



Night shift work and breast cancer: from etiopathology to precision risk analysis



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The increasing prevalence of night shift work (NSW) in our current professional landscape has raised significant public health concerns, particularly regarding its potential role in breast cancer (BC) development among women. Recognized by the International Agency for Research on Cancer (IARC) as a probable human carcinogen, NSW is believed to contribute to carcinogenesis primarily through circadian disruption induced by exposure to light at night. This review explores three key areas: (1) the biological mechanisms potentially linking NSW to BC, including melatonin suppression, oxidative stress, immune dysregulation, chronic inflammation, clock gene alterations, epigenetic modifications, telomere shortening, estrogen signaling disruption, vitamin D deficiency, and gut microbiome imbalance; (2) the emergence of novel putative biomarkers with might be relevant to early detection and precision risk analysis; and (3) the latest epidemiological evidence from case-control and cohort studies evaluating BC risk in female night shift workers, while considering the heterogeneity caused by exposure misclassification and other confounding factors. Altogether, these insights underscore the importance of integrating mechanistic, molecular, and epidemiological data, not only to deepen our understanding of the strength and nature of the relationship between NSW and BC, but also to support a precision medicine framework. This integrated approach is essential for improving individual risk stratification, guiding occupational health policies, and developing targeted preventive strategies for high-risk workers.

In industrialized societies, the demand for around-the-clock productivity has led to the widespread adoption of novel workplace dynamics, such as night shift work (NSW), across essential sectors including healthcare, transportation, manufacturing, and security¹. This shift in labor patterns, although vital to the functioning of our 24 h global economy, has introduced substantial public health concerns, especially for women². Indeed, a growing body of evidence suggests a potential association between long-term NSW and an increased risk of developing breast cancer (BC), a hypothesis with far-reaching implications for occupational medicine and cancer prevention³. BC remains the most commonly diagnosed malignancy and the leading cause of cancer-related mortality among women worldwide⁴. The contribution of occupational factors to this burden could translate into a significant number of preventable cases, making the elucidation of this association a priority for the occupational medicine sector⁵.

Since 2019, the International Agency for Research on Cancer (IARC) has classified NSW as “probably carcinogenic to humans” (i.e., Group 2 A carcinogen), based on strong mechanistic evidence and suggestive epidemiological data⁶. This decision reflects increasing recognition of circadian disruption as a biologically plausible contributor to cancer development⁷.

Hence, several epidemiological studies demonstrated an elevated incidence of BC among women exposed to long-term or rotating night shifts (NS). However, these findings have often been inconsistent and limited by challenges in exposure classification, retrospective design, and/or inadequate control of confounding factors¹. Pivotaly, BC is a prototypical multifactorial disease influenced by a complex interplay of genetic, hormonal, reproductive, environmental, and lifestyle factors⁸. Hence, isolating the specific contribution of NSW within this context is inherently difficult⁹. Many factors, such as age, menopausal status, hormonal therapy, lactation, family history, and high-penetrance germline mutations (e.g., BRCA1/2), all shape individual risk¹⁰. Moreover, gene-environment interactions, often mediated by epigenetic modifications, may further modulate susceptibility to carcinogenic effects of NSW, complicating causal inference¹¹.

At the mechanistic level, the hypothesized link between NSW and BC is grounded in the concept of circadian disruption¹². Circadian rhythms are endogenously generated 24 h cycles in physiology and behavior that are orchestrated by the suprachiasmatic nucleus (SCN) of the hypothalamus, commonly referred to as the central clock¹³. These rhythms regulate a wide range of biological processes including hormonal secretion, immune

function, DNA repair, metabolism, and cell cycle progression¹⁴. Synchronization of the central clock with the external environment relies on time cues known as zeitgebers (derived from the German “time-givers”)¹⁵. The most dominant zeitgeber is the light-dark cycle, although other factors such as feeding times, temperature changes, and social cues also contribute¹⁶. NSW disturbs this alignment by exposing individuals to artificial light at night (LAN), in turn altering both sleep-wake and feeding rhythms. This misalignment, often termed circadian desynchrony, may have far-reaching effects on hormonal and metabolic homeostasis¹⁷.

One of the most critical pathways implicated in the NSW-BC axis involves the suppression of nocturnal melatonin secretion¹⁸. Melatonin, a neuro-hormone produced by the pineal gland in darkness, possesses potent oncostatic properties¹⁹. It regulates cell proliferation, promotes apoptosis in malignant cells, scavenges reactive oxygen species, and, importantly, it modulates estrogen receptor (ER) expression and activity²⁰. Its suppression through LAN exposure is hypothesized to facilitate estrogen-dependent carcinogenesis²¹. Preclinical studies in rodent models have shown that LAN exposure, increases oxidative DNA damage, disrupts estrogen signaling and accelerates mammary tumor growth²². These effects that can be partially reversed by exogenous melatonin administration²³. Altogether, these findings support the so-called melatonin-estrogen hypothesis of breast carcinogenesis²⁴.

In addition to melatonin-related mechanisms, there is growing interest in uncovering the role played by circadian clock genes (such as CLOCK, BMAL1, PER1/2/3, CRY1/2, and NPAS2), which not only govern circadian timing but also regulate processes central to tumorigenesis, including cell cycle control, DNA damage response, metabolism, and immune regulation²⁵. Alterations either in the expression or in the epigenetic regulation of these genes have been observed in BC and are increasingly linked to exposure to chronic NSW²⁶. For instance, reduced expression of PER1 and CRY1 has been associated with higher tumor grade and poor prognosis²⁷.

Epidemiological efforts are likewise evolving. While earlier studies were limited by crude exposure assessments and lack of chronobiological context (e.g., individual chronotype, sleep quality), newer cohorts are integrating objective measurements such as actigraphy, light sensors, and sleep diaries²⁸. Concurrently, genetic and epigenetic profiling is being incorporated into large-scale prospective studies to further clarify gene-environment interactions, identify biologically vulnerable subgroups, and build individualized risk models²⁹. Some of these investigations are also testing interventional strategies, such as melatonin supplementation or circadian-friendly lighting systems, to mitigate biological disruptions in NS workers³⁰.

Recent studies have begun to identify novel molecular biomarkers capable of capturing early biological effects of NSW exposure³¹. Among the most promising markers there are: urinary melatonin metabolites (such as 6-sulfatoxymelatonin or aMT6s), salivary or serum cortisol profiles, DNA methylation signatures in clock and tumor suppressor genes, telomere elongation patterns, and altered circulating microRNAs (miRNAs) levels^{32,33}. These novel biomarkers may eventually provide precision tools for risk stratification, early detection, and intervention among NSW-exposed individuals, but further validation in longitudinal cohort studies is essential³⁴.

Despite considerable progress, multiple challenges remain. For instance, the absence of standardized definitions for NSW across studies, the difficulty in quantifying long-term exposure retrospectively, and the often-subtle nature of biological changes, all hinder the establishment of causality and the development of occupational exposure limits³⁵. Nevertheless, the convergence of mechanistic, epidemiological, and biomarker-based evidence suggests that the link between NSW and BC is biologically plausible and clinically relevant¹. In this context, moving from risk description to precision risk prediction represents both a scientific and ethical imperative¹¹.

This review aims to report the most recent and significant advances in our understanding of the relationship between NSW and BC, with a particular focus on female occupational exposure. By integrating mechanistic evidence, biomarker discoveries, and emerging data from human studies, it

will be examined how chronic circadian disruption may drive breast carcinogenesis, how novel molecular tools could refine individual risk stratification, and, finally, how these insights might reshape regulatory frameworks and preventive strategies¹⁷. Although NSW is currently as a Group 2 A carcinogen, this assessment is primarily based on mechanistic evidence and limited epidemiological data¹. By critically reviewing recent scientific developments, the final goal is to assess whether new findings might reinforce this classification or offer alternative perspectives that may refine or challenge it. In fact, as we are living the era of precision medicine, there is a pressing need to identify high-risk workers, establish robust exposure-effect relationships, and develop targeted interventions to mitigate occupational cancer risk³⁶.

Literature search and outcomes

A keyword search was carried out in PubMed, Web of Science, and Scopus databases. Keywords were organized into major thematic groups to ensure systematic coverage of all relevant aspects. Boolean operators (“AND,” “OR”) were used to combine terms across groups (Supplementary Table 1). In addition, a MEDLINE search using Medical Subject Headings (MeSH) was conducted. Each MeSH term was combined with relevant subheadings and qualifiers to refine the search (Supplementary Table 2).

The search was limited to peer-reviewed articles in English. Inclusion criteria were: (1) original research or review articles examining the association between NSW and BC in women; (2) studies addressing biological mechanisms, epidemiological associations, or biomarker discovery; (3) observational studies (cohort, case-control, cross-sectional) or experimental/preclinical evidence relevant to NSW-BC pathways. Exclusion criteria included: (1) preprints, editorials, commentaries, or conference abstracts without peer review; (2) articles without full-text availability; (3) studies not directly relevant to the predefined thematic topics.

As shown in the flow diagram (Supplementary Fig. 1), the initial database search identified 2494 records. After removing 1799 duplicates, 695 records remained for screening. Of these, 384 articles were excluded due to non-English language, unavailable full text, preprints, or lack of relevance. Ultimately, 312 records were included in the final review. Among these, 151 were used for general review across all sections, while 161 peer-reviewed studies met the inclusion criteria and were retained for qualitative analysis. Within this subset, 127 studies were discussed in the section on mechanistic studies in worker cohorts, while 34 studies were analyzed in the section focusing on worker populations assessing BC risk.

Night shift work-related breast carcinogenesis: mechanistic pathways and biomarker discovery

The etiopathogenesis of BC in the context of NSW is increasingly understood through mechanistic studies that explore the biological consequences of circadian disruption³⁷. Experimental models have been instrumental in elucidating how perturbations in circadian rhythms may foster a pro-tumorigenic environment, particularly through hormonal, immune, genetic, and epigenetic pathways³⁸. Furthermore, human evidence strengthened the current conclusions³⁹.

A substantial body of research conducted in animals has consistently demonstrated that the disruption of the normal light-dark cycle may significantly increase cancer incidence, tumor growth, and progression^{40,41}. In particular, it was observed that either phase shifts in the light-dark schedule or manipulated photoperiods may promote the initiation and advancement of mammary tumors^{42–44}.

In detail, over six decades of research have progressively deepened our understanding of the role of LAN and circadian disruption in BC development. Foundational rodent studies in the 1960s, were among the first to correlate either constant light exposure or surgical pinealectomy (which disrupts melatonin production) in heightening mammary tumor risk, highlighting the central role of the pineal gland in circadian-driven hormonal regulation^{45,46}. Subsequently, research from the 1980s and 1990s consistently found that suppression of melatonin via continuous light exposure increased susceptibility to chemically induced mammary tumors,

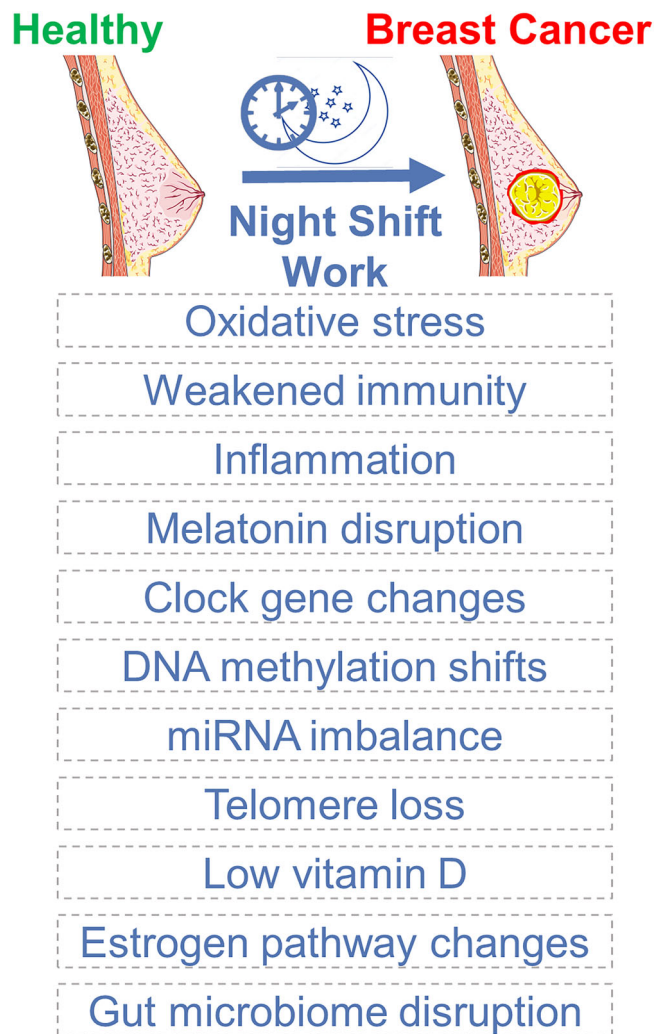


Fig. 1 | Schematic representation of night shift work as a contributor to breast cancer development. The diagram illustrates the transition from a healthy breast to breast cancer, driven by chronic exposure to night shift work. Key biological mechanisms implicated in this process are reported. Selected elements adapted from Servier Medical Art (<https://smart.servier.com>, last accessed on 09 November 2025), licensed under Creative Commons Attribution 4.0 International License (CC BY 4.0; <https://creativecommons.org/licenses/by/4.0/>, last accessed on 09 November 2025).

while exogenous melatonin conferred protective effects^{47–51}. The 2000s marked a mechanistic leap, as it was demonstrated that suppression of nocturnal melatonin through LAN enhanced BC cellular metabolism and growth in breast cancer xenografts, providing compelling evidence of a link between circadian biology and tumor progression^{52–54}. Additional studies confirmed that constant light exposure accelerated tumorigenesis in chemically induced mammary cancer models^{44,55}.

More recent work has expanded these insights. For instance, It was demonstrated that circadian disruption induced by jet lag or shifted light cycles not only accelerates tumor development but also disrupts endocrine, metabolic, and immune pathways that are central to breast carcinogenesis^{40,56}. Furthermore, emerging evidence reveals that circadian misalignment promotes metastasis by enhancing tumor stemness and modifying the tumor immune microenvironment, while also impairing the efficacy of cancer therapies such as cisplatin^{57,58}. Also, it was shown that dietary quercetin mitigates the metastasis-promoting effects of disrupted light-dark cycles, suggesting that holistic dietary or chrono-modulated interventions may offer therapeutic benefit in circadian-compromised oncologic contexts⁵⁹.

Taken together, the results underscore circadian rhythm disruption as a significant and modifiable environmental risk factor in BC development

and progression. Importantly, the majority of these experimental findings have been further corroborated in human NS workers, with clear differences compared to day workers observed across multiple biological mechanisms (including oxidative stress, immune dysfunction, inflammation, melatonin disruption, clock gene alterations, epigenetic changes, telomere attrition, and hormonal imbalances). These key aspects are summarized in Fig. 1 and thoroughly discussed in the sections below.

Oxidative stress

Multiple preclinical findings have demonstrated that LAN exposure induces oxidative stress responses, leading to the accumulation of reactive oxygen species (ROS), DNA damage, and altered expression of oxidative stress-related genes^{60–62}. In turn, these oxidative insults contribute to genomic instability and may act as an early driver of BC development⁶³.

In line with the preclinical evidence, a number of clinical observations in NS and rotating shift workers across various populations and settings consistently suggest that NSW is associated with elevated oxidative stress and DNA damage, along with alterations in antioxidant defense systems⁶⁴. For a summary of the worker findings, see Table 1. In particular, urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, has been found higher in NS workers compared to day shift (DS) workers, although its levels may vary depending on timing relative to work and sleep periods^{65–68}. Consistently, a recent long-term cohort study in hospital NS workers using a '1-1-3' rotating schedule reported significantly increased serum and urinary levels of 8-OHdG during the NS period, which remained elevated even one month after returning to DS, indicating persistent oxidative DNA damage induced by chronic NSW⁶⁹. Also, the expression of the DNA repair enzyme OGG1 is often reduced in NS workers, with increased DNA strand breaks and lowered repair-genes expression measured in blood mononuclear cells from NS physicians compared to DS ones^{70,71}.

Other blood markers such as superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) are frequently found elevated in NS workers, indicating enhanced oxidative stress^{72,73}. Similarly, total oxidative status (TOS), oxidative stress index (OSI), and urinary 8-isoprostane levels (a non-enzymatic peroxidation product of arachidonic acid) tend to increase after extended or continuous NSW, with concurrent decreases or alterations in total antioxidant capacity (TAC) and total antioxidant status (TAS), highlighting an imbalance in favor of oxidative damage^{74–77}. Moreover, a recent randomized crossover study in healthy women demonstrated that prolonged mild sleep restriction markedly increased endothelial oxidative stress without activating compensatory antioxidant responses, due to reduced expression of DCUN1D3 gene, a regulator of the Nrf2-mediated antioxidant pathway, thereby suggesting a mechanistic link between insufficient sleep and vascular oxidative injury⁷⁸. Finally, some antioxidant enzymes like glutathione peroxidase (GSH-Px) appear upregulated in rotating NS workers, possibly as a compensatory mechanism⁷⁹.

However, findings are not uniformly consistent across all populations and biomarkers. Certain studies, such as those involving control room operators and refinery workers, report no significant differences in antioxidant enzymes (i.e., CAT and TTG), TAC, or lipid peroxidation markers (i.e., MDA) in correlation with NSW, although a borderline increase in OSI was noted in rotating NS healthcare workers^{80–82}. Similarly, a recent study among Turkish nurses found no significant differences in oxidative stress parameters (TOS, TAS, cortisol, epinephrine, and norepinephrine) between day and NS workers, although higher perceived stress and anxiety sensitivity were associated with increased TOS and cortisol levels, suggesting that psychological stress may contribute to oxidative imbalance even in the absence of direct shift-related biochemical changes⁸³. This variability might reflect differences in study designs, populations, sample types, or covariate controls, which often included age, sex, (body mass index) BMI, smoking, alcohol consumption, sleep duration, and additional work-related factors. In addition, a retrospective study in men seeking fertility assessment found that NS workers exhibited significantly higher systemic oxidative stress (measured by reactive oxygen metabolites species) compared to day

Table 1 | Human studies on NSW and oxidative stress

NSW Effect	Parameter(s)	Outcome	Year	Ref.
↑ Oxidative DNA damage	Urinary 8-OH-dG	↑ 8-OH-dG	2001	68
↑ Oxidative DNA damage linked to stress/depression (females)	Urinary 8-OH-dG	↑ 8-OH-dG	2008	67
↑ Oxidative stress	Erythrocyte SOD activity, plasma MDA	↑ SOD, ↑ MDA	2008	72
↓ Antioxidant capacity	Plasma total antioxidant capacity (TAC)	↓ TAC	2008	77
↑ Oxidative stress	Serum TOS, OSI; ↓ TAS	↑ TOS, ↑ OSI, ↓ TAS	2010	74
↑ Oxidative stress/burnout	Serum SOD1, MDA	↑ SOD1, ↑ MDA	2011	73
↑ Oxidative stress during shifts	Serum TAS, TOS, OSI	↓ TAS, ↑ TOS, ↑ OSI	2012	75
Altered antioxidant response (menopause-dependent)	Erythrocyte/plasma GPx	↑ GPx (pre-M), ↓ GPx (post-M)	2013	79
No overall difference; possible compensatory effect	Plasma TAC, TOS, OSI	↔ OS markers; TAC↔cognition	2013	81
Association with mild depression	Plasma TAC, MDA	↓ TAC (depressed), ↔ MDA	2014	80
↓ DNA repair capacity	OGG1 gene expression	↓ OGG1	2015	70
Circadian variation in oxidative stress	Urinary 8-OH-dG	↑ 8-OH-dG (day sleep vs night sleep)	2016	65
Impaired repair under melatonin suppression	Urinary 8-OH-dG	↑ 8-OH-dG (night work vs night sleep)	2017	66
↑ Lipid peroxidation	Urinary 8-isoprostane	↑ 8-isoP	2017	76
No light effect on oxidative markers	Salivary antioxidant capacity, thiols, CAT	↔ parameters	2018	82
↑ DNA damage, ↓ repair genes	DNA damage (Comet assay), OGG1, ERCC1, XRCC1 expression	↑ DNA damage, ↓ repair	2019	71
↔ Oxidative stress	Serum TOS, TAS	↔ TOS/TAS	2021	83
↑ Endothelial oxidative stress after sleep restriction	Endothelial OS markers	↑ OS, no ↑ antioxidants	2023	78
↑ Oxidative DNA damage persisting post-shift	Serum and urinary 8-OH-dG	↑ 8-OH-dG (during and post NSW)	2023	69
↑ DNA repair during melatonin supplementation	Urinary 8-OH-dG	↑ 8-OH-dG (day sleep), ↔ night shift	2024	30
↑ Baseline OS; improved with antioxidants	Serum D-ROMs	↑ D-ROMs (baseline), ↓ after supplement	2025	84

NSW night shift work, 8-OH-dG 8-hydroxy-2'-deoxyguanosine, 8-oxodG 8-oxo-2'-deoxyguanosine, 8-isoP 8-isoprostane, CAT catalase, GPx glutathione peroxidase, MDA malondialdehyde, OS oxidative stress, OSI oxidative stress index, SOD superoxide dismutase, TAC total antioxidant capacity, TAS total antioxidant status, TOS total oxidant status, D-ROMs derivatives of reactive oxygen metabolites, pre-M premenopausal, post-M postmenopausal. Arrows indicate direction of change relative to day workers (↑ increase; ↓ decrease; ↔ no change).

workers. In the NS group, antioxidant supplementation over three months led to a greater reduction in oxidative stress and improved semen quality, suggesting a potential benefit of targeted antioxidant therapy in mitigating NSW-related oxidative damage and infertility issues⁸⁴.

Overall, the majority of evidence indicates that NSW significantly contribute to increased oxidative stress and DNA damage, along with diminished DNA repair capacity and altered antioxidant defenses, potentially underlying the elevated health risks, predisposing to several chronic diseases including cancer, observed in NS-working populations^{64,69,85,86}. Intriguingly, melatonin supplementation improves oxidative DNA capacity (measured as urinary excretion of 8-OHdG) in NS workers, suggestive of a possible preventive intervention³⁰.

Immune system impairment

Closely linked to oxidative stress is the deregulation of immune functions. Both preclinical and clinical studies have revealed that circadian misalignment compromises immune surveillance, reducing the capacity of the innate and adaptive immune systems to detect and eliminate emerging tumor cells⁶³.

Experimental studies in rodents and other small mammals consistently demonstrate that disruptions to normal light-dark cycles (e.g., via phase shifts, continuous light or darkness, or dim LAN) can impair various components of the immune system, including natural killer (NK) cell activity, cytokine production, balance in white blood cell populations, and immune responses to pathogens, tumors, or injury⁸⁷. For instance, repeated light-dark cycles reduced NK cell cytotoxicity and increased lung tumor burden in rats, while alternating light-dark cycles durations impaired both delayed-type hypersensitivity (DTH) responses and phagocytosis^{88,89}. Always in rats, continuous light exposure led to diminished humoral

responses and interleukin-2 (IL-2) secretion, as well as persistent disruption of circadian leukocyte rhythms and decreased number and activation of NK cells^{90,91}. However, certain light-deprived conditions may enhance some immune parameters. For example, it has been proven that extended continuous darkness increased circulating T and B lymphocytes in rats⁹².

In diurnal animals like the Nile grass rat, dim LAN paradoxically enhanced immune markers including plasma IgG and DTH, whereas in nocturnal rodents, such as Siberian hamsters and C57BL/6 mice, dim light exposure and phase shifts typically led to immune suppression, delayed wound healing, reduced febrile responses to lipopolysaccharide (LPS), and diminished bactericidal activity^{93–96}.

Additionally, in mice, phase shifts or LAN exposure altered cytokine expression and increased interleukin-6 (IL-6) levels, both systemically and following LPS stimulation^{97,98}. Interestingly, it was recently found that chronic shift lag induces NK cells aging and impairs immunosurveillance⁹⁹. Overall, the findings strongly support the conclusion that chronic or acute disruptions of circadian light-dark patterns impair immune competence, particularly innate immunity, cytokine signaling, with potential effects on BC development^{100–102}.

Cross-sectional studies examining immune function in NS workers confirmed functional impairments in immune cell activity, even when total lymphocyte counts remain unchanged. For a summary of the worker findings, see Table 2. Independent studies across countries, including the USA, Japan, Germany, and Italy, reported no significant differences in total lymphocyte counts between DS and NS workers^{103–106}. However, more detailed measures of immune competence reveal NSW-related suppression. For instance, several observations consistently proven that T-cell and lymphocyte proliferative responses were significantly reduced in NS workers compared to DS ones^{107–110}. This reduced proliferation may indicate

Table 2 | Human studies on NSW and immune system impairment

NSW Effect	Parameter(s)	Outcome	Year	Ref.
Impaired innate immunity	NK cell activity (PB)	↓ NK activity	2008	104
Altered immune regulation	nTreg suppressive function (PB)	↓ nTreg activity	2009	109
No chronic immune activation	IL-6, TNF- α , lymphocyte counts (PB)	↔ Cytokines; ↔ Lymphocytes	2010	105
Transient cytokine suppression	IL-1 β , TNF- α , cortisol (PB, saliva)	↓ Cytokines (transient); ↔ Cortisol	2011	106
Fatigue-related immune changes	NK activity, CD4 + T cell counts (PB)	↓ NK; ↑ CD4+	2011	114
No leukocyte differences	Total leukocytes (PB)	↔ Counts	2018	103
Mild immune activation	Monocytes, lymphocytes, CD8 + T cells (PB)	↑ Counts; ↔ Function	2019	107
Impaired T cell adhesion	β 2-integrin activation on CD8 + T cells (PB)	↓ Adhesion; ↓ Adaptive response	2019	110
Suppressed adaptive immunity	CD4 + T, pDCs, Treg, IL-10, antibody response (PB)	↓ CD4 + /pDCs; ↑ Treg/IL-10; ↓ Humoral response	2020	117
Reduced vaccine durability	Immune cell circadian rhythm; antibody persistence	↓ Vaccine-induced immunity	2022	118
Disrupted immune rhythmicity	Lymphocytes, Th, Tc, memory B, neutrophils, IL-6 (PB)	Phase-shifted peaks; ↑ Infection risk	2022	119
Increased viral susceptibility	SARS-CoV-2 infection risk (epidemiologic data)	↑ Infection risk	2022	122
Suppressed cytokine milieu	Broad cytokine and chemokine profile (PB)	↓ Pro/anti-inflammatory mediators	2022	113
Heightened inflammatory activation	Leukocytes, monocytes, lymphocytes, basophils (PB)	↑ Immune cell counts	2022	112
Reduced humoral response	Anti-SARS-CoV-2 IgG (serum)	↓ IgG levels	2023	120
Enhanced cellular immunity	SARS-CoV-2-specific antibodies, IFN- γ + T cells (PB)	↔ IgG; ↑ IFN- γ response	2024	121
Increased infection prevalence	Self-reported infections; sleep debt (survey)	↑ Common cold, pneumonia, sinusitis, GI infections	2025	123

NSW night shift work, PB peripheral blood, NK natural killer, nTreg natural regulatory T cells, Th T helper cells, Tc cytotoxic T cells, pDCs plasmacytoid dendritic cells, IL interleukin, IFN- γ interferon gamma, GI gastrointestinal. Arrows indicate direction of change relative to day workers (↑ increase; ↓ decrease; ↔ no change).

a diminished ability to trigger effective immune responses, including anti-tumor surveillance¹¹¹. Supporting these findings, a large cross-sectional analysis from the Lifelines Cohort (11,000 individuals) demonstrated that NS workers exhibited significantly higher counts of leukocytes, monocytes, lymphocytes, and basophil granulocytes compared to non-shift workers, suggesting that frequent or consecutive NSW may acutely stimulate immune cell mobilization and contribute to systemic inflammation¹¹².

In line with this, a study in rotating shift workers from the automotive industry found that NSW significantly reduced plasma concentrations of multiple immune markers, including pro- and anti-inflammatory cytokines (TNF- α , IL-2R, IL-1RA, IL-4, IL-12, IL-17), chemokines (IP-10, MIP-1 α , MIP-1 β , RANTES), and growth factors (EGF, FGF, VEGF), indicating a broad suppression of immune pathways linked to circadian disruption and higher susceptibility to infection and cancer¹¹³. Of particular relevance to cancer, NK cell activity, which plays a central role in recognizing and destroying tumor cells, was found to be significantly decreased in NS workers compared to DS¹¹⁴. This suppression of NK function has been hypothesized as a mechanistic link between NSW and increased cancer risk, especially for hormone-sensitive cancers such as BC^{115,116}. Although these studies did not directly measure cancer incidence, the consistent finding of impaired NK cytotoxicity in NS workers supports the experimental evidence that links circadian disruption to reduced anti-tumor immunity¹⁰⁷. Collectively, these findings align with broader epidemiological data suggesting that long-term NSW may contribute to BS risk, in part via suppressed immune surveillance mechanisms¹¹.

Given the effects on immune health, NSW and sleep disruption can affect vaccine efficacy. Following meningococcal vaccination, it was observed that NS workers exhibit reduced deep and REM sleep, elevated inflammatory cytokines (TNF- α , IL-6), and diminished humoral immune responses. These immune deficits were also associated with altered circadian rhythms, reduced CD4 + T cells, and lower prolactin levels¹¹⁷. In a large hospital cohort, it was further investigated the impact of NSW on hepatitis B virus (HBV) vaccine response. NS workers showed reduced serum protection rates; however, after adjusting for demographic and occupational variables, NSW was no longer significantly associated with anti-HBs titers. This suggests that while NSW may influence immune status, its effects may

be confounded by other risk factors¹¹⁸. Also, NS workers exhibited greater social jet lag, shorter sleep, and disrupted diurnal patterns in immune biomarkers such as IL-6, lymphocytes, and T-helper cells, thereby reducing vaccine efficacy¹¹⁹.

In the COVICAT study, which assessed antibody responses to COVID-19 vaccines in a general population cohort, all participants reporting short sleep duration had significantly reduced IgM levels. These results suggest sleep quantity, rather than NSW alone, may play a more direct role in modulating humoral immune responses¹²⁰. In contrast, in a study aimed to characterize immune responses to mRNA COVID-19 vaccines among NS workers, despite significantly reduced sleep duration in NSW post-vaccination, no differences were observed in antibody titers compared to day workers. Notably, NS workers demonstrated stronger SARS-CoV-2-specific T-cell responses, and sleep metrics were not significantly associated with vaccine immunogenicity, suggesting that primary immune responses may be resilient to short-term sleep loss in healthy individuals¹²¹. Recent evidence further supports this link, showing that NS workers had a significantly higher risk of testing positive for SARS-CoV-2 compared to DS workers, suggesting that circadian disruption and sleep loss may increase susceptibility to infectious diseases through impaired immune regulation¹²². Consistent with these findings, a study of 1335 Norwegian nurses reported that NSW was associated with a higher prevalence of self-reported common cold, pneumonia/bronchitis, sinusitis, and gastrointestinal infections, highlighting the role of disrupted sleep and circadian misalignment in increasing susceptibility to infections¹²³.

Inflammation

Together with immune deregulation, both LAN and NSW have also been associated with chronic low-grade inflammation, a recognized hallmark of cancer, including BC, as evidenced by elevated levels of pro-inflammatory cytokines and inflammatory markers in both animal models exposed to LAN and NS workers¹²⁴ (Fig. 2).

Experimental studies conducted in animals over the past two decades consistently demonstrate that disruptions in the light-dark schedule induce inflammatory responses across a range of biological systems, with several findings suggesting potential relevance to BC. For instance, it was shown

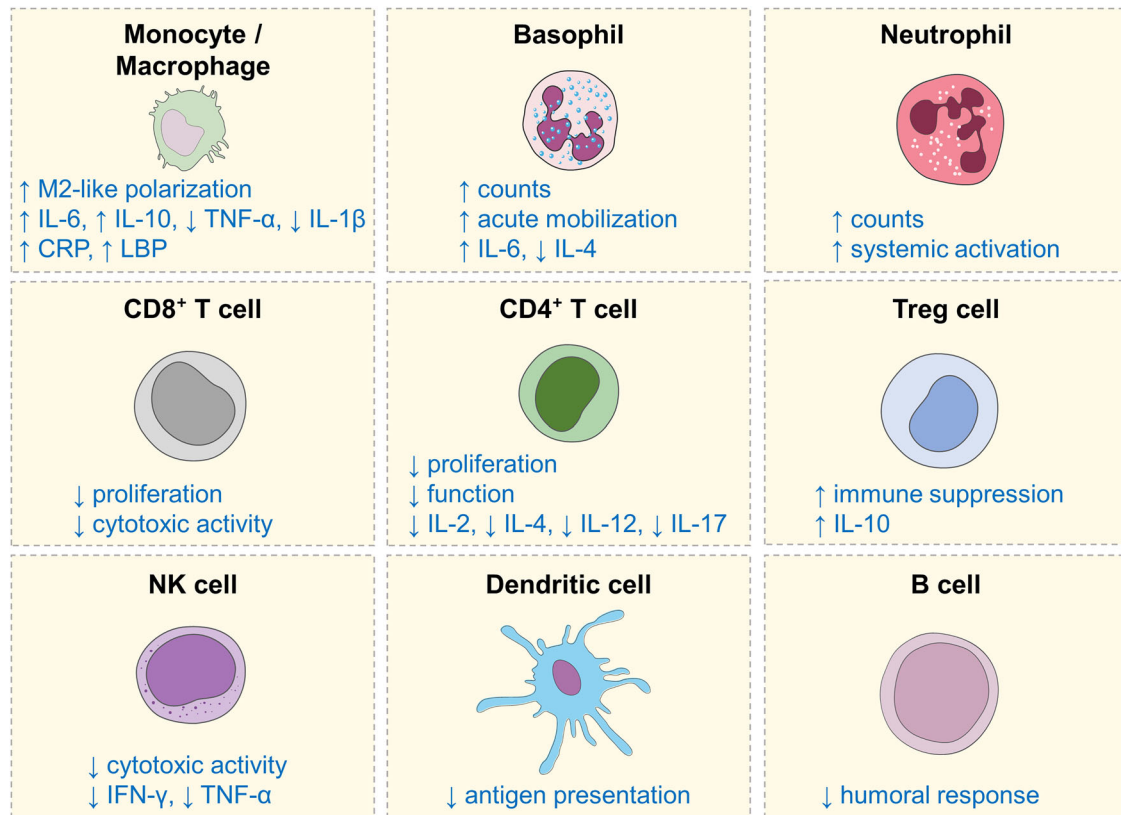


Fig. 2 | Immune cell populations modulated by night shift work. Schematic representation of immune cell subsets affected by circadian disruption in night shift work. Each box depicts a cell type with altered functions and secreted molecules, including cytokines. Monocytes/macrophages: M2-like polarization. Basophils: acute mobilization. Neutrophils: systemic activation. CD8⁺/CD4⁺ T cells: reduced proliferation and activity. Treg cells: enhanced immunosuppression. NK cells: decreased cytotoxicity. Dendritic cells: impaired antigen presentation. B cells:

diminished humoral response. These alterations promote chronic low-grade inflammation and reduced antitumor immune surveillance in long-term night shift work exposure. Selected elements adapted from Servier Medical Art (<https://smart.servier.com>, last accessed on 09 November 2025), licensed under Creative Commons Attribution 4.0 International License (CC BY 4.0; <https://creativecommons.org/licenses/by/4.0/>, last accessed on 09 November 2025).

that repeated light-dark shifts in male C57BL/6 mice exacerbated colonic inflammation and tissue damage, pointing to heightened systemic inflammatory responses¹²⁵. Further studies in Per2Luc knock-in mice revealed that chronic light-dark disruption elevated pro-inflammatory cytokines (e.g., IL1 β , IL6, GM-CSF) and impaired host resilience to endotoxin challenge¹²⁶. Also, it was confirmed persistent IL6 elevation, which is a pro-inflammatory cytokine strongly linked to BC progression^{127,128}. Always in mice, long-term light-dark shifts compromised intestinal integrity and amplified alcohol-induced endotoxemia and hepatic inflammation, highlighting the involvement of the gut-liver-immune axis in circadian disruption¹²⁹.

Complementary findings reported that mice exposed to LAN increased expression of IL6, IL1 β , and TNF α , again highlighting mechanisms of inflammation with known cancer associations^{93,130,131}. This was confirmed in rats, where continuous light significantly upregulated Stat3, IL1 α , and IL17 α , gene products associated with pro-inflammatory immune regulation and BC oncogenesis¹³². Also, it was proven that light-induced circadian disruption primed a heightened pro-inflammatory TNF α response to LPS challenge, while immune imbalance, with increases in pro-inflammatory M1 macrophages and reductions in regulatory T cells, suggesting compromised anti-tumor immune surveillance^{133,134}. A recent study further demonstrated that circadian regulation of mitochondrial function governs time-of-day-dependent activation of the NLRP3 inflammasome in macrophages, with peak inflammatory responses occurring when mitochondrial membrane potential is highest¹³⁵. Collectively, these findings provide compelling experimental evidence that circadian disruption promotes chronic inflammation and associated immune system dysregulation¹³⁶. In particular, the upregulation of IL6, IL1 β , and Stat3, along with alterations in

immune cell populations such as MDSCs and regulatory T cells, and inflammasome suggest plausible mechanistic links to BC pathogenesis¹³⁷.

Several studies in workers further confirmed that NSW is associated with inflammatory alterations that are mechanistically relevant to BC pathogenesis. For a summary of the worker findings, see Table 3. These include elevated levels of c-reactive protein (CRP), IL-6, and increased monocyte counts, suggesting that circadian disruption through NSW may promote a pro-inflammatory and pro-tumorigenic immune environment¹³⁸. In several independent studies it was observed elevated leukocyte counts in NS workers compared to DS workers, indicative of increased systemic inflammation^{139,140}. Also, significantly lower TNF- α and IL-1 β levels were measured in NS nurses compared to DS, and increased leukocyte counts and CRP levels were found in blood of Finnish airline workers engaged in rotating NS^{106,141}.

Furthermore, significantly higher leukocyte counts and CRP levels were reported in both current and former NS workers, highlighting the long-term inflammatory effects of NSW¹⁴². Similarly, significantly increased IL-6 and LPS-binding protein levels were observed in US NS workers, indicating time-dependent immune activation¹⁴³. In Italian nurses exposed to NSW elevated monocyte counts and CRP were reported. These findings are especially relevant to BC due to the involvement of chronic inflammation and monocyte-derived macrophages in tumor development¹⁴⁴. A crossover study in Iranian NS workers demonstrated that night work significantly elevated pro-inflammatory markers, including IL-6, CRP, white blood cells, neutrophils, lymphocytes, and platelets, compared to DS workers, supporting a link between circadian disruption and triggering of systemic inflammation¹⁴⁵. A transcriptomic study in healthcare NS workers

Table 3 | Human studies on NSW and inflammation

NSW Effect	Parameter(s)	Outcome	Year	Ref.
Poor sleep quality linked to elevated WBC rather than NSW itself	WBC (blood)	↑ Systemic inflammation (sleep-mediated)	2007	139
Circadian disruption induces leukocytosis	Total leukocytes (blood)	↑ Systemic inflammation	2007	140
No sustained inflammatory changes with rotational NSW	CRP, ILs, immune cell profile (blood)	↔ No significant effect	2011	106
Multi-shift work increases CRP and WBC (reversible after cessation)	CRP, WBC (blood)	↑ Mild systemic inflammation	2011	141
Elevated IL-6, CRP, WBC, neutrophils, lymphocytes, platelets	Multiple inflammatory markers (blood)	↑ Systemic inflammation	2011	145
Higher hs-CRP and leukocyte count vs. day workers	hs-CRP, WBC (blood)	↑ Systemic inflammation	2016	142
Circadian misalignment amplifies alcohol-induced inflammation	LBP, LPS, IL-6 (blood)	↑ Basal inflammation, loss of rhythmicity	2016	143
NSW increases CRP and monocytes independent of CVD risks	CRP, monocytes (blood)	↑ Systemic inflammation	2017	144
Night-only work elevates IL-6 but not hs-CRP	IL-6, hs-CRP (blood)	↑ IL-6; ↔ hs-CRP	2018	148
Disrupted circadian variation in IL-6, partial for TNF/IL-1β	IL-6, TNF, IL-1β (saliva)	Altered cytokine rhythmicity	2019	149
CRP not elevated by NSW alone, but amplifies pain risk	CRP (blood)	↔ CRP; ↑ Pain risk (synergistic)	2021	150
Increased leukocytes, hs-CRP, and differential counts	hs-CRP, WBC, neutrophils, lymphocytes, monocytes (blood)	↑ Systemic inflammation	2022	151
Male NSW workers show higher GlycA (BMI-related)	GlycA (serum)	↑ Low-grade inflammation	2022	153
NSW elevates WBC, LBP, TNF-α, IL-10; impairs cytokine response	WBC, LBP, TNF-α, IL-10 (blood)	↑ Low-grade inflammation; ↓ cytokine responsiveness	2023	31
NSW and poor sleep ↑ BP, correlated with CRP, WBC, neutrophils	CRP, WBC, neutrophils (blood); BP	↑ Low-grade inflammation, ↑ BP	2023	147
Elevated hs-CRP and TLC; predictors include NSW, age, BMI	hs-CRP, TLC (blood)	↑ Systemic inflammation	2024	152
Low-grade inflammation mediates IBS risk (INFLA-score)	INFLA-score (5 biomarkers, plasma)	↑ IBS risk	2024	154
NSW increases psoriasis severity and IL-8	IL-8 (blood); psoriasis index	↑ Chronic inflammation	2024	156
NSW ↑ CAR and NLR vs. day workers	CAR, NLR (blood)	↑ Chronic low-grade inflammation	2025	124
NSW activates innate immune/inflammatory pathways (TLR/MyD88)	IL-1β, IL-2, IL-6, IFN-γ, CSF2 (blood)	↑ Cytokine activation	2025	146
NSW ↑ INFLA-score (CRP, PLT, WBC, NLR) mediating IBS risk	INFLA-score (blood)	↑ Low-grade inflammation; ↑ IBS risk	2025	155

NSW night shift work, WBC white blood cell count, CRP C-reactive protein, hs-CRP high-sensitivity CRP, IL interleukin, TNF-α tumor necrosis factor alpha, BP blood pressure, CVD cardiovascular disease, LBP lipopolysaccharide-binding protein, LPS lipopolysaccharide, GlycA glycoprotein acetylation, CAR CRP-to-albumin ratio, NLR neutrophil-to-lymphocyte ratio, TLC total leukocyte count, PLT platelet count, INFLA-score composite systemic inflammation score, IFN-γ interferon gamma, CSF2 colony-stimulating factor 2, TLR toll-like receptor. Arrows indicate direction of change relative to day workers (↑ increase; ↓ decrease; ↔ no change).

demonstrated that a single NS can activate innate immune and inflammatory pathways, including Toll-like receptor signaling and cytokines such as IL-6 and IL-1 β . Gene expression changes also involved pathways linked to autoimmune diseases, cancer, and major depressive disorder, indicating that even brief circadian disruption can trigger systemic inflammatory responses with potential long-term health consequences¹⁴⁶.

Recent studies have continued to reinforce these associations. For instance, in healthcare workers, NSW was associated with elevated low-grade systemic inflammation, evidenced by higher leukocytes, TNF- α , IL-10, and LBP levels. Endotoxin tests further showed that long-term exposure impaired TNF- α reactivity, suggesting cumulative NSW increases inflammation-related disease risk, including cancer³¹. Furthermore, a UK Biobank study of over 350,000 adults found that poor sleep quality, extreme sleep durations, and permanent NSW were linked to higher blood pressure and elevated pro-inflammatory markers, including CRP and leukocyte counts. Although largely independent of age and sex, these effects were partially mediated by BMI¹⁴⁷. Higher IL-6 levels were measured in NS workers compared to DS, implicating NSW in immune dysregulation¹⁴⁸. Another study in contrast not report significant differences associated with NSW, possibly because of methodological limitations, such as the use of salivary cytokine measurements¹⁴⁹.

Additionally, a 7-year prospective study found that while NSW alone was not significantly associated with chronic pain or CRP levels, the combination of NSW and moderately elevated CRP was associated with an increased risk of chronic pain and pro-inflammatory background¹⁵⁰. A large cross-sectional study in electronics manufacturing workers further showed that NSW was associated with significantly elevated serum CRP levels, suggesting systemic inflammation and potential cardiovascular disease risk¹⁵¹. Consistently, NS healthcare workers were shown to have significantly higher levels of pro-inflammatory markers (i.e., CRP and TLC) than DS workers, with NSW, age, and BMI identified as independent predictors of elevated CRP¹⁵². Long-term NS workers exhibited significantly higher CRP-albumin and neutrophil-lymphocyte ratios compared to DS workers, supporting the presence of chronic low-grade inflammation associated with circadian rhythm disruption¹²⁴.

A recent metabolomics study found that male NSW exhibited higher glycoprotein acetyls (GlycA), which is a marker of chronic low-grade inflammation, along with elevated triglycerides and fatty acids, indicating a potential sex-specific link between NSW, systemic inflammation, and metabolic deregulation¹⁵³. Similarly, a UK Biobank study of 240,000 workers showed that long-term NSW increased the risk of irritable bowel syndrome (IBS), with higher shift frequency and duration predicting greater incidence¹⁵⁴. A follow-up cohort study further demonstrated that this association is partly mediated by low-grade systemic inflammation, highlighting inflammation as a mechanistic link between chronic NSW and IBS risk¹⁵⁵. Low grade inflammation in NS workers is also associated to other pathologies as demonstrated by a case-control study in psoriatic patients subjected to NSW, which showed higher disease severity and elevated IL-8 levels compared to DS workers. In these subjects, long-term NSW was also associated with higher BMI and hormonal changes, confirming that circadian disruption may exacerbate chronic inflammatory-linked conditions¹⁵⁶. Collectively, these findings strongly suggest that exposure to NSW, induces systemic low-grade inflammation, likely through circadian disruption, and may contribute to the development of chronic diseases, including BC, highlighting the importance of occupational health strategies to mitigate these risks.

Disruption of melatonin chronobiology

As discussed above, a central mechanism that might trigger BC development, involves the suppression of melatonin synthesis by LAN¹⁵⁷. Melatonin, secreted by the pineal gland in response to darkness, plays a key role in maintaining circadian homeostasis and exhibits broad oncogenic properties¹⁹. These include inhibition of tumor cell proliferation, promotion of apoptosis, suppression of angiogenesis, regulation of estrogen receptor expression, and modulation of immune activity¹⁵⁸.

Results in rodent models have shown that exposure to LAN accelerates mammary tumor development, increases estrogenic activity, and reduces circulating melatonin levels¹⁵⁹. In particular, the disruption of melatonin chronobiology due to alterations in the light-dark schedule can significantly affect tumor etiology, by altering cell proliferation, cell death, and nutrient supply¹⁶⁰.

Across multiple experimental systems, primarily involving rodent models with implanted breast or liver tumors, the exposure to abnormal lighting conditions (e.g., continuous light, dim LAN, or intermittent light during the dark phase) consistently led to suppressed melatonin production and enhanced tumor growth dynamics¹⁶¹. For instance, in athymic nude rats with MCF-7 breast cancer xenografts, continuous light exposure significantly increased tumor growth and uptake of linoleic acid, which was metabolized into a mitogenic compound⁵². In a related study, rats exposed to varying intensities of nocturnal light showed dose-dependent melatonin suppression and increased tumor cell proliferation when perfused with blood collected from humans after nighttime light exposure. This effect was reversed by a melatonin receptor antagonist, indicating that melatonin directly mediated tumor-suppressive effects via MT1/MT2 receptors signaling⁵⁴. Similarly, dim light during the dark phase accelerated tumor onset and growth in tamoxifen-treated breast cancer xenografts, alongside reduced melatonin levels¹⁶². However, not all studies supported a direct link between melatonin suppression and tumorigenesis. For example, in F344/N rats exposed to brief light pulses during the night following Neuromedin U treatment, melatonin levels were reduced, but no significant changes in mammary tumor development were observed¹⁶³.

Numerous animal studies demonstrate that the administration of melatonin, alone or in combination with other agents, exerts significant anti-cancer effects in various animal models. Melatonin alone consistently reduces tumor growth, cell proliferation, and angiogenesis, as evidenced by decreased VEGF expression and micro-vessel density in xenograft and murine adenocarcinoma models^{161,164}. It also promotes apoptosis through upregulation of p53-dependent pathways and cell cycle arrest at G1 phase, mediated by modulation of key downstream targets, such as FoxO1, miR-215, miR-96, CD44, survivin, and VEGF, thereby limiting tumor survival and metastasis¹⁶⁵.

Intriguingly, when combined with other compounds, the anticancer efficacy of melatonin is enhanced synergistically. For example, its combination with thymoquinone induces extensive tumor necrosis, increases apoptosis, suppresses angiogenesis, and activates a T helper 1 (TH1) immune response, resulting in significant tumor size reduction and improved cure rates without liver toxicity¹⁶⁶. Similarly, co-administration of melatonin with polyunsaturated fatty acids from walnuts modulates COX and LOX enzymatic activities, enhancing apoptosis, tumor immune infiltration, and survival in mammary adenocarcinoma¹⁶⁷. Furthermore, melatonin combined with a ketogenic diet overcomes drug resistance to cisplatin and vincristine in BC-bearing mice by inducing apoptosis, inhibiting angiogenesis, lowering blood glucose, increasing ketone bodies, and downregulating resistance genes, achieving cure rates up to 70%¹⁶⁸. Additional studies highlight the role of melatonin in enhancing the antitumor efficacy of chemotherapeutic agents administered at suboptimal circadian times, stabilizing tumors without added toxicity¹⁶⁹.

Collectively, these preclinical findings demonstrate that melatonin bears multifaceted anticancer properties, ranging from direct tumor suppression and apoptosis induction to immune modulation and overcoming chemotherapy resistance. Hence this neuro-hormone may be positioning as a promising adjuvant in BC therapy. Importantly, these findings strongly support the “melatonin-estrogen hypothesis”, suggesting that melatonin suppression might facilitate estrogen-driven breast carcinogenesis¹⁷⁰.

A substantial body of clinical evidence accumulated over the past two decades, consistently demonstrates that NSW is able to significantly suppress endogenous melatonin secretion, as measured through urinary and salivary metabolites, primarily aMT6s and 6-hydroxymelatonin sulfate (6-OHMS)¹⁷¹. For a summary of the worker findings, see Table 4. Early investigations in diverse occupational groups, including oil rig workers,

Table 4 | Human studies on NSW and melatonin

NSW Effect	Parameter(s)	Outcome	Year	Ref.
Circadian adaptation after ~1 week of sustained NSW	Urinary 6-SMT	→ Phase delay (successful entrainment)	1998	172
Seasonal dependence of MEL rhythm adjustment	Urinary 6-SMT	→ Phase advance (spring); ↔ no adaptation (winter)	1998	173
Disrupted MEL rhythm and poor sleep	Urinary 6-OHMS ratio (sleep:work)	↓ MEL; ↓ sleep quality; ↑ fatigue	2005	177
Non-significant reduction in MEL	Urinary aMT6s	slight MEL ↓	2006	176
Persistent suppression during and after NSW	Urinary 6-SMT (nurses)	↓ MEL on work and off days	2006	178
Reduced post-shift MEL, timing unchanged	Urinary MEL	↓ Levels after night shifts	2009	179
Light-dependent desynchrony	Urinary 6-SMT	↓ Total MEL; → circadian misalignment	2012	174
Chronic suppression and potential cancer risk	Urinary 6-SMT (male NSW)	↓ MEL across sleep periods	2013	181
No measurable change (few NSW exposures)	Urinary aMT6s	↔ MEL	2014	180
Delayed and reduced MEL peak	Urinary MEL	↓ MEL; → delayed acrophase	2014	182
Progressive decline with simulated NSW	Urinary MEL	↓ Nighttime and 24-h secretion	2014	183
Suppressed amplitude with consecutive NSW	Salivary MEL	↓ Amplitude ≈ 4.9% day ⁻¹ ; ↔ phase	2016	186
Reduced mesor and phase advance	Urinary aMT6s	↓ MEL; → earlier peak	2016	187
Global summary of MEL disruption	Review	↓ Overall production; → gradual misalignment	2017	171
Transient, light-mediated suppression	Salivary MEL	↓ 15–16% on workdays; ↔ off-days	2017	193
Gene-shift interaction in BC risk	MTNR1A, CRY2, RORA polymorphisms	NSW ↑ BC risk in high-risk genotypes	2019	195
Lower MEL linked to metabolic risk	Plasma MEL (nurses)	↓ MEL; possible metabolic impact	2019	175
Social jetlag correlated with suppression	Urinary aMT6s	↓ MEL with ↑ SJL	2021	184
Epigenetic alterations in circadian genes	Urinary 6-SMT + DNA methylation (RORA, MTNR1A, PER3)	Altered methylation; ↓ MEL	2022	196
Light-intensity-dependent suppression	Salivary MEL	↓ with high nocturnal light; → delayed rhythm	2023	185
MEL supplementation improved sleep and cognition	Oral MEL 5 mg post-shift	↑ Sleep quality; ↑ cognitive performance	2024	189
MEL improved oxidative DNA repair	Urinary 8-OH-dG + aMT6s	↑ 8-OH-dG excretion; ↑ aMT6s	2025	30
No effect on metabolism	Oral MEL 0.3 mg q.o.d.	↔ Body composition; ↔ intake	2025	190
No alteration in salivary MEL rhythm	Salivary MEL (day vs night)	↔ Circadian rhythm	2025	188

NSW night shift work, MEL melatonin, aMT6s 6-sulfatoxymelatonin, 6-SMT 6-sulfatoxymelatonin, 6-OHMS 6-hydroxymelatonin sulfate, BC breast cancer, SJL social jetlag, 8-OH-dG 8-hydroxy-2'-deoxyguanosine. Arrows indicate direction of change relative to day workers (↑ increase; ↓ decrease; ↔ no change).

telecommunications employees, and nurses, utilized 24-hour urine collections to compare melatonin output between DSW and NSW, often revealing reduced melatonin levels during NS or rotating shift schedules^{172–174}. Similarly, a recent cross-sectional study in healthy nurses found that NS workers had significantly lower plasma melatonin levels compared to DS workers, alongside trends toward altered leptin, ghrelin, and metabolic parameters, supporting the role of circadian disruption in predisposing to metabolic syndrome, which is a risk factor for BC development¹⁷⁵.

Large cross-sectional studies among healthcare professionals have repeatedly confirmed these findings. For instance, in premenopausal nurses it was reported a significant inverse relationship between the number of NS worked and urinary melatonin metabolite levels¹⁷⁶. Similar reductions were observed across various workers cohorts, including manufacturing workers and healthcare workers. Importantly, these studies controlled for important covariates such as age, BMI, smoking, chronotype, and timing of sample collection, reinforcing the robustness of this association^{177–182}.

Further, controlled clinical studies simulating NSW provide causal evidence that melatonin secretion diminishes rapidly upon night work exposure and remains suppressed with continued circadian disruption¹⁸³. A study on hospital workers found that NS workers exhibited significantly greater social jetlag and correspondingly lower urinary aMT6s levels on workdays than days off. This reduction correlated with increased circadian misalignment¹⁸⁴. Additionally, different lighting patterns on eight adults working night overtime were tested. Lower circadian stimulus increased melatonin and sleepiness, improving sleep quality, whereas higher stimulus

suppressed melatonin but caused more morning sleepiness, suggesting that careful lighting design can reduce circadian disruption and enhance safety during NSW¹⁸⁵.

Interestingly, the magnitude of melatonin suppression appears to correlate with both the frequency and duration of NS exposure^{186,187}. For instance, in hospital nurses following a clockwise rapidly rotating shift schedule, salivary melatonin levels were maintained across DS and NS, indicating preservation of the normal circadian phase despite NSW¹⁸⁸.

Recent researches have further examined the effects of melatonin supplementation and circadian factors on NS workers. A randomized, double-blind, placebo-controlled trial with shift workers suffering from sleep disorders demonstrated that 5 mg melatonin taken before sleep over four weeks significantly improved sleep quality and cognitive performance, with notable reductions in fatigue and good tolerability, supporting the role of melatonin in managing NSW-related sleep issues¹⁸⁹. More recently, a randomized placebo-controlled trial on NS workers assessed melatonin supplementation before day sleep for four weeks, showing a significant increase in urinary 8-OHdG, biomarker of oxidative DNA damage repair, during day sleep³⁰. Interestingly, a recent clinical trial in climacteric women working fixed shifts found that low-dose exogenous melatonin administered for three months did not significantly alter dietary intake or body composition, although postmenopausal participants showed modest reductions in fat mass, suggesting that physiological melatonin doses may have limited impact on metabolic outcomes in NS workers¹⁹⁰.

Collectively, these studies underscore the potential role of the neurohormone melatonin in mitigating circadian disruption and improving physiological and cognitive outcomes in NS workers, emphasizing the importance of implementing chronobiological strategies in occupational health¹⁹¹. However, despite these promising findings, it remains unclear how chronic melatonin supplementation affects long-term health outcomes in shift workers. Melatonin is popular for its sleep-regulating effects, and short-term use appears safe, but further research is needed to evaluate its broader impacts, including cardiovascular health, before it can be considered a “wonder pill” for NS workers¹⁹². Furthermore, while most findings indicate lower melatonin levels during NS compared to DS, some variability arises due to factors such as seasonality and specific shift schedules^{173,193}. Nevertheless, the majority of data supports that NSW disrupts normal circadian melatonin rhythms, which may underlie increased health risks, including BC, associated with chronic circadian misalignment. This highlights the urgent need for identification of effective targeted strategies to protect shift workers from the adverse biological effects of circadian disruption¹⁹⁴. Supporting the genetic basis of these associations, a recent hospital-based case-control study in Korean women identified variants in the melatonin receptor gene *MTNR1A* that were linked to BC risk, with certain polymorphisms interacting with NSW to further elevate susceptibility, highlighting a gene-environment interplay in circadian disruption-mediated breast carcinogenesis¹⁹⁵. Additionally, recent evidence suggests that melatonin secretion patterns in NS workers are associated with differential DNA methylation in melatonin receptor gene *MTNR1A* and other circadian genes, potentially linking altered melatonin rhythms to increased cancer susceptibility¹⁹⁶.

Intriguingly, growing evidence suggests that circadian rhythm disruption plays a critical role not only in BC progression but also in therapeutic response, with particular implications for melatonin as a therapeutic target. A recent study revealed that triple-negative breast cancer (TNBC) exhibits the most severe circadian rhythm disruption among all subtypes, marked by loss of rhythmic gene expression, metabolic reprogramming, and an immunosuppressive microenvironment. Notably, TNBC tumors with high-circadian rhythm disruption score were associated with decreased melatonin biosynthesis and increased tryptophan metabolism, associating melatonin suppression with immune evasion and poor relapse-free survival¹⁹⁷. These findings align with preclinical data showing that circadian disruption enhances TNBC tumor growth and impairs the time-dependent efficacy of cisplatin therapy, suggesting that both intact circadian signaling and melatonin rhythms are crucial for optimizing chemotherapeutic outcomes⁵⁸.

Meanwhile, in luminal A tumors, circadian rhythms remain partially intact but reprogrammed. Paradoxically, stronger tumor-intrinsic circadian rhythmicity was associated with worse five-year survival, mediated by rhythmic expression of epithelial-to-mesenchymal transition (EMT)-related genes and enhanced metastatic potential²⁷. On the therapeutic side, a recent clinical study on BC survivors found that chronotype-tailored light therapy, which can modulate melatonin production, significantly reduced fatigue and improved sleep quality¹⁹⁸. Altogether, these studies suggest that both melatonin and treatments aimed to remodulate circadian homeostasis hold promise not only in enhancing treatment efficacy and quality of life, but also in potentially counteracting immunosuppressive and pro-metastatic mechanisms driven by circadian disruption in BC patients.

Clock genes alterations

In addition to melatonin-dependent mechanisms, recent research has highlighted the importance of core circadian clock genes (e.g., *CLOCK*, *BMAL1*, *PER1/2/3*, *CRY1/2*, and *NPAS2*) in the regulation of pathways central to tumor biology¹⁹⁹. These genes govern cell cycle checkpoints, DNA damage repair, metabolic regulation, and apoptosis. Experimental disruption of these genes in cellular and animal models has been shown to enhance genomic instability and promote malignant transformation. For example, knockdown of *PER1* or *CRY2* in mammary epithelial cells impairs cell cycle control and facilitates oncogenic behavior, while mouse models with

mutations or deletions in circadian genes develop spontaneous tumors or exhibit heightened sensitivity to mammary carcinogens²⁰⁰.

Experimental studies investigating clock gene disruption and carcinogenesis have demonstrated several mechanisms relevant to BC risk. For instance, disruption of circadian rhythms by repeated advances in the light-dark schedule in rodent models consistently altered expression of core clock genes such as *Per2* and *Bmal1*, leading to impaired immune function, including reduced NK cell cytotoxicity and decreased expression of cytolytic factors like IFN γ , perforin, and granzyme B. This immunosuppression was linked to increased tumor incidence⁸⁸. In Wistar rats, shifts in light exposure altered peripheral clock gene expression and increased inflammatory markers and estrogen receptor expression in white adipose tissue, suggesting that circadian disruption may enhance estrogen-mediated pathways, which are known to be involved in breast carcinogenesis²⁰¹.

Mice exposed to altered light-dark cycles also exhibited disrupted *Per2* rhythms alongside chronic inflammation characterized by elevated IL-1 β , IL-6, and TNF- α , and changes in macrophage populations favoring pro-inflammatory phenotypes^{126,202}. Moreover, advanced light-dark schedules in genetically predisposed mice (e.g., *K-ras/p53* mutants) led to enhanced tumor growth and cell proliferation with increased oncogene *c-Myc* expression, while clock gene knockouts have tumor suppressive effects²⁰³. Altogether, these experimental data reveal that circadian disruption affects multiple BC-related hallmarks including immunosuppression, chronic inflammation, altered hormone receptor expression, and dysregulated cell proliferation²⁰⁴.

Studies examining clock gene sequence, expression and methylation in NS workers reveal notable disruptions linked to BC-related pathways. For a summary of the worker findings, see Table 5. Several cross-sectional studies among nurses and healthcare workers consistently found that NSW alters the expression of key clock genes in blood mononuclear cells. For example, NS nurses in Poland showed increased *PER1* expression, especially after ≥ 15 years of NSW²⁰⁵. Italian nurses on rotating NS had higher expression of *BMAL1*, *CLOCK*, *NPAS2*, *PER1*, *PER2*, and *REV-ERBa* but lower expression of *PER3*, *CRY1*, and *CRY2* than DS nurses²⁰⁶. Similar findings of increased *PER2* expression in short-term rotating NS were observed in American hospital residents²⁰⁷. Conversely, some studies reported decreased amplitude of *PER1* expression following NSW schedules, indicating complex effects depending on shift duration and design²⁰⁸. Consistent with these studies, a recent study in healthy NS workers demonstrated that moderate alcohol consumption further altered peripheral clock gene amplitude in *CLOCK*, *BMAL1*, *PER1*, *CRY1*, and *CRY2*, and it was associated with increased colonic permeability, highlighting the compounding effects of environmental circadian disruption and lifestyle factors on peripheral clock gene regulation¹⁴³.

Further supporting these findings, a recent study on Japanese male workers demonstrated that clock gene expression rhythms in beard hair follicle cells varied with shift work type. Specifically, *PER3* expression was significantly reduced and circadian rhythmicity disrupted in workers on multiple consecutive NS compared to those on a single NS or daytime shifts²⁰⁹. Additional molecular evidence indicates NSW induces a state of circadian misalignment marked by upregulation of key clock genes including *BMAL1*, *CLOCK*, *PER1*, *PER2*, and *PER3*. This dysregulation correlates with elevated night-time blood pressure and disrupted nocturnal dipping patterns, linking circadian gene dynamics also to cardiovascular risk in shift workers²¹⁰. Importantly, the EuRhythDia study explored the effects of timed light therapy on circadian and metabolic regulation in rotating NS workers. While 12 weeks of bright light exposure did not produce measurable improvements in sleep quality, glucose metabolism, or inflammatory markers, it significantly altered clock gene expression in peripheral blood mononuclear cells (PBMCs, specifically decreasing *REV-ERBa* levels and increasing the *REV-ERBa/BMAL1* ratio). These gene expression changes partially persisted after the intervention, suggesting that light therapy can modulate molecular circadian rhythms in NS workers, underscoring the potential use of circadian gene markers as early indicators of intervention efficacy in occupational chrono-medicine²¹¹. A recent

Table 5 | Human studies on NSW and clock genes

NSW Effect	Parameter(s)	Outcome	Year	Ref.
Epigenetic alterations in core clock genes	DNA methylation: CLOCK (promoter), CRY2 (promoter)	CLOCK ↓meth; CRY2 ↑meth	2011	216
Altered circadian gene expression (not significant after adjustment)	mRNA (PER1, PER3) in peripheral blood leukocytes	PER1, PER3 ↑ (ns)	2013	205
No significant difference in clock gene expression	Peripheral lymphocyte clock gene mRNA	↔ unchanged gene expression	2013	206
Shift-dependent variation of PER2	mRNA (PER2, NR1D1, ESR2) in blood	PER2 ↑ (night), ↓ (day); NR1D1, ESR2 ↔	2015	207
Genome-wide circadian gene hypomethylation	DNA methylation: CLOCK, BMAL1, PER1–3, CSNK1D/E, NPAS2, RORA	↓meth (multiple)	2015	214
Alcohol modulated peripheral clock gene rhythm	mRNA (CLOCK, BMAL1, PER1, CRY2, PER2) in blood	↑amplitude CLOCK, BMAL1, PER1, CRY2	2016	143
Differential methylation of clock genes linked to BC risk	DNA methylation: CLOCK, BMAL1, PER1, CRY1	CLOCK/BMAL1/PER1 ↑meth; CRY1 ↓meth	2017	213
Dampened amplitude and phase misalignment of rhythmic transcripts	mRNA (PER1, DBP, BMAL1, NR1D1, NR1D2) in PBMCs	↓amplitude, phase shift	2018	208
Reduced expression of PER3 and NR1D2	mRNA in beard follicle cells	PER3, NR1D2 ↓	2019	209
Gene–environment interaction in BC risk	CLOCK, CRY2, RORA SNPs	CRY2 (rs2292912) + NSW ↑ BC risk; RORA (rs1482057) ↑ BC risk	2019	195
Light therapy modulated circadian gene markers	mRNA (REV-ERBa, BMAL1) in PBMCs	REV-ERBa ↓; REV-ERBa/BMAL1 ratio ↑	2022	211
Differential methylation of circadian genes by shift duration	DNA methylation: CSNK1E, NR1D1, BMAL1	CSNK1E ↑meth; NR1D1 ↓meth; BMAL1 ↑meth	2022	215
Melatonin-linked methylation patterns	DNA methylation: RORA, MTNR1A, PER3	RORA ↑meth; MTNR1A ↑/↓; PER3 ↓meth	2022	196
Increased clock gene expression post-shift with BP rise	mRNA (BMAL1, CLOCK, PER1–3) in blood; BP	All ↑; ↑SBP/DBP, non-dipping	2023	210
NSW-genotype interaction in T2D risk	CLOCK rs1801260, MTNR1A/B variants	Gene-environment ↑T2D risk	2023	217
Epigenetic alterations in core clock genes	DNA methylation: CRY2, CSNK1D, RORA, RORC	Differential meth (↑/↓)	2023	33
Dysregulated clock gene expression linked to BC susceptibility	mRNA (PER1, TEF, CLOCK)	PER1, TEF ↓; CLOCK ↑	2024	26
Disrupted rhythmicity of core clock genes	mRNA (PER2, PER3, BMAL1, ESR1/2) in PBMCs	PER2/BMAL1 rhythm lost; PER1, ESR1 altered	2024	212
Genetic protection vs hypertension under NSW	CLOCK rs1801260, BMAL1 rs11022775	GG (CLOCK), TT (BMAL1) → ↓ HTN risk	2024	218

NSW night shift work, BC breast cancer, BP blood pressure, SBP/DBP systolic/diastolic blood pressure, PBMCs peripheral blood mononuclear cells, mRNA messenger RNA, meth. DNA methylation, CpG cytosine–phosphate–guanine dinucleotide, SNP single-nucleotide polymorphism, TSS transcription start site, UTR untranslated region, T2D type 2 diabetes, BMAL1 (ARNTL), brain and muscle ARNT-like 1, REV-ERBa (NR1D1) nuclear receptor subfamily 1 group D member 1, CRY1/CRY2 cryptochrome circadian regulator 1/2, PER1–3 period circadian regulator 1–3, CLOCK circadian locomotor output cycles kaput, NPAS2 neuronal PAS domain protein 2, CSNK1D/E casein kinase 1 delta/epsilon, RORA/RORC retinoic acid receptor-related orphan receptor α/γ , TEF thyrotroph embryonic factor, DBP D-box binding PAR bZIP transcription factor, ESR1/ESR2 estrogen receptor α/β , MTNR1A/B melatonin receptor 1A/1B, ↑, increase/upregulation/hypermethylation; ↓, decrease/downregulation/hypomethylation; ↔, no significant change.

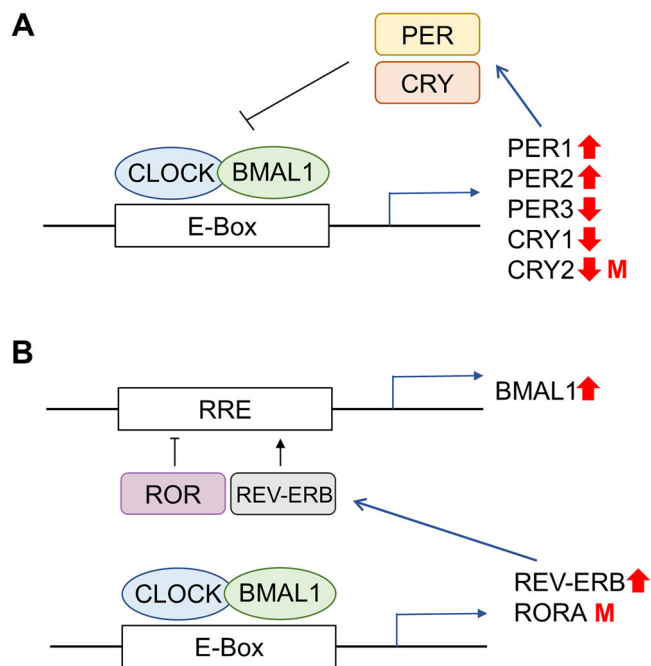


Fig. 3 | Night shift work-associated gene expression and DNA methylation changes in circadian clock genes. Schematic representation of the core circadian clock feedback loops showing how night shift work alters clock gene expression and DNA methylation. **A** The CLOCK-BMAL1 complex activates PER and CRY transcription via E-box elements. **B** ROR and REV-ERB regulate BMAL1 expression through RRE sites. Red arrows indicate changes in gene expression, while red “M” denotes altered DNA methylation in clock genes, both associated with night shift work.

clinical study further confirmed that NSW disrupts 24-hour rhythms of PER2, PER3, BMAL1, and ESR2 in PBMCs, impairs the inverse correlation between PER2 and BMAL1, and alters PER1 and ESR1 expression patterns, highlighting a misalignment in the peripheral circadian network associated with NSW schedules²¹². Altogether, these findings highlight the broader systemic impact of circadian disruption induced by NSW, which encompasses cancer susceptibility, metabolic disorders, and cardiovascular dysfunction.

Regarding epigenetic modifications, methylation patterns of clock genes differ between NS and DS workers, with potential implications for BC risk. Female healthcare workers showed significant hypomethylation of multiple clock genes including CLOCK, PER1, PER2, PER3, and NPAS2 in NS workers, which may reflect altered gene regulation²¹³. Genome-wide methylation comparisons between 65 DS and 59 NS healthcare workers revealed 21 loci in circadian genes, including PER3 and CSNK1E, to be significantly hypomethylated among NSW, highlighting potential epigenetic mechanisms underlying shift work-associated disease risk²¹⁴. Similarly, a study focusing on CLOCK-related circadian regulators in nurses, found that hypermethylation in BMAL1 gene bodies was associated with working ≥ 3 consecutive NS per week, suggesting possible epigenetic silencing affecting CLOCK transcriptional activity. Also, prolonged NSW (≥ 10 years) correlated with hypomethylation in NR1D1, and hypermethylation of CSNK1E, a kinase regulating PER proteins downstream of CLOCK²¹⁵. In line with these findings, a Danish pilot study demonstrated promoter hypomethylation of CLOCK and hypermethylation of CRY2 in peripheral blood DNA, potentially mediating the relationship between NSW and BC susceptibility²¹⁶. Complementing these targeted analyses, a recent epigenome-wide association study demonstrated sex-specific DNA methylation changes and epigenetic age acceleration, including the differential methylation in both CRY2 and RORA, linking NSW to epigenetic alterations in circadian regulators³³. Additionally, melatonin secretion patterns in NS workers were associated with differential methylation of key

circadian genes, including RORA, MTNR1A, and PER3, suggesting a potential link between circadian disruption, epigenetic regulation, and cancer risk¹⁹⁶.

Moreover, genetic studies among Korean women revealed significant associations between circadian gene variants and BC risk. The CLOCK gene variant rs11133373 was linked to increased BC risk, while the MTNR1A gene variant rs2119882 was associated with reduced risk. Crucially, interactions between NSW and specific polymorphisms in CRY2 and RORA further elevated BC risk, underscoring the complex interplay of genetic susceptibility and environmental circadian disruption in BC etiology¹⁹⁵. Beyond cancer-related effect, it was also observed within a cohort of steelworkers that the presence of CLOCK rs1801260 and MTNR1A rs2119882 polymorphisms combined with rotating NSW, significantly increased the risk of type 2 diabetes²¹⁷. Additionally, a study on healthcare workers investigated CLOCK, BMAL1, and PER1 polymorphisms in relation to hypertension, finding that the CLOCK rs1801260 GG and BMAL1 rs11022775 TT genotypes were associated with a reduced risk of hypertension, whereas PER1 rs2735611 showed no significant association, highlighting how circadian gene variants may influence cardiovascular outcomes in NS workers²¹⁸.

Collectively, these data demonstrate that NSW disrupts both the expression and methylation status of core circadian clock genes involved in BC etiology, such as PER1, PER2, BMAL1, and CLOCK (Fig. 3). These molecular alterations likely affect circadian regulation of hormone receptor pathways, cell cycle control, and DNA repair, potentially contributing to increased BC susceptibility observed in epidemiological studies²⁶. Additionally, specific polymorphisms in clock genes may affect individual vulnerability to circadian disruption, thereby influencing the extent to which NSW impacts BC risk and related molecular pathways²¹⁹. The integration of genetic, epigenetic, and gene expression data across diverse populations and models may provide a more comprehensive understanding of the molecular mechanisms linking circadian disruption with cancer and other NSW-related health risks.

Epigenetic changes

Growing evidence from both experimental models and human studies reveals that circadian disruption caused by altered light-dark cycles and NSW may lead to diverse epigenetic alterations. For a summary of the worker findings, see Table 6. For instance, changes in DNA methylation patterns, histone modifications, as well as circulating miRNA expression alterations may affect critical biological pathways, thereby influencing health outcomes in NS workers²²⁰. In experimental studies exploring the epigenetic effects of disrupted light-dark cycles, various types of epigenetic alterations (e.g., DNA methylation, histone modifications, and miRNA expression) have been observed across different animal models. In detail, it was observed that repeated 3 h phase advances in the light-dark cycle over several days increased DNMT1 protein levels in rat liver²²¹. Furthermore, exposure to dim light during the dark phase in rats with MCF-7 tumors induced Stat3 gene expression via promoter methylation of ARH1²²². Histone modifications were also reported. In particular, rapid trimethylation of histone H3 at lysine 4 (H3K4me3) was observed in mouse livers after weekly 6-hour light-dark phase advances²²³.

Additionally, miRNA alterations have been found as well, as it were shown decreased miR-127 and miR-146-b levels in rat mammary tissues following both acute and prolonged light-dark alterations²²¹. Subsequently, it was observed that altered lighting (i.e., abrupt light-dark shifts and light intensity changes) in Syrian hamsters modulated expression of miR-132, miR-212, and their target MeCP2 in SCN tissue²²⁴. Finally, increased liver expression of several Rev-erba-targeting miRNAs (miR-140-5p, miR-185-5p, miR-326-5p, and miR-328-5p) in mice exposed to continuous light for 8 weeks were also reported²²⁵. These findings collectively demonstrate that circadian disruption via altered light exposure can induce diverse epigenetic responses depending on tissue, timing, and species.

Multiple studies have investigated the effects of NSW on DNA methylation patterns in blood cells, revealing a range of epigenetic

Table 6 | Human studies on NSW and epigenetic changes

NSW Effect	Parameter(s)	Outcome	Year	Ref.
Epigenetic alterations linked to inflammation	DNAme of <i>Alu</i> , <i>IFN-γ</i> , <i>GCR</i> , <i>TNF-α</i> (blood)	↓ <i>Alu</i> , ↓ <i>IFN-γ</i> , ↑ <i>GCR</i> , ↓ <i>TNF-α</i> → inflammation-related DNAme changes	2010	226
Circadian and cancer-related gene methylation	Promoter DNAme of <i>CLOCK</i> , <i>CRY2</i> , <i>ESR1</i> , <i>CDKN2A</i> , <i>MBD2</i> , <i>HDAC2</i> (blood)	↓ <i>CLOCK</i> , ↑ <i>CRY2</i> , ↑ <i>ESR1</i> / <i>CDKN2A</i> / <i>MBD2</i> / <i>HDAC2</i> → circadian disruption and BC risk ↑	2011	216
miRNA promoter methylation	DNAme of 31 miRNA promoters (esp. miR-219) (blood)	↑ miR-219 DNAme → ↓ <i>Jak-STAT</i> / <i>NF-κB</i> immune-antitumor signaling → BC risk ↑	2013	228
Imprinted gene methylation alterations	DNAme at 50 CpG sites in 26 imprinted genes (<i>DLX5</i> , <i>IGF2AS</i> , <i>TP73</i>)	Mixed ↑/↓ DNAme → altered imprinting and cancer-related pathways	2013	229
Global and circadian gene hypomethylation	DNAme of circadian genes (<i>PER1-3</i> , <i>CSNK1E/D</i> , <i>CLOCK</i> , <i>ARNTL</i>)	↓ DNAme across circadian and immune/oxidative stress genes → systemic epigenetic dysregulation	2015	214
Tumor-suppressive miRNA silencing (female workers)	DNAme of miR-34b promoter (blood)	↑ miR-34b DNAme → ↓ expression → ↓ antiproliferative/tumor-suppressive activity → BC risk ↑	2015	232
No methylation change in tumor suppressors	Promoter DNAme of <i>BRCA1</i> , <i>BRCA2</i>	No significant change → no detectable epigenetic effect	2017	230
Tumor-suppressor gene hypomethylation	DNAme of <i>ESR1</i> , <i>TP53</i> , <i>BRCA1</i> , <i>LINE-1</i> , <i>Alu</i>	↓ <i>ESR1</i> / <i>TP53</i> / <i>BRCA1</i> , no change in <i>LINE-1</i> / <i>Alu</i> → specific epigenetic disruption	2019	227
Epigenetic age acceleration	DNAme age (PhenoAge) and CpG methylation (<i>ZFHx3</i> , <i>ATXN7</i>)	↑ epigenetic age (+ 3 y), ↓ <i>ZFHx3</i> , ↑ <i>ATXN7</i> → accelerated aging and circadian disruption	2019	231
Reversible methylation linked to recovery (SWD cases)	DNAme in <i>GRIN2C</i> , <i>CREB1</i> , <i>CAMK2B</i>	↓ DNAme during work, reversible ↑ during recovery → <i>CREB</i> – <i>CaMKII</i> pathway modulation	2021	235
Melatonin-linked circadian gene methylation (female workers)	DNAme of <i>RORA</i> , <i>MTNR1A</i> , <i>PER3</i>	↑ <i>RORA</i> , mixed <i>MTNR1A</i> , ↓ <i>PER3</i> → circadian disruption and potential BC risk ↑	2022	196
Shift-dependent methylation in circadian genes	DNAme of <i>CSNK1E</i> , <i>NR1D1</i> , <i>ARNTL</i>	↑ <i>CSNK1E</i> (current), ↓ <i>NR1D1</i> (≥ 10 y NSW), ↑ <i>ARNTL</i> (≥ 3 consecutive NS/week) → epigenetic shift-pattern dependence	2022	215
Sex-specific DNAme and epigenetic aging	DNAme of <i>CRY2</i> , <i>CSNK1D</i> , <i>RORA</i> , <i>RORC</i> , <i>LIN54</i> , <i>E2F1</i> , <i>ETS1</i> ; epigenetic clocks	↑ DNAme in women after 10–16 y NSW; short-term NSW → ↑ epigenetic age (Horvath, Hannum, PhenoAge, GrimAge)	2023	33
HERV and immune gene methylation (female nurses)	DNAme of <i>ERVFRD-1</i> , <i>ERVL</i> , <i>HERV-P</i> , <i>ERVW-1</i> , <i>BIRC2</i> , <i>FLRT3</i> , <i>MIG6</i> , <i>SIRT1</i>	Differential DNA me; effects modified by BMI → inflammation and immune epigenetic modulation	2023	234

NSW night shift work, SWD shift work disorder, DNAme DNA methylation, CpG cytosine-phosphate-guanine site, miRNA microRNA, HERV human endogenous retrovirus, BC breast cancer, BMI body mass index, IFN-γ interferon gamma, TNF-α tumor necrosis factor alpha, GCR glucocorticoid receptor, ESR1 estrogen receptor 1, TP53 tumor protein p53, BRCA1/2, breast cancer gene 1/2, CDKN2A cyclin-dependent kinase inhibitor 2A, MBD2 methyl-CpG binding domain protein 2, HDAC2 histone deacetylase 2, PER1–3 period circadian regulators 1–3, CSNK1D/E casein kinase 1 delta/epsilon, ARNTL, BMAL1 CRY2 cryptochrome circadian regulator 2, NR1D1 nuclear receptor subfamily 1, group D, member 1 (REV-ERBα)/RORA/RORC RAR-related orphan receptor A/C, ZFHx, zinc finger homeobox 3, ATXN7 ataxin 7, CREB1 cAMP response element-binding protein 1, CAMK2B calcium/calmodulin-dependent protein kinase II beta, GRIN2C glutamate ionotropic receptor NMDA type subunit 2C, MTNR1A melatonin receptor 1A, DLX5 distal-less homeobox 5, IGF2AS insulin-like growth factor 2 antisense RNA, TP73 tumor protein p73, BIRC2 baculoviral IAP repeat-containing protein 2, FLRT3 fibronectin leucine-rich transmembrane protein 3, MIG6 mitogen-inducible gene 6, SIRT1 sirtuin 1, ERVFRD-1/ERVW-1/HERV-P/ERVE human endogenous retrovirus family elements; NF-κB nuclear factor kappa-light-chain-enhancer of activated B cells, Jak-STAT Janus kinase-signal transducer and activator of transcription pathway; PhenoAge/Horvath/Hannum/GrimAge, epigenetic aging clocks based on DNA methylation. Arrows indicate direction of change relative to day workers (↑ increase; ↓ decrease; → consequential change).

alterations associated with circadian disruption, immune regulation, and aging. These changes can be categorized broadly into global/repetitive element methylation, gene-specific methylation, miRNA methylation, epigenetic clock genes alterations (see above), and methylation of immune- and retroviral-related elements²⁰⁴.

Early cross-sectional research in Italian chemical plant workers found no significant differences in methylation of repetitive elements such as Alu and LINE-1, or in inflammatory genes like TNF α and IFN γ , despite exposure to NSW²²⁶. Similarly, no significant changes in LINE-1 and Alu methylation among female nurses was observed, although several gene-specific hypomethylation effects to the estrogen receptor gene ESR1 (associated with heightened estrogen sensitivity), to the tumor suppressor genes TP53 and BRCA1 (indicating possible disruption in cell cycle regulation and DNA repair mechanisms) were reported. These findings suggest that repetitive element methylation may be less sensitive or not consistently altered by NS exposure compared to specific gene methylation²²⁷. Indeed, significant gene-level methylation changes have been consistently reported in various cohorts. For example, it was demonstrated hypermethylation in multiple CpG sites in NS workers²¹⁶. Additionally, always in NS workers it was observed hypermethylation in 48 CpGs across 29 miRNAs, as well as 20 hyper- and 30 hypo-methylated CpGs within 56 imprinted genes^{228,229}. In line with these findings, overall lower DNA methylation was reported in NS healthcare workers compared to DS workers in an American cohort, upon adjustments for confounders such as immune cell composition²¹⁴. Contrariwise, no methylation differences specific to BRCA1/BRCA2 gene promoters were observed among rotating NS nurses²³⁰.

More recent work linked long-term NS work (≥ 10 years) to significant hypomethylation in 85 CpG sites, including loci in ZFH3, a gene involved in circadian rhythm regulation and tumor suppression, and ATXN7, associated with transcriptional control and cancer susceptibility²³¹. The same group demonstrated that NSW is associated with biological age acceleration, indicating an average age advancement of over three years in women with ≥ 10 years of NSW²³¹. These findings were further corroborated within the Lifelines Cohort, where results showed sex- and duration-specific DNA methylation changes in women exposed to intermediate-term NSW (10–16 years), enriched in pathways related to circadian regulation and cellular senescence. Notably, short-term NS workers (2–6 years) of both sexes exhibited increased epigenetic age acceleration, possibly reflecting early biological stress with adaptation over time³³.

Among the earliest specific findings, a total of 50 loci corresponding to 31 miRNA promoters were found differentially methylated in NS workers compared to DS workers, including the circadian miR-219²²⁸. In line with these results, also the promoter of miR-34b, a miRNA relevant in BC susceptibility and involved in tumor suppression and cell cycle control, was found hyper-methylated in women exposed to NSW compared to non-exposed controls²³².

Going deeply into mechanistic advances, it was demonstrated that altered melatonin secretion patterns in NS workers correlate with DNA methylation changes in circadian genes. In particular, it was observed increased methylation in the gene body of RORA clock gene and decreased methylation in the promoter of MTNR1A melatonin receptor gene¹⁹⁶. Supporting these findings, a recent study in female healthcare workers demonstrated that NSW parameters, including current status, duration, and intensity, were associated with differential methylation in key circadian genes such as CSNK1E, NR1D1, and ARNTL²¹⁵. These epigenetic modifications suggest a mechanistic link between melatonin disruption, NSW features, circadian gene regulation, and potential BC risk²³³.

Additionally, NSW affects methylation of immune-modulatory genes and human endogenous retroviral elements such as ERVFRD-1, HERV-L, and HERV-P, indicating combined effects of circadian and metabolic stressors on immune epigenetic regulation²³⁴. Interestingly, DNA methylation dynamics vary during recovery from shift work disorder (SWD). It was found that vacation periods reversed hypomethylation accumulated during NSW, particularly in genes involved in glutamatergic neurotransmission and immune pathways, including CREB1, CAMK2B, and

GRIN2C, highlighting the plasticity of epigenetic landscape in response to circadian stress and recovery²³⁵.

Overall, the body of research indicates that NSW induces complex, multi-level epigenetic changes encompassing miRNAs, circadian and tumor suppressor genes, melatonin-related genes, immune regulators, and endogenous retroviral elements. These methylation alterations are often duration- and sex-dependent and relate to accelerated biological aging, disrupted circadian regulation, immune dysfunction, and potentially increased BC risk. However, the heterogeneity in study designs, populations, and covariates underscores the need for larger, longitudinal, and mechanistically focused investigations to fully elucidate the epigenetic consequences of NSW exposure and deriving health issues.

Telomere shortening

Shorter telomere length is associated with a higher risk of BC recurrence, suggesting its potential as a prognostic biomarker for disease-free and recurrence-free survival²³⁶. In this context, the relationship between NSW and telomere length has been examined in several human studies with mixed results. For a summary of the worker findings, see Table 7. Large cross-sectional analyses from the USA, including the Sister Study with 608 women and the Nurses Health Study (NHS) with over 2400 nurses, found no significant association between the duration of rotating NSW and telomere length after adjusting for multiple covariates such as age, BMI, smoking, and stress. These findings suggest that moderate or shorter-term exposure to NSW may not substantially affect telomere length^{237,238}. In contrast, evidence from a Norwegian nested case-control study involving hospital nurses demonstrated statistically significant telomere shortening associated with long-term NS (≥ 6 consecutive nights, for ≥ 5 years). This indicates that extended exposure to consecutive NS may accelerate telomere erosion, consistent with the hypothesis that chronic circadian disruption can promote cellular aging²³⁹. Complementary with these findings, it was also demonstrated a non-linear association between NS duration and telomere length among female hospital nurses. In this study, it was observed that telomere length increased with NSW exposure up to about 12 years, but then significantly decreased with longer exposures (≥ 12 years). This biphasic pattern could reflect initial compensatory telomere maintenance or cellular stress responses in early years of NS work, followed by accelerated telomere shortening as cumulative damage and circadian disruption intensify over time²²⁷.

Supporting these results, a recent cross-sectional study of hospital workers reported that leukocyte telomere length was positively associated with plasma PTX3 levels, suggesting that efficient regulation of inflammatory processes may protect telomeres from attrition, even in NS workers¹⁴⁴. Also, evidence from a population-based study indicates that obstructive sleep apnea is consistently associated with accelerated epigenetic ageing, while the impact of insomnia and sleep deprivation appears to be modulated by the duration of NSW schedule, highlighting a complex interplay between sleep disturbances, NSW, and biological aging²⁴⁰. Consistent with these observations, a study in older workers reported that current NS workers had significantly shorter telomeres than non-shift workers, while former NS workers showed partial recovery of telomere length with increasing time since cessation, suggesting that NS-induced telomere attrition may be at least partially reversible²⁴¹.

Collectively, these data imply that the impact of NSW on telomere length may depend on the duration and intensity of exposure, with short to moderate durations having a limited effect, but long-term or consecutive NS contributing to telomere shortening and potentially premature cellular aging. This progressive telomere shortening in long-term NS workers could partially explain the observed increases in age-related diseases and cancer risk in this population. However, discrepancies across studies highlight the need for robust longitudinal research with precise exposure assessment and consideration of specific confounders, such as lifestyle and chronotype.

Table 7 | Human studies on NSW and telomeres length and aging

NSW Effect	Parameter(s)	Outcome	Year	Ref.
Slight shortening of TL, more pronounced in postmenopausal women	Leukocyte relative TL (LTL, blood)	↓ TL	2011	237
Trend toward shorter TL with longer NSW history (not significant)	Peripheral blood leukocyte TL (PBL TL, blood)	↓ TL (ns)	2011	238
Intensive consecutive NSW (≥ 4–6 nights for ≥5 yrs) associated with shortened TL and higher BC risk	TL (saliva DNA)	↓ TL	2017	239
NSW associated with higher CRP (systemic inflammation), LTL positively correlated with PTX3 (protective)	LTL (blood), plasma PTX3, CRP	↑ (PTX3), ↑ CRP → potential ↓ TL	2017	144
Cumulative NSW: <12 yrs associated with longer TL, ≥12 yrs with shorter TL	TL (blood)	↑ (< 12 yrs), ↓ (≥ 12 yrs)	2019	227
NSW modifies effect of sleep disturbances on epigenetic ageing	AgeDevGrim and DunedinPACE clocks	↑ TL (with insomnia/sleep deprivation)	2025	240
Prolonged NSW associated with shorter TL; partial reversal after cessation; DNA methylation AA unchanged	TL (blood), DNA methylation-based age acceleration	↓ TL, no change AA	2025	241

NSW night shift work, TL telomere length, LTL leukocyte TL, PBL TL peripheral blood leukocyte TL, BC breast cancer, CRP C-reactive protein, PTX3 pentraxin 3, AA age acceleration, AgeDevGrim Age Deviation GrimAge epigenetic clock, DunedinPACE Dunedin PoAm/ Accelerated Pace of Aging epigenetic clock. Arrows indicate direction of change relative to day workers (↑ increase, ↓ decrease, ↔ no change, → consequential change).

Other factors

Vitamin D deficiency has also emerged as a potential contributor to the elevated BC risk observed in NS workers. For a summary of the worker findings, see Table 8. Reduced sunlight exposure due to altered sleep-wake patterns and nighttime work schedules can lead to suboptimal cutaneous synthesis of vitamin D, a hormone with well-documented anti-proliferative, pro-apoptotic, and immunomodulatory effects in breast tissue²⁴². Observational studies have reported lower serum vitamin D levels among NS workers^{188,243–246}. Furthermore, results in NS workers support a role for vitamin D in regulating circadian gene expression, while also interacting with estrogen pathways, inflammatory signaling, and sleep quality^{247,248}. Also, evidence from male rotating shift workers indicates that vitamin D deficiency is associated with a higher risk of obstructive sleep apnea, highlighting another potential health consequence of low vitamin D in shift-working populations²⁴⁹. These findings suggest that vitamin D deficiency may act as both a consequence and a mediator of circadian misalignment, potentially compounding the carcinogenic effects of LAN exposure and reinforcing the need to consider vitamin D status in risk stratification and prevention strategies for NSW-exposed individuals²⁵⁰.

In addition, over the past decade, numerous cross-sectional studies have investigated the effects of NSW work on several other hormonal levels among female healthcare workers, revealing complex and sometimes contradictory patterns²⁵¹. For a summary of the worker findings, see Table 9. Early research from the American NHS I found increased estradiol but not estrone, among postmenopausal women with prolonged rotating NS exposure, along with a modest inverse correlation between follicular estradiol and melatonin metabolite levels, suggesting circadian regulation of estrogen synthesis^{176,252}. Similarly, within Japanese nurses with NSW history, it was reported elevated estrone in postmenopausal²⁵³. In Italian premenopausal nurses on rapid forward-rotating NS, it was also observed significantly higher 17-β-estradiol, especially among those who skipped naps during shifts^{180,206}.

Contrastingly, a Canadian study found no clear estrogen associations in nurses working NS, while in American nurses it was documented elevated estrogen, LH, and FSH levels following daytime sleep after NSW^{254,255}. In Spanish female healthcare workers was found an association between rotating NS and increased estradiol, as well as progesterone, modulated by menstrual phase, whereas in Greek hospital NS workers elevated progesterone and androgens, but not estrogen, were confirmed^{256,257}. These findings were further refined by findings demonstrating that estradiol increased with years of NSW only in postmenopausal mid-ages female nurses who show a morning chronotype, highlighting chronotype as a possible modulating factor²⁵⁸.

More recent investigations evidenced how women working whole NS exhibit suppressed estradiol levels compared to DS workers, coinciding with disrupted sleep and melatonin rhythms, while half-NS did not significantly affect estradiol synthesis²⁵⁹. At the molecular level, healthy DS nurses showed robust circadian oscillations of the estrogen receptor gene ESR2 in PBMCs, peaking in the morning. However, this rhythm was lost in NS nurses, indicating that NSW disrupts normal temporal regulation of estrogen receptor expression and may contribute to altered estrogen signaling²¹². Finally, in a cohort of female hospital workers, rapid rotating NS was linked to increased serum release of stress-related steroids-cortisol, 11-deoxycortisol, DHEA, and androstenedione, whereas estradiol levels were slightly decreased, and salivary estradiol remained unchanged, suggesting a complex and multifaceted influence of NSW on estrogen regulation¹⁸⁸.

Together, these findings underscore that NSW alters estrogenic activity in women, primarily through elevated estradiol in certain populations, with stronger effects in postmenopausal women and modulation by chronotype and circadian disruption²⁵¹. However, the suppression of estradiol observed in whole-NS workers and the loss of estrogen receptor rhythmicity highlight the nuanced and context-dependent nature of endocrine disruption caused by shift work²⁵¹. This complexity suggests that altered estrogen signaling may represent a key mechanistic pathway linking NSW to BC risk and

Table 8 | Human studies on NSW and vitamin D

NSW Effect	Parameter(s)	Outcome	Year	Ref.
No effect	Serum 25(OH)D	↔ VIT D	2011	243
↓ VIT D in NSW	Serum 25(OH)D	↓ VIT D	2015	250
↓ VIT D in females; dose-dependent by intensity/duration NSW; no change in males	Serum 25(OH)D	↓ VIT D females, ↔ males	2016	247
Slightly ↓ VIT D in NSW; not significant	Serum 25(OH)D	↔ VIT D	2018	242
↓ VIT D in NSW; partially mediates depressive symptoms; no sex difference	Serum 25(OH)D	↓ VIT D	2019	248
Consistently ↓ VIT D in r-NSW, especially in summer	Serum 25(OH)D	↓ VIT D	2020	244
↓ VIT D, especially in females and Mexican Americans; long hours (> 40 h/week) worsen	Serum 25(OH)D	↓ VIT D	2021	246
VIT D deficiency (< 20–30 ng/mL) in NSW, ↑ OSA risk in males ≥40 y	Serum 25(OH)D; Berlin questionnaire; NoSAS score	↓ VIT D	2022	249
↓ VIT D; NSW workers 65% ↑ risk deficiency (< 20 ng/mL), 17% ↑ deficiency/insufficiency (< 30 ng/mL)	Serum 25(OH)D	↓ VIT D	2025	245
Slight ↓ VIT D in female nurses with rapid clockwise r-NSW	Serum 25(OH)D	↓ VIT D	2025	188

NSW night shift work, r-NSW rotating night shift work, 25(OH)D serum 25-hydroxyvitamin D, OSA obstructive sleep apnea, NoSAS Neck, Obesity, Snoring, Age, Sex (OSA screening score), VIT D vitamin D. Arrows indicate direction of change relative to day workers (↑ increase; ↓ decrease).

warrants further investigation considering individual circadian profiles and shift patterns.

Although beyond the primary scope of this review, it is worth mentioning that emerging evidence highlights the gut microbiome as a potential mediator of circadian disruption-associated breast carcinogenesis, with studies indicating that NSW can alter microbial composition and diurnal rhythmicity, thereby modulating systemic inflammation, estrogen metabolism, and immune function, all of which are relevant to BC risk²⁶⁰.

Precision biomarkers for women exposed to night shift work

Emerging evidence underscores the urgent need to incorporate novel biomarkers to identify women who are particularly susceptible to the adverse effects of NSW, especially those at increased risk of developing BC or who already exhibit early biological signs of exposure-related harm²⁶¹. Historically, most biomonitoring in NSW contexts has relied either on the detection of melatonin synthesis suppression or general markers of inflammation or biological signs of sleep disturbance^{262–264}. In light of the recent mechanistic findings, a more refined panel of biomarkers might be proposed to enhance early risk stratification, prevention, and personalized interventions in occupational health (Fig. 4)²⁶⁵.

For instance, oxidative stress is a well-documented mediator of LAN-induced carcinogenesis. Specific biomarkers like 8-OHdG and 8-isoprostane are validated indicators of oxidative DNA and lipid damage, respectively, and are consistently elevated in blood from female NS workers²⁶⁶. Additional markers such as SOD, CAT, MDA, TOS, and TAC/TAS support a comprehensive profile of oxidative burden⁸⁶. Longitudinal monitoring of these biomarkers may help identify individuals with persistent redox imbalance and stratify risk by exposure duration and chronotype.

Also, chronic circadian misalignment leads to immune suppression and low-grade systemic inflammation, both of which contribute to impaired tumor immunosurveillance¹⁴⁶. In addition to elevated CRP, IL-6, and TNF- α , shift workers show altered immune profiles such as a reduced CD4 + / CD8 + T-cell ratio, increased monocyte counts, and impaired NK cell cytotoxicity. Measuring these markers may help detect early immune dysregulation in at-risk women²⁶².

Melatonin remains a cornerstone biomarker of circadian health²⁶⁷. Decrease of urinary aMT6s and 6-OHMS is associated with increased BC risk and is easily measured via non-invasive urine assays²⁶⁸. Disruption of MT1 and MT2 melatonin receptors, implicated in antiproliferative signaling, has also been reported in LAN-exposed tissues²⁶⁹. These markers,

measured for example in peripheral blood cells, could provide a dynamic measure of melatonin pathway disruption over time.

Furthermore, dysregulation of core clock genes such as CLOCK, BMAL1, PER1/2, CRY1/2, and NPAS2, both at the transcriptional and post-translational levels, is consistently observed in PBMC samples of shift workers and may serve as early predictors of disrupted cell cycle control, DNA repair, and apoptosis. Specific polymorphisms, such as CLOCK rs11133373, PER3 VNTR, and MTNR1A rs2119882, have been associated with differential susceptibility to circadian misalignment and cancer risk²⁷⁰, hence they can be used for risk stratification purposes.

Recent findings reveal that NSW disrupts the gut microbiota's diurnal oscillations, impairing estrogen metabolism via altered expression of bacterial β -glucuronidase and contributing to systemic inflammation²⁷¹. Markers of microbial dysbiosis, such as shifts in the Firmicutes/Bacteroidetes ratio, reduction in butyrate-producing species, and increased LPS levels, may serve as early indicators of circadian-gut axis dysfunction²⁶⁰.

Concurrent deficiencies in vitamin D, likely due to reduced sunlight exposure, and fluctuations in estradiol, progesterone, and DHEAS, especially among premenopausal women and extreme chronotypes, further exacerbate endocrine disruption²⁷². The detection of all these parameters through non-invasive or minimally-invasive measurements could help risk stratification among female NS workers.

To date, occupational health assessments for NS workers, particularly women, have underutilized mechanistic biomarkers capable of detecting early and personalized signs of biological disruption²⁷³. The integration of specific, validated markers will enable early detection of high-risk individuals, dynamic monitoring of exposure effects, and, finally, the development of chrono-medicine-dictated interventions aimed at prevention, mitigation, and tailored policy-making²⁷⁴. By integrating biomarker profiles with genetic susceptibility and exposure history, future studies may develop predictive models that could implement targeted surveillance and preventive interventions tailored to the unique biological impact of occupational circadian disruption²⁷³.

Correlation between night shift work and breast cancer risk in female workers

The potential association between NSW and BC risk has been extensively investigated over the past three decades through population-based case-control and large prospective cohort studies¹. While several studies suggest that chronic circadian disruption from NSW may modestly elevate BC risk, findings have been heterogeneous and at times conflicting, reflecting

Table 9 | Human studies on NSW and hormonal levels

NSW Effect	Parameter(s)	Outcome	Year	Ref.
↑ E2 and ↓ morning urinary aMT6s in long-term NSW vs day workers	Plasma E2, Morning urinary aMT6s	↑ E2, ↓ aMT6s	2004	252
NS changes in sex steroids in NSW vs day workers	Circulating sex steroids (E2, P, E1, E1S, DHEA, DHEAS, T, androstenedione), morning urinary aMT6s	NS trend ↓ aMT6s	2006	176
↑ E2 and ↑ E1 in graveyard shift / awake ≥1 a.m. vs day workers	Serum E2, E1, T, DHEAS	↑ E2, ↑ E1; T, DHEAS NS	2008	253
NS associations in rotating NSW vs day workers	Urinary aMT6s, E2, E1, P, prolactin	NS	2012	254
↓ aMT6s, ↑ FSH/LH during NSW vs day workers	Urinary aMT6s, urinary E1C, FSH, LH	↓ aMT6s; ↑ FSH, ↑ LH; E1C NS	2012	255
↑ E2 in premenopausal NSW nurses without naps vs napping and day workers	Serum 17β-E2	↑ E2	2013	206
↑ E2 in rotating NSW vs day workers	Serum 17β-E2, urinary aMT6s	↑ E2; aMT6s NS	2014	180
↓ aMT6s; ↑ E2 and P (follicular), ↓ T (phase-specific) in rotating NSW vs day workers	Urinary aMT6s, serum E2, P, T, cortisol, DHEA/S	↓ aMT6s; ↑ E2, ↑ P; ↓ T; cortisol, DHEA/S NS	2015	256
↑ androgens and progestagens; delayed peaks in NSW vs day workers	24 h urinary androgens (T, DHEA, androstosterone, OH-androstenedione, androstenediol), progestagens	↑ androgens and progestagens; delayed peak	2015	257
↑ E2 and DHEAS in long-term rotating NSW (> 15 y) postmenopausal vs day workers	Serum E2, DHEAS, T	↑ E2, ↑ DHEAS; T NS	2016	258
↓ E2 and melatonin; ↑ leptin, ↑ TNF-α, ↑ IL-1β, ↑ IL-6 in whole-night NSW vs day workers	Melatonin, E2, leptin, TNF-α, IL-1β, IL-6	↓ melatonin, ↓ E2; ↑ leptin, ↑ TNF-α, ↑ IL-1β, ↑ IL-6	2020	259
↓ ESR1/ESR2 gene expression in NSW vs day workers	ESR1 and ESR2 gene expression in PBMCs	↓ ESR1, ↓ ESR2	2024	212
↑ corticosterone, ↑ 11-deoxycortisol, ↑ DHEA, ↑ androstenedione; ↓ E2 in NSW vs day workers	Serum corticosterone, 11-deoxycortisol, DHEA, androstenedione, E2	↑ corticosterone, ↑ 11-deoxycortisol, ↑ DHEA, ↑ androstenedione; ↓ E2	2025	188

NSW/night shift work, E2 estradiol, E1 estrone, P progesterone, T testosterone, DHEA dehydroepiandrosterone, DHEAS dehydroepiandrosterone sulfate, aMT6s 6-sulphatoxymelatonin, E1S estrone sulfate, FSH follicle-stimulating hormone, LH luteinizing hormone, NS non-significant, ESR1 estrogen receptor α gene, ESR2 estrogen receptor β gene, TNF-α tumor necrosis factor α, IL-1β interleukin-1β, IL-6 interleukin-6, PBMCs peripheral blood mononuclear cells. Arrows indicate direction of change relative to day workers (↑ increase; ↓ decrease).

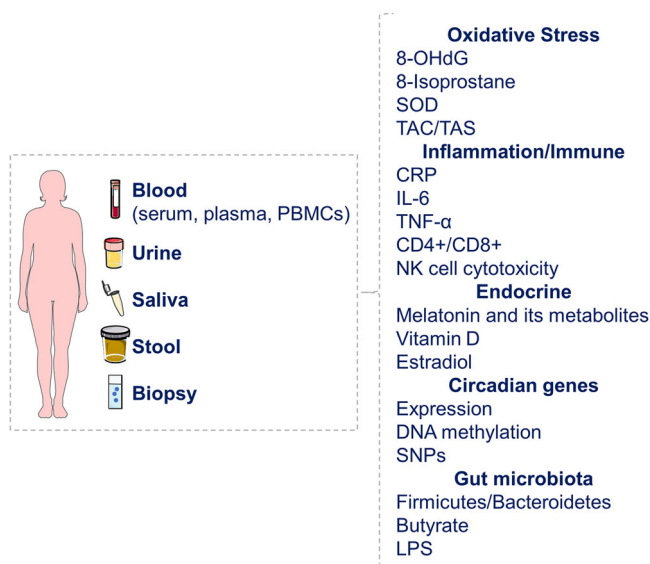


Fig. 4 | Biological sampling and biomarker overview in female night shift workers. The figure illustrates key sample types (blood, urine, saliva, stool, biopsy) and associated biomarkers, including indicators of oxidative stress, inflammation, immune function, endocrine disruption, circadian-relevant gene alterations (including clock genes and melatonin pathway genes), and gut microbiota imbalance. Selected elements adapted from Servier Medical Art (<https://smart.servier.com>, last accessed on 09 November 2025), licensed under Creative Commons Attribution 4.0 International License (CC BY 4.0; <https://creativecommons.org/licenses/by/4.0/>, last accessed on 09 November 2025).

variation in study design, exposure definitions, and adjustment for reproductive and lifestyle factors (Tables 10–11).

This section summarizes the evolution of epidemiologic evidence, from 1996 to the present time²⁷⁵. The epidemiologic literature before 2019 presented mixed evidence linking NSW to BC. Two major authoritative assessments synthesized these findings: the U.S. National Toxicology Program (NTP) and the IARC^{276,277}. Both concluded that human evidence for the carcinogenicity of NSW was limited. In detail, the NTP determined there was strong, though not sufficient, evidence for persistent NSW (long-term, frequent, or beginning at an early age), supported by mechanistic data on circadian disruption, while IARC classified NSW as probably carcinogenic to humans (Group 2 A) based on limited human and sufficient animal evidence^{276,277}.

As summarized in Table 10, individual studies reported variable results. For instance, early case-control analyses yielded both positive and inverse associations, while more recent pooled analyses suggested only borderline increased risk^{278–280}. Occupational cohorts of Norwegian and Danish nurses and militaries, as well as the Swedish Work, Lipids and Fibrinogen (WOLF) cohort indicated higher BC risk with ≥ 20 –30 years of NSW^{281–287}. Additionally, two Chinese cohort studies found that NSW was associated with increased risk of BC, along with a dose-response relationship per cumulative hour-year of exposure^{288,289}. Whereas large prospective cohorts, such as the Shanghai Women's Cohort, the UK Million Women Study, the Swedish twin cohort, and the Dutch National Cohort reported null associations^{290–294}.

The NHS II observed elevated risk with ≥ 20 years of NSW, while NHS I and payroll-based cohorts were mixed or null^{295–298}. Intriguingly, risk appeared greater among premenopausal women, as well as for estrogen receptor-positive BC cases^{280,296}. Genetic susceptibility has also been explored, reporting a possible interaction between CLOCK gene polymorphism and NSW²⁹⁹. Nonetheless, multiple sources of bias likely influenced results, including exposure misclassification (es., current shift status used as proxy for lifetime exposure), survivor bias and left truncation in cohorts, and incomplete control for reproductive or behavioral

confounders²⁷⁶. Heterogeneous NSW definitions (i.e., rotating versus permanent schedules, varying duration thresholds) further limited comparability among studies²⁷⁷.

Since 2020, several clinical studies have further evaluated the association between NSW and BC risk using improved exposure characterization and analytical designs. Among the twelve studies identified, six were included in the 2022 systematic review and meta-analysis by Hong et al. while six more recent investigations (published after that review) are newly discussed in the present work¹³⁸. Definitions of NSW differed among studies but generally involved working during night hours for specified durations or frequencies, commonly ≥ 10 years or ≥ 10 NS per month. Data collection methods ranged from structured interviews and questionnaires to registry-based employment records and JEM. The studies were variably adjusted for potential confounders including age, reproductive history, smoking status, BMI, breastfeeding, socioeconomic status, and family history of BC (Table 11).

Case-control studies from Mexico, Korea, Poland, China, and Spain reported significant associations between long-term NSW and BC risk. In Mexican women, Bustamante-Montes et al. found markedly increased risk for ≥ 10 years of NSW (OR = 8.58, 95% CI 2.19–33.8), with early menarche amplifying risk and breastfeeding conferring protection³⁰⁰. Korean data linked NSW with circadian gene variants in CLOCK, CRY2, and RORA, while Polish studies demonstrated dose-response correlations and higher risks for backward-rotation schedules (OR = 3.31)^{195,301,302}. Similar positive findings emerged among Chinese healthcare workers and Spanish nurses^{303,304}.

By contrast, cohort studies reported more shaded results. Among Norwegian offshore petroleum workers, NSW was not associated with BC (HR = 0.87, 95% CI 0.52–1.46)³⁰⁵. The U.S. Sister Study observed a modest risk with short-term NSW (HR = 1.12, 95% CI 1.00–1.26) but none for long-term exposure³⁰⁶. The Canadian Census Health and Environment Cohort also found no increased risk (HR = 0.94, 95% CI 0.90–0.99), although exposure estimates relied on job-exposure matrices lacking behavioral detail³⁰⁷.

Nordic registry studies provided mixed evidence. In Finnish public-sector employees, risk doubled among women ≥ 50 years with ≥ 10 years of NSW (OR = 2.05, 95% CI 1.04–4.01)³⁰⁸. The Swedish Healthcare Worker study identified elevated postmenopausal BC risk with ≥ 8 years of NSW (HR = 4.33, 95% CI 1.45–10.57), although based on few cases³⁰⁹. The Older Finnish Twin Cohort found increased BC risk (HR = 1.58, 95% CI 1.16–2.15), particularly in younger women (born 1950–1957; HR = 2.08, 95% CI 1.32–3.28). Whereas, direct co-twin comparisons suggested effects independent of shared genetics or environment³¹⁰.

Overall, the novel body of evidence on exposed workers supports an association between NSW and increased BC risk, with risk magnitude influenced by duration, shift rotation patterns, and genetic susceptibility. Several studies, particularly from Poland, Mexico, Korea, and Spain, report ORs ranging from approximately 2 to over 8, with stronger associations for long-term NSW (≥ 10 years) and specific genetic polymorphisms in clock genes and melatonin receptors genes. Indeed, protective factors such as breastfeeding also emerged in some studies^{195,300,303,304}. Conversely, large prospective cohorts and population-based studies, like for the past population studies, present more nuanced findings, with some reporting no association, possibly due to “healthy worker effects”, differential exposure misclassification, or lack of detailed shift characteristics. Importantly, the impact of shift intensity, timing, and chronotype remains underexplored^{306,308,309}.

Common limitations include the reliance on self-reported NSW exposure, potential recall bias, small sample sizes in case-control studies resulting in wide confidence intervals, and incomplete adjustment for confounders such as alcohol intake, hormone therapy, and other lifestyle factors. Also, some cohort studies lacked detailed exposure intensity and duration data or were unable to evaluate cumulative effects over extended periods²⁷³. The heterogeneity in study designs, populations, and NSW definitions complicates direct comparisons. Genetic and sociodemographic

Table 10 | Past studies in night shift workers assessing breast cancer risk (from 1996 to 2019)

Study	BC Risk Outcome (associated with NSW)	BC Risk Direction	Year	Ref.
Norwegian radio/telegraph operators	Age ≥ 50 : OR = 4.3 (0.7–26); small n, wide CI	Positive ($\uparrow$)	1996	281
US Cancer Surveillance System	Ever NS: OR = 1.6 (95% CI: 1.0–2.5). Dose-response with duration/intensity	Positive ($\uparrow$)	2001	278
US LIBCSP Study	Ever NS: OR = 0.55 (95% CI: 0.32–0.94); inverse association, small n, potential bias	Negative ($\downarrow$)	2006	279
Norwegian nurses	Long-term NS ≥ 30 yrs: OR = 2.21 (1.10–4.45)	Positive ($\uparrow$)	2006	282
Shanghai (China) Women's Cohort	HR = 1.0 (JEM), HR = 0.9 (self-report)	Null (–)	2010	290
Norwegian follow-up studies	≥ 5 yrs with ≥ 6 consecutive NS: elevated risk. ≥ 12 yrs NS: OR = 1.3; ≥ 30 yrs, ≥ 3 shifts/month: OR = 0.8	Mixed	2011	284
Danish nurses	Ever rotating NS: OR = 1.8; permanent + rotating: OR = 2.9; ≥ 20 yrs: OR = 2.1	Positive ($\uparrow$)	2012	285
Military women	Ever NS: OR = 1.4; ≥ 3 NS/week for ≥ 15 yrs: OR = 2.5	Positive ($\uparrow$)	2012	286
Norwegian follow-up studies	≥ 5 yrs with ≥ 6 consecutive NS: elevated risk. ≥ 12 yrs NS: OR = 1.3; ≥ 30 yrs, ≥ 3 shifts/month: OR = 0.8	Mixed	2013	283
Swedish WOLF cohort	NS vs day: HR = 2.02 (1.03–3.95); small cases	Positive ($\uparrow$)	2013	287
GENICA study	CLOCK gene AA vs GG among NS: OR = 3.53 (95% CI: 1.09–11.42); small n, tentative	Positive ($\uparrow$)	2014	299
Dutch National Cohort	Null or slightly decreased risk	Null (–)	2014	294
Guangzhou (China) Cohort	Ever NS: OR = 1.34 (95% CI: 1.05–1.72)	Positive ($\uparrow$)	2015	288
Shanghai (China) Textile Workers	>28 yrs NS: HR = 0.88; $>2,880$ NS: HR = 0.89	Null (–)	2015	291
Swedish Twin Cohort	Ever NS: HR = 0.94; 21–45 yrs: HR = 1.68 (0.98–2.88)	Null (–)	2015	292
UK Million Women Study	Ever NS: RR = 1.00 (0.92–1.08); no trend by duration	Null (–)	2016	293
US NHS II	≥ 20 yrs cumulative NS: HR = 1.40; baseline: HR = 2.15; ER-/PR+ tumors: HR = 1.62	Positive ($\uparrow$)	2017	296
US NHS I	≥ 30 yrs NS: HR = 0.95	Null (–)	2017	295
BCEES, CBCS, CECILE, GENICA, MCC Studies	Ever NS: OR = 1.12 (1.00–1.25); recent exposure (0–2 yrs): OR = 1.26; ≥ 20 h/week: OR = 1.28; premenopausal ≥ 3 NS/week: OR = 1.80	Positive ($\uparrow$)	2018	280
Jinjiang (China) Cohort	NS workers: OR = 1.38 (95% CI: 1.04–2.71); dose-response with cumulative hour-years: OR = 1.15 per hour-year	Positive ($\uparrow$)	2019	289
Danish payroll-based cohort	Ever NS: RR = 0.90; weak elevation in HER2 + BC	Mixed	2019	297
UK Generations Study	Past 10 yrs NS: HR = 1.00; no association with duration	Null (–)	2019	298

NSW night shift work, NS night shift, BC breast cancer, OR odds ratio, HR hazard ratio, RR relative risk, CI confidence interval, ER-/PR+ estrogen receptor-negative / progesterone receptor-positive; n, number of participants, JEM job-exposure matrix, $\uparrow$, positive association / increased risk; $\downarrow$, negative association / decreased risk, – no association / null, Mixed mixed results.

differences may limit generalizability. Additionally, the “healthy worker effect” and other biases may attenuate observed associations in prospective designs^{306,311}. Based on these limitations, future research should integrate detailed exposure metrics, genetic profiling, and chronotype assessments to better clarify mechanisms and identify high-risk subgroups.

Several ongoing investigations will clarify these relationships. The Italian Night-shift Work and Breast Cancer study (NCT06786949) is prospectively examining healthcare workers, integrating biospecimens for circadian-related epigenetic markers. In Canada, Improving Cancer-Related Outcomes in Shift Workers (NCT02609373) evaluated sleep-behavior interventions to reduce circadian disruption. Mechanistic studies such as the U.S. Sleep, Circadian Hormonal Dysregulation, and Breast Cancer Survival (NCT00519168) and the French Awareness About Breast Cancer Screening (NCT03777397) are linking epidemiologic findings with biological and behavioral pathways.

Collectively, current research reinforces a multifactorial relationship between NSW and BC risk. Evidence increasingly supports an elevated risk associated with persistent, intensive NSW, particularly when initiated early in life or combined with genetic susceptibility. Across the broader epidemiologic record, analysis of human cohort studies conducted between 1996 and the present shows that more than half (53%) reported a positive association between NSW and BC risk, with an apparent increase from 50% before 2019 to 58% after 2019. However, this difference was not statistically significant ($\chi^2 = 1.45$, $p = 0.694$; Supplementary Fig. 2). Despite growing evidence, variability in exposure assessment and potential biases continue to limit causal

inference, emphasizing the need for integrative molecular-epidemiologic approaches to better identify vulnerable subgroups and refine quantitative risk estimates.

Conclusions

In conclusion, the complex relationship between NSW and BC is increasingly supported by mechanistic, preclinical and clinical evidence, underscoring its relevance for occupational medicine and public health³¹². Disruption of circadian rhythms through exposure to LAN appears to trigger a cascade of biological effects, ranging from melatonin suppression and oxidative stress to immune dysregulation, telomere shortening, and epigenetic alterations. Such effects may contribute to BC etiopathology²⁷⁵. The identification of novel biomarkers, such as melatonin metabolites, oxidative catabolites, DNA methylation patterns, and circulating miRNAs, represents a critical step toward developing reliable tools for early detection and individualized risk stratification².

From an occupational medicine perspective, these findings highlight the urgent need to better define and monitor exposure to NSW, improve workplace lighting policies, and consider individual susceptibility factors such as chronotype and genetic predisposition. Incorporating biomarker-based screening and precision risk models into occupational health protocols could enhance preventive efforts and enable timely interventions. Ultimately, integrating mechanistic insights with robust epidemiological data is essential to establish evidence-based guidelines and regulatory frameworks that effectively protect vulnerable workers and reduce the long-term burden of work-related BC.

Table 11 | Recent studies in night shift workers assessing breast cancer risk (from 2019 to 2024)

Study Design / Population	Country	Age (years)	Night Shift Definition	Breast Cancer Cases	Controls	Job Type	Breast Cancer Risk	Breast Cancer Risk	Key Notes / Limitations	Ref.
Case-control	Mexico	50.1 (mean)	Night work during usual sleep hours; subgroup ≥ 10 years	101	101	Security institute workers	OR = 8.58 (95% CI: 2.19–33.8; higher in early menarche women)	↑Risk	Small sample size, Wide CIs, Self-reported exposure, No shift pattern data, Limited generalizability	300
Case-control	Korea	47.5 (mean)	≥ 2 months of regular night shifts (9 PM–8 AM)	941	959	NS	OR = 1.98–2.20 (SNP interaction)	↑Risk (genetics)	Binary exposure (ever/never), Limited duration detail, Recall bias, Small night shift subgroup	195
Case-control	Poland	>35	≥ 10 night shifts/month for >10 years	494	515	NS	OR = 2.20 (95% CI: 1.57–3.08)	↑Risk	Recall bias, Large sample, Multifactorial analysis	301
Prospective cohort	USA	35–74	≥ 1 hour between 12 AM–2 AM; includes rotating/irregular shifts	3191	48451	NS	HR = 1.12 (≤ 5 yrs NSW); 1.30 (rotating); 0.81 (> 5 yrs NSW)	↑Risk (limited to time and rotation)	No chronotype data, Healthy worker bias, Exposure misclassification, Familial risk cohort	306
Prospective cohort	Canada	25–74	JEM-based probability of night/rotating shifts by occupation	30775	939520	Retail, healthcare, hospitality	HR = 0.94 (95% CI: 0.90–0.99)	No Risk	Indirect exposure, No timing data, Misclassification risk, Healthy worker effect	307
Population-based case-control	Poland	>35	≥ 6 months; intensity measured (consecutive, duration, frequency, rotation)	494	515	NS	OR = 2.03–3.31 (intensity dependent)	↑Risk	Self-reported, Recall bias, No chronotype, Residual confounding	302
Case-control	China	20–84	Night shift intensity measured (consecutive, duration, frequency, rotation)	495	478	Healthcare (nurses)	OR = 1.53 (95% CI: 1.11–2.11)	↑Risk	Recall/selection bias, Limited geography, No age data	303
Cross-sectional	Spain	41.2 (mean)	>500 nights; ≥ 3 nights/month for 10 years	56	502	Nurses	OR = 2.71 (95% CI: 1.55–4.74)	↑Risk	Self-reported, Cross-sectional, Limited causality	304
Case-control	Norway	44 (mean)	Night/rollover shifts	86	514	Offshore petroleum workers	HR = 0.87 (95% CI: 0.52–1.46, not significant)	No Risk	Low power, Self-reported exposure, Imputed missing data	305
Prospective cohort	Finland	40.6 (mean), stratified $<50/\geq 50$	Shift work with/without nights (rotating/permanent)	1129	32230	Healthcare, social services, education	OR = 2.05 (95% CI: 1.04 to 4.01, ≥ 50 yrs)	↑Risk (limited to older age)	Self-reported, No intensity data, Missing confounders	308
Prospective cohort	Sweden	Mostly <50 ; stratified by menopausal status	≥ 3 hours/night between 22:00–06:00	299	25286	Healthcare (nurses, midwives)	HR = 4.33 (95% CI: 1.45–10.57, ≥ 8 yrs NSW, post-menopause)	↑Risk (limited to time and post-menopause)	Short follow-up, Small long-term exposure group	309
Prospective cohort	Finland	43.6 (mean)	Rotating night shifts	407	5781	NS	HR = 1.58–2.08 (stronger for younger birth cohort)	↑Risk	Self-reported, Incomplete shift/hormone data, Birth cohort effect (twin study)	310

BC Breast Cancer, CI Confidence Interval, HR Hazard Ratio, JEM Job Exposure Matrix, NS Not Specified, NSW Night Shift Work, OR Odds Ratio, PBMCS Peripheral Blood Mononuclear Cells, SNP Single Nucleotide Polymorphism.

Data availability

No datasets were generated or analysed during the current study.

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Competing interests

The authors declare no competing interests.

Additional information

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