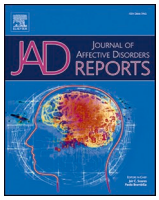
Journal of Affective Disorders Reports 15 (2024) 100699

Contents lists available at ScienceDirect

Journal of Affective Disorders Reports

journal homepage: www.sciencedirect.com/journal/journal-of-affective-disorders-reports



Research Paper

Unique contributions of anxiety, stress and depression to immunity: A cross-cultural investigation

Catherine Breeze [a], Oleg N. Medvedev [a,*], Matti Cervin [b], Anna Sutton [a], Barbara Barcaccia [c,d], Alessandro Couyoumdjian [c], Susanna Pallini [c], Moana Billot [a], Rebecca Chalmers [a], Naved Iqbal [e], Vincent Reid [a], Nirbhay N. Singh [f]

[a] University of Waikato, School of Psychology, Hamilton, New Zealand
[b] Lund University, Lund, Sweden
[c] Department of Psychology, Sapienza University of Rome, Rome, Italy
[d] Department of Education, Roma Tre University, Roma, Rome, Italy
[e] Jamia Millia Islamia Central University, Department of Psychology, New Delhi, India
[f] Augusta University, United States of America

ARTICLE INFO

Keywords:
Anxiety
Stress
Depression
Immunity
Stress-disease model
Dominance analysis

ABSTRACT

While immunity and psychological distress are strongly associated, studies seldom consider how different types of distress relate to immune functioning. The literature tends to emphasis the impact of stress on immunity. The present cross-sectional study estimated the unique contributions of depression, anxiety, and stress on immune function in culturally diverse samples of adults from Italy, New Zealand and India. Participants were Italian (n = 1061), New Zealand (n = 1037), and Indian (n = 384) volunteers. Stepwise multiple linear regression and dominance analysis were used to analyse differences in immunity uniquely explained by anxiety, depression, and stress. While samples from the three countries differed significantly, anxiety consistently explained the greatest proportion of differences in immunity. After accounting for the effect of anxiety, stress and depression explained only negligible variation in immune functioning. This association of anxiety with immune functioning was consistent across three different countries and this unique impact was further confirmed by the results of dominance analysis. These findings suggest a clear link between anxiety and immunity, which advances the prevailing stress-disease model and foster further experimental and longitudinal research into the impact of anxiety on immunity.

1. Introduction

The relationship between affective conditions and immune dysfunction has garnered significant attention in recent studies, especially considering the adverse health outcomes resulting from immune system disruptions due to stress (Andreasson et al., 2018; Momen et al., 2020; Lasselin, 2021; Seiler et al., 2020). While the general impact of negative affect on immune imbalances is understood, there remains a paucity of research focusing on specific negative affect facets, such as depression and anxiety, and their unique roles on immune functioning.

Subjective reports of infectious symptoms, such as fever and cough, serve as pivotal indices in immune functioning and play a crucial role in the preliminary diagnosis of infections like flu or COVID-19 (Alghamdi et al., 2021; Libet, 2004). These patient-centred reports, though cost-effective, sometimes yield inconsistent results when mapped with inflammatory biomarkers, stressing the complexity of the immune system and the challenges of having clear clinical thresholds for these biomarkers (O'Connor et al., 2021; Lodin et al., 2017). Depression, anxiety, and stress, while often coexisting, manifest distinct patterns and impacts on immune function (Barlow et al., 2014; Eysenck and Fajkowska, 2018; Mariani et al., 2021; Momen et al., 2020). While depression's association with immune deregulation is well-documented, the nuances of anxiety's relationship with immunity require more comprehensive research (Dantzer et al., 2008; Culpepper, 2009; Renna et al., 2018).

The comorbidity of depression, anxiety, and stress is evident, with shared symptoms like repetitive negative thinking. However, their unique emotional and cognitive domains highlight the need to

* Corresponding author at: University of Waikato, School of Psychology, Gate 1, Knighton Road, Hamilton 3240, New Zealand.
E-mail addresses: oleg.medvedev@waikato.ac.nz (O.N. Medvedev), vincent.reid@waikato.ac.nz (V. Reid).

https://doi.org/10.1016/j.jadr.2023.100699
Received 25 August 2023; Received in revised form 16 November 2023; Accepted 2 December 2023
Available online 9 December 2023

understand them as separate entities (Spinhoven et al., 2018; Eysenck and Fajkowska, 2018). Various models, such as Clark and Watson's tripartite model, have sought to explain their interrelationships, emphasizing their distinct conditions and the importance of analyzing their individual impacts on immune functioning (Clark and Watson, 1991; Lasselin, 2021).

Given the variability of affective responses across populations and the cultural nuances in expressing negative affect, this study undertakes a comparison among Italy, India, and New Zealand (NZ), leveraging their diverse cultural backgrounds to provide richer insights. In response to the existing literature, our research seeks to delineate the unique contributions of depression, anxiety, and stress to immune status by examining a cross-country non-clinical sample from these three countries.

2. Method

2.1. Participants

The total sample (n = 2482) was composed of Italian university students and general population, NZ general population and Indian university students above the age of 18. The Italian sample was composed of 97.64 % of people who identified as European, Asiatic (0.26 %), African (0.56 %), American (0.56 %), with the remainder (0.94 %) made up of other ethnicities not specified. The NZ sample was composed of NZ European (64.6 %), Māori (14.8), Pacific nations (3.9 %), Asian (12.6 %) and (4.1 %) of other ethnicities not specified. All India student participants identified as Indian.

2.2. Procedure

The study was approved by the ALPSS Divisional Human Research Ethics Committee of the University of Waikato (FS2021–58), New Zealand, which is following the internationally recognised ethical standards consistent with APA 7 edition. Written informed consent was obtained from all participants prior to completing the questionnaire. Participation was anonymous, and participants gave their consent after reading information about the study. Data collection took place through an online survey and no time limit was imposed. Participants were recruited by a combination of techniques. The Italian students were recruited in October 2022 through a convenient sample technique. Further participants were recruited through outreach by snowballing techniques. All Italian participants accessed the survey through the university based Qualtrics website. Dynata distributed the NZ survey online through their network in January-February 2022, and participants obtained compensation up to the value of $5 for completing the form. Further NZ participants were recruited through outreach by snowballing techniques. The Indian students were recruited in March-May 2022, through a convenient sample technique. The link of the study was shared with teachers in different universities and shared with associated classes. Moreover, the link of the study was also shared with different students' group. No compensation was provided to the Italian or Indian participants for taking part in the study because these data collection methods did not involve monetary compensation, unlike Dynata in New Zealand. To ensure the reliability and integrity of our data, we implemented specific quality control measures. Although participants were not confined to a strict time limit for survey completion, we recognized the potential risk of hastily filled-out or random responses. Using the Qualtrics software, we estimated the expected completion time for our survey to be between 10 and 30 min. Based on this estimation, we made the decision to exclude any data from participants who completed the survey in less than 10 min. This approach was adopted to filter out potential responses from participants who might have skimmed through questions or selected options without genuine contemplation, thereby ensuring the validity of the collected data.

2.3. Measures

2.3.1. Depression, anxiety and stress scales (DASS-21)

The DASS-21 is an abbreviated 21-item version of the original self-report measure that captures perceptions of emotions over the past week with strong reliability (Lovibond and Lovibond, 1993). It consists of three subscales: depression, anxiety, and stress, with reliability indices ranging from 0.83 to 0.94 across scales and countries (Lightburn et al., 2023). In our study, the scores from these subscales were used as continuous variables.

2.3.2. Immune status questionnaire (ISQ)

The ISQ is a seven-item self-report tool that gauges the frequency of infectious symptoms experienced over the past twelve months and overall health perceptions (Wilod Versprille et al., 2019). Its reliability metrics in our study were between 0.70 to 0.76 across countries, showcasing its consistent research reliability.

2.3.3. MacArthur scale of subjective social status (SSS)

The SSS is a self-assessment measure where participants indicate their perceived social standing within their community on a 10-point scale (Adler et al., 1994). It offers insights into socio-economic status nuances and has been shown to correlate well with objective measures in industrial cultures (Oswald and Wu, 2010).

2.4. Data analyses

Data analyses were undertaken using IBM SPSS Statistics (Version 27). The required sample size was estimated using G*Power software 3.1 (Faul et al., 2009) and indicated that with the maximum number of predictors limited to 10 to detect a small effect size (0.01) under the α=0.05 with a certainty of 95 % (1-β=0.95), the required sample size would be $\geq$254 participants. The dataset exceeded this figure, reflecting strong statistical power. During data preparation, we noted that less than 4 % of our dataset values were missing. Despite its low rate, we carefully evaluated methods for handling missing data, including multiple imputations, mean imputation, and model-based methods. Ultimately, we chose to exclude missing values for several reasons: the missingness was minimal and unlikely to affect our analysis's power or generalizability given our sample's size and diversity; our preliminary checks indicated the data was missing completely at random (MCAR), ensuring no systematic bias; and this direct exclusion approach provided simplicity, transparency, and ease of interpretation or replication by peers. This approach underpins the integrity and clarity of our results.

The data met the assumptions for multiple linear regression. with variables being normally distributed and with skewness and kurtosis falling within the acceptable range between +2 and – 2 (West et al., 1995). There was no multicollinearity among predictor variables, according to the criterion of Variance Inflating Factors (VIF) below 5 (Roemer et al., 2021; Podsakoff et al., 2021). For linearity, we visually inspected scatter plots of the observed versus predicted values. Additionally, we used the Partial Regression Plots for individual predictors to ensure that the relationship was linear with the outcome variable. Homoscedasticity, or the assumption of equal variance of the errors, was assessed using the residuals-versus-fits plot. We looked for any evident patterns in these plots; a random distribution of points suggested that the assumption was met. Pearson correlation coefficients were calculated between age, SSS, the ISQ and the subscales of depression, anxiety, and stress.

Stepwise multiple linear regression on the outcome variable the ISQ (immunity), combining forward and backward entry to ensure robustness was used to examine the unique contributions of depression, anxiety, and stress on immune status (Babyak, 2004). Stepwise multiple linear regression extracts and lists the strongest predictor which explains most of the variance first, using mathematical criteria (Roemer et al., 2021).

Demographic variables including sex, age and SSS were controlled for and entered together in the first block. Analysis was undertaken by country, as although socio-demographic differences were controlled for, cross-country variation was to be explored. Following this, depression, anxiety, and stress were entered using combined forward and backward stepwise entry, by country. In this method, the strongest significant predictor that explains the greatest amount of the variance is extracted first based on mathematical criteria.

Subsequent predictors, which explain the unique variance not explained by the first predictor, appear in order of their unique predictability and were extracted sequentially, while reiteratively accounting for and deducting the variance of predictors which were already extracted, until no more significant predictors remained (Roemer et al., 2021). The threshold for inclusion was $p < .05$ and for removal was $p \geq .10$. These analyses were conducted individually for the Italian, Indian and NZ samples due to significant differences between samples demographics for the countries.

To determine the unique variance explained by each of the predictors, dominance analysis was conducted (Budescu, 1993). In dominance analysis, all subsets of predictors are examined in relation to the dependent variable and the unique contribution of each predictor is estimated. Dominance analysis reduces potential distortions through multicollinearity and enables direct comparisons of predictors within a model. To conduct dominance analysis in the present study, that included three predictors, seven regression analyses were run using SPSS, containing all possible combinations of predictors. Following these analyses, the ΔR^2 values were averaged and compared to identify the variable with the highest mean incremental contribution to variance explained, using Excel (Tonidandel and LeBreton, 2011). The percentage value of relative importance of predictors is derived by dividing the overall average incremental R^2 by the contribution of average predictors to the model.

3. Results

Demographic characteristics of the sample are shown in Table 1. When age was viewed by country, the average NZ sample age showed greater variation relative to the more youthful Italian and Indian sample. A chi-square test of independence examined distribution of sex by country, and noted significant differences in two of the countries, with the Italian and Indian sample being weighted towards significantly more females compared to males ($p < .01$). Descriptive statistics are represented in Table 2, with skewness and kurtosis values being in the acceptable range. The Indian and Italian participants were significantly younger, and their indices of negative affect levels, notably stress, were significantly higher compared to the NZ sample. The NZ sample reported lower perceived SSS and lower average negative affect levels.

Table 3 presents the results of stepwise multiple linear regression predicting immunity in each sample. Within the Italian sample, sociodemographic features accounted for 11 % of the variance, with age showing the strongest impact on immunity. After accounting for the effect of demographics, anxiety was significantly associated with immunity and the strongest affective predictor and accounted for a further 14 % of the variance.

In the Indian sample, sociodemographic characteristics accounted for 3.6 % of the variation with SSS being the only significant predictor. After controlling for demographics anxiety was significantly associated with immunity and the strongest affective predictor and accounted for a further 15 % of the variance.

In the NZ sample, sociodemographic characteristics accounted for 9.3 % of the variation with SSS and age being a significant predictors. After accounting for demographics, anxiety was significantly associated with immunity and the strongest affective predictor and accounted for a further 20 % of the variance.

Table 4 shows the results of the dominance analyses; full results are available by request from the first author. Anxiety had the greatest dominance value in all samples, showing general dominance over the other affect facets and accounting for between 42 % to 50 % of the total variance explained. Stress was the second most important contributor in the NZ and Indian samples, while depression was the second most important second contributor for the Italian sample.

Table 1
Demographic Characteristics of Participants.

Country	*n*	Sample	Age *M* (*SD*)	% Females
NZ	1037	General Population	49.00 (17.63)	51.0
Italy	1061	University Students & General Population	29.99 (13.85)	78.6
India	384	University Students	23.67 (7.68)	67.0

4. Discussion

This study focused on investigating the unique contributions of depression, anxiety and stress to immune functioning within a large sample drawing participants from Italy, India and NZ. When disentangling the three constructs, anxiety consistently demonstrated the strongest negative impact associated with immune functioning. After accounting for the effects of anxiety, stress had only negligible association with immunity in the Indian and NZ samples. Depression had a modest impact on the Italian sample, indicating variation between countries. While this study found an association between stress and immunity as proposed by the stress immune model (O'Connor et al., 2021; McEwen, 2017; Morey et al., 2015), the association was notably stronger for anxiety and immunity.

The present findings indicate that associations between stress, depression and immunity in previous studies may have been partially explained by unmeasured anxiety, which tends to be strongly associated and frequently comorbid with depression and stress (Patriquin and Mathew, 2017; Renna et al., 2018). This is irrespective of whether negative affect types are differentiated between, or whether disorders are controlled for (Barlow et al., 2014; Budescu, 1993). The results suggest that when the frequently comorbid and co-varying constructs of stress and depression are controlled for, anxiety is shown to have the greatest impact on immunity. As such, an anxiety-disease model, as outlined in Fig. 1, would appear to outline the relationship more accurately between types of negative affect, immunity, and adverse health outcomes, which is supported by this study. While relatively scant attention is provided to the association of anxiety and immunity, these results suggest that anxiety is the strongest affective condition contributing to differences in immune deregulation across different countries (Culpepper, 2009; Renna et al., 2018).

There are similarities in this study's findings to those of a recent cross-sectional study in Saudi Arabia (Alghamdi et al., 2021). Those reporting mild to moderate anxiety were two to four times more likely to report reduced immune status. Anxiety and immunity also showed the strongest relationship, with a trend for severe anxiety to be associated with poorer immune functioning, in a dose dependent manner (Alghamdi et al., 2021).

The study also found significant associations with stress and immunity (Alghamdi et al., 2021). However, the variance explained by anxiety while estimating contribution of stress was not controlled for. Therefore, the association with stress might be due to shared variance between anxiety and stress. In the current study, we controlled for anxiety and only found weak associations between stress and immune functioning within the Indian and NZ samples. This highlights the importance of using appropriate methodology that allows identification of the unique effects of specific predictors, such as anxiety. The limitation of the Saudi Arabian study is related to using methodology that involved dichotomization of continuous variables, namely immune

Table 2
Means (M), 95 % confidence intervals (CI) of the samples by country and combined, also including skewness and kurtosis data for the total sample.

	Italian (n = 1061)		Indian (n = 384)		New Zealand (n = 1037)		Total sample (n = 2482)			
Variable	*M*	Confidence intervals	*M*	Confidence intervals	*M*	Confidence intervals	*M*	Confidence intervals	Skewness	Kurtosis
Age	29.99	[29.16, 30.83]	23.67	[22.84, 24.50]	49.00	[47.92, 50.07]	42.88	[41.87, 43.90]	0.778	−0.609
SSS	6.56	[6.45, 6.66]	6.52	[6.29, 6.75]	5.60	[5.49, 5.72]	5.83	[5.72, 5.93]	−0.357	0.040
Immunity	5.86	[5.70, 6.03]	6.41	[6.10, 6.72]	6.92	[6.77, 7.08]	6.80	[6.66, 6.94]	−0.659	−0.342
Depression	6.81	[6.51, 7.12]	6.99	[6.45, 7.53]	5.70	[5.36, 6.03]	6.01	[5.72, 6.30]	0.805	−0.071
Stress	9.12	[8.84, 9.40]	7.36	[6.92, 7.80]	6.01	[5.73, 6.30]	6.34	[6.09, 6.58]	0.478	−0.275
Anxiety	5.95	[5.66, 6.23]	6.81	[6.31, 7.30]	4.36	[4.09, 4.63]	4.95	[4.70, 5.19]	0.889	0.193

Note. n = sample size, M = mean.

Table 3
Summary of multiple linear stepwise regression analyses predicting immunity and the impact of affective subscales from the Italian sample (n = 1061), Indian sample (n = 384) and NZ sample (n = 1039).

Outcome	Step	Block and Predictors		R^2	R^2 change	Standardized β	p
Italian sample	Demographics			.106	.106		
		1	Age			.266[.205, 0.321]	< 0.001
		2	SSS			.122[.064, 0.180]	< 0.001
		3	Sex			−0.080[−0.138,−0.022]	.007
	Affective scales	1	Anxiety	.249	.143	−0.400[−0.455,−0.345]	< 0.001
		2	Depression	.257	.011	−0.162[−0.243,−0.083]	< 0.001
Indian sample	Demographics			.036	.036		
		1	Age			.099[−0.008, 0.206]	.072
		2	SSS			.135[.029, 0.242]	.013
		3	Sex			−0.048[−0.156, 0.060]	.382
	Affective scales	1	Anxiety	.186	.151	−0.405[−0.507,−0.303]	< 0.001
		2	Stress	.197	.011	−0.142[−0.274,−0.010]	.036
NZ sample	Demographics			.093	.093		
		1	Sex			−0.107[−0.047, −0.0167]	< 0.001
		2	SSS			.193[.135, 0.251]	< 0.001
		3	Age			.166[.111, 228]	< 0.001
	Affective scales	1	Anxiety	.293	.200	−0.480[−0.535,−0.425]	< 0.001
		2	Stress	.298	.005	−0.117[−0.202,−0.031]	.007

Table 4
Dominance analysis: average R^2, general dominance and rescaled dominance across negative affect facets and countries.

Outcome	Numbers of predictors in the model	Depression	Anxiety	Stress
Italy	1	0.181	0.212	0.172
	2	0.022	0.050	0.016
	3	0.006	0.032	0.003
	General dominance	0.070	0.098	0.064
	Rescaled dominance%	30.09	42.42	27.49
India	1	0.100	0.169	0.132
	2	0.005	0.058	0.023
	3	0.000	0.036	0.003
	General dominance	0.035	0.088	0.053
	Rescaled dominance%	20.00	50.00	30.00
NZ	1	0.156	0.268	0.215
	2	0.003	0.087	0.036
	3	0.000	0.057	0.007
	General dominance	0.053	0.137	0.086
	Rescaled dominance%	19.14	49.76	31.10

dysfunction, which inevitably leads to losing information and consequently, accuracy of estimates. This may have reduced the power of the analysis and contributed to differences compared with our findings (Roemer et al., 2021). However, it is striking that anxiety exhibited the strongest relationship to immunity in both studies.

Anxiety was the strongest factor associated with immunity across countries. This persisted despite discrepancies in culture, age and other demographic factors, and this consistency is salient. Comparisons of types of affective relationships do show some differences between countries (De Vaus et al., 2018; Long et al., 2022). Stress levels were detected in the Indian students and NZ citizens. Depression was found to be the second most important feature for the Italians. Again, despite the high recent prevalence of depression and anxiety in Italy, the methodology showed a negligible association between immunity and depression (Caldirola et al., 2022; Fioravanti et al., 2022). It is unclear though interesting to speculate to what degree this may be a culturally mediated

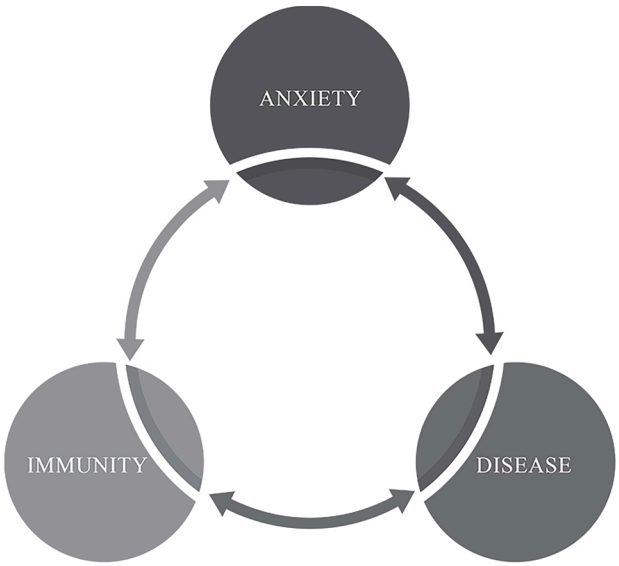


Fig. 1. Anxiety-disease model.

effect. There are Eastern cultural traditions that increase mind and body awareness, in addition to other features (De Vaus et al., 2018; Khambaty and Parikh, 2022; Long et al., 2022). Alternatively, it may simply reflect recent geopolitical events and conditions, which may have contributed to a relative increase in mood languor within Italy (Caldirola et al., 2022; Fioravanti et al., 2022).

The regression results indicated that after accounting for the effect of anxiety, stress and depression did not appear to have a strong association with immunity. This is despite the literature emphasising the close association between depression, stress, immunity and sickness (Andreasson et al., 2018; Binder and Nemeroff, 2010). Since depression shares common variance with both anxiety and stress, studies on stress and depression may not be able to control for effects of anxiety and stress when measuring solely depression (Medvedev et al., 2018; Roemer et al., 2021; West et al., 1995). It does highlight the advantages of using appropriate methodology including stepwise multiple linear regression and dominance analysis when examining unique contributions of psychological symptoms on immunity, since the effects of stress and depression are forced away when analysed simultaneously (Babyak, 2004; Budescu, 1993).

The lack of association between stress and immune functioning after accounting for the effect of anxiety was salient given the high statistical power of this sample. There are a couple of possible explanations. Firstly, the method of stepwise multiple linear regression deducts the variance of predictors already explained by the model, and as noted, anxiety accounted for most of the variance (Budescu,1993; Roemer et al., 2021). Secondly, acute stress-response hormesis can have some beneficial effects on immunity, which may weaken the broader association of stress and immunity within this study, since the measurement used may not distinguish acute from chronic stress, as is true of many studies considering stress (Aschbacher et al., 2013); Lovibond and Lovibond, 1993). For example, theme parks may induce stress levels through illusions of imminent danger, but the acute stress response from the fear produced may not necessarily lead to illness, as this type of stress is still compatible with positive affect (Aschbacher et al., 2013). Anxiety, with features of incessant worry and the negative appraisals of one's ability to cope with looming potential threats possesses clearer features of negative affect, and is less compatible with mood stability (Spinhoven et al., 2018; Taylor and Snyder, 2021).

Our study emphasizes the intricate relationships between psychological constructs, such as depression, anxiety, and stress, and immune functioning across populations from Italy, India, and New Zealand. Most strikingly, anxiety was identified as having a consistent negative link with immune functioning. On the other hand, depression demonstrates a robust connection with immune deregulation, with some immunological reactions differing from those elicited by stress (Dantzer et al., 2008; Lasselin, 2021; Mariani et al., 2021; Miller and Raison, 2016). Our results supported a modest link between depression and immune functioning in the Italian sample. Contrastingly, the relationship between stress and immunity in our study was weaker than suggested by the stress-immune model. This deviation from established literature, which often underscores a robust connection between stress and immune response, invites speculation on potential protective biological mechanisms or other moderating biomarkers. Such factors might be influenced by genetic, environmental, or lifestyle differences inherent to the populations studied. In sum, delving deeper into the biological mechanisms, such as hormonal, genetic, and inflammatory pathways, could elucidate our findings and account for the observed inter-country variations.

4.1. Strengths, limitations and directions of future research

Naturally, this study has strengths and limitations. The strengths lie in the size of the study, statistical power, cross-country participants which encompasses great cultural diversity and participant heterogeneity, the use of appropriate statistical techniques to undertake preliminary analysis. However, some of the common limitations are related to the cross-sectional nature of the study, which does not permit causal inferences, so the association found across countries cannot be deemed deterministic. Psychological disorders and immune dysfunction may share other commonalities, such as adverse experiences early in life (Dieleman et al., 2015). Also, temporal snapshots lack the ability to detect time related nuances. For example, the impact of negative affect on immunity may vary during time-sensitive windows, which could be further explored through longitudinal analysis, such as the Environmental Risk Longitudinal Twin Study (Baldwin et al., 2018).

Considering that psychological symptoms from participants who were members of the general population was generally skewed toward being subclinical, the study may benefit from the inclusion of a broader psychiatric range (Antony et al., 1998). Further research which encompasses and targets certain features, such as patients in primary medical health care, would augment the study. The study would also benefit from further refinement by examination with other assessments, such as anxiety sensitivity (Lasselin et al., 2016; Taylor et al., 2007).

From biological perspective, it would be interesting to observe whether greater levels of anxiety impacted known immunological responses, in a dose dependent pattern. For example, whether higher anxiety levels correspond to reduced immune responses to deliberate viral exposure challenges could support or falsify these findings. Alternatively, the immune responses of non-clinical participants to standard immunisation can be compared with placebo in randomised controlled trials while accounting for anxiety levels. This research may prove very useful, as the effectiveness of interventions, such as vaccinations, are based on the assumptions of typical immune functioning.

The study results may also be affected by common method bias (Podsakoff et al., 2012). The fact that multiple constructs are measured using common methods (e.g., multiple-item scales presented within the same survey) leads to spurious effects due to the assessment instruments rather than to the constructs being measured. For example, the fact that subjects are asked to report their own perceptions or impressions on two or more constructs in the same survey is likely to produce spurious correlations among the items measuring these constructs owing to response styles, social desirability, and priming effects, which may be independent from the true correlations among the constructs being measured.

In addition, there was no consistency time periods indicated in the measures for participants to consider, with the DASS referring to the past week, the ISQ referring to the past year, and the SSS referring to the participants' current point in life (Adler et al., 1994; Lovibond and Lovibond, 1993; Wilod Versprille et al., 2019). While common, such discrepancy could be criticised due to recall bias, and variation. However, the DASS subscales were well validated as a trait measures while changes in the SSS are very unlikely withing a short period of time (Antony et al., 1998; Lightburn et al., 2023). The ISQ, while increasingly globally used, including in English, Indian and Italian studies has been validated to the lesser extent (Nesari et al., 2022; Tarantino et al., 2021; Verster et al., 2022).

Given the highly comorbid nature of depression, stress and anxiety, it is not possible to conclude that individual conditions of anxiety, depression and stress in isolation are contributing to immunity, because shared factors between the conditions may drive this association. While the DASS-21 has been employed as a reliable instrument to assess the severity of these conditions, it is inherent to the tool's design to measure domains that have shared variance. Our analyses sought to discern unique contributions of each domain. However, we recognize the challenge posed by their interrelatedness. It's conceivable that shared factors among depression, anxiety, and stress might influence the observed associations with immunity. Thus, while our findings shed light on the differential contributions of these psychological states, the potential for underlying shared factors to drive these associations remains. In the context of our study, it's essential to interpret the results with an understanding of this overlap and the limitations of any single tool, including the DASS-21, in fully isolating the unique effects of

intertwined psychological domains. Future research employing a multi-faceted assessment approach, perhaps incorporating multiple tools and methodologies, could provide additional clarity on this matter.

Among the limitations of our study, a significant concern relates to the sampling strategy employed. While drawing comparisons across countries, we noted marked differences in factors such as age, sex, and negative affect levels between members of the general public and university students. The participants were predominantly youthful with a disproportionate representation of females. This skewness towards younger females could partially explain the elevated rates of negative affect observed, considering the heightened mental unwellness typically associated with this demographic (Medvedev et al., 2018; Spence et al., 1987). Moreover, the student participants tended to score higher on negative affect indices, underscoring the prevalent negative affect challenges faced by females and students (Spence et al., 1987). However, it's essential to highlight that findings specific to university students might not generalize to the broader population, as student cohorts often exhibit unique attributes like enhanced reported parenting and psychological resilience compared to their non-university attending peers (Cvetkovski et al., 2019).

Furthermore, we acknowledge the potential for volunteer bias, which may influence our results. Individuals more inclined to participate in such studies might possess particular characteristics or be more motivated by specific factors, potentially skewing our sample. Our recruitment strategies varied across countries, and only participants from New Zealand were compensated for their time. Such variations in recruitment and compensation might have led to differential participation motivations and could introduce biases in the sample composition. The lack of compensation in other regions, compared to New Zealand, might affect the motivation and the demographic mix of the participants, possibly influencing their responses. Future research would benefit from more standardized recruitment strategies and broader, more representative participant populations to bolster cross-country comparisons.

5. Conclusions

Studies which have examined the relationship between immunity and affective conditions have greatly emphasised stress and depression. The impact of anxiety on immunity has remained relatively neglected. Within the present study, anxiety consistently explained the greatest proportion (between 14 % and 20 %) of differences, when investigating the unique contributions of affective symptoms on immune status. After accounting for effects of anxiety, other affective symptoms explained only negligible variation in immune function. While preliminary, these cross-country results highlight a relationship between anxiety and immunity, and strongly encourage greater examination of the impact of anxiety on immune functioning, with a view to improving health outcomes.

Statement of Ethics

The study was approved by the ALPSS Divisional Human Research Ethics Committee of the University of Waikato, New Zealand, which is following the internationally recognised ethical standards consistent with APA 7 edition.

Informed Consent

All participants provided their informed consent before participating in the study.

Use of Artificial Intelligence

Artificial intelligence was not used in the creation of this manuscript.

Funding Sources

This study was funded by the University of Waikato Strategic Research Project Grant

Data availability

The data are deposited in the OSF and accessible through the following link https://osf.io/k6su3/?view_only=6722b87d00324446ae3cb128bc9b197a

CRediT authorship contribution statement

Catherine Breeze: Conceptualization, Formal analysis, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing. **Oleg N. Medvedev:** Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Writing – review & editing. **Matti Cervin:** Conceptualization, Methodology, Writing – review & editing. **Anna Sutton:** Funding acquisition, Project administration, Writing – review & editing. **Barbara Barcaccia:** Data curation, Project administration, Writing – review & editing, Conceptualization. **Alessandro Couyoumdjian:** . **Susanna Pallini:** Conceptualization, Data curation, Project administration, Writing – review & editing. **Moana Billot:** Data curation, Project administration, Writing – review & editing. **Rebecca Chalmers:** Data curation, Project administration, Writing – review & editing. **Naved Iqbal:** Data curation, Project administration, Writing – review & editing. **Vincent Reid:** Conceptualization, Funding acquisition, Project administration, Resources, Writing – review & editing. **Nirbhay N. Singh:** Conceptualization, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest in regards of this manuscript.

Acknowledgements

We wish to express our sincere gratitude to all the participants in New Zealand, Italy, and India who devoted their time and shared their experiences in contributing to our study. The generosity and openness exhibited by each of you have truly been a cornerstone to the completion of this research.

References

Adler, N.E., Boyce, T., Chesney, M.A., Cohen, S., Folkman, S., Kahn, R.L., Syme, S.L., 1994. Socioeconomic status and health: the challenge of the gradient. Am. Psychol. 49 (1), 15–24. https://doi.org/10.1037//0003-066x.49.1.15.

Alghamdi, B.S., Alatawi, Y., Alshehri, F.S., Tayeb, H.O., Tarazi, F.I., 2021. Relationship between public mental health and immune status during the COVID-19 pandemic: cross-sectional data from Saudi Arabia. Risk Manage. Healthc. Policy 14, 1439–1447. https://doi.org/10.2147/RMHP.S302144.

Andreasson, A., Wicksell, R.K., Lodin, K., Karshikoff, B., Axelsson, J., Lekander, M., 2018. A global measure of sickness behaviour: development of the sickness questionnaire. J. Health Psychol. 23 (11), 1452–1463. https://doi.org/10.1177/1359105316659917.

Antony, M.M., Bieling, P.J., Cox, B.J., Enns, M.W., Swinson, R.P., 1998. Psychometric properties of the 42-item and 21-item versions of the Depression Anxiety Stress Scales in clinical groups and a community sample. Psychol. Assess. 10 (2), 176–181. https://doi.org/10.1037/1040-3590.10.2.176.

Aschbacher, K., O'Donovan, A., Wolkowitz, O.M., Dhabhar, F.S., Su, Y., Epel, E., 2013. Good stress, bad stress and oxidative stress: insights from anticipatory cortisol reactivity. Psychoneuroendocrinology 38 (9), 1698–1708. https://doi.org/10.1016/j.psyneuen.2013.02.004.

Babyak, M.A., 2004. What you see may not be what you get: a brief, nontechnical introduction to overfitting in regression-type models. Psychosom. Med. 66 (3), 411–421.

Baldwin, J.R., Arseneault, L., Caspi, A., Fisher, H.L., Moffitt, T.E., Odgers, C.L., Pariante, C., Ambler, A., Dove, R., Kepa, A., Matthews, T., Menard, A., Sugden, K., Williams, B., Danese, A., 2018. Childhood victimization and inflammation in young

adulthood: a genetically sensitive cohort study. Brain Behav. Immun. 67, 211–217. https://doi.org/10.1016/j.bbi.2017.08.025.
Barlow, Sauer-Zavala, S., Carl, J.R., Bullis, J.R., Ellard, K.K, 2014. The nature, diagnosis, and treatment of neuroticism: back to the future. Clin. Psychol. Sci. 2 (3), 344–365. https://doi.org/10.1177/2167702613505532.
Binder, E., Nemeroff, C., 2010. The CRF system, stress, depression and anxiety: insights from human genetic studies. Mol. Psychiatry 15 (6), 574–588. https://doi.org/10.1038/mp.2009.141.
Budescu, D.V., 1993. Dominance analysis: a new approach to the problem of relative importance of predictors in multiple regression. Psychol. Bull. 114 (3), 542–551. https://doi.org/10.1037/0033-2909.114.3.542.
Caldirola, D., Daccò, S., Cuniberti, F., Grassi, M., Alciati, A., Torti, T., Perna, G., 2022. First-onset major depression during the COVID-19 pandemic: a predictive machine learning model. J. Affect. Disord. 310, 75–86. https://doi.org/10.1016/j.jad.2022.04.145.
Clark, L.A., Watson, D., 1991. Tripartite model of anxiety and depression: psychometric evidence and taxonomic implications. J. Abnorm. Psychol. 100 (3), 316–336. https://doi.org/10.1037/0021-843X.100.3.316.
Culpepper, L., 2009. Generalized anxiety disorder and medical illness. J. Clin. Psychiatry 70 (2), 20–24. https://doi.org/10.4088/JCP.s.7002.04.
Cvetkovski, S., Jorm, A.F., Mackinnon, A.J., 2019. An analysis of the mental health trajectories of university students compared to their community peers using a national longitudinal survey. Stud. Higher Edu. 44 (1), 185–200. https://doi.org/10.1080/03075079.2017.1356281.
Dantzer, R., O'connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat. Rev. Neurosci. 9 (1), 46–56. https://doi.org/10.1038/nrn2297.
De Vaus, J., Hornsey, M.J., Kuppens, P., Bastian, B., 2018. Exploring the East-West divide in prevalence of affective disorder: a case for cultural differences in coping with negative emotion. Personality Soc. Psychol. Rev. 22 (3), 285–304. https://doi.org/10.1177/1088868317736222.
Dieleman, G.C., Huizink, A.C., Tulen, J.H., Utens, E.M., Creemers, H.E., van der Ende, J., Verhulst, F.C., 2015. Alterations in HPA-axis and autonomic nervous system functioning in childhood anxiety disorders point to a chronic stress hypothesis. Psychoneuroendocrinology 51, 135–150. https://doi.org/10.1016/j.psyneuen.2014.09.002.
Eysenck, M.W., Fajkowska, M., 2018. Anxiety and depression: toward overlapping and distinctive features. Cognit. Emotion 32 (7), 1391–1400. https://doi.org/10.1080/02699931.2017.1330255.
Faul, F., Erdfelder, E., Buchner, A., Lang, A.-G., 2009. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav. Res. Methods 41, 1149–1160.
Fioravanti, G., Benucci, S.B., Prostamo, A., Banchi, V., Casale, S., 2022. Effects of the COVID-19 pandemic on psychological health in a sample of Italian adults: a three-wave longitudinal study. Psychiatry Res. 315, 114705 https://doi.org/10.1016/j.psychres.2022.114705.
Khambaty, M., Parikh, R.M., 2022. Cultural aspects of anxiety disorders in India. Dialogues Clin. Neurosci. 19 (2), 117–126. https://doi.org/10.31887/DCNS.2017.19.2/rparikh.
Lasselin, J., 2021. Back to the future of psychoneuroimmunology: studying inflammation-induced sickness behavior. Brain, Behav. Immun.-Health 18 (18), 100379. https://doi.org/10.1016/j.bbih.2021.100379.
Lasselin, J., Elsenbruch, S., Lekander, M., Axelsson, J., Karshikoff, B., Grigoleit, J.-S., Engler, H., Schedlowski, M., Benson, S., 2016. Mood disturbance during experimental endotoxemia: predictors of state anxiety as a psychological component of sickness behavior. Brain Behav. Immun. 57, 30–37. https://doi.org/10.1016/j.bbi.2016.01.003.
Libet, B., 2004. Mind Time the Temporal Factor in Consciousness. Harvard University Press. https://doi.org/10.4159/9780674040168.
Lightburn, S.J., Pratscher, S.D., Bettencourt, B.A., Hartstone, J.M., Medvedev, O.N., 2023. Evaluating depression anxiety and stress assessment before and during the COVID-19 pandemic using generalisability theory. Int. J. Psychol. https://doi.org/10.1002/ijop.12907.
Lodin, K., Lekander, M., Syk, J., Alving, K., Andreasson, A., 2017. Associations between self-rated health, sickness behaviour and inflammatory markers in primary care patients with allergic asthma: a longitudinal study. NPJ Prim. Care Respir. Med. 27 (1), 67. https://doi.org/10.1038/s41533-017-0068-0.
Long, D., Bonsel, G.J., Lubetkin, E.I., Janssen, M.F., & Haagsma, J.A. (2022). Anxiety, depression, and social connectedness among the general population of eight countries during the COVID-19 pandemic. *archives of public health = archives belges de santé publique,* 80(1), 237–237. https://doi.org/10.1186/s13690-022-00990-4.
Lovibond, S.H., Lovibond, P.F., 1993. Manual For the Depression, Anxiety, Stress Scales (DASS). University of New South Wales. Psychology Foundation Monograph.
Mariani, N., Cattane, N., Pariante, C., Cattaneo, A., 2021. Gene expression studies in depression development and treatment: an overview of the underlying molecular mechanisms and biological processes to identify biomarkers. Trans. Psychiatry 11 (1), 1–23. https://doi.org/10.1038/s41398-021-01469-6.
McEwen, B.S., 2017. Neurobiological and systemic effects of chronic stress. Chronic. Stress 1, 2470547017692328. https://doi.org/10.1177/2470547017692328.
Medvedev, O.N., Norden, P.A., Krägeloh, C.U., Siegert, R.J., 2018. Investigating unique contributions of dispositional mindfulness facets to depression, anxiety, and stress in general and student populations. Mindfulness 9 (6), 1757–1767. https://doi.org/10.1007/s12671-018-0917-0.
Miller, Raison, C.L, 2016. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat. Rev. Immunol. 16 (1), 22–34. https://doi.org/10.1038/nri.2015.5.
Momen, N.C., Plana-Ripoll, O., Agerbo, E., Benros, M.E., Børglum, A.D., Christensen, M. K., McGrath, J.J., 2020. Association between mental disorders and subsequent medical conditions. N Engl. J. Med. 382 (18), 1721–1731. https://doi.org/10.1056/NEJMoa1915784.
Morey, J., Boggero, I., Scott, A., Segerstrom, S., 2015. Current directions in stress and human immune function. Curr. Opin. Psychol. 5, 13–17. https://doi.org/10.1016/j.copsyc.2015.03.007.
Nesari, T., Kadam, S., Vyas, M., Huddar, V.G., Prajapati, P.K., Rajagopala, M., More, A., Rajagopala, S.K., Bhatted, S.K., Yadav, R.K., Mahanta, V., Mandal, S.K., Mahto, R.R., Kajaria, D., Sherkhane, R., Bavalatti, N., Kundal, P., Dharmarajan, P., Bhojani, M., Tripathi, R., 2022. AYURAKSHA, a prophylactic ayurvedic immunity boosting kit reducing positivity percentage of IgG COVID-19 among frontline Indian Delhi police personnel: a non-randomized controlled intervention trial. Front. Public Health 10, 920126. https://doi.org/10.3389/fpubh.2022.920126.
O'Connor, D.B., Thayer, J.F., Vedhara, K., 2021. Stress and health: a review of psychobiological processes. Annu. Rev. Psychol. 72, 663–688. https://doi.org/10.1146/annurev-psych-062520-122331.
Oswald, A.J., Wu, S., 2010. Objective confirmation of subjective measures of human well-being: evidence from the U.S.A. Science 327 (5965), 576–579. https://doi.org/10.1126/science.1180606.
Patriquin, M.A., Mathew, S.J., 2017. The neurobiological mechanisms of generalized anxiety disorder and chronic stress. Chronic. Stress 1, 2470547017703993.
Podsakoff, P.M., MacKenzie, S.B., Podsakoff, N.P., 2012. Sources of method bias in social science research and recommendations on how to control it. Annu. Rev. Psychol. 63 (1), 539–569. https://doi.org/10.1146/annurev-psych-120710-100452.
Renna, M., O'Toole, M.S., Spaeth, P.E., Lekander, M., Mennin, D.S., 2018. The association between anxiety, traumatic stress, and obsessive–compulsive disorders and chronic inflammation: a systematic review and meta-analysis. Depress. Anxiety 35 (11), 1081–1094. https://doi.org/10.1002/da.22790.
Roemer, A., Sutton, A., Grimm, C., Medvedev, O.N., 2021. Differential contribution of the five facets of mindfulness to well-being and psychological distress. Mindfulness 12 (3), 693–700. https://doi.org/10.1007/s12671-020-01535-y.
Seiler, A., Fagundes, C.P., Christian, L.M., 2020. The Impact of Everyday Stressors on the Immune System and Health. In: Choukèr, A. (Ed.), Stress Challenges and Immunity in space: From mechanisms to Monitoring and Preventive Strategies. Springer. https://doi.org/10.1007/978-3-030-16996-1_6.
Spence, J., Helmreich, R., Pred, R., 1987. Impatience versus achievement strivings in the Type A Pattern: differential effects on students' health and academic achievement. J. Appl. Psychol. 72 (4), 522–528. https://doi.org/10.1037/0021-9010.72.4.522.
Spinhoven, P., van Hemert, A.M., Penninx, B.W., 2018. Repetitive negative thinking as a predictor of depression and anxiety: a longitudinal cohort study. J. Affect. Disord. 241, 216–225. https://doi.org/10.1016/j.jad.2018.08.037.
Tarantino, V., Tasca, I., Giannetto, N., Mangano, G.R., Turriziani, P., Oliveri, M., 2021. Impact of perceived stress and immune status on decision-making abilities during COVID-19 pandemic lockdown. Behav. Sci. 11 (12), 167. https://doi.org/10.3390/bs11120167.
Taylor, M.M., Snyder, H.R., 2021. Repetitive negative thinking shared across rumination and worry predicts symptoms of depression and anxiety. J Psychopathol. Behav. Assess. 43, 904–915. https://doi.org/10.1007/s10862-021-09898-9.
Taylor, S., Zvolensky, M.J., Cox, B.J., Deacon, B., Heimberg, R.G., Ledley, D.R., Abramowitz, J.S., Holaway, R.M., Sandin, B., Stewart, S.H., Coles, M., Eng, W., Daly, E.S., Arrindell, W.A., Bouvard, M., Cardenas, S.J., 2007. Robust dimensions of anxiety sensitivity: development and initial validation of the anxiety sensitivity Index-3. Psychol. Assess. 19 (2), 176–188. https://doi.org/10.1037/1040-3590.19.2.176.
Tonidandel, S., LeBreton, J.M., 2011. Relative importance analysis: a useful supplement to regression analysis. J. Bus. Psychol. 26 (1), 1–9. https://doi.org/10.1007/s10869-010-9204-3.
Verster, J.C., Kraneveld, A.D., Garssen, J., 2022. The assessment of immune fitness. J. Clin. Med. 12 (1), 22. https://doi.org/10.3390/jcm12010022. –.
West, S.G., Finch, J.F., Curran, P.J., 1995. Structural equation models with nonnormal variables: problems and remedies. In: Hoyle, R.H. (Ed.), Structural Equation modeling: Concepts, issues, and Applications. Sage Publications, Inc, pp. 56–75.
Wilod Versprille, L.J., van de Loo, A.J., Mackus, M., Arnoldy, L., Sulzer, T.A., Vermeulen, S.A., Verster, J.C, 2019. Development and validation of the immune status questionnaire (ISQ). Int. J. Environ. Res. Public Health 16 (23), 4743. https://doi.org/10.3390/ijerph16234743.