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Longitudinal Assessment of Sleep Disturbance and Systemic Inflammatory Responses in Breast Cancer Patients Undergoing Curative Treatment

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ABSTRACT: Sleep disturbances are frequently seen in people who have been diagnosed with breast cancer. Yet the connection between these sleep disturbances and systemic inflammatory responses or stress biomarkers during treatment is not fully understood. The present prospective, longitudinal case-control study was carried out to examine the associations between sleep disturbances (specifically insomnia) and haematological inflammatory indices, circulating inflammatory markers, stress hormones, and immune function. Initially, 218 participants were enrolled, consisting of 118 healthy controls and 100 women who had just been diagnosed with breast cancer. Following eligibility

screening, the final study group included 104 participants: 40 healthy controls, 32 breast cancer patients without clinically significant insomnia (the non-insomnia group), and 32 breast cancer patients who did have insomnia (the insomnia group). Sleep disturbance was assessed using the Insomnia Severity Index (ISI), and haematological and inflammatory biomarkers were measured at three different times: before surgery, during treatment, and at the first follow-up. The results showed that patients with insomnia had a more marked inflammatory and stress-related biomarker pattern than those without insomnia and also than the healthy controls, although the size and significance of the differences varied at different clinical time points. The findings indicate a longitudinal association between insomnia and systemic inflammation in breast cancer patients and underline the need for further multicentre studies that include objective sleep assessments.

1. INTRODUCTION

Breast cancer (BC) is the most frequently diagnosed malignancy among women globally, and it remains one of the top causes of cancer-related death (Sung et al., 2021). Despite great breakthroughs in early identification and treatment, improvements in surgery, chemotherapy, radiation, targeted therapy, and endocrine therapy have dramatically increased survival rates (Waks and Winer, 2019). However, many patients continue to experience a considerable symptom burden throughout the disease course. Among these symptoms, sleep disturbance has emerged as one of the most prevalent yet under-recognized complications, adversely affecting both physical and psychological well-being (Savard and Morin, 2001; Howell et al., 2013; Büttner-Teleagă et al., 2021).

Almost 30 to 60 per cent of women with breast cancer suffer from insomnia, a figure which is much higher than that found in the general population (Savard et al., 2001; Grassi et al., 2023). Sleep disturbances can appear before treatment as a result of the psychological stress caused by the diagnosis and may continue or get worse throughout surgery, chemotherapy, radiotherapy, endocrine therapy, and the survivorship period (Savard et al., 2015; Fleming et al., 2019). There is a link between chronic insomnia and a reduced quality of life, greater fatigue, anxiety, depression, cognitive difficulties, lower adherence to treatment, and worse overall clinical results (Howell et al., 2013; Büttner-Teleagă et al., 2021). Although insomnia is very common, it is frequently not properly diagnosed and inadequately managed in everyday oncology practice (Dahiya et al., 2013).

Increasing amounts of research indicate that the clinical effects of insomnia go beyond simply having poor sleep quality. Chronic sleep disturbance has been connected to the activation of systemic inflammatory and neuroendocrine pathways, which could have an impact on cancer growth. Experimental and clinical studies both demonstrate that insufficient sleep causes activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system, leading to a continuous release of stress hormones and pro-inflammatory mediators. These physiological changes may result in ongoing immune dysregulation, thus affecting patients' well-being and possibly influencing the course of their disease (Irwin, 2019; Besedovsky et al., 2012; Irwin et al., 2016; Coussens and Werb, 2002; Hanahan, 2022).

Inflammation is generally seen as an indication of cancer progression. Cytokines that have a pro-inflammatory effect, such as IL-6, TNF- α , and IFN- γ , play a role in regulating immune responses, tumour cell proliferation, angiogenesis, and metastatic progression. The nuclear factor kappa B (NF- κ B) signalling pathway, when activated, controls the expression of inflammatory genes and thus connects chronic inflammation to both tumour growth and resistance to treatment. Additionally, a disruption of the hypothalamic-pituitary-adrenal (HPA) axis can lead to altered cortisol secretion, which in turn maintains inflammatory responses and results in immunological dysfunction. Together, these inflammatory and neuroendocrine changes offer a reasonable biological basis by which sleep disturbance could affect breast cancer biology (Greten and Grivennikov, 2019; Grivennikov et al., 2010; Taniguchi and Karin, 2018; Yu et al., 2009; Lupien et al., 2009).

In addition to circulating cytokines, haematological inflammatory indices from routine complete blood count analysis have emerged as simple, accessible, and cost-effective indicators of systemic inflammation. The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR), systemic immune-inflammation index (SII), and C-reactive protein (CRP) are all strongly linked to disease severity, treatment response, and prognosis in breast cancer. These biomarkers, which indicate the balance between systemic inflammatory activity and host immunological competence,

may provide vital insight into the molecular repercussions of sleep disruption during breast cancer treatment (Templeton et al., 2014a, 2014b; Ethier et al., 2017; Yang et al., 2018; Roxburgh and McMillan, 2010; Diakos et al., 2014).

While a number of studies have found associations between sleep disturbance and inflammatory biomarkers in cancer patients, most of these investigations have been cross-sectional or have focused on only one stage of treatment. As a result, the way in which inflammatory and stress-related biomarkers change over time in relation to insomnia during breast cancer treatment is not fully understood. Moreover, longitudinal studies looking at the repeated changes in both haematological inflammatory indices and circulating inflammatory biomarkers in patients receiving curative-intent treatment are scarce (Savard et al., 2015).

This study was carried out with the aim of examining the long-term relationship between sleep disturbance and various systemic inflammatory, immunological, and stress-related markers in women who have breast cancer and are receiving curative treatment. Sleep disturbance was assessed using the Insomnia Severity Index (ISI). At three clinically important time points—before surgery, during treatment, and at the first follow-up appointment—the hematological inflammatory indices, the levels of the circulating cytokines (IL-6, TNF- α , and IFN- γ), NF- κ B, cortisol, and CRP were measured. We assumed that breast cancer patients suffering from insomnia would show a continuously elevated inflammatory profile as compared to those without insomnia and to healthy controls, thus enabling a better understanding of the connection between sleep disturbance and systemic inflammation while patients are undergoing treatment for breast cancer.

2. MATERIALS AND METHODS

2.1 Study Design

This study employed a prospective, longitudinal, case-control design in order to examine the association between sleep disruption and various systemic inflammatory, immunological, and stress-related biomarkers in women with breast cancer who were receiving curative treatment. By combining clinical evaluation with biochemical and haematological investigations, the study aimed at assessing the changes over time in the biomarker profiles during treatment.

The level of sleep disturbance was measured with the Insomnia Severity Index (ISI), a self-report questionnaire which has been validated (Bastien et al., 2001), and peripheral blood samples were taken at specific clinical time points over a period. This approach allowed changes in both sleep status and biomarker levels to be evaluated within the same participants during treatment, thus reducing variability between individuals because of the repeated longitudinal assessments.

2.2 Study Setting

The clinical aspect of the study took place at the Breast Screening Unit of the Malabar Cancer Centre (MCC) in Thalassery, Kerala, India, and the laboratory tests were carried out in the Division of Biochemistry, which is part of the Department of Clinical Laboratory Services and Translational Research at the Malabar Cancer Centre, where the biochemical and molecular analyses were performed.

2.3 Ethical Considerations

The study adhered to the ethical principles set out in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board and the Institutional Ethics Committee of the Malabar Cancer Centre (MCC) in Thalassery (Approval No. 1617/IRB-IEC/13/MCC/29-01-2025/2; Date: February 17, 2025). Furthermore, the Institutional Human Ethics Committee of the Avinashilingam Institute for Home Science and Higher Education for Women in Coimbatore, India, granted additional ethical clearance (Clearance No. AUW/IHEC/24-25/BCBT/FHP-07). Written informed consent was given by all participants before they were enrolled and had their samples collected.

2.4 Study Period

The study ran from 1st March 2025 to 28th February 2026, and during that time participants were recruited, clinical data were gathered, and blood samples from the periphery were obtained in accordance with the predetermined longitudinal sampling schedule.

2.5 Study Population

The study started with 218 people taking part, consisting of 118 healthy controls and 100 women who had just been diagnosed with breast cancer. The Breast Screening Unit at the Malabar Cancer Centre in Thalassery, India, selected the breast cancer patients in accordance with established inclusion and exclusion criteria, while the healthy female volunteers acted as the control group. Baseline information regarding the participants' socio-demographic, lifestyle, medical, and family history features was obtained through the use of a standardized questionnaire administered by the investigators. At the beginning, the participants were divided into two main categories: healthy controls and patients with breast cancer. After the baseline evaluation had been carried out using the Insomnia Severity Index (ISI), the group of breast cancer patients was split into a non-insomnia subgroup (ISI 0–7) and an insomnia subgroup (ISI >7).

From the 118 healthy controls who were initially screened, 78 were excluded because they met the predetermined criteria and 40 were included in the final study group. Of the 100 breast cancer patients who were screened, 36 were excluded on the basis of the predefined exclusion criteria and 64 patients remained eligible. Using the baseline ISI assessment, the 64 eligible breast cancer patients were divided into two groups: 32 being in the non-insomnia group and 32 in the insomnia group. The final longitudinal study cohort therefore consisted of 104 people, namely 40 healthy controls, 32 in the non-insomnia group, and 32 in the insomnia group.

2.6 Participant Recruitment

The process we followed for recruiting participants included a predetermined screening and eligibility assessment. At first, we screened 118 healthy controls to see if they were eligible. Of these, 78 were excluded, consisting of 45 who failed to meet the inclusion criteria and 33 who refused to take part or who did not give informed consent. As a result, 40 healthy controls were enrolled.

We also screened 100 patients who had just been diagnosed with breast cancer. Out of these, 36 were excluded: 20 did not satisfy the eligibility criteria and 16 refused to take part or did not give informed consent. The other 64 breast cancer patients were then enrolled. After the initial ISI evaluation, the 64 eligible breast cancer patients were divided into two groups, consisting of 32 in the non-insomnia group and 32 in the insomnia group.

The study therefore included a total of 104 participants, comprising 40 healthy controls, 32 from the non-insomnia group, and 32 from the insomnia group.

2.7 The criteria for inclusion and exclusion

Inclusion Criteria

Participants were eligible if they fulfilled the following criteria:

- The women involved were between 20 and 70 years of age.
- The ability to understand the study procedures and to give written informed consent.
- For the group with breast cancer: this refers to cases of newly diagnosed breast cancer that has been confirmed by histopathology and for which curative-intent treatment is scheduled.
- The control group consisted of healthy female volunteers who were of similar age and had no history of cancer or any clinically significant sleep disturbance.
- The ability to fill out the study questionnaires and to provide the clinical information required.

Exclusion Criteria

- Participants were excluded if they had:
- The way breast cancer was previously treated.
- A primary sleep disorder that was diagnosed previously, apart from insomnia.
- Psychiatric or neurological disorders.

- Conditions that are autoimmune, chronic inflammatory, or immunological.
- Conditions that are seriously concurrent or infections that are currently active, as these could affect levels of sleep or inflammatory biomarkers.
- The inability to finish the study questionnaires or to give reliable information.
- Clinical or laboratory records that are incomplete.

2.8 The evaluation of sleep disturbance

Sleep disturbance was examined with the use of the Insomnia Severity Index (ISI), a self-report questionnaire consisting of seven items and having been validated for use in assessing the nature, severity, and impact of insomnia symptoms. The questionnaire looks at problems concerning sleep initiation, sleep maintenance, early-morning awakenings, satisfaction with the present sleep pattern, the extent to which it interferes with daily activities, how noticeable the sleep problems are, and the level of distress caused by difficulties with sleep.

Each item is assessed using a five-point Likert scale, ranging from 0 to 4, which results in a total score between 0 and 28. The participants were classified in accordance with the established ISI scoring criteria:

- 0–7: No clinically significant insomnia
- 8–14: Subthreshold insomnia
- 15–21: Moderate insomnia
- 22–28: Severe insomnia

At the baseline stage, the ISI was given to all eligible participants following the baseline characterisation. The ISI score was used to evaluate the participants' sleep状况 and to divide them into groups for the final study cohort; healthy controls who had ISI scores between 0 and 7 were kept as the control group, and breast cancer patients were divided into a non-insomnia group and an insomnia group in accordance with the predefined study criteria.

For the women taking part in the longitudinal evaluation, the ISI was given at three specific clinical time points: before surgery (at baseline), during treatment, and at the first follow-up appointment after treatment had been completed. This arrangement allowed for a longitudinal assessment of changes in sleep disturbance along the treatment course. The mean ISI scores rose during treatment and then partly decreased at the first follow-up.

2.9 Blood Sample Collection

Blood samples from the peripheral veins of the breast cancer patients were taken at three specific clinical time points: at the pre-surgical baseline, during treatment, and at the first follow-up. The baseline sample was obtained one day prior to surgery and before the initiation of curative-intent treatment. In the treatment phase, the patients received chemotherapy, radiotherapy, or both, in accordance with their personal clinical treatment plans. The second blood sample was collected during the period of active treatment, that is, after surgery and while undergoing chemotherapy and/or radiotherapy. The third blood sample was taken at the first follow-up visit after the planned treatment had been completed in order to examine the levels of the biomarkers following treatment. The same biomarker parameters were evaluated at the three clinical time points within the same longitudinal cohort in order to examine changes over time in individual participants. Healthy control participants gave a single peripheral venous blood sample at the time of enrolment, and this sample was used as the reference measurement for comparison with the groups affected by breast cancer. Since the current study mainly focused on the longitudinal changes in sleep disturbance and in biomarkers relating to systemic inflammation, the immune system, and stress, the different treatment modalities were not analysed separately.

The blood samples were analysed using standard laboratory procedures; the serum was obtained by centrifugation and then kept at -80°C until the biochemical analysis was carried out. A complete blood count was conducted on the whole blood which had been collected in EDTA tubes, and the haematological inflammatory indices—namely the neutrophil-to-lymphocyte ratio

(NLR), the platelet-to-lymphocyte ratio (PLR), the lymphocyte-to-monocyte ratio (LMR) and the systemic immune-inflammation index (SII)—were calculated from the relevant haematological parameters.

For the individuals taking part in the breast cancer study, the ISI evaluations were carried out at each of the three blood sampling time points in order that there might be a correspondence over time between the measurements of sleep disturbance and the biomarkers.

2.10 Haematological Analysis

A complete blood count (CBC) was carried out using an automated haematology analyser as instructed by the manufacturer. The parameters that were assessed consisted of the total leukocyte count, the absolute neutrophil count, the lymphocyte count, the monocyte count, the platelet count, and the haemoglobin concentration.

On this basis we calculated inflammatory indices which indicate the body's systemic immune status and the level of inflammation.

The following indices were calculated:

Neutrophil-to-Lymphocyte Ratio (NLR)

Platelet-to-Lymphocyte Ratio (PLR)

Lymphocyte-to-Monocyte Ratio (LMR)

Systemic Immune-Inflammation Index (SII)

The SII value was calculated using the formula (platelet count $\times$ neutrophil count) divided by the lymphocyte count (Hu et al., 2014).

The indices served as surrogate indicators of systemic inflammation and immune status and were compared in the healthy controls, in the breast cancer patients without insomnia, and in the breast cancer patients with insomnia at the three clinical time points.

2.11 The estimation of inflammatory and stress biomarkers

The levels of inflammatory and stress-related biomarkers in the serum were determined by using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the instructions provided by the manufacturer (Progen Biolab Pvt. Ltd., India). This company supplied ELISA kits which were used for the measurement of the serum concentrations of IL-6, TNF- α , IFN- γ , NF- κ B, and cortisol. Serum CRP levels were measured with the AFINION™ CRP system. We regarded the NF- κ B measurement as one based on circulating immunoreactivity rather than as a direct evaluation of intracellular NF- κ B activation.

We heated the reagents and serum samples to room temperature before carrying out the analysis and then produced the standard curves for each assay in accordance with the manufacturer's instructions.

The amounts of cytokines (IL-6, TNF- α , IFN- γ) and NF- κ B were measured by means of sandwich ELISA, while cortisol was determined using competitive ELISA.

To sum up, the standards and serum samples were put into the antibody-coated microplates and then left to stand under the specified conditions. The plates were then washed, after which the enzyme-conjugated detection antibodies were added. Following this incubation, a substrate solution was added to produce colour, and the reaction was stopped using the stop solution. The optical density was read at 450 nm with a microplate reader, and the biomarker concentrations were calculated using the standard calibration curves.

The laboratory analyses were carried out in accordance with the standard operating procedures; when possible, the samples were tested in duplicate, the calibration methods were applied as suggested by the manufacturer, and the data were verified independently prior to being subjected to statistical analysis in order to ensure analytical accuracy and reproducibility.

2.12 Statistical Analysis

The data were analysed with the use of GraphPad Prism software (version 8.0.2). For the continuous variables, the mean together with the standard deviation (SD) was given. The distribution of the data was evaluated by means of the Shapiro–Wilk test and homogeneity of variances was examined using Levene’s test. In order to compare the three groups at each clinical time point, one-way analysis of variance (ANOVA) was performed, followed by Tukey’s post hoc test for multiple comparisons. A p-value less than 0.05 for a two-sided test was regarded as statistically significant. For the statistical analyses, the available observations for each biomarker and each clinical time point were used.

3. RESULTS

3.1 The basic characteristics of the group studied

The assessment initially involved 218 participants, of whom 118 were healthy controls and 100 were patients who had recently been diagnosed with breast cancer (BC). After carrying out the eligibility screening, 104 participants were included in the final longitudinal cohort, consisting of 40 healthy controls, 32 breast cancer patients without clinically significant insomnia, and 32 breast cancer patients who did have insomnia. Baseline socio-demographic, lifestyle, medical, and family-history characteristics were recorded using a standardized questionnaire administered by the investigators. Table 1 shows how these baseline characteristics were distributed among the participants who were initially assessed.

Of the 100 patients with breast cancer, invasive ductal carcinoma was the most common histological type (65%), 35% of the cases being invasive lobular carcinoma. With respect to histological grade, 35% of the patients had Grade I disease, 55% had Grade II disease and 10% had Grade III disease. As for tumour stage, 55% of the patients were regarded as being in Stage I and 45% in Stage II (Table 1).

The baseline characteristics of the study participants (N = 218) are given in Table 1.

A. Sociodemographic characteristics

Age group (years)	20–35	9 (7.6%)	11 (11.0%)
	36–50	27 (22.9%)	28 (28.0%)
	51–65	58 (49.2%)	47 (47.0%)
	66–70	24 (20.3%)	14 (14.0%)
Educational level	Lower primary	0 (0.0%)	6 (6.0%)
	Upper primary	6 (5.1%)	21 (21.0%)
	High school	21 (17.8%)	0 (0.0%)
	Higher secondary	42 (35.6%)	44 (44.0%)
	Graduation	39 (33.1%)	23 (23.0%)
	Post-graduation	10 (8.5%)	6 (6.0%)
Marital status	Married	108 (91.5%)	84 (84.0%)

	Never married	10 (8.5%)	8 (8.0%)
	Widowed	0 (0.0%)	8 (8.0%)
Household income	Below poverty line (BPL)	57 (48.3%)	48 (48.0%)
	Above poverty line (APL)	61 (51.7%)	52 (52.0%)

B.Lifestyle characteristics

Exercise frequency	Yes	88 (74.6%)	84 (84.0%)
	No	30 (25.4%)	16 (16.0%)
Physical activity	Active	69 (58.5%)	78 (78.0%)
	Sedentary	49 (41.5%)	22 (22.0%)
Sleep duration	<4 h	0 (0.0%)	0 (0.0%)
	4–5 h	30 (25.4%)	58 (58.0%)
	6–8 h	88 (74.6%)	42 (42.0%)
Processed food & drinks intake	Too much	0 (0.0%)	9 (9.0%)
	Moderate	91 (77.1%)	51 (51.0%)
	Very less	27 (22.9%)	40 (40.0%)
Water intake	Too much	43 (36.4%)	50 (50.0%)
	Moderate	53 (44.9%)	38 (38.0%)
	Very less	22 (18.6%)	12 (12.0%)
Salt/sugar intake	Too much	0 (0.0%)	15 (15.0%)
	Moderate	80 (67.8%)	55 (55.0%)
	Very less	20 (16.9%)	30 (30.0%)

As for the baseline observations: none of the participants were vegetarians and, in the previous month, no participant had used any anti-inflammatory drugs or had been engaged in stressful job or shift work.

C. Medical, familial history and clinicopathological features

Diabetes	Yes	11 (9.3%)	8 (8.0%)
	No	107 (90.7%)	92 (92.0%)
Hypertension	Yes	14 (11.9%)	16 (16.0%)
	No	104 (88.1%)	84 (84.0%)
Thyroid disorder	Yes	6 (5.1%)	7 (7.0%)
	No	112 (94.9%)	93 (93.0%)
Cardiovascular disease	Yes	0 (0.0%)	0 (0.0%)
	No	118 (100%)	100 (100%)
Other comorbidities	Yes	0 (0.0%)	0 (0.0%)
	No	118 (100%)	100 (100%)
Family history of breast cancer	Yes	0 (0.0%)	2 (2.0%)
	No	118 (100%)	98 (98.0%)
Family history of other cancer	Yes	3 (2.5%)	8 (8.0%)
	No	115 (97.5%)	92 (92.0%)

Clinicopathological characteristics — BC only			
Histological type	Invasive ductal carcinoma	—	65 (65%)
	Invasive lobular carcinoma	—	35 (35%)
Histological grade	Grade I	—	35 (35%)
	Grade II	—	55 (55%)
	Grade III	—	10 (10%)

Tumour stage	Stage I	—	55 (55%)
	Stage II	—	45 (45%)

The data are given as numbers (n) and percentages (%). The table provides a summary of the baseline socio-demographic, lifestyle, medical, family-history, and clinicopathological features of the study participants; for the socio-demographic, lifestyle, medical, and family-history characteristics, information is given for both healthy controls (n=118) and breast cancer patients (n=100), whereas for the clinicopathological characteristics only those of the breast cancer patients are given.

3.2 The assessment of sleep disturbance and the classification of the ISI

The Insomnia Severity Index (ISI) was used for a baseline assessment of sleep disturbance and this revealed clinically relevant differences in sleep problems among the participants in the study. At the start, the study population was divided into two broad categories: healthy controls and breast cancer patients. Following the initial baseline ISI assessment, the group of breast cancer patients was split into a non-insomnia group (with an ISI score of 0–7) and an insomnia group (with an ISI score greater than 7). According to the predefined criteria for classifying ISI scores, scores from 0 to 7 meant that there was no clinically significant insomnia, while scores between 8 and 14, 15 and 21, and 22 and 28 indicated subthreshold, moderate, and severe insomnia, respectively (Bastien et al., 2001). After the baseline screening and the application of the selection criteria, the final study group consisted of 40 healthy controls (32 in the non-insomnia group and 8 in the insomnia group). None of the participants had an ISI score in the range of severe insomnia. The longitudinal changes in individual ISI scores at the three clinical time points are shown in Figure 1.

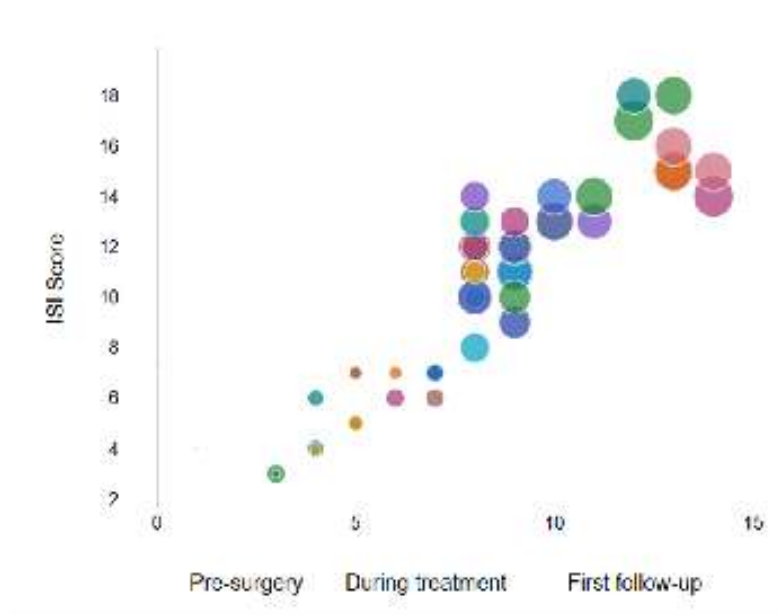


Figure 1. Longitudinal changes in ISI scores from pre-surgery to during treatment and first follow-up



3.3 A longitudinal assessment of the hematological inflammatory indices

At the baseline (before surgery), the haematological examination showed considerable differences in the inflammatory markers between the various research groups (see Table 2). The breast cancer patients in the insomnia group had a markedly lower lymphocyte-to-monocyte ratio (LMR) when compared with the healthy controls (6.07 ± 1.60 versus 7.42 ± 0.67 ; $p < 0.0001$) and with those in the non-insomnia group (7.00 ± 1.70 ; $p = 0.0030$). The insomnia group also had a much higher platelet-to-lymphocyte ratio (PLR) than the control group (123.41 ± 29.20 compared with 91.92 ± 51.00 ; $p = 0.005$). Nevertheless, there was no significant difference between the two groups of breast cancer patients. Likewise, the neutrophil-to-lymphocyte ratio (NLR) was substantially higher in the insomnia group than in the healthy controls (2.14 ± 0.48 versus 1.67 ± 0.48 ; $p = 0.0321$). However, the difference between the breast cancer groups was not statistically significant.

The systemic immune-inflammation index (SII) was found to be significantly higher in the insomnia group than in both the control group and the non-insomnia group (in both cases $p < 0.0001$), which shows that there was greater inflammatory activity. At baseline, the levels of CRP were significantly elevated in the insomnia group as compared to those in healthy controls and in the non-insomnia group (in both cases $p < 0.0001$); however, no significant difference was detected between healthy controls and the non-insomnia group ($p = 0.8281$) (Table 5). Taken as a whole, the baseline results show that the breast cancer patients in the insomnia group had a more marked systemic inflammatory response than those in the non-insomnia group, especially when looking at LMR and SII. The CRP findings are given separately in Table 5.

Table 2. Haematological inflammatory indices in healthy controls, the non-insomnia group, and the insomnia group at pre-surgery

Haematological inflammatory indices	Controls	Non-insomnia	Insomnia	P values	P values	P values
N	40	32	32	Control vs non-insomnia	Control vs Insomnia	Non-insomnia vs Insomnia
LMR	7.42 ± 0.67	7.00 ± 1.70	6.07 ± 1.60	0.5576	<0.0001	0.0030
PLR	91.92 ± 51	115.04 ± 22.01	123.41 ± 29.20	0.0987	0.005	0.9871
NLR	1.67 ± 0.48	1.86 ± 0.54	2.14 ± 0.48	0.9195	0.0321	0.5929
SII	479.81 ± 102.08	433.73 ± 32.60	579.47 ± 48.17	0.0719	<0.0001	<0.0001

The data are given as the mean $\pm$ standard deviation (SD); the table provides a summary of the various haematological inflammatory indices, namely the lymphocyte-to-monocyte ratio (LMR), the platelet-to-lymphocyte ratio (PLR), the neutrophil-to-lymphocyte ratio (NLR), and the systemic immune-inflammation index (SII). The SII was calculated by the formula (platelet count $\times$ neutrophil count) / lymphocyte count. The comparisons between the groups are expressed in the form of p-values for the following pairs of groups: healthy controls versus the non-insomnia group, healthy controls versus the insomnia group, and the non-insomnia group versus the insomnia group. The statistical analysis was carried out using GraphPad Prism version 8.0.2.

While being treated, the systemic inflammatory changes became more marked (see Table 3). The breast cancer patients in the insomnia group showed an additional decrease in their LMR, this decrease being significantly less than that observed in both the healthy controls and in the non-insomnia group (in both cases $p < 0.0001$). Moreover, the PLR increased considerably in the insomnia group and was found to be significantly higher than that in both the other groups ($p < 0.0001$ and $p = 0.0147$, respectively).

While NLR was still higher in the insomnia group, the difference was not found to be statistically significant at this time point. On the other hand, SII showed a marked increase in the insomnia group and the difference was highly significant when compared with both the healthy controls and the non-insomnia group (both $p < 0.0001$). Likewise, CRP levels were found to be significantly raised in the insomnia group as compared to both the healthy controls and the non-insomnia group (both $p < 0.0001$) (Table 5). These results indicate that inflammatory dysregulation was still present during active treatment and was more evident in the insomnia group.

Table 3. Haematological inflammatory indices in healthy controls, the non-insomnia group, and the insomnia group during treatment

Haematological inflammatory indices	Controls	Non-insomnia	Insomnia	Pvalues	Pvalues	Pvalues
N	40	32	32	Control vs non-insomnia	Control vs Insomnia	Non-insomnia vs Insomnia
LMR	7.42 $\pm$ 0.67	6.05 $\pm$ 0.70	4.47 $\pm$ 1.09	<0.0001	<0.0001	<0.0001
PLR	91.92 $\pm$ 51	131.71 $\pm$ 25.30	161.50 $\pm$ 40	0.0001	<0.0001	0.0147
NLR	1.67 $\pm$ 0.48	1.77 $\pm$ 0.53	2.11 $\pm$ 0.88	0.9989	0.0702	0.3810
SII	479.81 $\pm$ 102.08	418.61 $\pm$ 43.99	823.58 $\pm$ 64.60	0.0043	<0.0001	<0.0001

The data are given as the mean $\pm$ standard deviation (SD); the table provides a summary of the various haematological inflammatory indices, namely the lymphocyte-to-monocyte ratio (LMR), the platelet-to-lymphocyte ratio (PLR), the neutrophil-to-lymphocyte ratio (NLR), and the systemic immune-inflammation index (SII). The SII was calculated by the formula (platelet count $\times$ neutrophil count) / lymphocyte count. The comparisons between the groups are expressed in the form of p-values for the following pairs of groups: healthy controls versus the non-insomnia group, healthy controls versus the insomnia group, and the non-insomnia group versus the insomnia group. The statistical analysis was carried out using GraphPad Prism version 8.0.2.

In the first follow-up, several systemic inflammatory changes remained (see Table 4). The breast cancer patients in the insomnia group still showed a significantly lower LMR when compared to both the healthy controls and the non-insomnia group (in both cases $p < 0.0001$). Likewise, the PLR remained significantly higher than that of both groups (in both cases $p < 0.0001$), which shows that there was ongoing platelet-related inflammatory activity.

The level of NLR was still much higher in the insomnia group than in healthy controls ($p = 0.0487$), although there was no significant difference between the two groups of breast cancer patients. SII was also much higher in the insomnia group than in the non-insomnia group ($p = 0.0019$), even though the difference when compared to healthy controls was not statistically significant. In a similar way, the CRP values remained considerably elevated in the insomnia group as compared to both healthy controls and the non-insomnia group (both $p < 0.0001$) (Table 5). Altogether, these results indicate that several inflammatory

abnormalities continued after treatment, inflammatory changes being more marked in the insomnia group than in the other two groups.

The longitudinal trends of the haematological inflammatory indices are shown in Figure 2. In the insomnia group, the LMR decreased during treatment and then showed partial recovery at the first follow-up. The PLR increased during treatment in both breast cancer groups, with an additional increase in the insomnia group at the first follow-up. SII exhibited a marked rise during treatment in the insomnia group before decreasing at the first follow-up, while NLR showed only small and less consistent changes throughout the various clinical time points.

Table 4. Haematological inflammatory indices in healthy controls, the non-insomnia group, and the insomnia group at first follow-up

Haematological inflammatory indices	Controls	Non-insomnia	Insomnia	<i>P</i> values	<i>P</i> values	<i>P</i> values
<i>N</i>	40	32	32	Control vs non-insomnia	Control vs Insomnia	Non-insomnia vs Insomnia
LMR	7.42±0.67	7.80±0.65	5.12±0.75	0.6795	<0.0001	<0.0001
PLR	91.92±51	126.15±32.80	193.12±31.27	0.0018	<0.0001	<0.0001
NLR	1.67±0.48	2.05±0.67	2.12±1.25	0.1652	0.0487	>0.9999
SII	479.81±102.08	432.42±35.99	501.05±44.66	0.0580	0.8949	0.0019

The data are given as the mean ± standard deviation (SD); the table provides a summary of the haematological inflammatory indices, namely the lymphocyte-to-monocyte ratio (LMR), the platelet-to-lymphocyte ratio (PLR), the neutrophil-to-lymphocyte ratio (NLR), and the systemic immune-inflammation index (SII). The SII was calculated by the formula (platelet count × neutrophil count) / lymphocyte count. The comparisons between the groups are expressed in the form of p-values for the following pairs of groups: healthy controls versus the non-insomnia group, healthy controls versus the insomnia group, and the non-insomnia group versus the insomnia group. The statistical analysis was carried out using GraphPad Prism version 8.0.2.

Figure 2. The longitudinal patterns of the haematological inflammatory indices in healthy controls, in the non-insomnia group, and in the insomnia group.

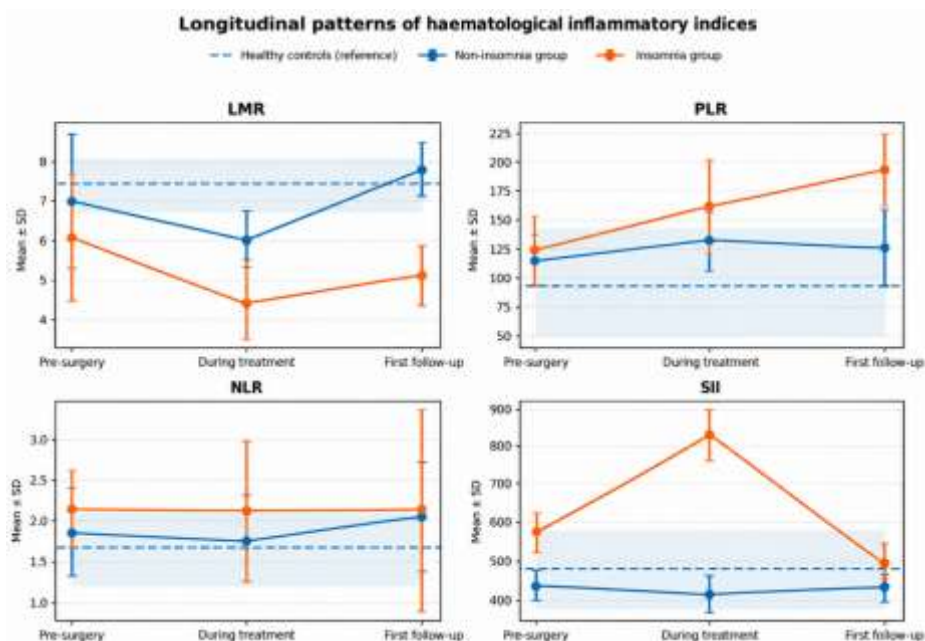


Table 5. Longitudinal Assessment of C-Reactive Protein (CRP) Levels

CRP mg/L	Controls	Non-insomnia	Insomnia	<i>P</i> values	<i>P</i> values	<i>P</i> values
N	40	32	32	Control vs non-insomnia	Control vs Insomnia	Non-insomnia vs Insomnia
Pre surgery	6.22±0.44	7.66±1	18.6±1.7	0.8281	<0.0001	<0.0001
During treatment	6.22±0.44	8±1.06	20.45±1.16	0.6546	<0.0001	<0.0001
First follow up	6.22±0.44	7.5±0.90	13.6±0.40	0.1685	<0.0001	<0.0001

The data are given as the mean $\pm$ standard deviation (SD). The table provides a summary of the longitudinal C-reactive protein (CRP) levels in the group of healthy controls (n = 40), the non-insomnia group (n = 32), and the insomnia group (n = 32) at the pre-surgical stage, during treatment, and at the first follow-up. The comparisons between the groups are expressed in the form of p-values for the following pairs of groups: healthy controls versus the non-insomnia group, healthy controls versus the insomnia group, and the non-insomnia group versus the insomnia group. The statistical analysis was carried out using one-way ANOVA followed by Tukey's multiple-comparisons test with GraphPad Prism version 8.0.2.

3.4 A longitudinal assessment of circulating inflammatory and stress biomarkers

In the baseline (pre-surgical) phase, the serum levels of inflammatory and stress-related biomarkers are shown in Figure 3. The breast cancer patients who had insomnia showed significantly higher levels of IL-6, TNF- α , and cortisol than did the healthy controls. IL-6 levels were found to be significantly increased in the insomnia group as compared to the controls ($p < 0.0001$) and also when compared to the non-insomnia group ($p < 0.01$). Likewise, TNF- α levels were significantly higher in the insomnia group than in the controls ($p < 0.05$) and than in the non-insomnia group ($p < 0.0001$). The cortisol levels were also found to be significantly greater in the insomnia group than in the controls ($p < 0.0001$) and than in the non-insomnia group ($p < 0.01$).

On the other hand, there was no significant difference in the levels of circulating NF- κ B among the three groups at the baseline time point. Similarly, although the levels of IFN- γ seemed higher in both breast cancer groups compared to those in the control group, no statistically significant difference was found between the insomnia group and the non-insomnia group. This shows that elevated levels of inflammatory cytokines and cortisol were already present at baseline in the patients in the insomnia group, while the measurement of circulating NF- κ B did not reveal a significant difference between the groups.

While being treated, marked changes were seen in the levels of inflammatory and stress-related biomarkers (see Figure 3). The breast cancer patients in the insomnia group had significantly higher levels of IL-6, TNF- α , cortisol, NF- κ B, and IFN- γ than both the healthy controls and those in the non-insomnia group. IL-6 levels were found to be significantly increased in the insomnia group as compared to the controls ($p < 0.0001$) and also when compared to the non-insomnia group ($p < 0.0001$). Similarly, TNF- α levels were also significantly higher than those in the controls ($p < 0.01$) and the non-insomnia group ($p < 0.0001$).

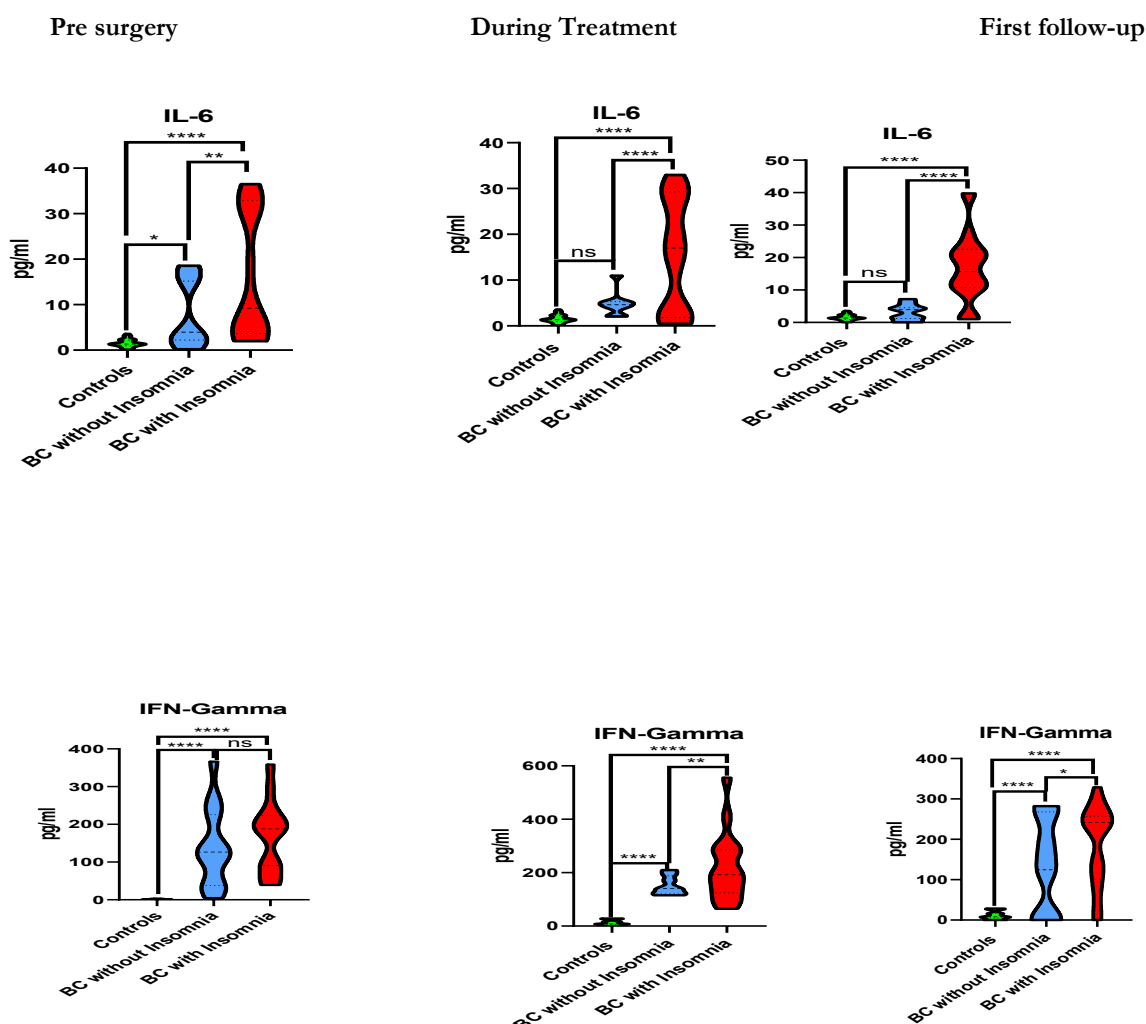
Cortisol levels were at their highest during the treatment time point and were found to be significantly higher in the insomnia group than in both the control group and the non-insomnia group ($p < 0.0001$). Circulating NF- κ B levels were also found to be significantly elevated in the insomnia group as compared to healthy controls ($p < 0.0001$) and the non-insomnia group ($p < 0.05$). In a similar manner, IFN- γ concentrations were markedly increased in the insomnia group when compared with those in the controls ($p < 0.0001$) and the non-insomnia group ($p < 0.01$). In general, the treatment time point was marked by significant increases in circulating inflammatory cytokines, cortisol, and NF- κ B-related measurements, especially in breast cancer patients who had insomnia.

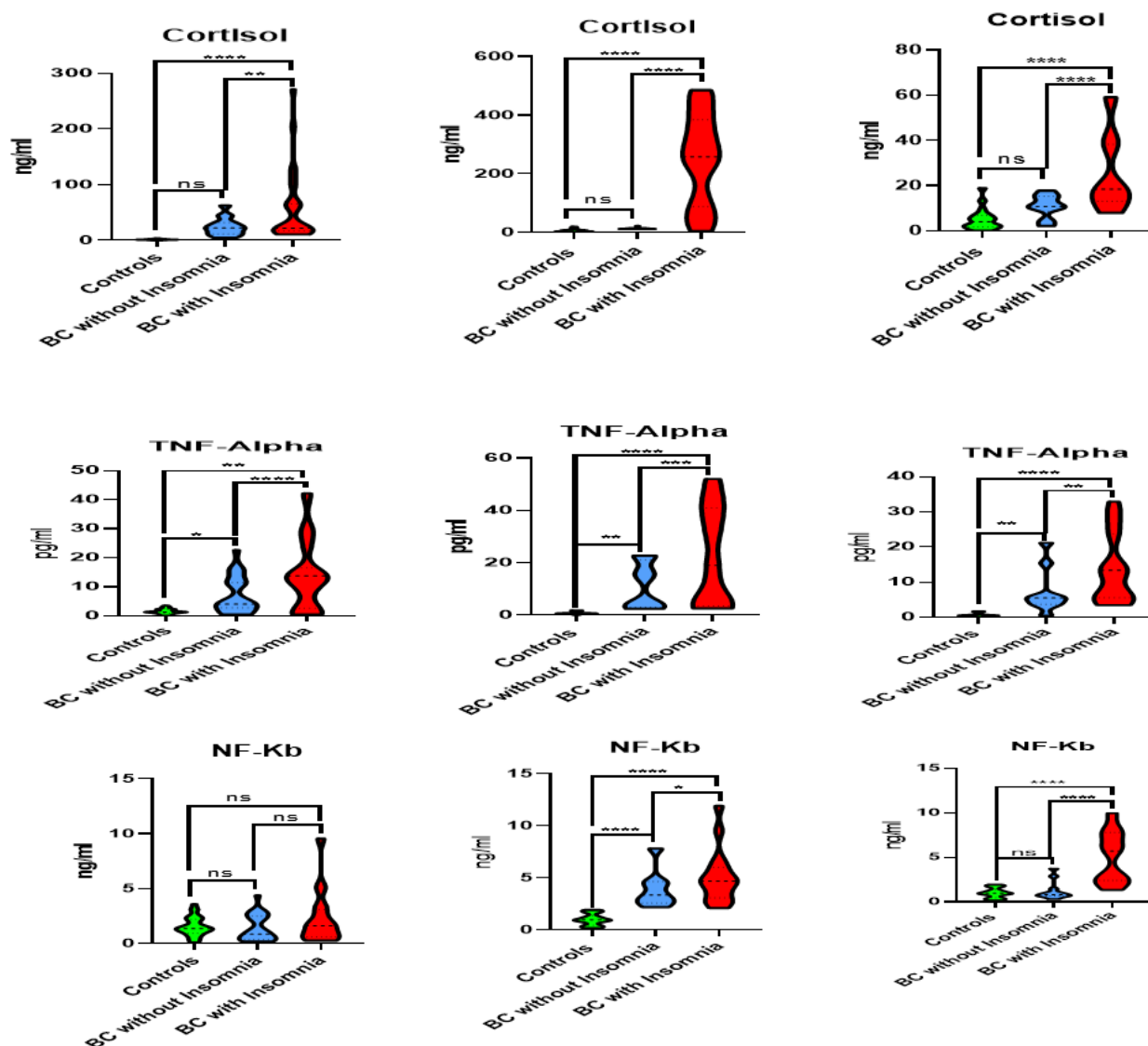
In the first follow-up assessment (Figure 3), the breast cancer patients in the insomnia group still showed significantly higher levels of circulating inflammatory and stress biomarkers than both the healthy controls and those in the non-insomnia group.

Serum IL-6 was still found to be significantly higher in the insomnia group than in the control group ($p < 0.0001$) and than in the non-insomnia group ($p < 0.0001$). Likewise, the concentrations of TNF- α were still significantly elevated when compared with those of the controls ($p < 0.01$) and with those of the non-insomnia group ($p < 0.01$).

In the insomnia group, cortisol levels were found to have increased persistently, the difference being highly significant when compared with both the healthy controls and the non-insomnia group (both $p < 0.0001$). Compared to both the control group and the non-insomnia group, NF- κ B levels in the circulation were still significantly higher in the insomnia group (both $p < 0.0001$). Moreover, while the concentrations of IFN- γ were still significantly greater in the patients in the insomnia group than in both of the other groups, the extent of the difference between the two breast cancer groups was less marked than it had been at the treatment time point. Together, these results show that the inflammatory and stress-related biomarker pattern associated with insomnia continued beyond the period of active treatment, with elevated cytokine concentrations and circulating NF- κ B levels still being apparent at the first follow-up assessment.

Figure 3 shows the longitudinal patterns of serum inflammatory and stress-related biomarkers in healthy controls, in the non-insomnia group, and in the insomnia group throughout the treatment trajectory.





3.5 Overall Longitudinal Trends

An assessment carried out over time of the haematological indices together with the levels of inflammatory and stress-related biomarkers showed a general association between insomnia and a modified systemic inflammatory response in women with breast cancer, thus achieving the study's purpose of clarifying the link between sleep disturbance and systemic inflammation during treatment. In the group with insomnia, the LMR was generally lower and the PLR and SII were generally higher, which was in line with the research objective of identifying the changes in inflammatory biomarkers associated with insomnia. Several of the inflammatory and stress-related biomarkers also had higher levels in patients who had insomnia, especially during treatment and in the follow-up period. However, the extent of the changes and the statistical significance of the individual biomarkers differed at the three clinical time points. Overall, these results support the study's aim by showing that there is a continuous association between sleep disturbance and systemic inflammatory and stress-related changes throughout the breast cancer treatment process.

4. DISCUSSION

In the study's results, the insomnia group always had a lower lymphocyte-to-monocyte ratio (LMR), a higher platelet-to-lymphocyte ratio (PLR), a higher systemic immune-inflammation index (SII), and elevated levels of C-reactive protein (CRP), together with significantly increased concentrations of the inflammatory and stress-related biomarkers (including IL-6, TNF- α , cortisol, IFN- γ , and NF- κ B) when compared with the non-insomnia group and healthy controls. These differences were already present before surgery, became more marked during active treatment, and remained at the first follow-up. Furthermore, the mean ISI scores showed that sleep disturbance got worse during treatment and only partially improved afterwards. Taken together, these findings show that insomnia was linked to ongoing systemic biological changes throughout the entire treatment process, not just at a single point in time. Since the study was observational, the results should be seen as associations and not as proof of causation.

The longitudinal pattern of sleep disturbance is an important aspect when interpreting the biomarker results. The mean ISI scores rose from the pre-surgical baseline level while active treatment was underway and then fell at the first follow-up, even though sleep disturbance did not completely revert to baseline. This indicates that sleep problems may get worse during active cancer treatment and still be a factor after treatment has finished (Savard et al., 2015; Fleming et al., 2019). The fact that sleep disturbance worsened together with more significant systemic inflammatory and stress-related changes during treatment underscores the need to regard sleep as part of the overall symptom and biological burden suffered by patients with breast cancer.

The haematological inflammatory markers showed a consistent, though time-dependent, relationship with insomnia. At the time of surgery, people in the insomnia group had a lower LMR and higher PLR and SII than those in the respective control groups, indicating that the differences in their inflammatory and immune-cell profiles occurred before treatment began. During active treatment, the differences became significant, with the insomnia group showing further reductions in LMR and greater increases in both PLR and SII. At the first follow-up, the LMR was still lower and the PLR remained high in the insomnia group, while SII had dropped from its level during treatment but was still above that of the non-insomnia group. Nevertheless, NLR did not show a consistent pattern, since statistically significant differences were observed in some of the clinical comparisons at the three time points. This indicates that the connection between insomnia and the body's systemic inflammatory state was more clearly reflected in the LMR, PLR and SII than in the NLR, which can be easily obtained from standard haematology tests and have been commonly used as markers of systemic inflammation and immune function in cancer (Guthrie et al., 2013; Templeton et al., 2014a, 2014b; Nishijima et al., 2015; Yang et al., 2018; Hu et al., 2014). The fact that the LMR, PLR and SII remained altered throughout the study period suggests a relationship between sleep disturbances and a continuing inflammatory imbalance; it is also possible that effects related to treatment and the disease itself played a role.

In this study NLR exhibited a relatively less stable pattern. While the insomnia group had higher values at a number of time points, statistical significance was not consistent in all the comparisons. This variation might be due to NLR's sensitivity to treatment-induced physiological changes, to the characteristics of the disease, and to individual inflammatory responses. Therefore, the current results indicate that NLR by itself may not reflect the full relationship between sleep disturbance and systemic inflammation. The more consistent patterns seen for LMR, PLR and SII may better show the altered balance between inflammatory cell populations and immune competence among the breast cancer patients in the insomnia group (Templeton et al., 2014a; Ethier et al., 2017).

CRP revealed one of the most consistent results in the study. At the time before surgery, during treatment, and at the first follow-up, the levels in the insomnia group were higher than those in both the healthy controls and the non-insomnia group, while no significant difference was found between the healthy controls and the non-insomnia group. The fact that this difference remained present at all three clinical time points shows that the link between insomnia and systemic inflammatory activity was not merely due to the active treatment phase. The substantial rise in CRP during treatment, coupled with the continued elevation at follow-up, suggests that the inflammatory burden associated with the insomnia group was still evident along the entire treatment course. Since CRP is a non-specific acute-phase marker and can be affected by a number of clinical and physiological factors, its increase cannot be specifically attributed to sleep disturbance. Yet the persistent CRP pattern observed in this study is in line with an association between insomnia and increased systemic inflammatory activity (Irwin, 2019; Irwin et al., 2016).

The cytokine results also supported this explanation. IL-6 was found to be elevated throughout the insomnia group at all three clinical time points, with significant differences compared to both healthy controls and the group without insomnia. The fact that IL-6 levels remained higher from baseline through to active treatment and the first follow-up indicates that the link between insomnia and systemic inflammatory activity lasted throughout the whole of the longitudinal study. IL-6 is a pleiotropic cytokine that plays a role in inflammatory signalling, immune regulation, angiogenesis, and processes related to tumour progression. Earlier clinical studies have reported associations between sleep disturbance and higher IL-6 concentrations in both cancer and non-cancer populations (Irwin, 2019; Irwin et al., 2016). The present longitudinal findings are thus in agreement with the existing evidence which links sleep disturbance to systemic inflammatory activity. Nevertheless, the study cannot establish whether sleep disturbance leads to increased IL-6, whether systemic inflammation causes sleep disturbance, or whether both are affected by common disease- or treatment-related factors.

TNF- α also displayed a similarly persistent pattern. At baseline, during treatment, and at the first follow-up, higher concentrations of TNF- α were found in the insomnia group. The fact that this difference was present before treatment and continued afterwards indicates that the association between insomnia and TNF- α was not limited to the acute phase of treatment. Since TNF- α is a major pro-inflammatory mediator that plays a role in immune regulation, apoptosis, and the activation of downstream inflammatory pathways, and as earlier studies have associated elevated levels of circulating TNF- α with a greater burden of cancer-related symptoms and sleep disturbance (Irwin, 2019; Irwin et al., 2016), the current findings thus point to a link between insomnia and ongoing pro-inflammatory activity in patients with breast cancer. However, because the study is observational, it is not possible to draw a conclusion about a direct causal relationship.

The pattern of IFN- γ was more dependent on time than that of IL-6 and TNF- α . At baseline, the levels of IFN- γ were higher in the breast cancer groups compared to those in healthy controls, but the difference between the insomnia group and the non-insomnia group was not statistically significant. During active treatment, on the other hand, IFN- γ was considerably higher in the insomnia group than in both the healthy controls and the non-insomnia group. At the first follow-up, IFN- γ remained elevated in the insomnia group, although the difference between the two breast cancer groups was less marked than during active treatment. This indicates that the link between insomnia and altered immune-related cytokine responses may become more evident under the physiological stress caused by active treatment. Since IFN- γ plays an important role in antitumour immune responses and immune regulation (Grivennikov et al., 2010), the increased levels in the circulation should not be seen simply as evidence of either favourable or adverse tumour biology. Rather, the present findings may indicate altered immune activation resulting from the combined effects of breast cancer, treatment, and sleep disturbance.

There was a clear pattern relating to stress over time. At the beginning of the study, the levels of cortisol were already higher in the group with insomnia, they became most elevated during the period of active treatment, and the levels remained high at the first follow-up. The rise in cortisol associated with treatment can be explained by the combined impact of psychological stress caused by cancer, the physiological burden of treatment, and sleep disturbance on the activity of the hypothalamic-pituitary-adrenal axis (Besedovsky et al., 2012; Lupien et al., 2009). Chronic sleep disturbance might disrupt the normal circadian regulation of cortisol and thus lead to a prolonged activation of the body's stress-response mechanisms. Higher cortisol levels may also affect the function of immune cells and inflammatory signalling. The fact that the levels remained elevated at follow-up indicates that the neuroendocrine aspect of the link between sleep disturbance and systemic stress may last beyond the period of active treatment. However, since a number of factors can affect cortisol levels, this study is unable to establish the independent contribution of insomnia to these changes.

A distinct time-dependent pattern was seen in the circulating NF- κ B-related measurement. At baseline there were no significant differences between the healthy controls, the non-insomnia group, and the insomnia group, but during treatment and at the first follow-up the insomnia group had significantly higher levels; the difference therefore became apparent during treatment and remained after treatment had finished. Since NF- κ B is a major regulator of inflammatory gene expression and of cellular stress responses, it provides a reasonable biological basis for the link between sleep disturbance and inflammatory signalling (Taniguchi and Karin, 2018; Greten and Grivennikov, 2019). In this study, the insomnia group had higher levels of both IL-6 and TNF- α together with higher circulating NF- κ B-related measurements, indicating coordinated changes in systemic inflammation. However, the NF- κ B measurement was taken from peripheral blood and was based on immunoreactivity in the circulation rather than representing a direct measurement of intracellular NF- κ B activation. It must therefore be regarded as

indicating an association with systemic inflammatory changes and not as proof of activation of the NF- κ B pathway within the breast tumour microenvironment.

When looking at the connection between insomnia and changes in the body's systemic biology, it was not limited to just one inflammatory or stress indicator. On the contrary, the group with insomnia displayed a more widespread pattern, including a lower LMR, a higher PLR and SII, persistently raised CRP, and higher levels of IL-6, TNF- α , cortisol, IFN- γ , and NF- κ B-related values at various stages of the treatment process. Importantly, the greatest difference between the insomnia group and healthy controls, as well as between the insomnia group and the group without insomnia, was seen during active treatment, a period when sleep disturbance also increased. Several of the abnormalities were still apparent at the first follow-up visit even though there had been some improvement in sleep disturbance and a partial reduction in the inflammatory markers. This longitudinal pattern indicates that the biological links between insomnia may be intensified during treatment and may last on beyond the active phase of therapy. At the same time, the variation among the different biomarkers shows that sleep disturbance is probably not connected to systemic inflammation via a single, uniform pathway.

The results are consistent with interactions between sleep disturbance, neuroendocrine stress responses, immune regulation, and systemic inflammation. It is possible that chronic sleep disturbance could affect the activity of the hypothalamic-pituitary-adrenal and sympathetic nervous systems, thereby influencing cytokine production, leukocyte trafficking, and platelet activation. On the other hand, the inflammatory signalling linked to breast cancer and the physiological stress caused by treatment might lead to a worsening of sleep disturbance. These mutual interactions may account for the fact that both sleep disturbance and changes in inflammation were seen throughout the treatment period. The current study thus offers a longitudinal clinical framework illustrating the association between sleep disturbance and systemic inflammatory and stress-related responses, although the exact biological mechanisms involved have not yet been established experimentally.

The results obtained could have important consequences for clinical practice. The fact that patients have persistent sleep disturbances together with abnormal levels of inflammatory and stress-related factors suggests that regular sleep assessment should be carried out in cases of breast cancer (Howell et al., 2013; Grassi et al., 2023). If clinically significant insomnia is detected, this might cause clinicians to realise that the patients are undergoing a greater burden of symptoms and biological effects as a result of treatment; yet this study did not set out to find out whether treating insomnia leads to changes in the levels of inflammatory biomarkers. Future prospective intervention studies should investigate whether evidence-based interventions such as cognitive behavioural therapy for insomnia, education on good sleep habits, or other appropriate sleep management interventions can improve sleep and whether any such improvement is linked to changes in systemic inflammatory and stress-related biomarkers.

A key advantage of the current study is its use of a prospective longitudinal design, which permitted the repeated evaluation of sleep disturbance together with biomarkers relating to inflammation, the immune system, and stress at three clinically important stages: before surgery, during treatment, and at the first follow-up. Because of this method, it was possible to identify temporal patterns that could not be detected by a single cross-sectional measurement. The combination of ISI assessment with haematological inflammatory markers, including CRP, circulating cytokines, cortisol, and a circulating NF- κ B-related measure also offered a more comprehensive evaluation of the systemic biological changes associated with sleep disturbance.

When interpreting the findings, several limitations should be taken into account. Firstly, since the study is based on an observational approach it can only identify associations and cannot prove causality. Secondly, the number of patients in the final subgroups was fairly small, there being 32 breast cancer patients in each group, and this may result in limited statistical power and reduced generalizability together with an increased risk of a type II error in which real associations fail to be detected. Moreover, although we recorded tumour stage, histological grade, and other clinicopathological features, the sample size was not large enough to allow for adequately powered stratified or interaction analyses according to these variables or molecular subtypes. This limitation means that we are unable to assess whether the relationships between sleep disturbance and inflammatory biomarkers vary among the subgroups defined by cancer pathology or by treatment regimen. We also did not carry out a detailed analysis of differences in treatment exposure, including variations in chemotherapy, radiation, and endocrine therapy, and these factors might introduce heterogeneity in the inflammatory and sleep-related responses that the main analyses do not take into account.

The study gathered information regarding selected comorbidities, medication use, and baseline lifestyle characteristics, but it was not set up to make detailed longitudinal adjustments for these factors. Since there was no repeated assessment of comorbidity or systematic recording of the start or stopping of new medications during the follow-up period, it is not possible to assess how changing exposures might affect biomarker dynamics. The medication exposure was not examined in enough depth or frequency to establish its independent or interactive effects on systemic inflammation, so residual confounding or drug-biomarker interactions cannot be ruled out. In a similar way, psychological distress—which is known to contribute to both sleep disturbances and inflammation—was not evaluated using validated longitudinal mental health measures, which in turn limits the ability to take it into account or adjust for its effects on the observed outcomes.

Lifestyle factors such as diet, physical activity, and substance use were evaluated at baseline only and not monitored at any later time. Since these behaviours could alter as a result of being diagnosed or receiving treatment, the fact that changes in them have not been measured may still lead to bias when looking at associations over time. Insomnia was measured only by means of the self-reported ISI, not through objective sleep measures such as actigraphy or polysomnography, which could result in reporting bias or misclassification.

Other limitations involve selection bias resulting from non-participation or attrition, as well as unmeasured confounding factors which were not taken into account in the analytical method. Altogether, these limitations highlight the importance of conducting future multicentre longitudinal studies using larger and more diverse groups, including objective measurement of sleep, carrying out comprehensive and repeated assessments of clinical, pharmacological, psychological and lifestyle variables, and carrying out stratified analyses in order to clarify the temporal and possibly mechanistic relationships between sleep disturbance and systemic inflammatory responses in breast cancer.

In summary, this study shows that the group of patients with insomnia had a more widespread and lasting pattern of systemic inflammatory, immune-related, and stress-related biomarkers among breast cancer patients who were receiving curative treatment. The fact that the changes were more evident during active treatment and that several abnormalities remained at the first follow-up visit indicates that sleep disturbance may be a clinically important part of the systemic burden encountered throughout the breast cancer treatment process. In order to move forward in this area, future research should involve multicentre, longitudinal studies using larger and more diverse groups of patients in order to investigate the causal links between sleep disturbance and systemic inflammation. Clinical trials that examine whether targeted treatment of insomnia leads to measurable changes in inflammatory and stress-related biomarkers could help to clarify the underlying mechanisms. Moreover, it would be useful to include objective methods of assessing sleep such as actigraphy or polysomnography, together with more thorough longitudinal monitoring of psychological, lifestyle, and medication factors, in order to improve the understanding of the mechanisms involved. However, the findings should still be regarded as indicating association rather than as proof that insomnia directly causes changes in systemic inflammation.

5. CONCLUSION

In this ongoing prospective longitudinal study, an association was found between insomnia and various haematological, inflammatory, immune, and stress-related biomarker levels in women with breast cancer who were receiving treatment with the aim of curing the disease. Patients who suffered from insomnia as a whole had a lower lymphocyte-to-monocyte ratio but a higher platelet-to-lymphocyte ratio, a higher neutrophil-to-lymphocyte ratio, higher levels of C-reactive protein and a number of other circulating inflammatory and stress-related biomarkers, although the strength and significance of specific biomarkers varied at the different clinical time points. The frequency of sleep disturbances rose during the course of treatment and then fell after treatment had been completed; nevertheless, this reduction was only partial. The findings support the idea of incorporating regular sleep screening into the care of patients with breast cancer and suggest that assessments of sleep disorders and of inflammatory biomarkers may provide mutually informative insights into the body's systemic response to treatment. Since the study is observational, it is not possible to establish causality.

Ethics Statements

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board and Institutional Ethics Committee of Malabar Cancer Centre (MCC), Thalassery (Approval No. 1617/IRB-IEC/13/MCC/29-01-2025/2; Date: February 17, 2025), with additional ethical clearance from the Institutional Human Ethics Committee of the Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore (Clearance No. AUW/IHEC/24-25/BCBT/FHP-07). Written informed consent was obtained from all participants prior to enrolment and sample collection.

Data Availability

The datasets generated and/or analysed during the current study are available upon reasonable request, subject to institutional, ethical, and participant confidentiality restrictions.

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Author Contributions

Jilsheena P.K.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review and editing.

Sumathi S.: Project administration, Supervision, Validation, Methodology, Conceptualization, Writing – review and editing.

Sindhu Edakkadath Raghavn: Conceptualization, Supervision.

Jisha Abraham: Conceptualization.

Conflict of Interest

The authors declare that they have no conflicts of interest.

References

- [1] Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al., 2021. GLOBOCAN's estimates of the incidence and mortality worldwide for 36 cancers in 185 countries as presented in Global Cancer Statistics 2020. *CA Cancer J Clin* 71(3), 209–249. 10.3322/caac.21660.
- [2] Waks, A.G., Winer, E.P., 2019. Breast Cancer Treatment: A Review. *JAMA* 321(3), 288–300. 10.1001/jama.2018.19323.
- [3] Savard, J., Morin, C.M., 2001. Insomnia in the context of cancer: A review of a neglected problem. *J Clin Oncol* 19(3), 895–908. 10.1200/JCO.2001.19.3.895.
- [4] Howell, D., Oliver, T.K., Keller-Olaman, S., Davidson, J.R., Garland, S., Samuels, C., et al., 2013. A Pan-Canadian practice guideline: prevention, screening, assessment, and treatment of sleep disturbances in adults with cancer. *Support Care Cancer* 21(10), 2695–2706. 10.1007/s00520-013-1823-6.
- [5] Büttner-Teleagă, A., Kim, Y.T., Osel, T., Richter, K., 2021. Sleep Disorders in Cancer—A Systematic Review. *Int J Environ Res Public Health* 18(21), 11696. 10.3390/ijerph182111696.
- [6] Savard, J., Simard, S., Blanchet, J., Ivers, H., Morin, C.M., 2001. Prevalence, clinical characteristics, and risk factors for insomnia in the context of breast cancer. *Sleep* 24(5), 583–590. 10.1093/sleep/24.5.583.
- [7] Grassi, L., Zachariae, R., Caruso, R., Palagini, L., Campos-Ródenas, R., Riba, M.B., et al., 2023. Insomnia in adult patients with cancer: ESMO Clinical Practice Guideline. *ESMO Open* 8(6), 102047. 10.1016/j.esmoop.2023.102047.
- [8] Savard, J., Ivers, H., Savard, M.H., Morin, C.M., 2015. Cancer treatments and their side effects are associated with aggravation of insomnia: Results of a longitudinal study. *Cancer* 121(10), 1703–1711. 10.1002/cncr.29244.
- [9] Fleming, L., Randell, K., Stewart, E., Espie, C.A., Morrison, D.S., Lawless, C., Paul, J., 2019. Insomnia in breast cancer: a prospective observational study. *Sleep* 42(3), zsy245. 10.1093/sleep/zsy245.
- [10] Dahiya, S., Ahluwalia, M.S., Walia, H.K., 2013. Sleep disturbances in cancer patients: Underrecognized and undertreated. *Cleve Clin J Med* 80(11), 722–732. 10.3949/ccjm.80a.12170.

- [11] Irwin, M.R., 2019. Sleep and inflammation: Partners in sickness and in health. *Nat Rev Immunol* 19(11), 702–715. 10.1038/s41577-019-0190-z.
- [12] Besedovsky, L., Lange, T., Born, J., 2012. Sleep and immune function. *Pflugers Arch* 463(1), 121–137. 10.1007/s00424-011-1044-0.
- [13] Irwin, M.R., Olmstead, R., Carroll, J.E., 2016. Sleep disturbance, sleep duration, and inflammation: A systematic review and meta-analysis of cohort studies and experimental sleep deprivation. *Biol Psychiatry* 80(1), 40–52. 10.1016/j.biopsych.2015.05.014.
- [14] Coussens, L.M., Werb, Z., 2002. Inflammation and cancer. *Nature* 420(6917), 860–867. 10.1038/nature01322.
- [15] Hanahan, D., 2022. Hallmarks of Cancer: New Dimensions. *Cancer Discov* 12(1), 31–46. 10.1158/2159-8290.CD-21-1059.
- [16] Greten, F.R., Grivnickov, S.I., 2019. Inflammation and Cancer: Triggers, Mechanisms, and Consequences. *Immunity* 51(1), 27–41. 10.1016/j.immuni.2019.06.025.
- [17] Grivnickov, S.I., Greten, F.R., Karin, M., 2010. Immunity, inflammation, and cancer. *Cell* 140(6), 883–899. 10.1016/j.cell.2010.01.025.
- [18] Taniguchi, K., Karin, M., 2018. NF- κ B, inflammation, immunity and cancer: Coming of age. *Nat Rev Immunol* 18(5), 309–324. 10.1038/nri.2017.142.
- [19] Yu, H., Pardoll, D., Jove, R., 2009. STATs in cancer inflammation and immunity: A leading role for STAT3. *Nat Rev Cancer* 9(11), 798–809. 10.1038/nrc2734.
- [20] Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci* 10(6), 434–445. 10.1038/nrn2639.
- [21] Templeton, A.J., McNamara, M.G., Šeruga, B., Vera-Badillo, F.E., Aneja, P., Ocaña, A., et al., 2014a. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: A systematic review and meta-analysis. *J Natl Cancer Inst* 106(6), dju124. 10.1093/jnci/dju124.
- [22] Ethier, J.L., Desautels, D., Templeton, A., Shah, P.S., Amir, E., 2017. Prognostic role of neutrophil-to-lymphocyte ratio in breast cancer: A systematic review and meta-analysis. *Breast Cancer Res* 19(1), 2. 10.1186/s13058-016-0794-1.
- [23] Yang, R., Chang, Q., Meng, X., Gao, N., Wang, W., 2018. Prognostic value of systemic immune-inflammation index in cancer: A meta-analysis. *J Cancer* 9(18), 3295–3302. 10.7150/jca.25691.
- [24] Roxburgh, C.S.D., McMillan, D.C., 2010. Role of systemic inflammatory response in predicting survival in patients with primary operable cancer. *Future Oncol* 6(1), 149–163. 10.2217/fon.09.136.
- [25] Diakos, C.I., Charles, K.A., McMillan, D.C., Clarke, S.J., 2014. Cancer-related inflammation and treatment effectiveness. *Lancet Oncol* 15(11), e493–e503. 10.1016/S1470-2045(14)70263-3.
- [26] Bastien, C.H., Vallières, A., Morin, C.M., 2001. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. *Sleep Med* 2(4), 297–307. 10.1016/S1389-9457(00)00065-4.
- [27] Guthrie, G.J., Charles, K.A., Roxburgh, C.S., Horgan, P.G., McMillan, D.C., Clarke, S.J., 2013. The systemic inflammation-based neutrophil-to-lymphocyte ratio: Experience in patients with cancer. *Crit Rev Oncol Hematol* 88(1), 218–230. 10.1016/j.critrevonc.2013.03.010.
- [28] Templeton, A.J., Ace, O., McNamara, M.G., Al-Mubarak, M., Vera-Badillo, F.E., Hermanns, T., et al., 2014b. Prognostic role of platelet-to-lymphocyte ratio in solid tumors: A systematic review and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 23(7), 1204–1212. 10.1158/1055-9965.EPI-14-0146.

- [29] Hu, B., Yang, X.R., Xu, Y., Sun, Y.F., Sun, C., Guo, W., et al., 2014. Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. *Clin Cancer Res* 20(23), 6212–6222. 10.1158/1078-0432.CCR-14-0442.
- [30] Nishijima, T.F., Muss, H.B., Shachar, S.S., Tamura, K., Takamatsu, Y., 2015. Prognostic value of lymphocyte-to-monocyte ratio in patients with solid tumors: A systematic review and meta-analysis. *Cancer Treat Rev* 41(10), 971–978. 10.1016/j.ctrv.2015.10.003.

Figure legends

Figure 1. Longitudinal changes in individual ISI scores from pre-surgery to during treatment and first follow-up. Individual points represent breast cancer participants assessed at the three clinical time points. The mean ISI score increased from 7.13 at pre-surgery to 9.25 during treatment and subsequently decreased to 8.48 at first follow-up.

Figure 2. Longitudinal patterns of haematological inflammatory indices in healthy controls, breast cancer patients without insomnia (non-insomnia group), and breast cancer patients with insomnia (insomnia group). Mean $\pm$ SD values of lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), and systemic immune-inflammation index (SII) at pre-surgery, during treatment, and first follow-up. Healthy controls were assessed at a single enrolment time point and are shown as a reference for comparison. Breast cancer patients were assessed longitudinally at all three clinical time points.

Figure 3. Longitudinal patterns of serum inflammatory and stress-related biomarkers in healthy controls, breast cancer patients without insomnia (non-insomnia group), and breast cancer patients with insomnia (insomnia group) across the treatment trajectory. Violin plots show the distribution of IL-6, cortisol, circulating NF- κ B, IFN- γ , and TNF- α levels in healthy controls, breast cancer patients without insomnia (non-insomnia group), and breast cancer patients with insomnia (insomnia group) at pre-surgery, during treatment, and first follow-up. Healthy controls were assessed at a single enrolment time point and are shown as a reference for comparison, whereas breast cancer patients were assessed longitudinally at all three clinical time points. The y-axis represents the respective concentration units indicated in each plot. Statistical significance between groups is indicated by asterisks: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns, not significant. Statistical analysis was performed using GraphPad Prism version 8.0.2.