C. M. (2017). Eating disorders, autoimmune, and autoinflammatory disease. *Pediatrics*, *140*, e20162089. https://doi.org/10.1542/peds.2016-2089

Zozulya, A. A., Gabaeva, M. V., Sokolov, O. Y., Surkina, I. D., & Kost, N. V. (2008). Personality, coping style, and constitutional neuroimmunology. *Journal of Immunotoxicology*, *5*, 221–225. https://doi.org/10.1080/15476910802131444

How to cite this article: Vaz-Leal FJ, Ramos-Fuentes MI, Guisado-Macías JA, et al. Coping strategies and stress-induced natural killer cell redistribution in women with eating disorders. *Int J Eat Disord*. 2020;53:964–971. https://doi.org/10.1002/eat.23278