ORIGINAL ARTICLE

An immunological age index in bipolar disorder: A confirmatory factor analysis of putative immunosenescence markers and associations with clinical characteristics

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Abstract

Objectives: The study aims to generate an immunological age (IA) trait on the basis of immune cell differentiation parameters, and to test whether the IA is related to age and disease characteristics.

Methods: Forty-four euthymic type I bipolar disorder patients were included in this study. Five immunosenescence-related parameters were assessed: proportions of late-differentiated cells (e.g., CD3+CD8+CD28-CD27- and CD3-CD19+IgD-CD27-), and the expression of CD69, CD71, and CD152 after stimulation. Confirmatory factor analysis was applied to generate an IA trait underlying the 5 measures.

Results: The best-fit model was constituted by 4 parameters that were each related to an underlying IA trait, with 1 cell population positively correlated (CD3+CD8+CD28-CD27- [$\lambda = 0.544$, where λ represents the loading of the parameter onto the IA trait] and 3 markers negatively correlated (CD69 [$\lambda = -0.488$], CD71 [$\lambda = -0.833$], and CD152 [$\lambda = -0.674$]). The IA trait was associated with chronological age ($\beta = 0.360$, $p = .013$) and the number of previous mood episodes ($\beta = 0.426$, $p = .006$). In a mediation model, 84% of the effect between manic episodes, and IA was mediated by body mass index.

Conclusion: In bipolar disorder type I, premature aging of the immune system could be reliably measured using an index that validated against chronological age, which was related to adverse metabolic effects of the disease course.

KEYWORDS

aging, bipolar, confirmatory factor analysis, neuroimmunology

1 | INTRODUCTION

In the recent years, the immune system has received increased attention in the study of the pathophysiology of mood disorders, especially inflammatory changes in bipolar disorder (BD). There is a well-described relationship between BD and general medical comorbidities with an inflammatory component, such as autoimmunity, heart diseases, respiratory diseases, type 2 diabetes, and the metabolic syndrome (Kilbourne et al., 2004; Osby, Brandt, Correia, Ekbom, & Sparen, 2001), and new findings have emerged elucidating the cross talk between the brain and the immune system that may mediate these associations (Soczynska, Kennedy et al. 2011). Among the most relevant, it is the discovery of bidirectional pathways connecting the nervous and immune systems (e.g., indoleamine 2,3-dioxygenase activation, the vagal reflex, active transport of cytokines across the blood-brain barrier, and others; Raison, Capuron, & Miller, 2006).

Several molecular and cellular immune abnormalities have been documented in BD, with elevated levels of proinflammatory cytokines demonstrated more prominently during mood episodes, especially those of manic polarity (Brietzke et al., 2009). Euthymic patients also show differences when compared with healthy volunteers (Modabbernia, Taslimi, Brietzke, & Ashrafi, 2013). Changes in the proportions of different cellular subsets, such as increased circulating monocytes and activated cells, and decreased regulatory T cells have been reported; as have changes in pathways downstream of immune cell signaling such as increased phosphorylation of NF κ B and ERK proteins, and decreased sensitivity to glucocorticoids (Breunis et al., 2003; do Prado et al., 2013; Wieck et al., 2013).

Similarities between immune abnormalities in BD and the continuous shaping of immune characteristics during natural aging, termed *immunosenescence*, have been noted (Maurya et al., 2016; Rizzo et al., 2014; SayuriYamagata, Brietzke, Rosenblat, Kakar, & McIntyre, 2017). Immunosenescence affects both innate and adaptive arms of immunity, with reduced production of B and T cells in bone marrow and the thymus, and diminished function of mature lymphocytes in secondary lymphoid tissues. As a result, elderly individuals respond less robustly to immune challenge than the young (Montecino-Rodriguez, Berent-Maoz, & Dorshkind, 2013), but they are prone to chronic low-grade inflammatory activity, described collectively by some researchers as “inflammaging” (Baylis, Bartlett, Patel, & Roberts, 2013).

From these markers, it might be reasonable to assume an underlying factor, “immunological age” (IA), and useful to use this factor to estimate the extent of immunosenescence in BD. Specifically, immunosenescence has been associated with an increased proportion of late-differentiated T (e.g., CD3+CD8+CD28-CD27-; Aiello et al., 2016) and B cells (e.g., CD3-CD19+IgD-CD27-; 14), a reduced proportion of activated T cells after stimulation (i.e., reduced expression of CD69 and CD71 proteins on the T cell surface; Lio et al., 1996) and with decreased regulatory T (Treg) cell suppressive capacity, partially due to decreased expression of CD152 (Tsaknaris et al., 2003). Therefore, we hypothesize that a latent trait for IA would be related positively to CD3+CD8+CD28-CD27- and CD3-CD19+IgD-CD27- cells, and negatively to cells bearing CD69, CD71, and CD152. We consider that this latent measure (i.e., IA) might be correlated with clinical features of the disorder, and with patient outcomes.

The main objective of this work was to provide evidence of a measurement model for IA on the basis of five putative cellular and molecular parameters obtained by flow cytometry. Underlying the observed indicators, we hypothesize a single IA factor. Evidence of convergent validity with chronological age was also sought. To probe the potential for clinical relevance, the IA was compared with characteristics of the course of BD type I between participants, including the number of previous mood episodes.

2 | MATERIALS AND METHODS

2.1 | Sample

The sample consisted of 44 male and female euthymic patients with BD type I, aged 18 to 65 years, attending a regular follow-up in a secondary outpatient clinic. Psychiatric diagnosis was based on clinical interview and confirmed with the *Structured Clinical Interview for DSM-IV-Axis I (SCID-I)*. Manic and depressive symptoms severities were assessed using the Young Mania Rating Scale and the Hamilton Depression Rating Scale-17 items, respectively. To be included euthymic, patients did not fulfill DSM-IV criteria for any current mood episode and they presented with Young Mania Rating Scale and Hamilton Depression Rating Scale below 8. All the patients were under a stable medication treatment regimen (taking the same medications in the same doses for a minimum of 2 months prior to the study inclusion). Exclusion criteria were: (a) recent use of anti-inflammatories (up to and including the previous 2 weeks); (b) treatment with immunomodulatory drugs; and (c) acute or chronic general medical condition associated with imbalances in immune-inflammatory pathways, such as infections, HIV infection, allergies, and autoimmune diseases (e.g., rheumatoid arthritis and inflammatory bowel disease). In addition, women were excluded if they were pregnant or postpartum.

2.2 | Blood collection and cell isolation

A sample of 15 ml of peripheral blood was obtained from each subject by arm venipuncture. The blood collection was made in a standardized time of day (between 08:00 and 10:00 a.m.) and following a 12-hr period of fasting. Peripheral blood mononuclear cells (PBMCs) were isolated from BD Vacutainer® EDTA tubes by density gradient centrifugation for 30 min at 900 $\times$ g (Ficoll® Paque Plus, GE Healthcare Life Sciences, SP, Brazil). Cells were counted by means of microscopy, and viability confirmed to exceed 95%, as judged from their ability to exclude Trypan Blue (Sigma).

2.3 | Flow cytometry

To examine lymphocyte subsets, 2×10^5 PBMCs were stained directly for 30 min with combinations of anti-CD3 FITC, -CD4 APCy7, -CD8 BV510, -CD71 PE, -CD69 PeCy7, -CD28 PE Cy5, -CD27 PE, -CD19 APCCy7, -IgD BV42, -CD25 BV510, and -CD152 PECy5 monoclonal antibodies (BD Biosciences). For intracellular FoxP3 staining, the Human FoxP3 Buffer Set (BD Biosciences) was used after surface staining, including cell fixation, permeabilization, and staining with the monoclonal antibody anti-FoxP3 Alexa647.

An additional aliquot of PBMCs were stimulated in 96-well plates coated with 5 µg/ml anti-human CD3 antibody (OKT3, eBioscience) (previously prepared, overnight at 4°C). The PBMCs (1×10^5 cells) were added to each well with antihuman CD28 antibody (CD28.2, eBioscience) in a final concentration of 5 µg/ml. The PBMCs were cultivated in RPMI 1640 culture medium supplemented with L-glutamine, 10% fetal bovine serum, antibiotics, and antimycotics (Life-Thermo Fischer). The cells were allowed to grow for 3 days at 37 °C in a 5% CO₂ atmosphere after which time the T helper (Th) and T cytotoxic (Tc) activation markers CD69 and CD71 and the Treg functional molecule CD152 were counted and stained (as above).

Cells were analyzed by flow cytometry immediately after staining. At least 50,000 lymphocytes were identified by size (forward scatter; FSC) and granularity (side scatter; SSC) and they were acquired with a FACSCantoII flow cytometer (BD Biosciences). Isotype controls for all fluorophores were used as negative controls. Data were analyzed using the FlowJo 7.2.5 software (Tree Star Inc.).

2.4 | Statistical analysis

Confirmatory factor analysis (CFA) is a statistical approach to test a measurement model by analyzing to what extent observed indicators (i.e., the five immunosenescence-related parameters) indicate a common cause (i.e., a common factor, latent variable, or construct) that is not directly observed. Herein, the observed indicators are continuous measures assumed to have two causes: a single latent factor (i.e., IA) that the indicators are intended to measure and measurement error (Kline, 2011).

We conducted CFA aiming to provide evidence of a latent IA trait underlying five immunosenescence parameters and a parsimonious solution constituted of one dimension (i.e., comprised of one factor) was tested. To evaluate the statistical model goodness of fit, the following indices were used: chi-square (χ^2), Confirmatory Fit Index (CFI), Tucker-Lewis Index (TLI), root mean square error approximation (RMSEA), and standardized root mean square residual (SRMR). The following cutoff criteria were used to determine a well-fit model: not statistically significant chi-square ($p > .05$), RMSEA near or less than 0.05 (Browne, Cudeck, Bollen, & Long, 1993) and a nonsignificant Cfit ($p > .05$), a statistical test of closeness of model fit using RMSEA. Lastly, for a well-fitted model, it is expected CFI and TLI to be near to or greater than 0.95 and SRMR less than 0.08 (L. t. Hu & Bentler, 1999).

Beyond the fit indices, CFA evaluates the strength of associations between the cellular indicators and the IA trait factor loading (λ), which, under its standardized forms, is interpreted in the same way as a Pearson correlation where values close to one would indicate a perfect correlation between the indicator and the IA trait. Because immunological indicators are used, and our model is a reduction of a complex biological phenomenon where there are other intercorrelated indicators (and traits) not included in the model, the indicators may not be expected to exhibit very strong correlations with the latent trait as often observed in psychometric analysis (e.g., higher than 0.8).

A CFA with covariates, also called “multiple-indicator, multiple-cause” (MIMIC; B. O. Muthén, 1989), was used to investigate invariance of the IA trait in relation to chronological age by allowing a direct

path from age to each observed variable. MIMIC is a flexible way of invariance testing at the indicator level (Finch, 2005; Fleishman, Spector, & Altman, 2002), offering advantages over the traditional multigroup CFA and item response theory due to its simplicity and flexibility (B. O. Muthén, 2002; Thompson & Green, 2006); MIMIC does require splitting the data for each group as multigroup CFA and item response theory do in the context of categorical covariates—it runs a single model as a whole (Kim & Yoon, 2011). Establishing invariance indicates that the trait is stable over the characteristic, and, therefore, statistical comparisons between the variable and the amount of the trait are meaningful. As described in Brown (Brown, 2015), a significant direct effect of the covariate on the latent variable indicates population heterogeneity (relation between age and IA values), and a significant direct effect of the covariate on an indicator represents differential item functioning. An important limitation of MIMIC is its ability to examine just two potential sources of invariance: indicators intercept and factor means.

In the context of MIMIC, invariance was evaluated in an exploratory fashion, because we have no specific hypothesis about likely noninvariance behavior of immunosenescence-related parameters underlying to IA. Then, we fixed all direct effects between the covariate and the indicators to zero, and then we inspected modification indices (and associated expected parameter change values) to determine whether salient direct effects may be present in the data. Modification indices are the amount chi-square will drop the parameter is estimated as part of the model. The chi-square value of 3.84 is the value that should be exceeded at the 0.05 level for one degree of freedom. Therefore, we consider a salient modification indices being at least 3.84 and making sense to free the parameter.

Relationships between IA and participant characteristics (i.e., age, body mass index (BMI), and number of previous manic and depressive episodes) were assessed in path analysis, and their effects expressed as unstandardized and standardized regression coefficients (represented by B and β , respectively); it is important to state that the p values differ for unstandardized and standardized parameters because the parameters have different sampling distributions.

Lastly, to estimate a likely indirect effect of mood episodes on IA via BMI, we used a bias-corrected bootstrap confidence interval (Hayes, 2013). The hypothesis of this indirect effect stems from observations that BMI increase during manic episodes (Fiedorowicz, Andersen, Persons, & Calarge, 2015), which is *path a*. The second step in the mediation, *path b*, is based on the observation that BMI can be related to inflammatory markers in BD (Lee et al., 2013). Moreover, this mediation model hypothesis was based on previous studies, which have found that obesity is associated with inflammation, and a meta-analysis (Howren, Lamkin, & Suls, 2009) suggested that this relationship was mediated in part by BMI. Therefore, we hypothesized that immunological changes in BD might similarly be related to BMI. Similarly, manic episodes were predicted by inflammation in a study of men with depressive symptoms (Becking et al., 2013). Interestingly, previous studies have suggested that periods of illness during bipolar cycling are associated with immunological and metabolic changes, leading us to test the hypothesis post hoc that BMI might mediate the relationship between manic episodes and the IA. Because all analyses in the current manuscript are cross-sectional, the direction of

causation cannot be inferred; instead, we intend only to demonstrate how these features are related to IA.

The estimator used in all analyses was the robust maximum likelihood, and the level of statistical significance was 0.05. All analyses were run using Mplus version 8 under maximum likelihood estimation with robust standard error (L. K. Muthén & Muthén, 2012).

3 | RESULTS

3.1 | Sample characteristics

Descriptive statistics are shown in Table 1 ($n = 44$). The mean number of mood episodes was 5.93 ($SD = 3.51$) for any polarity, three for manic polarity ($SD = 2.59$), and 1.9 ($SD = 2.13$) for depressive polarity.

3.2 | Building and evaluating a unidimensional immunosenescence model

The correlation/covariance matrices for the unidimensional model with four indicators are reported in Table 2 ($n = 39$). The covariance coverage for missing values ranged from 0.564 to 0.897. A unidimensional model constituted by five continuous cellular indicators returned excellent fit indices: $\chi^2(5) = 0.749$, $p = .9802$; RMSEA = 0.000, 90% CI [0.000, 0.000], Cfit = 0.984; CFI = 1.000; TLI = 1.608, and SRMR = 0.044. In the standardized factor solution (Figure 1, on left side) shows the magnitude of the standardized correlation between each indicator and IA trait. The highest factor loading was the expression of CD71 ($\lambda = -0.812$, $p < .001$), indicating that this cellular parameter is strongly and positively correlated to IA. Because CD3-CD19+IgD-CD27- showed the smallest factor loading ($\lambda = 0.242$, $p = .271$), where the IA accounts for less than 6% of the variance of that manifest measure, we excluded it and reran the model with only the other four measures with factor loadings superior to 0.4 (Figure 1, on right side) returning excellent fit indices: $\chi^2(2) = 0.677$,

TABLE 1 Characteristics of the studied sample ($n = 44$)

	Mean (SD) or count (%)
Age (years)	44.50 (12.29)
Sex (female)	30 (68.2%)
Ethnicity (White)	33 (75%)
Education (years)	11.33 (3.67)
Physical exercise (hr/week)	1.28 (2.06)
Current smoking	12 (25.53%)
Years of illness	17.27 (13.36)
YMRS score	1.13 (1.88)
HDRS score	2.19 (2.68)
GAF score	78.75 (10.30)
Medications	
Lithium	19 (43.2%)
Antidepressants	8 (18.2%)
Benzodiazepine	13 (29.5%)
Valproate	19 (43.2%)
Carbamazepine	6 (13.6%)

Note. GAF = Global Assessment of Functioning; HDRS = Hamilton depression rating scale; YMRS = Young mania rating scale.

TABLE 2 Correlation/covariance matrix for the four-indicator confirmatory factor model ($n = 39$)

Covariance matrix	(1)	(2)	(3)	(4)
CD3+ CD69+ (1)	247.854			
CD3+ CD71+ (2)	204.931	1038.613		
Treg CD152+ (3)	79.666	282.029	239.515	
CD28- CD27- (4)	-52.887	-126.554	-41.909	74.073
Correlation matrix	(1)	(2)	(3)	(4)
CD3+ CD69+ (1)	1.000			
CD3+ CD71+ (2)	0.404	1.000		
Treg CD152+ (3)	0.327	0.565	1.000	
CD28- CD27- (4)	-0.390	-0.456	-0.315	1.000

$p = .7127$; RMSEA = 0.000, 90% CI [0.000, 0.230], Cfit = 0.735; CFI = 1.000; TLI = 1.218, and SRMR = 0.044.

3.3 | Convergent validity and invariance of IA with chronological age

In a MIMIC model (Figure 2), no differential item functioning (i.e., no modification indices greater than 3.84) with respect to age was observed; the direct effects between age and the five cellular indicators (depicted by dashed lines) was not significant controlling for IA, therefore each cellular marker was functioning properly over the range of ages in the sample. There was a significant correlation between IA and chronological age ($B = 0.03$, $\beta = 0.360$, $p = .013$), suggesting that the IA has validity as an age-related phenomenon; the older the patient, the greater the IA values.

3.4 | Associations between IA and clinical characteristics

Table 3 shows the correlation/covariance matrix for all the used variables for the subsequent analysis. In a path analysis controlling for age (Figure 3), the IA was positively associated with the total number of previous mood episodes ($B = 0.139$, $p = .023$; $\beta = 0.426$, $p = .006$). In unadjusted post hoc models, the IA was associated with the number of previous manic episodes ($B = 0.194$, $p = .073$; $\beta = 0.418$, $p = .036$), but the model testing for depressive episodes did not converge, such that the effect could not be estimated.

In a mediation model (Figure 4), BMI mediated the effect of previous number of manic episodes on the IA (unstandardized $B = 0.220$, 95% bootstrap CI [0.008, 1.035] and standardized estimate $\beta = 0.349$, 95% bootstrap CI [0.066, 0.811]; Figure 3). The direct effect of the number of previous manic episodes on the IA was not significant ($B = 0.048$; $p = .867$; $\beta = 0.129$, $p = .607$). In terms of magnitude of the indirect effect, because the indirect and direct effects were in the same direction, we calculated the proportion of the mediated effect, which is given by unstandardized indirect effect/(indirect effect + direct effect). Then, $(0.220/[0.220 + 0.0418])$, resulted in 0.840, which means that 84.0% was mediated by BMI.

FIGURE 1 Confirmatory factor analysis of five (left) and four (right) immune markers. Markers related to an IA latent variable. Factor loadings of each marker are shown with their respective standard errors in parentheses. Four markers were found to have meaningful factor loadings, and they were used in the final model. IA = immunological aging

FIGURE 2 Multiple-indicators, multiple-causes model evaluating invariance of the immunological aging trait with age. The biological markers did not function differently across different ages (i.e., no evidence of measurement invariance was found). Age was correlated with the IA trait. This indicates that the correlation between age and IA is a true correlation and not an artefact of differential item functioning. IA = immunological aging

4 | DISCUSSION

Here we tested a measurement model for “immunological aging” factor informed by five cellular immunosenescence markers measured using flow cytometry. Using CFA, we obtained evidences for the construct and convergent validity of such measurement model which returned good fit indices (i.e., preconceived model adherence to the data and acceptability of the solution) and meaningful parameters estimates (i.e., substantive relation between the indicators and the IA and the latter with clinical features).

An association between the number of previous mood episodes and IA suggests that the IA may be clinically relevant and related to the course of the disorder. The observed association between IA and BMI would be consistent with the suggestion that metabolic dysfunction is related to immunosenescence in BD (Kilbourne et al., 2004; Osby et al., 2001; Yamagata et al., 2017). Because BMI has also been related to chronicity, disability, and less likelihood of achieving complete remission with treatment (Galvez et al., 2014; C. Hu et al., 2017), IA might also be assessed as

a prospective predictor of these outcomes in further studies (Calkin et al., 2009). Further, we offer evidence that higher BMI is responsible for the relationship between manic episodes and immunological aging. The finding is consistent with the previous prospective study suggesting that manic episodes represent high-risk periods for the accumulation of adiposity (Fiedorowicz et al., 2015).

The observed indicator with the highest absolute contribution to the IA model was CD3+CD71+ ($\lambda = -0.833$, $p < .001$) known as transferrin receptor protein 1 (TfR1), an early/middle molecular marker in the process of cellular activation (Caruso et al., 1997). Lio et al. (1996) showed that the expression of CD71 in T cells could be also used to follow their proliferative capacity. The lack of proper T cell proliferation is a well replicated finding of the immunosenescence process (Candore et al., 1992; Ferguson, Wikby, Maxson, Olsson, & Johansson, 1995; Potestio et al., 1998).

The percentage of Tregs expressing CD152 (CD4+CD25+FoxP3+CD152+) was the variable with the second highest absolute factor loading ($\lambda = -0.674$, $p < .001$). The CD152 is one of the main molecules by which Tregs exert their cell-to-cell anti-inflammatory ability. It presents on the surface of Tregs and it competes with CD28 for binding to CD80 and CD86 in APCs, consequently impairing the immune response (Sakaguchi, Miyara, Costantino, & Hafler, 2010). Studies in mice and humans have reported a decrease in Treg suppressive capacity in the elderly (Tsaknaris et al., 2003; Zhao et al., 2007), which was partially explained by a decrease in CD152 expression (Tsaknaris et al., 2003).

The percentage of late differentiated B cells (CD3-CD19+IgD-CD27-) was poorly related to the IA factor ($\lambda = 0.242$) in the measurement model constituted by five indicators. We excluded this indicator and reran the model leaving the measurement model only with those indicators with meaningful parameter estimates. Colonna-Romano et al. (2009) first described this cell population as probably senescent cells because it shows shorter telomeres and decreased proliferative capacity, and it expressed less CD40, HLA-DR and CD80 (Colonna-Romano et al., 2009). They also implicated these senescent B cells in immune aging because they were increased in elderly people (Colonna-Romano et al., 2009), particularly in unsuccessful aging, as defined by the presence of moderate or severe Alzheimer's disease (Bulati et al., 2015; Martorana et al., 2014), and decreased in a population with a genetic advantage for longevity (Martorana et al., 2014). It is possible that this marker reflects specific pathological processes that are also more common with aging (e.g., Alzheimer's

TABLE 3 Correlation/covariance matrix for all the used variables (Figures 1–4; $n = 44$)

Covariance matrix	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
CD3+ CD69+ (1)	264.586							
CD3+ CD71+ (2)	224.804	1.044.862						
Treg CD152+ (3)	83.366	275.345	234.913					
CD28- CD27- (4)	-75.488	-134.501	-35.916	86.069				
Age (5)	-40.758	-114.959	-29.630	34.089	147.705			
Number of manic episodes (6)	-19.265	-42.881	-3.044	7.728	8.503	6.679		
BMI (7)	-43.338	-85.520	-18.830	35.140	25.732	6.184	30.946	
Number of episodes in total (8)	-7.228	-55.621	-9.756	6.475	18.375	5.532	4.733	12.064
Correlation matrix	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
CD3+ CD69+ (1)	1.000							
CD3+ CD71+ (2)	0.428	1.000						
Treg CD152+ (3)	0.334	0.556	1.000					
CD28- CD27- (4)	-0.500	-0.449	-0.253	1.000				
Age (5)	-0.206	-0.293	-0.159	0.302	1.000			
Number of manic episodes (6)	-0.458	-0.513	-0.077	0.322	0.271	1.000		
BMI (7)	-0.479	-0.476	-0.221	0.681	0.381	0.430	1.000	
Number of episodes in total (8)	-0.128	-0.495	-0.183	0.201	0.435	0.616	0.245	1.000

FIGURE 3 Path analysis of number of mood episodes and age on IA. This model describes the effects of number of episodes and age on the IA latent trait. IA = immunological aging

disease) rather than aging itself per se; however, our data cannot speak to this possible interpretation.

The CD3+CD8+CD28-CD27- cells have lost the expression of the CD28 and CD27 proteins, which are necessary for their complete stimulation and clonal expansion and maturation. These cells have short telomeres, impaired cytotoxic function, and they are resistant to apoptosis (Derhovanessian, Larbi, & Pawelec, 2009; Weng, Akbar, & Goronzy, 2009). The proportion of CD3+CD8+CD28-CD27- cells increases with aging and a high frequency in the elderly correlates with a less effective

response to influenza virus vaccination (Goronzy et al., 2001) and with a higher risk of mortality (Olsson et al., 2000; Wikby et al., 2002). Fletcher and collaborators (2005) demonstrated that expansion of CD3+CD8+CD28-CD27- can be related to CMV infectious burden (Fletcher et al., 2005). Human CMV has increasingly been implicated in accelerated immunosenescence in aging studies (Spyridopoulos et al., 2009). It is usually present as a latent and asymptomatic infection, generating a permanent alert state of the immune system, leading to overwork of the immune system, and the observed changes in CD3+CD8+CD28-CD27- cell populations (Fulop, Larbi, & Pawelec, 2013).

As potential limitations, the study was cross-sectional in design, necessitating further longitudinal studies and limiting our ability to comment on the utility of the IA trait as a predictive biomarker. As a relatively small study, inferences as to effects of covariates on the IA were limited, and estimates should be viewed as preliminary. In addition, examination of missing data (e.g., missingness mechanism and different approaches to deal with missing data [e.g., multiple imputation]) was not undertaken and results should be interpreted accordingly; the mediation model deals with missing values using a full information maximum likelihood estimator.

All subjects were assessed in euthymia and the majority was using medication, and therefore, the results may not generalize to BD patients in acute mania or depression, or to patients who are unmedicated; however,

FIGURE 4 Mediation model. An indirect effect of the number of manic episodes on the IA trait was mediated by BMI. B are the unstandardized parameters, and β the standardized parameters. BMI = body mass index; IA = immunological aging

the results are useful because they do not merely reflect the acute fluctuations in inflammatory markers known to accompany changes in mood states. BD has been associated with shortened telomere length (Barbe-Tuana et al., 2016) corroborating the present observations of immunological aging; larger studies might assess the relationship between these two phenomena in BD, or attempt to integrate telomere length into a more inclusive IA trait model.

This study confirmed that a latent IA trait contributes to several cellular immunosenescence markers, and that this IA trait correlates with chronological age in BD patients. The association between the IA trait and greater cumulative episode burden lends preliminary support to the theory that accelerated aging of the immune system is part of the pathophysiology of BD. The association between the IA trait and BMI further implicates metabolic dysfunction in accelerating immunological aging in BD. Confirmatory and prospective studies are warranted.

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