HHS Public Access

Author manuscript

Cancer. Author manuscript; available in PMC 2019 August 01.

Published in final edited form as:

Cancer. 2018 August 01; 124(15): 3240–3248. doi:10.1002/cncr.31538.

Cells, cytokines, chemokines, and cancer stress: A biobehavioral study of patients with chronic lymphocytic leukemia

Barbara L Andersen, Ph.D.[1], **Neha Godiwala Goyal, Ph.D.**[2], **David M. Weiss, M.A.**[1], **Travis Westbrook, M.A.**[1], **Kami Maddocks, M.D.**[3], **John C. Byrd, M.D.**[3], and **Amy J. Johnson, Ph.D.**[3]

[1]The Ohio State University

[2]Wake Forest School of Medicine

[3]The Ohio State University Wexner Medical Center

Abstract

Purpose—Chronic lymphocytic leukemia (CLL) is the most prevalent adult leukemia, with profound disease-related cellular, humoral, and innate immune suppression. The relationship between stress and disease-specific negative prognostic cellular, cytokine, and chemokine markers in CLL patients is studied.

Patients and methods—A single-group, observational design was used. Relapsed/refractory CLL patients (N=96) entering a Phase II trial of an experimental therapy (ibrutinib) were studied. Before the first dose, a validated self-report measure of stress (Impact of Event Scale) was completed and blood was drawn for absolute lymphocyte counts (ALCs) and cytokine and chemokine ELISA assays. Multiple linear regression models tested stress as a concurrent predictor of ALCs, cytokines [tumor necrosis factor alpha (TNFα), a proliferation-inducing ligand (APRIL), B-cell activating factor (BAFF), Interleukins (IL)-6, 10, 16, VEGF] and the chemokine (C-C motif) ligand 3 (CCL3).

Results—Controlling for relevant demographic variables, comorbidities, CLL genetic risk (del17p), and correlates of inflammation, stress predicted higher ALCs ($p<.05$), and higher levels of TNFα ($p<.05$), IL-16 ($p<.01$), and CCL3 ($p<.05$). Stress was not associated with APRIL, BAFF, IL-6, IL-10, or VEGF.

Conclusions—Novel biobehavioral data for relapsed/refractory patients show stress is related to heightened levels of cellular, cytokine, and chemokine markers that have been previously shown to

Correspondence to: Barbara L. Andersen, Ph.D., Department of Psychology, The Ohio State University, 1835 Neil Avenue, Columbus, OH 43210, Andersen.1@osu.edu; Telephone: 614-292-4236.

Trial Registration: NCT01589302 clinicaltrials.gov

Disclaimers: This manuscript contains original work that is not currently being considered for publication elsewhere. Author KM has received honoraria and research funding from Pharmacyclics. Author JB has received research funding from Pharmacyclics.

Author Contributions: Barbara Andersen: Conceptualization, funding acquisition, writing – original draft and review and editing; Neha Godiwala Goyal: Data curation, writing – original draft and review and editing; David Weiss: Data curation, formal analysis, writing – review and editing; Travis Westbrook: Data curation, formal analysis, writing – review and editing; Kami Maddocks: Conceptualization, funding acquisition, writing –review and editing John Byrd: Conceptualization, funding acquisition, writing – review and editing Amy Johnson: Conceptualization, funding acquisition, project management, writing –review and editing

be associated with progressive disease in CLL. Results indicate that stress is related to immune and inflammatory processes that contribute to cancer cell proliferation and survival. These data provide a first look into these processes.

Keywords

Chronic lymphocytic leukemia; psychological stress; cytokines; chemokines

Introduction

Patients with chronic lymphocytic leukemia (CLL), which accounts for about one-third of adult leukemia in the United States,[1] may be vulnerable to psychological stress as chemo-immunotherapy is not curative. At diagnosis, patients may be asymptomatic excepting for high lymphocyte counts (> 5,000/mm^3). Closer inspection finds small, mature appearing lymphocytes with progressive B lymphocyte accumulation in the blood stream, making CLL a disease in which a key biologic system—immunity—is compromised[2]. Treatments may bring remission but relapses are likely to occur with successive treatments often having greater toxicity and reduced effectiveness[3]. Thus, the relapsing/refractory nature of CLL place patients at risk of stress and perhaps, stress-induced immune impairments.

Previous work has provided empirical support for how stress impacts immunity to initiate or facilitate an ongoing pathological process[4]. The immune system is viewed as acting like a sensory organ, informing the brain of immune challenges, and further, immune activation may be interpreted by the central nervous system as a stressor[5]. Cytokines may be a part of the regulatory loop[6]. Indeed, “sickness behaviors,” such as heightened negative emotions are seen as the products of pro-inflammatory cytokines[5]. Conversely, data from psychological intervention trials show that when patients’ stress lowers, inflammation is reduced and health and cancer outcomes are improved[7-9].

Examining how stress interacts with the immune system in CLL is critical as CLL cells develop in the same environment as normal immune cells[10]. Specifically, malignant cells interact with stromal cells as well as T cells in the lymphoid tissue, which collectively create a microenvironment conducive to the survival and proliferation of malignant cells[11]. CLL cells can be stimulated directly by elements of the microenvironment or through cytokines and chemokines[12]. Additionally, lymphatic tissues are the principle site of B-cell receptor (BCR) activation for both normal and malignant B-cells[13]. In response to BCR activation, CLL cells secrete chemokines, a type of cytokine that directs white blood cells to infected tissues to help create a microenvironment conducive to disease progression.[11] Additional research has indicated that BCR signaling results in malignant B cell proliferation and may impede the immune system’s ability to destroy malignant cells.[13] Considering these literatures, we studied how stress might covary with absolute lymphocyte count (ALC), the hallmark CLL indicator[14], and cytokine and chemokine factors important to the interaction of CLL cells and the microenvironment.

Tumor necrosis factor alpha (TNFα) is a cytokine involved in systemic inflammation and is produced by activated macrophages, although other cell types such as CD4+ lymphocytes and natural killer (NK) cells also produce TNFα. TNFα is increased in CLL patients

compared to healthy individuals[15], and is correlated with higher white cell counts at diagnosis and shorter progression-free survival[16, 17]. In vitro data show CLL neoplastic lymphocytes release TNFα spontaneously[15] and further, exposure to TNFα increases the proliferation and viability of leukemic lymphocytes[18].

A proliferation-inducing ligand (APRIL) and the B-cell-activating factor of the TNF family (BAFF) are cytokines that aid in the survival and proliferation of CLL cells[11, 19]. In vitro data demonstrate APRIL and BAFF aid CLL cell survival through upregulation of CD40 ligands[20]. Clinical data has shown patients with lower APRIL/BAFF expression in CLL cells had longer survival times compared to those with greater expression of APRIL/BAFF in CLL cells[21].

Furthermore, inflammatory cytokines, such as Interleukins-6 (IL-6) and -10 (IL-10), are involved in promoting angiogenesis in CLL[22]. Researchers have demonstrated that B cells and CLL cells produce IL-10 and suggest that IL-10 creates an immunosuppressive niche that allows CLL cells to proliferate[23, 24]. Elevated levels of IL-6 and IL-10 have been observed in CLL patients compared to controls and are associated with shorter survival times[16]. A meta-analysis has shown IL-10 expression to predict worse disease-free survival among those with hematological malignancies[25]. Also relevant is IL-16, a pro-inflammatory cytokine that contributes to the pathogenesis of CLL through the regulation of CD4+ cell recruitment and activation at sites of inflammation[26].

CLL cells produce high levels of angiogenic factors that support cell accumulation and proliferation[27]. Among the most studied angiogenic factors, vascular endothelial growth factor (VEGF) is secreted by malignant B-cells[28]. Research has demonstrated that CLL patients with higher levels of VEGF have 3 times the increased risk of progressive disease[29]. Additionally, malignant B cells secrete chemokine (C-C motif) ligand 3 (CCL3) which fosters the interaction between CLL cells and the leukemia microenvironment[30] and has been associated with advanced stage disease[31].

Patients with relapsed/refractory CLL in a Phase II (open label) trial of a new targeted agent, ibrutinib, were studied. Past research has shown relapsed/refractory patients to have lower quality of life compared to treatment-naïve patients[32, 33] and may be vulnerable to stress. Previous research has shown a measure of cancer-specific stress[34] can predict lower NK cell cytotoxicity, diminished response of NK cells to recombinant interferon gamma, and decreased T-cell blastogenesis in breast cancer patients awaiting chemotherapy[35]. Therefore, in a CLL sample, heightened stress may be associated with heightened CLL-specific cellular (ALC), cytokine (TNFα, APRIL, BAFF, IL-6, IL-10, IL-16, and VEGF), and chemokine (CCL3) markers while controlling for disease-relevant and behavioral correlates of inflammation.

Methods

Design

A single group, observational design was used. Patients with relapsed/refractory CLL were screened and accrued to a phase II study of ibrutinib at the Ohio State University (OSU) Medical Center.

Participants and procedures

The Institutional Review Board granted ethical approval for the study with an accrual goal of 154 patients. Informed written consent was obtained from 152 patients with relapsed/refractory CLL with accrual occurring from May 2012 to July 2014. Descriptive data are displayed in Table 1. In the clinic on day 1 of initial treatment, patients independently completed a self-report measure of stress (Impact of Event; IES) and provided descriptive information and blood was drawn.

Funding was sought to conduct immune assays. Of the 152 patients, 144 patients had adequate samples. Patients within sex were rank ordered from highest to lowest on his/her stress score (IES) with every other subject selected for the first batch of cytokine/chemokine assays (n=72). Later funding provided for a second batch where 24 patients with the highest stress scores were selected. A total of 96 patient samples were available for the cytokine/chemokine assays.

Measures

Stress

The Impact of Event Scale-Revised (IES)[34, 36] assesses stress reactions in the form of intrusive thoughts (8 items), avoidant thoughts or behaviors (8 items), and hyperarousal (6 items) with items tailored to read “cancer stress” or “cancer”. Previous research has found the IES to covary with immunity in breast cancer patients[35]. Patients rate the frequency of feelings or events in the past week on a scale ranging from 0 (not at all) to 4 (extremely) and all items are summed. Possible scores range from 0 to 88 with higher scores indicative of greater stress. Coefficient alpha reliability was 0.89.

Immune

Absolute Lymphocyte Count (ALC)—Nursing staff collected 6-mL potassium EDTA [lavender-top vacutainer] peripheral blood. Complete blood cell counts with ALC were quantified by the hospital laboratories.

Cytokine and chemokine—Plasma samples were centrifuged and aliquoted into 4 samples and frozen at −80C. Later, samples were thawed, batched by participant, and spun to remove debris. Assessment of plasma levels of cytokines (TNFα, APRIL, BAFF, IL-6, IL-10, and IL-16, VEGF) and chemokine CCL3, were quantified using individual enzyme-linked immunosorbent assays (ELISA) in triplicate per manufacturer’s specifications for each of the cytokines and chemokines (R&D Systems, Minneapolis, MN). Detection limits were 1.6 pg/mL for TNFα, 7.1 pg/mL for APRIL, 2.7 ng/mL for BAFF, 0.7 pg/mL for IL-6,

3.9 pg/mL for IL-10, 6.2 pg/mL for IL-16, 9.0 pg/mL for VEGF, and 10.0 pg/mL for CCL3. The inter- and intra-assay coefficients of variation for all cytokines tested were 6.26% and 14.29%, respectively.

Covariates

Older age, male sex, presence of multiple comorbidities, number of prior treatments, and presence of del17p were included as covariates as these factors are predictive of poorer outcomes in CLL[37-41]. Four areas were considered. 1) Demographic: age and gender. 2) Comorbidities: The Charlson Comorbidity Index (CCI)[42] is a measure of risk of death from current diseases or conditions. It has 19 items (e.g. liver disease, diabetes mellitus), each weighted from 1 to 6 based on severity and mortality risk, which are summed for a total score. All patients received 2 points for having cancer; a score > 2 indicates the presence of comorbid conditions. 3) CLL severity and risk: Number of prior therapies and genetic risk (presence of del17p). 4) Behavioral correlates of inflammation[43] were also considered: body mass index (BMI) and smoking status.

Analytic Plan

Preliminary analyses contrast patients in the cytokine/chemokine assay subgroup (n=96) and those not (n=56) on descriptive characteristics, covariates, and stress (IES), using independent-sample t-tests for continuous variables and chi-square tests for categorical variables. For cytokine and chemokine values below detectable limits, the MICE package[44] for statistical software R was used to generate random values between 0 and the minimum detectable level to increase variance. Multiple imputation has been previously used to impute cytokine and chemokine values[45]. Spearman correlations between each covariate and outcome variable were examined and only covariates associated ($p < 0.10$) with an outcome were included in analyses.

Analyses used multiple linear regressions (MLRs) to test the concurrent relationship between stress and ALC, cytokines, and chemokine responses following the entry of relevant covariates. For each outcome, step 1 included only the associated covariates as predictors while step 2 included associated covariates with the addition of IES as predictors of cellular, cytokine, and chemokine responses. The increment in the squared multiple correlation (R^2) from step 1 to step 2 provided variance attributable to stress beyond the covariates. In addition, standardized regression betas (β) in step 2 indicated the magnitude and direction of the influence of stress on outcomes. General assumptions of linear regression including multicollinearity, variance of errors, and normality of error distributions were examined. If positively skewed, variables were log transformed.

Results

Preliminary

Minimum detectable levels were imputed using the MICE package for TNFα (5 patients), APRIL (6 patients), and IL-10 (2 patients). Patients with (n=96) and without (n=56) cytokine/chemokine data did not differ (ps ≥.18) on any demographic, general health, CLL

risk, or behavioral correlates of inflammation variables. Patients with cytokine/chemokine data reported greater IES scores, [t(150) = 3.04, p<0.01].

Intercorrelations between covariates and ALC, cytokine, and chemokine markers were inspected. No outcome covaried with current smoking status. For the remainder, the following p<.10 associations were used in MLR analyses: ALC (age: ρ=.232), TNFα (prior therapies: ρ=.224, gender: ρ=−.190), APRIL (prior therapies: ρ=.218, gender: ρ=−.194), BAFF (CCI: ρ=−.203,), IL-6 (prior therapies: ρ=.338, gender: ρ=.192, del17p: ρ=−.177), IL-16 (gender: ρ=−.269, prior therapies: ρ=.229, BMI: ρ=−.200, del17p: ρ=.178),VEGF (prior therapies: ρ=−.271), CCL3 (gender: ρ=−.216, BMI: ρ=−.199). IL-10 was not associated with any covariates.

Primary

Results of MLRs are displayed in Table 2. Results indicated stress was a significant predictor of higher ALC (β=.208, p=.037) (Figure 1). Additionally, the R^2 change between steps 1 and 2 indicated that stress accounted for 4.3% of the variance in ALC, controlling for age. Models examining cytokines and chemokines showed stress to be a significant predictor of TNFα (β=.251, p=.016) and IL-16 (β=.413, p<.001), and CCL3 (β=.230, p=.027) such that higher levels of stress were associated with increased levels of these three outcomes (Figure 1). The R^2 change indicated stress accounted for 6.3%, 17.0%, and 5.2% of the variance in TNFα, IL-16, and CCL3, respectively. Analyses with stress and IL-6, IL-10, and VEGF were not significant (ps>.17).

Discussion

Novel data from patients with relapsed/refractory CLL show increased stress is associated with multiple poor prognosis CLL biomarkers. Heightened stress covaried with higher ALC, a salient marker of CLL burden, along with TNFα and IL-16. In the biobehavioral literature there are few studies of stress and chemokines[46, 47] and none of CCL3, which also covaried with stress. Effects were observed with outcome-specific controls for age, gender, BMI, comorbid illnesses, prior treatments, and/or CLL genetic risk.

Meta analyses of studies with adults experiencing general stressors, show heightened stress to covary with increases in lymphocyte numbers[48, 49], whereas meta-analyses of stress from acute stressors show a lowering of lymphocyte numbers[50]. Both human lymphocyte data and leukemic animal paradigms[51] illustrate the adverse effects of chronic stress, an effect that may be especially pernicious in CLL.

The association between stress and TNFα is particularly important in CLL as malignant cells release TNFα spontaneously[15] and TNFα increases their proliferation and viability[52]. The TNFα finding adds to the biobehavioral cancer literature, as most studies have found depression rather than stress to be related to higher levels of TNFα[53-55]. Stress and depression can co-occur in patients; future research may examine the independent and synergistic effects of depression and stress on inflammatory markers such as TNFα[56].

Higher levels of stress were also associated with higher levels of IL-16. It has been suggested that IL-16 may mediate communications between B cells and T cells within lymph node follicles[57, 58] and further, IL-16 may suppresses effector T-cell function[59-61]. To our knowledge, the only other stress/IL-16 data come from a study of a naturalistic stressor (students' academic examinations) in which IL-16 was significantly elevated pre-examination in contrast to post examination[62]. Interestingly, no covariates were needed for the IL-16 analyses and stress accounted for substantial variance (17%) in IL-16 prediction. Future research might explore the pathway of heightened stress, increased IL-16, and suppression of effector T-cell function which may be relevant to autoimmune diseases[63] or other cancers[64] in which Il-16 plays an important role.

While the function of CCL3 is not yet fully clarified[31], it has been hypothesized that increased CCL3 secretion may induce trafficking and homing of T cells to CLL cells in the tissue microenvironments[65]. Moreover, by attracting immune cells for interaction with CLL cells and their microenvironment, CCL3 creates a circumstance in which CLL cells interact with T cells providing survival and proliferation signals[66].

Some cytokines did not covary with stress: VEGF, BAFF, APRIL, IL-6, and IL-10. It is perhaps noteworthy that number of prior therapies was a significant negative predictor for all outcomes, except for IL-10 which was not associated with any covariates. This highlights the general importance of including treatment variables in tests of stress. It also suggests possible limiting factor(s) – the disease, repeated treatments, or some combination – in detecting stress effects on immunity for relapse/refractory CLL patients as manifest in these specific assays. When stress was a significant predictor, number of prior therapies was not correlated (entered) for ALC, IL-16, and CCL3 and was entered but was not significant for TNFα analysis. Data such as these are important for discerning under what specific conditions effects of stress may be detected. While the use of these covariates may account for null findings, results may be specific to CLL as previous research suggests stress is associated with increased levels of inflammatory cytokines in solid tumor cancers. For example, IL-6 may contribute to tumor suppression rather than immune suppression in CLL[67].

The strengths and limitations of the study are considered. The data come from complete accrual to a phase II trial of an experimental therapy, an important study context for biobehavioral studies. When both effectiveness and toxicities are unclear, insights into psychological or behavioral factors and their interaction with disease-specific biomarkers add to the overall contribution of a phase II trial. Internal validity was strong, but as a single-site trial at a comprehensive cancer center, patients generally have more resources, are younger, and are less diverse[68, 69]. Low incidence blood cancers tend to receive treatment at regional centers, making the sample geographically diverse (patients traveled over 300 miles on average to receive treatment). Limited funds prevented analysis of all trial patients (N=152) but a sample of 96 was comparatively large for an exploratory study[70]. The latter sample differed from the remainder only in terms of having a higher mean level of stress (IES) which was anticipated because of oversampling patients with higher stress for the second batch of assays. Of the covariate candidates, age, gender, and number of prior therapies were important, while del17p, comorbidity, and BMI played little, if any role in

analyses, providing knowledge relevant to future studies. The cytokine/chemokine outcomes were chosen for their relevance to CLL, but there are others important for study[11], such as those for the T cell compartment (CD8+, CD57), NK cells, and response to Bruton's tyrosine kinase (BTK) inhibition. Lastly, as an exploratory study, findings are not adjusted for multiple outcomes in parallel analyses.

Regarding the clinical contribution, other studies using the IES to assess cancer stress find higher stress covaries with higher levels of CLL signs/symptoms (e.g., fatigue, enlarged nodes, infections, and others)[71]. Stress is also an important individual difference variables that is predictive of future quality of life[71] and is a moderator of intervention effectiveness. Patients with moderate to severe stress, anxiety, and/or depressive symptoms are in need of early, evidence based, psychological treatment to address current difficulties and lower the likelihood of poorer quality of life which may otherwise follow[73]. Selected intervention trials have resulted in increased T cell immunity[74], greater production of Th1 cytokines (IL-2, IL-12, IFN–γ)[75, 76], and decreases in Th_2 cytokines (IL-4, IL-10)[76].

In conclusion, novel biobehavioral data for relapsed/refractory CLL patients show psychological stress is related to heightened levels of cellular, cytokine, and chemokine markers that are associated with progressive disease. Understanding the association between psychological stress and host factors, particularly immunity, is needed. Although previous research has examined stress associations with animal models[51], this is the first biobehavioral study to show stress to covary with four key biomarkers in patients with CLL. Data are consistent with the hypothesis that stress may be a negative interface to an already weakened immune system. Replication and longitudinal data will be needed to clarify the trajectories of these responses and any relevance to CLL relapse.

Acknowledgments

Research support: This research was supported by the National Cancer Institute K05 CA098133 and R25 CA122061, Pharmacyclics, a Pelotonia Idea Award from the Ohio State University Comprehensive Cancer Center.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA-Cancer J Clin. 2016; 66(1):7–30. [PubMed: 26742998]
2. Nabhan C, Rosen ST. Chronic lymphocytic leukemia: a clinical review. JAMA. 2014; 312(21): 2265–76. [PubMed: 25461996]
3. Byrd JC, Furman RR, Coutre SE, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med. 2013; 369(1):32–42. [PubMed: 23782158]
4. Reiche EM, Nunes SO, Morimoto HK. Stress, depression, the immune system, and cancer. Lancet Oncol. 2004; 5(10):617–25. [PubMed: 15465465]
5. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008; 9(1):46–56. [PubMed: 18073775]
6. Brebner K, Hayley S, Zacharko R, Merali Z, Anisman H. Synergistic effects of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha: central monoamine, corticosterone, and behavioral variations. Neuropsychopharmacol. 2000; 22(6):566–80.
7. Raison CL, Miller AH. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. Am J Psychiatry. 2003; 160(9):1554–65. [PubMed: 12944327]

8. Andersen BL, Yang HC, Farrar WB, et al. Psychologic intervention improves survival for breast cancer patients: a randomized clinical trial. Cancer. 2008; 113(12):3450–8. [PubMed: 19016270]
9. Thornton LM, Andersen BL, Schuler TA, Carson WE 3rd. A psychological intervention reduces inflammatory markers by alleviating depressive symptoms: secondary analysis of a randomized controlled trial. Psychosom Med. 2009; 71(7):715–24. [PubMed: 19622708]
10. Dhodapkar MV, Dhodapkar KM. Immune modulation in hematologic malignancies. Semin Oncol. 2015; 42(4):617–25. [PubMed: 26320065]
11. Burger JA, Gribben JG. The microenvironment in chronic lymphocytic leukemia (CLL) and other B cell malignancies: Insight into disease biology and new targeted therapies. Semin Cancer Biol. 2014; 24:71–81. [PubMed: 24018164]
12. Herishanu Y, Katz BZ, Lipsky A, Wiestner A. Biology of chronic lymphocytic leukemia in different microenvironments: clinical and therapeutic implications. Hematol Oncol Clin North Am. 2013; 27(2):173–206. [PubMed: 23561469]
13. Burger JA, Chiorazzi N. B cell receptor signaling in chronic lymphocytic leukemia. Trends Immunol. 2013; 34(12):592–601. [PubMed: 23928062]
14. Kipps TJ, Stevenson FK, Wu CJ, et al. Chronic lymphocytic leukaemia. Nat Rev Dis Primers. 2017; 3:17008. [PubMed: 28179635]
15. Foa R, Massaia M, Cardona S, et al. Production of tumor necrosis factor-alpha by B-cell chronic lymphocytic leukemia cells: a possible regulatory role of TNF in the progression of the disease. Blood. 1990; 76(2):393–400. [PubMed: 2114936]
16. Fayad L, Keating MJ, Reuben JM, et al. Interleukin-6 and interleukin-10 levels in chronic lymphocytic leukemia: correlation with phenotypic characteristics and outcome. Blood. 2001; 97(1):256–63. [PubMed: 11133769]
17. Lech-Maranda E, Grzybowska-Izydorczyk O, Wyka K, et al. Serum tumor necrosis factor-alpha and interleukin-10 levels as markers to predict outcome of patients with chronic lymphocytic leukemia in different risk groups defined by the IGHV mutation status. Arch Immunol Ther Exp (Warsz). 2012; 60(6):477–86. [PubMed: 22945689]
18. Digel W, Stefanic M, Schoniger W, et al. Tumor necrosis factor induces proliferation of neoplastic B cells from chronic lymphocytic leukemia. Blood. 1989; 73(5):1242–6. [PubMed: 2539214]
19. Haiat S, Billard C, Quiney C, Ajchenbaum-Cymbalista F, Kolb JP. Role of BAFF and APRIL in human B-cell chronic lymphocytic leukaemia. Immunology. 2006; 118(3):281–92. [PubMed: 16827889]
20. Cols M, Barra CM, He B, et al. Stromal endothelial cells establish a bidirectional crosstalk with chronic lymphocytic leukemia cells through the TNF-related factors BAFF, APRIL, and CD40L. J Immunol. 2012; 188(12):6071–83. [PubMed: 22593611]
21. Bojarska-Junak A, Hus I, Chocholska S, et al. BAFF and APRIL expression in B-cell chronic lymphocytic leukemia: correlation with biological and clinical features. Leuk Res. 2009; 33(10): 1319–27. [PubMed: 19395025]
22. Letilovic T, Vrhovac R, Verstovsek S, Jaksic B, Ferrajoli A. Role of angiogenesis in chronic lymphocytic leukemia. Cancer. 2006; 107(5):925–34. [PubMed: 16832815]
23. DiLillo DJ, Weinberg JB, Yoshizaki A, et al. Chronic lymphocytic leukemia and regulatory B cells share IL-10 competence and immunosuppressive function. Leukemia. 2013; 27(1):170–82. [PubMed: 22713648]
24. Alhakeem SS, Sindhava VJ, McKenna MK, et al. Role of B cell receptor signaling in IL-10 production by normal and malignant B-1 cells. Ann N Y Acad Sci. 2015; 1362:239–49. [PubMed: 26096907]
25. Zhao S, Wu D, Wu P, Wang Z, Huang J. Serum IL-10 predicts worse outcome in cancer patients: a meta-analysis. PLoS One. 2015; 10(10):e0139598. [PubMed: 26440936]
26. Atanackovic D, Hildebrandt Y, Templin J, et al. Role of interleukin 16 in multiple myeloma. J Natl Cancer Inst. 2012; 104(13):1005–20. [PubMed: 22745469]
27. Aguirre Palma LM, Flamme H, Gerke I, Kreuzer KA. Angiopoietins modulate survival, migration, and the components of the Ang-Tie2 pathway of chronic lymphocytic leukaemia (CLL) cells in vitro. Cancer Microenviron. 2016; 9(1):13–26. [PubMed: 26846110]

28. Piechnik A, Dmoszynska A, Omiotek M, et al. The VEGF receptor, neuropilin-1, represents a promising novel target for chronic lymphocytic leukemia patients. Int J Cancer. 2013; 133(6): 1489–96. [PubMed: 23447383]
29. Molica S, Vacca A, Ribatti D, et al. Prognostic value of enhanced bone marrow angiogenesis in early B-cell chronic lymphocytic leukemia. Blood. 2002; 100(9):3344–51. [PubMed: 12384436]
30. Choi MY, Kashyap MK, Kumar D. The chronic lymphocytic leukemia microenvironment: Beyond the B-cell receptor. Best Pract Res Cl Ha. 2016; 29(1):40–53.
31. Sivina M, Hartmann E, Kipps TJ, et al. CCL3 (MIP-1alpha) plasma levels and the risk for disease progression in chronic lymphocytic leukemia. Blood. 2011; 117(5):1662–9. [PubMed: 21115978]
32. Levin TT, Li Y, Riskind J, Rai K. Depression, anxiety and quality of life in a chronic lymphocytic leukemia cohort. Gen Hosp Psychiatry. 2007; 29(3):251–6. [PubMed: 17484943]
33. Pashos CL, Flowers CR, Kay NE, et al. Association of health-related quality of life with gender in patients with B-cell chronic lymphocytic leukemia. Support Care Cancer. 2013; 21(10):2853–60. [PubMed: 23748484]
34. Horowitz M, Wilner N, Alvarez W. Impact of Event Scale: A measure of subjective stress. Psychosom Med. 1979; 41(3):209–18. [PubMed: 472086]
35. Andersen BL, Farrar WB, Golden-Kreutz D, et al. Stress and immune responses after surgical treatment for regional breast cancer. J Natl Cancer Inst. 1998; 90(1):30–6. [PubMed: 9428780]
36. Weiss D, Marmar C. The Impact of Event Scale Revised. In: Wilson JP, Keane TM, editorsAssessing Psychological Trauma and PTSD. New York: The Guilford Press; 1997.
37. Baumann T, Delgado J, Santacruz R, et al. Chronic lymphocytic leukemia in the elderly: clinico-biological features, outcomes, and proposal of a prognostic model. Haematologica. 2014; 99(10): 1599–604. [PubMed: 24972773]
38. Catovsky D, Wade R, Else M. The clinical significance of patients' sex in chronic lymphocytic leukemia. Haematologica. 2014; 99(6):1088–94. [PubMed: 24658818]
39. Goede V, Cramer P, Busch R, et al. Interactions between comorbidity and treatment of chronic lymphocytic leukemia: results of German Chronic Lymphocytic Leukemia Study Group trials. Haematologica. 2014; 99(6):1095–100. [PubMed: 24584349]
40. Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000; 343(26):1910–6. [PubMed: 11136261]
41. Cramer P, Hallek M. Prognostic factors in chronic lymphocytic leukemia-what do we need to know? Nat Rev Clin Oncol. 2011; 8(1):38–47. [PubMed: 20956983]
42. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987; 40(5):373–83. [PubMed: 3558716]
43. O'Connor MF, Bower JE, Cho HJ, et al. To assess, to control, to exclude: effects of biobehavioral factors on circulating inflammatory markers. Brain Behav Immun. 2009; 23(7):887–97. [PubMed: 19389469]
44. Zhang Z. Multiple imputation with multivariate imputation by chained equation (MICE) package. Ann Transl Med. 2016; 4(2):30. [PubMed: 26889483]
45. Whitcomb BW, Mumford SL, Perkins NJ, et al. Urinary cytokine and chemokine profiles across the menstrual cycle in healthy reproductive-aged women. Fertil Steril. 2014; 101(5):1383–91. [PubMed: 24581581]
46. Carlsson E, Frostell A, Ludvigsson J, Faresjo M. Psychological stress in children may alter the immune response. J Immunol. 2014; 192(5):2071–81. [PubMed: 24501202]
47. Richtig E, Trapp EM, Avian A, et al. Psychological stress and immunological modulations in early-stage melanoma patients. Acta Derm Venereol. 2015; 95(6):691–5. [PubMed: 25587794]
48. Denson TF, Spanovic M, Miller N. Cognitive appraisals and emotions predict cortisol and immune responses: a meta-analysis of acute laboratory social stressors and emotion inductions. Psychol Bull. 2009; 135(6):823–53. [PubMed: 19883137]
49. Zorrilla EP, Luborsky L, McKay JR, et al. The relationship of depression and stressors to immunological assays: A meta-analytic review. Brain Behav Immun. 2001; 15(3):199–226. [PubMed: 11566046]

50. Segerstrom SC, Miller GE. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychol Bull. 2004; 130(4):601–30. [PubMed: 15250815]
51. Lamkin DM, Sloan EK, Patel AJ, et al. Chronic stress enhances progression of acute lymphoblastic leukemia via beta-adrenergic signaling. Brain Behav Immun. 2012; 26(4):635–41. [PubMed: 22306453]
52. Primo K, Compas BE, Oppedisano G, Howell DC, Epping-Jordan JE, Krag DN. Intrusive thoughts and avoidance in breast cancer: Individual differences and association with psychological distress. Psychol Health. 2000; 14(6):1141–53. [PubMed: 22175267]
53. Dowlati Y, Herrmann N, Swardfager W, et al. A meta-analysis of cytokines in major depression. Biol Psychiatry. 2010; 67(5):446–57. [PubMed: 20015486]
54. Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom Med. 2009; 71(2):171–86. [PubMed: 19188531]
55. Liu Y, Ho RC, Mak A. Interleukin (IL)-6, tumour necrosis factor alpha (TNF-alpha) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. J Affect Disord. 2012; 139(3):230–9. [PubMed: 21872339]
56. Jaremka LM, Lindgren ME, Kiecolt-Glaser JK. Synergistic relationships among stress, depression, and troubled relationships: insights from psychoneuroimmunology. Depress Anxiety. 2013; 30(4): 288–96. [PubMed: 23412999]
57. Kaser A, Dunzendorfer S, Offner FA, et al. B lymphocyte-derived IL-16 attracts dendritic cells and Th cells. J Immunol. 2000; 165(5):2474–80. [PubMed: 10946273]
58. Kramer MF, Mack B, Rasp G. Immunohistological expression of interleukin 16 in human tonsils. Arch Otolaryngol Head Neck Surg. 2001; 127(9):1120–5. [PubMed: 11556864]
59. Matsumoto Y, Fujita T, Hirai I, et al. Immunosuppressive effect on T cell activation by interleukin-16- and interleukin-10-cDNA-double-transfected human squamous cell line. Burns. 2009; 35(3):383–9. [PubMed: 18950955]
60. Newell MK, Haughn LJ, Maroun CR, Julius MH. Death of mature T cells by separate ligation of CD4 and the T-cell receptor for antigen. Nature. 1990; 347(6290):286–9. [PubMed: 2144894]
61. Cruikshank WW, Lim K, Theodore AC, et al. IL-16 inhibition of CD3-dependent lymphocyte activation and proliferation. J Immunol. 1996; 157(12):5240–8. [PubMed: 8955168]
62. Katsuura S, Kamezaki Y, Yamagishi N, et al. Circulating vascular endothelial growth factor is independently and negatively associated with trait anxiety and depressive mood in healthy Japanese university students. Int J Psychophysiol. 2011; 81(1):38–43. [PubMed: 21545819]
63. Skundric DS, Cruikshank WW, Montgomery PC, Lisak RP, Tse HY. Emerging role of IL-16 in cytokine-mediated regulation of multiple sclerosis. Cytokine. 2015; 75(2):234–48. [PubMed: 25703787]
64. Richmond J, Tuzova M, Cruikshank W, Center D. Regulation of cellular processes by interleukin-16 in homeostasis and cancer. J Cell Physiol. 2014; 229(2):139–47. [PubMed: 23893766]
65. Burger JA, Ghia P, Rosenwald A, Caligaris-Cappio F. The microenvironment in mature B-cell malignancies: a target for new treatment strategies. Blood. 2009; 114(16):3367–75. [PubMed: 19636060]
66. Burger JA. Chemokines and chemokine receptors in chronic lymphocytic leukemia (CLL): from understanding the basics towards therapeutic targeting. Semin Cancer Biol. 2010; 20(6):424–30. [PubMed: 20883788]
67. Li Y, Shi Y, McCaw L, et al. Microenvironmental interleukin-6 suppresses toll-like receptor signaling in human leukemia cells through miR-17/19A. Blood. 2015; 126(6):766–78. [PubMed: 26041742]
68. Eichhorst B, Goede V, Hallek M. Treatment of elderly patients with chronic lymphocytic leukemia. Leuk Lymphoma. 2009; 50(2):171–8. [PubMed: 19197731]
69. Molica S. Sex differences in incidence and outcome of chronic lymphocytic leukemia patients. Leuk Lymphoma. 2006; 47(8):1477–80. [PubMed: 16966256]
70. Lutgendorf SK, Andersen BL. Biobehavioral approaches to cancer progression and survival: mechanisms and interventions. Am Psychol. 2015; 70(2):186–97. [PubMed: 25730724]

71. Morrison EJ, Flynn JM, Jones J, Byrd JC, Andersen BL. Individual differences in physical symptom burden and psychological responses in individuals with chronic lymphocytic leukemia. Ann Hematol. 2016; 95(12):1989–1997. [PubMed: 27539615]

72. Pulgar A, Alcala A, Reyes Del Paso GA. Psychosocial predictors of quality of life in hematological cancer. Behav Med. 2015; 41(1):1–8. [PubMed: 24965511]

73. Mantegna G, Petrillo M, Fuoco G, et al. Long-term prospective longitudinal evaluation of emotional distress and quality of life in cervical cancer patients who remained disease-free 2-years from diagnosis. BMC Cancer. 2013; 13:127. [PubMed: 23506235]

74. McGregor BA, Antoni MH. Psychological intervention and health outcomes among women treated for breast cancer: A review of stress pathways and biological mediators. Brain Behav Immun. 2009; 23(2):159–66. [PubMed: 18778768]

75. Antoni MH, Lechner S, Diaz A, et al. Cognitive behavioral stress management effects on psychosocial and physiological adaptation in women undergoing treatment for breast cancer. Brain Behav Immun. 2009; 23:580–91. [PubMed: 18835434]

76. Witek-Janusek L, Albuquerque K, Chroniak KR, Chroniak C, Durazo-Arvizu R, Mathews HL. Effect of mindfulness based stress reduction on immune function, quality of life and coping in women newly diagnosed with early stage breast cancer. Brain Behav Immun. 2008; 22:969–81. [PubMed: 18359186]

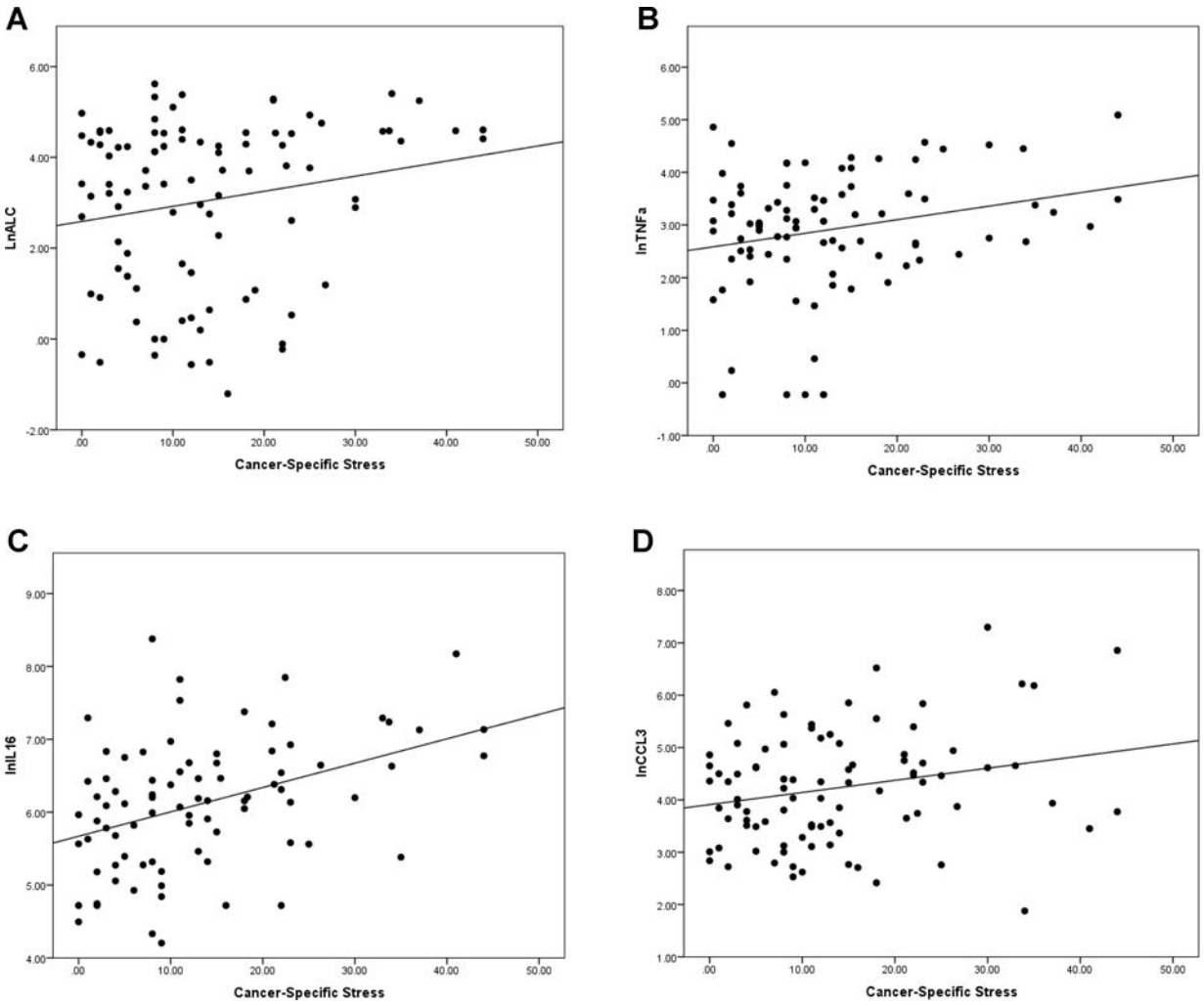

Figure 1.

Scatterplot of cancer-specific stress scores from CLL patients (N=92) and A) absolute lymphocyte counts B) TNFα C) IL-16, D) CCL3 at treatment initiation with line of best fit, in natural log.

Table 1

Sample characteristics: Demographic, general health, CLL risk, behavioral correlates of inflammation, and stress scores for trial patients (N=152) and subset with immune data (N=96)

Characteristic	Trial patients (n=152)		Patients with cytokine/chemokine data (n=96)	
Demographic	**M/SD**	**N (%)**	**M/SD**	**N (%)**
Age (in years), *M* (SD)	64.1 (10.80)		64.2 (10.40)	
Gender (Male)		107 (71%)		64 (67%)
Married/Partnered (Yes)		131 (86%)		83 (87%)
Distance from cancer center (in miles)	341.44 (438.05)		331.55 (422.58)	
Race				
Caucasian		147 (97%)		95 (99%)
African-American		5 (3%)		1 (1%)
Education				
High School/Technical School or Below		44 (29%)		18 (19%)
Some College/College Graduate		58 (38%)		44 (46%)
Some Graduate School/Graduate Degree		46 (30%)		32 (33%)
Missing		4 (3%)		2 (2%)
Household income (K)				
≤ 50		41 (27%)		29 (30%)
51-100		38 (25%)		23 (24%)
>100		46 (30%)		43 (45%)
Prefers Not to Answer		24 (16%)		10 (10%)
Missing		3 (2%)		2 (2%)
General Health				
Charlson Comorbidity Index (unadjusted)		2.5 (1.0)	2.49 (0.82)	
CLL Risk				
Number of prior therapies	3.5 (2.6)		3.27 (2.43)	
Del17p		78 (51%)		54 (56%)
Behavioral Correlates of Inflammation				
Body Mass Index	26.5 (4.8)		26.13 (5.10)	
Smoking Status (Current Smoker)		8 (5%)		5 (5%)
Stress (IES)	11.61 (10.55)		13.55 (10.62)	

Abbreviations: Del17p = presence of deletion of 17p; IES = Impact of Event Scale

Table 2

Results of multiple linear regression analyses with standardized betas. Step 1 enters covariates associated ($p < 0.10$) with outcomes, step 2 enters cancer-specific stress to predict log-transformed cellular, cytokine, and chemokine markers.

	Cellular			**Cytokine**					**Chemokine**
Step 1	ALC (n=96)	TNFα (n=88)	APRIL (n=94)	BAFF (n=95)	IL-6 (n=95)	IL-10 (n=74)	IL16 (n=86)	VEGF (n=96)	CCL3 (n=92)
Age	.253*	–	–	–	–	–	–	–	–
Gender	–	−.221*	−.168	−.152	.178	–	–	–	−.125
Number of prior therapies	–	.151	.267**	.264*	.321**	–	–	−.262*	–
Del17p	–	–	–	–	−.078	–	.150	–	–
CCI	–	–	–	−.016		–	–	–	
BMI	–	–	–	−.041		–	–	–	−.117
Adjusted R^2	.054	.073	.081	.066	.121	–	.011	.059	.019
Step 2									
Age	.262*	–	–	–		–	–	–	–
Gender	–	−.218*	−.171	−.160	.177	–	–	–	−.107
Number of prior therapies	–	.167	.263*	.256*	.320**	–	–	−.258*	–
Del17p	–	–	–	–	−.078	–	.173	–	–
CCI	–		–	−.018	–	–	–	–	.
BMI	–	–	–	−.034	–	–	–	–	−.126
Stress (IES)	.208*	.251*	−.049	−.119	−.008	.215	.413**	.051	.230*
Adjusted R^2	.088	.105	.074	.071	.111	.033	.173	.052	.062
R^2 Change	.043*	.063*	.002	.014	.000	–	.170**	.003	.052*

Note.

** = p <.01,

* = p <.05,

dashes indicate covariates that were not correlated (p>.01) with each outcome variable

Abbreviations: Del17p = presence of deletion of 17p; CCI = Charleson Comorbidity Index; BMI = Body Mass Index; IES = Impact of Event Scale