RESEARCH LETTER

Relation between serum interleukin 33 concentration, depressive symptoms, and sleep quality in inflammatory bowel disease

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Introduction Interleukin 33 (IL-33) is a pro-inflammatory cytokine belonging to the IL-1 family.[1] IL-33 receptor, suppression of tumorigenicity 2 (ST2), exists in 2 forms, a membrane-bound and a soluble one (sST2).[1] The latter sequestrates circulating IL-33, without exerting any biological effect.[1] The membrane-bound ST2, on the other hand, upon binding IL-33, forms a heterodimer with IL-1 receptor accessory protein and activates MyD88/NF-κB signaling pathway, modulating the immune response by stimulating the production of other proinflammatory cytokines by Th2 lymphocytes and type 2 innate lymphoid cells (ILC2).[1] Moreover, IL-33 was found to influence T regulatory cells, CD8+ T cells, and Th1 cells.[1] IL-33 can also regulate gene transcription; it contains a helix-turn-helix motif that can bind to heterochromatin.[1] IL-33 acts as an alarmin, meaning that it is released in an instance of cell death or injury and evokes inflammatory reactions.[1] Dysregulations in IL-33/ST2 signaling have been implicated in the pathophysiology of many immune-mediated diseases including inflammatory bowel diseases (IBDs).[1] IBD mainly encompasses 2 chronic inflammatory conditions: Crohn disease (CD) and ulcerative colitis (UC). These conditions differ in multiple clinical aspects, nevertheless, targeting inflammation with biologic drugs, such as anti–tumor necrosis factor (TNF) agents, which have the potential to hinder IL-33 synthesis, yields good results.[2-5] Pathophysiologic backgrounds of CD and UC might overlap to some extent, but it seems that UC is related to Th2 polarization, while in CD the cytokine profile is rather related to the Th1 and Th17 polarization.[1] The exact mechanisms by which IL-33 can influence the disease course have not yet been fully elucidated. IL-33/ST2 signaling might aid in the maintenance of the intestinal epithelium by promoting stem cell differentiation into secretory cells.[1] IL-33 also appears to play an important role in neuroimmune interactions in the intestines.[1] In a study on neurospheres,[1] IL-33 was shown to activate glial cells and induce the production of glial-derived neurotrophic factor family ligands via Myd88-dependent pathway.[1] This cytokine might also exert a damaging influence on the gut; IL-33 can promote the production of Th9 cells, which could advance the development of colitis by impairing tissue repair, barrier integrity, etc.[1] In studies on a murine colitis model, the animals with hindered IL-33/ST2 signaling suffered less damage than the wild-type controls.[1]

The role of immune response in psychiatric conditions is an active area of research. Affective disorders in particular tend to accompany chronic inflammatory diseases, such as IBD. It seems that IL-33 is consistently increased in patients with major depressive disorders, but studies regarding bipolar disorder are incongruent.[6] Inflammation and oxidative stress might be the interface between inflammatory diseases and poor mental health.[6] Moreover, in a murine model, an increase in IL-33 caused by lipopolysaccharide injection into the amygdala induced anxious behavior by repressing brain-derived neurotrophic factor (BDNF) production via NF-κB pathway.[7] IL-33 has not been studied in relation to sleep disturbances other than sleep apnea; nevertheless, considering that sleep disorders, such as insomnia, are comorbid with chronic inflammatory and psychiatric diseases, investigating this subject might elucidate some aspects of this relationship.

The aim of the study was to compare serum IL-33 concentrations of IBD patients and healthy

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controls (HCs) as well as to evaluate the association between the protein level, depression, sleep quality, and insomnia.

Patients and methods

Recruitment and patient eligibility Recruitment of participants (n = 125) was conducted at the Department of Digestive Tract Diseases (Medical University of Lodz, Poland). HCs (n = 44) were selected based on their similarity to the IBD group (n = 81) regarding demographic variables.

Out of all participants (n = 125, 44 HC, 81 IBD), only 52 individuals (22 HC, 30 IBD) were included in the analysis, as in 73 patients (22 HC, 51 IBD) serum IL-33 level was below the detection threshold.

Inclusion criteria involved age between 18 and 65 years, signing the informed consent, and CD or UC diagnosis based on endoscopic, clinical, and histopathologic criteria. Exclusion criteria comprised other chronic inflammatory diseases, psychotic disorders, substance abuse, abdominal surgery in the last 6 months, and malignant neoplasm except for basal cell carcinoma.

Material collection Questionnaires assessing the IBD severity, depression, sleep quality, insomnia symptoms, and excessive daytime sleepiness, as well as venous blood, were collected from all participants within the same time interval, that is, between 9.00 and 11.00 AM.

Assessment of disease severity and psychological variables Disease severity (active / inactive) was determined based on clinical findings, that is, the Harvey–Bradshaw Index (HBI) and the partial Mayo Score (PMS) in CD and UC, respectively.

The HBI evaluates the following aspects: intensity of abdominal pain, number of liquid or soft stools the day before the examination, patient's well-being, and complications such as arthralgia.[8] CD participants who did not receive steroid therapy and scored below 5 points were considered remitted.[8] The PMS assesses 3 domains, such as rectal bleeding, global assessment performed by a physician, and stool frequency.[9] The PMS score below 2 points qualified an individual for the UC remission group.[9] For standardization, the same person evaluated both scales in all participants.

The participants were asked to fill out questionnaires regarding their insomnia and depression symptoms, sleep quality, and excessive daytime sleepiness.

The Beck Depression Inventory (BDI) is an inventory evaluating depression symptoms, commonly used as a screening tool for this disorder.[10] It evaluates 21 items, each ranked from 0 to 3 points. A standard cutoff is set at 11 points. The Epworth sleepiness scale (ESS) evaluates the excessive daytime sleepiness.[11] The severity of this symptom is estimated by subjective assessment of the probability of falling asleep in 8 situations from daily life, each rated on a scale from 0 to 3 points. A score above 10 is suggestive of this symptom. The Athens Insomnia Scale (AIS) is an 8-item multiple-choice inventory used in diagnosing insomnia.[12,13] Each domain is awarded 0–3 points, and a score above 5 suggests the presence of insomnia. The Pittsburgh Sleep Quality Index (PSQI) is a 19-item questionnaire used in the evaluation of the subjective sleep quality.[14] A score above 5 indicates poor sleep quality.

Evaluation of serum protein concentration Serum concentration of IL-33 was evaluated with a kit based on the Luminex xMAP technology (Human IL-33 High Sensitivity Magnetic Luminex Performance Assay, R&D Systems, Austin, Texas, United States). Magnetic beads coated with IL-33 antibodies were added to the wells with standards and samples. After the first washing, IL-33–specific biotinylated antibodies were added. During the second washing, unbound antibodies were removed from the wells. Streptavidin-phycoerythrin (PE) conjugate was added to detect biotinylated antibodies. In the next step, free streptavidin-PE conjugate was washed away, and a buffer was added to the wells to suspend the magnetic beads. Fluorescence measurements were taken using Luminex MAGPIX analyzer (Luminex, Austin, Texas, United States). The IL-33 concentration was determined from the standard curve. The reference range for this parameter was 4.88–20 000 pg/ml. The laboratory tests were run in the Research Laboratory CoreLab, Medical University of Lodz.

Statistical analysis Statistical analysis was performed using Statistica 13.3 PL package (StatSoft, Tulsa, Oklahoma, United States). A value of P below 0.05 was considered significant. The distribution of continuous variables (normal vs non-normal) was assessed using the Shapiro–Wilk test. Due to non-normal distribution, data were presented as medians with interquartile ranges (IQRs). The differences between independent groups with non-normal distribution were analyzed with the Mann–Whitney test. The Wilcoxon test was used for repeated measures analysis of differences between dependent groups with distribution other than normal. Correlations were calculated with the Spearman correlation test. Qualitative data were evaluated with the χ^2 or the Fisher exact test, for groups above 10 individuals or smaller, respectively.

Ethics The Bioethical Committee of the Medical University of Lodz, Poland approved the study protocol (KE/1139/20). All participants provided their written informed consent to participate in the study.

Results

The control and the study group, regardless of exacerbation and remission, did not significantly differ in terms of demographic variables,

TABLE 1 Questionnaire scores and serum interleukin 33 concentration in the investigated participants

Variable	IBD (n = 30)	HC (n = 22)	*P* value	CD (n = 16)	UC (n = 14)	*P* value	Exacerbation (n = 17)	Remission (n = 13)	*P* value
Serum IL-33 concentration, pg/ml	26.2 (12.9–130.1)	33 (20.3–82.2)	0.6	23.6 (13.3–132.3)	28.2 (11.7–57.4)	0.92	21 (11.3–43.9)	47.7 (18.9–178.3)	0.11
BDI	5 (1–10)	3 (0–8)	0.18	6 (2–10)	4.5 (2–8)	0.63	8 (1–10)	3 (2–6)	0.28
PSQI	5 (4–7)	5 (2–6)	0.06	5.5 (5–8.5)	5 (4–7)	0.49	5 (5–8)	5 (4–7)	0.27
SE, %	86.6 (80–93)	96 (87.5–97.6)	0.004	87.5 (84.5–90.8)	81.8 (75–93)	0.2	85.7 (81.3–87.5)	87.5 (80–93)	0.93
AIS	5 (4–10)	3.5 (2–7)	0.052	6 (4–9.5)	4.5 (3–10)	0.57	7 (4–10)	4 (3–7)	0.15
ESS	6 (4–8)	4.5 (2–8)	0.26	5.5 (3.5–9)	6 (5–8)	0.71	6 (5–8)	5 (2–8)	0.49

Data are presented as median (interquartile range). P values <0.05 were considered significant.

Abbreviations: AIS, Athens Insomnia Scale; BDI, Beck Depression Inventory; CD, Crohn disease; ESS, Epworth Sleepiness Scale; HC, healthy control; IBD, inflammatory bowel diseases; IL-33, interleukin 33; PSQI, Pittsburgh Sleep Quality Index; SE, sleep efficiency; UC, ulcerative colitis

presence of sleep disorders, or depression (Supplementary material, *Table S1*).

There were no differences in IL-33 concentrations between IBD and HC patients ($P = 0.6$; TABLE 1). Poor sleep quality, symptoms of insomnia, depression, and excessive daytime sleepiness as measured by appropriate questionnaires were also similar in severity (TABLE 1). Sleep efficiency was higher in the HC than the IBD group ($P = 0.004$, TABLE 1). In the IBD, but not in the HC group, there was a positive correlation between IL-33 concentration and BDI, PSQI, and AIS (R = 0.521, $P = 0.003$; R = 0.458, $P = 0.01$; R = 0.4, $P = 0.03$, respectively; Supplementary material, *Table S2* and *Figure S1A–S1C*). On the contrary, sleep efficiency negatively correlated with serum level of IL-33 only in IBD patients (Supplementary material, *Table S2* and *Figure S1D*). Within the IBD group, the individuals who scored high in the ESS or the PSQI (ESS >10, PSQI >5) showed a significantly higher serum IL-33 level than the IBD patients without sleep problems (IL-33 concentration, 53.39 pg/ml [IQR, 3.07–313.02] vs 18.80 pg/ml [IQR, 11.79–26.18]; $P = 0.01$ for PSQI; 270.9 pg/ml [IQR, 156.23–438.68] vs 21.84 pg/ml [IQR, 11.87–47.72]; $P = 0.006$ for ESS).

The disease activity also did not affect the serum IL-33 levels, as no differences were found between the patients in remission and exacerbation ($P = 0.11$, TABLE 1). Severity of depression, insomnia symptoms, excessive daytime sleepiness, sleep efficiency and quality were also comparable between the 2 subgroups (all $P > 0.05$, TABLE 1).

No differences were noted between the CD and the UC group regarding either the IL-33 concentration or the questionnaire scores (TABLE 1). In the CD group, IL-33 positively correlated with the severity of depression symptoms and poor sleep quality, as measured by the BDI and the PSQI scales (R = 0.581, $P = 0.02$; R = 0.531, $P = 0.03$, respectively; Supplementary material, *Table S2*). Fittingly, there was a negative correlation between serum IL-33 and sleep efficiency (R = –0.667, $P = 0.005$; Supplementary material, *Table S2*) in the CD, but not the UC group.

Discussion The importance of interleukin IL-33 in IBD remains unclear; it seems to have a dichotomous role, exerting both protective and detrimental effects.

Similarly to several other studies, we found that serum IL-33 level was often indetectable, which suggests the need for using high-sensitivity protein detection kits.[15,16]

In this study, there were no differences in the serum IL-33 concentrations between the IBD patients and HCs, the exacerbation and remission group, or the individuals with CD or UC. Similarly, Ajdukovic et al[15] also noted that there were no significant differences between the IBD patients and the controls regarding the serum IL-33 levels.

Those findings contrasted with those emerging from other studies. Beltran et al[16] reported that IBD patients showed higher IL-33 levels than controls. In the group-based analysis, both CD and UC patients had a higher level of the discussed cytokine than the controls.[16] Nevertheless, UC activity did not affect the IL-33 concentration, as the patients with active disease tended to have a higher IL-33 level, but the difference between them and the remission group was insignificant.[16] Pastorelli et al[2] revealed that both UC and CD patients with the active disease had increased serum levels of IL-33 in comparison with control subjects. There was also a tendency toward higher levels of this cytokine among UC patients, but it remained insignificant.[2] Seo et al[17] observed lower IL-33 concentration in the IBD patients than in HCs, with the difference being particularly pronounced in the UC patients.

Differences between the studies might stem from the quantity of their participants. The number of patients in our study was similar to that in Ajdukovic's et al,[15] but notably smaller than in the other discussed works[2,16,17]. Additionally, a high percentage of patients and controls had undetectable IL-33 levels. Moreover, we have not studied the concentration of IL-33 decoy

receptor, sST2, which is crucial for the regulation of biological activity of this cytokine. Pastorelli et al[2] found that sST2 is upregulated in the serum of both CD and UC patients, as compared with HCs. The IL-33/sST2 ratio, which reflects the cytokine bioavailability, was increased in both CD and UC patients vs HCs, but the difference was significant only for the UC group.[2] In the study by Seo et al[17], the level of sST2 in the IBD patients was also higher than in HCs, but the IL-33/sST2 ratio in the UC group was markedly lower than that observed in the controls. Moreover, UC severity was associated with a higher sST2 concentration.[17] Beltran et al[16] obtained similar results; however, they did not calculate the IL-33/sST2 ratio.

Thus, studies on the serum IL-33 levels in IBD are not fully congruent. In contrast, analyses of the IL-33 protein and transcript in intestinal biopsies are quite consistently showing that the production of this cytokine is higher in inflamed tissues of UC patients, as compared with CD ones, who did not differ from controls.[18] Altogether, IL-33 appears to have a more important role in UC than in CD, on a local and systemic level.

The novelty of this study lies in the evaluation of sleep and mood disturbances in relation to IL-33. Our results seem to corroborate the association between disturbances of mood, sleep, and IL-33 levels. It is difficult to compare our findings with other studies on the IBD patients, as psychological variables usually were not considered in the methodology. It could be hypothesized that such changes in mental health stem from neuroinflammation caused by IL-33 increase.[19] A lack of a relationship between the investigated cytokine, depression, and sleep parameters in the control group could be ascribed to a small number of such participants with sleep disturbances, as well as no underlying cause for inflammation. The IL-33 concentration closer to the upper limit of normal could be a constitutive trait, without significant impact on the other aspects of health. In a study on obstructive sleep apnea (OSA) patients[19], treatment in a form of continuous positive airway pressure decreased serum IL-33 levels and other inflammatory markers, as well as improved daytime sleepiness and cognitive functions. Nevertheless, the OSA model is not directly comparable with IBD due to hypoxia and low-grade chronic inflammation. Thus, studies on this phenomenon in IBD patients before and after anti-inflammatory treatment would be desirable to elucidate the impact of IL-33 on their mental health.

An important limitation of this study is the small number of participants. It seems that a larger sample size might allow us to obtain significant differences between the groups. Moreover, we have not analyzed the serum sST2 level, which could limit the activity of IL-33. We also did not study the protein production and transcripts in biopsy materials from our participants. However, studies regarding this subject are rather consistent, and local inflammatory processes might not necessarily affect mental health.

To conclude, IL-33 might be associated with sleep and mood disorders in IBD. Its influence is probably related to systemic and neuroinflammation, which impair the central nervous system, thus negatively affecting the patient's well-being. Studies on the subject of mental health and serum IL-33 changes during anti-inflammatory treatment would be desirable to assess whether the mental faculties of IBD patients improve along with alleviation of inflammation. Future projects should be performed on larger samples, use high-sensitivity assays, and analyze IL-33 in the context of sST2.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

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CONFLICT OF INTEREST None declared.

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