Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Shaoqi Fan Date

Night shift work and alteration in leucocyte levels: A meta-analysis

By

Shaoqi Fan
Master of Public Health

Environmental Health

_____

Kyle Steenland, PhD
Committee Chair

_____

Paige Tolbert, PhD
Committee Member

Night shift work and alteration in leucocyte levels: A meta-analysis

By

Shaoqi Fan

B.S.
B.A.
Southern Medical University
2014

Thesis Committee Chair: Kyle Steenland, PhD

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Environmental Health
2016

Abstract

Night shift work and alteration in leucocyte levels: A meta-analysis

By Shaoqi Fan

Objectives. Objectives. Though night shift work has been studied in relation to various diseases, there has yet to be any systematic review or meta-analysis indicating how long-term and short-term night shift works affect the immune system on the cellular level. This meta-analysis aimed to synthesize the effect of sleep disturbance from long-term and acute short-term night shift work implementing on total leucocyte count.

Methods. Searching strings comprised of MeSH terms and keywords were developed. PubMed, EMBASE, Web of Science databases were searched with different searching logics. Inclusion and exclusion criteria were determined in selecting eligible references. A PRISMA flow diagram visualized both the screening and selection processes.

Results. Out of total 486 searched references, twelve were determined eligible—five were cross-sectional studies and the other seven were experimental studies. Only four studies include female as study subjects, and the total number of male subjects was greater than that of female subjects. Definition of night shift work or sleep deprivation/restriction were various across studies. Studies were grouped into two subgroup meta-analyses: i) five cross-sectional studies; ii) five cross-sectional studies plus two experimental studies which provide between-subjects measurements. The overall effects from the results showed that total leucocyte counts among night shift workers or sleep deprivation/restriction groups were significantly higher.

Conclusions. Chronic sleep disturbance could activate the systemic immunization and elevate peripheral total leucocyte count. This effect might be intensified when sleep disturbance was acute and short-term. No significant heterogeneity was found among studies included into the meta-analyses. Further studies are needed to investigate the mechanism of how the interaction between on-site occupational exposures and night shift work affect the immune system.

Keywords night shift work; sleep deprivation; sleep restriction; total leucocyte count; meta-analysis

Night shift work and alteration in leucocyte levels: A meta-analysis

By

Shaoqi Fan

B.S.
B.A.
Southern Medical University
2014

Thesis Committee Chair: Kyle Steenland, PhD

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Environmental Health
2016

Table of Contents

Introduction

Shift work refers to working schedules that cover the usual working hours (i.e. 6:00 am - 17:00 pm) and unusual working hours (i.e. 19:00 pm - 6:00 am)[2]. Shift work is commonly seen in certain industries, i.e. transportation, emergency services, healthcare, retailing. However, more working schedules are designed to make use of 24 hours, seven days a week by international society focusing on elevation of production and customers. Around 15% of all employees in Chile perform night shifts work.[3] 17.5% employees in China and 24% employees in Czech Republic do night shift work at least once a month, while 20% employees in Senegal work night shifts at least once a week[3]. Western countries also widely employ night shift work. Nearly 15 million Americans work a permanent night shift or regularly rotate in and out of night shifts[4]. Compared to developed countries, night shift workers in developing countries are reported to work for longer hours while in poorer working conditions[5].

Shift work is known to disrupt the relationship between the body's internal circadian clock and environmental cues, leading to a possible cascade of disease, including but not limited to sleeping disorder, cancer, metabolic syndrome, cardiovascular disease, insulin resistance, and stroke[6]. A growing evidence body has been focusing on whether sleep disruption from shift work would increase the risk of diseases mentioned above. On the cellular level, sleep disruption intervenes the chronobiology of circulating blood cells in the immune system.[7] Total leucocytes, or white blood cells, have been proved to have circadian rhythm across populations of different ethnics, living habits, and geographic locations[8-10]. Studies have shown the total leucocyte count peaks during the nocturnal

period at around 23:00 and reduces to the minimum level during the diurnal period at around 8:00[7, 11]. Under the natural rhythm of fluctuation in counts, leucocytes' number is often used as an indicator of infectious diseases. Elevated total leucocyte counts are reported in breast cancer[12], metabolic syndrome[13], vascular diseases[14], inflammatory disorders, and abnormal production as in leukemia[15].

Despite the seemingly abundance of information on the deleterious effects of night shift work on health, there has yet to be any systematic review or meta-analysis indicating how long-term and short-term night shift work affects the leucocyte count level. Thus the primary objective of this meta-analysis was to quantitatively synthesize the studies that report any alterations in leucocyte count levels due to the long-term or short-term night shift work. A second objective was to decide whether the effect on leucocyte count level varies between the long-term and the short-term night shift work. A third objective was to evaluate the overall quality of included studies. Based on the results, future directions for biological roles played by leucocytes in etiology might be suggested. Two hypotheses were made in this meta-analysis: i) the total leucocyte counts are significantly higher among night shift workers compared to non-night shift worker; ii) the effect on total leucocyte count level due to the short-term night shift work is more significant than that from the long-term night shift work, after adjusting for any confounders (e.g. infection, obesity, BMI, light exposure etc.). In this meta-analysis, the acute short-term effect of night shift work was defined as either sleep deprivation or sleep restriction.

Method

Searching logics and databases

The Cochrane online library (1956-2016) was searched before achieve the search strategy to confirm there was no systematic review or meta-analysis sharing a similar topic as in current study. Then a list of MeSH terms and keywords including the PICO (P-study population, I-intervention, C-comparison, O-outcome) four elements was developed (Table 1). PubMed (1982-2016), Web of Science (1956-2016), and EMBASE (1966-2016) databases were searched with different search logics (Appendix A, Table 8). To identify any additional qualified articles, literatures from reference list of each included study were extracted and hand searched using Google Scholar. ILLiad (Interlibrary loan) service provided by Health Science Library, Emory University, was also used to require full-text references.

Table 1 MeSH terms and keywords in searching process

MeSH term and keywords
Leucocyte*(all article)
Leukocyte* (all article)
Night work (tm)
Night shift work (tm)
Shift work (tm)
Rotating shift work (tm)
Overtime work (tm)
Sleep deprivation (tm)
Sleep restriction (tm)

all article: search in all article. tm: search on title and abstract.
*plural form.

Inclusion and exclusion criteria

Since only one researcher was responsible for every process in the current meta-analysis, a two-trail screening strategy was developed to determine the eligibility of each reference. Among the non-duplicate 73 out of total 120 references retrieved from the MeSH terms and keywords searching process, the selection first began with screening on titles and abstracts. Then, based on the results, references were divided into three groups: confirmed, suspected, and excluded. Then full-text screening was performed in each group to further confirm the references' eligibility. Screening process in each trail adopted the same pre-determined inclusion and exclusion criteria. Studies were included if they evaluate or at least report the association between night shift work (and/or shift work, rotating shift work) whose schedules cover or overlap with time interval from 24:00 to 05:00 am, and changes in total leucocyte count. Study designs could be either observational study (i.e. cohort study, case-control study, cross-sectional study) or experiment studies. There was no recruiting limitation on races or genders for any study population. Studies were excluded if, in observational studies, the desired exposure was neither night shift work nor rotating shift work that cover or overlap the time interval from 24:00 to 05:00 am; or if, in experimental studies, the desired exposure was neither sleep deprivation nor sleep restriction. Despite the association between night shift work (and/or sleep restriction) and total leucocyte count change were reported, studies without clear articulation on definition of night shift work schedules and/or sleep restriction designs were also excluded[16, 17]. The anticipated outcome, total leucocyte count and its changes, must be presented in cells per volume. Studies reporting leucocyte counts in percentile, median or grams per volume were excluded, unless provided sufficient data to

calculate the mean total leucocyte count in cells per volume. All eligible references should be written in English and be full-text available. References of editorials, comments, case reports were discarded. The selection process as a whole was summarized in a PRISMA flow diagram (Figure 1). The eligibility of any reference in question was discussed and solved by consensus between the research and the supervisor.

Experimental studies There were a number of experimental studies in which subjects were subjected to short-term sleep deprivation and served as their own controls. Unlike observational studies, experimental studies designed sleep deprivation or sleep restriction experiments to create the acute short-term sleep disruption in circadian rhythm. Also, experiment studies were carried out in a laboratory environment. Lighting, restricted physical activity, standard meal and drinking, prohibition of medication and smoking and alcohol consumption were strictly controlled under close supervision. Before the experiment, individuals with sleep disorder, mental illnesses, any other relevant diseases (e.g. cancer, hepatitis, nephritis etc.) or with positive immunological indicators (e.g. plasma C-creative protein <6mg/L, and white blood cell count<9/nl before and after participation) were excluded. Detailed sleep deprivation/restriction designs of each included experimental study were summarized in Table 3. An important issue to highlight was that generally experimental studies were not included in the meta-analysis because i) their paired design was fundamentally different from the cross-sectional studies, and ii) there was no appropriate paired variance data for the overall effect. However, there were two experimental studies which also included a control group not subjected to sleep deprivation[18, 19]. In this two experimental studies, subjects were randomized to be in the

sleep deprived group or the control group, hence controlling confounding. These two studies were analogous to the observational studies in design although sleep deprivation was shorter and controlled. In supplemental analyses, the author added these two studies in the meta-analysis of the cross-sectional observation studies.

Cross-sectional observational studies All observational studies in the literature were cross-sectional. They compared mean total leucocyte level in the shift workers and non-shift workers. Confounding could be present due to the lack of randomization. In some instances confounding was controlled in the analysis. All eligible observational studies were combined to conduct a meta-analysis using inversed-variance weighting.

Quality assessment

The checklist from Downs & Black was used to evaluate both methodological and reporting quality of each included reference[20]. The checklist consists of 27 items and five sub-scales with which a profile of scores could be provided on study quality, internal validity (i.e. bias and confounding), power, and external validity for both experimental and non-experimental studies[20]. Each included reference's profile of scores was determined and presented in the appendix (Appendix B, Table 9 &10). The total scores for experimental studies were 30 and for cross-sectional studies were19, for items 4, 8, 9, 13-15, 17, 19, 23, 24, 26 specifically pertained to the randomized, cohort, or case-control study design.

Data management and analysis

The screening and selection process was performed on EndNote X7.0.1 (Thomson Reuters, NY) which served as a literature management tool to store citations of all references. Data of eligible studies were then extracted (e.g. sample size, mean total leucocyte counts of night shift workers and non-night shift worker or sleep control groups and sleep deprivation groups, standard error of mean, 95% confidence interval). Unit of total leucocyte count was transformed and unified as cells per microliter (cell*μl^{-1}) across all eligible studies. Extracted data were used to calculate the effect measure (mean difference in total leucocyte mean of night shift workers vs. day workers, or of sleep deprivation/restriction group vs. sleep control group) and the corresponding weights, which were the inversed variance of the effect measure for each study.[1] Details of these calculations were presented in Table 2. Forest plots were made to visualize the overall effect of night shift work and/or sleep restriction on total leucocyte counts. The DerSimonian and Laird heterogeneity test (Q statistics)[1] was calculated via SAS 9.4 (SAS Institute Inc., NC). Results with *p* value less than 0.05 was considered statistically significant.

Table 2 Calculation of effect measure with inversed variance weighting (fixed effect) [1].

	Definition	Function	Denotation
Difference of mean leucocyte count	Difference between (combined) groups of night shift workers and non-night shift workers or sleep control group and sleep deprivation/restriction group.	$Diff = M_{i1} - M_{i0}$	$Diff$: difference of total leucocyte count. M_{i1}: mean total leucocyte count of *i*th study night shift workers or sleep deprivation group. M_{i0}: mean total leucocyte count of *i*th study day workers or sleep control group.
Variance of difference of means	Use either the provided standard error of mean (1) or the provided standard deviation of the population mean of each group (2).	1) Var = (standard error in night shift workers)^2 + (standard error in day workers)^2 2) Var = (standard deviation of population in night shift workers)^2/n + (standard deviation of population in day workers)^2/n	Var: variance. n: sample size of nigh shift workers/day workers/sleep deprivation group/sleep control group.
Weight of leucocyte mean difference	Inversed variance of difference of mean.	$W_{i0} = \frac{1}{Var_{i0}}$	W_{i0}: inversed variance of mean of each group within *i*th study.
Meta-analysis effect measure	The overall effect of night shift work, sleep deprivation, sleep restriction on mean total leucocyte count across studies.	$T = \frac{\sum_{i=1}^{m} Diff_i \times W_i}{\sum_{i=1}^{m} W_i}$	T: total effect measure of included studies. $Diff_i$: mean difference of total leucocyte count within *i*th study. m: number of included studies in meta-analysis.
Variance of effect measure	Summed weight for effect size.	$W_{Eff} = \frac{1}{\sum_{i=1}^{m} W_i}$	W_{Eff}: overall weight of included studies.

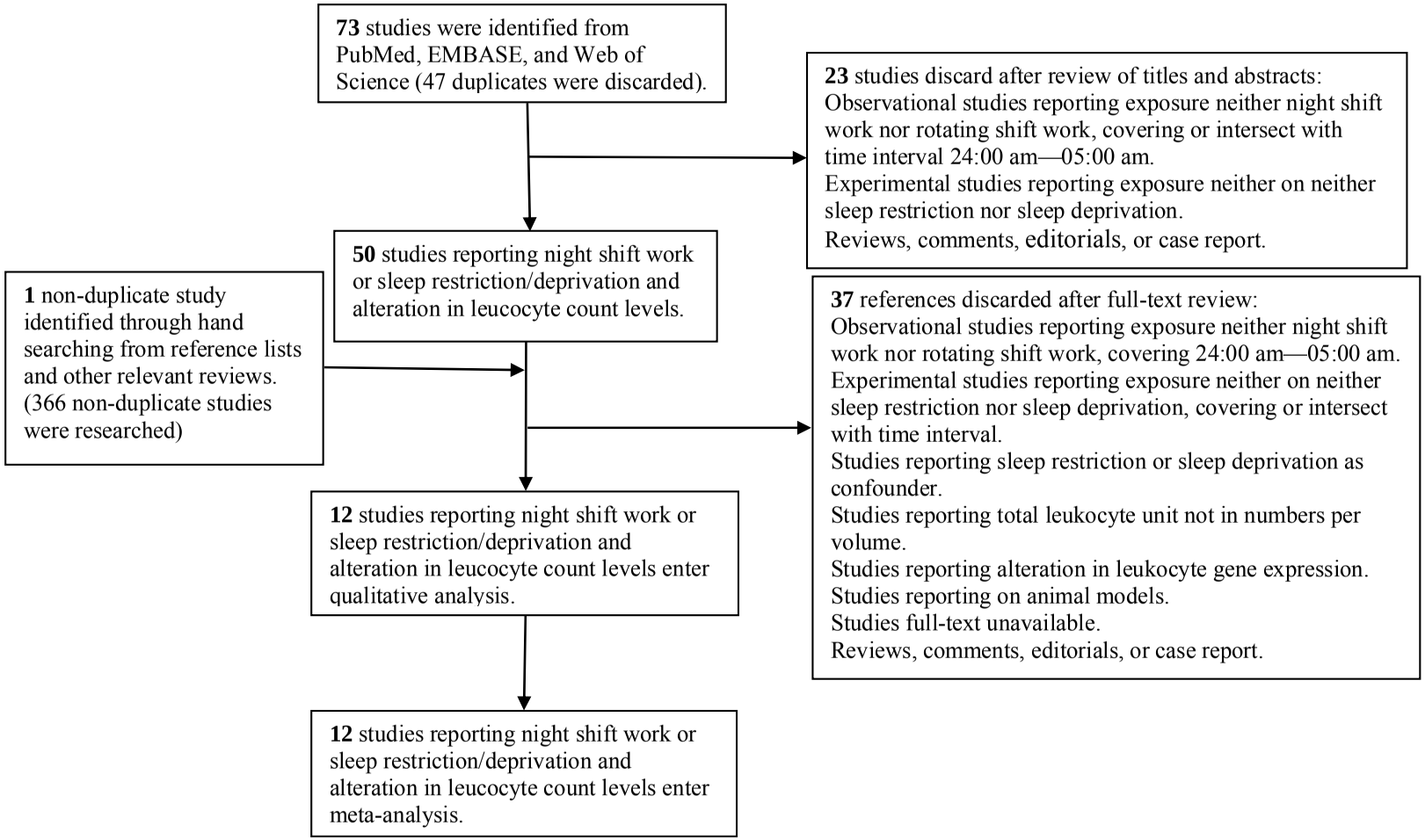

Figure 1 PRISMA flow diagram summarizing the two-trail selection process

Table 3 Summary of the main characters of included studies

Study	PMDI	Observational/ Experimental	Study design	Observation/Data collection period	Age and gender
Nishitani *et al.* 2006, Japan[21]	17485874	Observational	Cross-sectional study	March to May, 2003	33.7 ± 12.3 years, male
Sookoian *et al.* 2007, Argentina[22]	17305651	Observational	Cross-sectional study	2005	34.4 ± 8.4 years, male
Okamoto *et al.* 2008, Japan[23]	18403864	Observational	Cross-sectional study	January to June, 2005	24-49 years, male and female
Burgueno *et al,* 2009, Argentina[24]	20106477	Observational	Cross-sectional study	-	34.4 ± 8.6 years, male
Puttonen, *et al.* 2011, Finland[25]	21797781	Observational	Cross-sectional study	2006-2008	44.6 ± 8.9 years, male and female
Born *et al.* 1997, Germany[11]	9127011	Experimental	Within-subject design	96 hours (Two sections: Section I includes two 24h normal wake-sleep cycles; Section II include 1 complete restriction sleep night and 1 recovery 24h wake-sleep cycle; at least 10 days interval between the two sections)	25 ± 4 years, healthy male
Kerkhofs *et al.* 2006, Belgium[26]	16950577	Experimental	Within-subject design	5 days (1 normal 24h wake-sleep cycle, then 3 consecutive partial sleep restriction nights for sleeping between 1am and 5am, followed by 1 recovery 24h wake-sleep cycle)	55-65 years, healthy female

Table 3 *Continued*

Study	PMID	Observational/ Experimental	Study design	Observation/Data collection period	Age and gender
Boudjetia *et al.* 2008, Belgium[18]	19337560	Experimental	Within-/between-subject design	5 days (Study group: 1 normal 24h wake-sleep cycle, then 3 consecutive partial sleep restriction nights for sleeping between 1am and 5am, lastly 1 recovery 24h wake-sleep cycle; Control group: 5 consecutive normal 24h wake-sleep cycles.)	24.5 ± 3.3 years, healthy male
Costa Ricardo *et al.* 2009, UK[27]	19018559	Experimental	Within-subject design	2 days (Individual goes through two occasions. Occasion I: 30h sleep deprivation; Occasion II: 1 normal 24h wake-sleep cycle; two occasions are separated by seven days)	20 ± 3 years, healthy male
Liu *et al.* 2009, China[28]	18597046	Experimental	Within-subject design	3 days (1 normal 24h wake-sleep cycle, then 24h sleep deprivation)	19-23 years, male and female
Ruiz *et al.* 2010, Brazil[19]	20188046	Experimental	Within-/between-subject design	4 days (Sleep deprivation group: 1 night adaptation, 1 night baseline, 2 nights total sleep deprivation; Control groups: 1 night adaptation, 1 night baseline, 2 norm 24h wake-sleep cycles)	19-29, healthy male
Christoffersson *et al.* 2014, Sweden[29]	24878171	Experimental	Within-subject design	4 days (Individual goes through two experimental conditions. Condition I: 2 normal 24h wake-sleep cycles; Condition II: first 1 normal 24h wake-sleep cycle followed by 24h sleep deprivation; two occasions are separated by at least 28 days)	23 ± 1 years, healthy male

Table 4 Statistics from cross-sectional studies

Study	Groups	Sample size	Leucocyte Mean/ cell*μl^{-1}	Standard error of mean	Standard deviation of population mean	Variance of mean	Difference of mean	Variance of the difference
Nishitani *et al.* 2006, Japan[21]	Day worker	101	6100	-	1500	22277	700	49287
	Rotating shift worker	107	6800	-	1700	27009		
Sookoian *et al.* 2007, Argentina[22]	Day worker	474	6730	58	-	3364	300	10420
	Rotating shift worker	877	7030	84	-	7056		
Okamoto *et al.* 2008, Japan[23]	Day worker	8	4752	-	904	102063	1215	164510
	Shift worker	27	5967	-	1299	62448		
Burgueno *et al.* 2009, Argentina[24]	Day worker	184	6574.4	-	1640	14614	404.8	25453
	Rotating shift worker	255	6979.2	-	1663	10839		
Puttonen, *et al.* 2011, Finland[25]	Day worker	295	5478.6	370	-	1700	261.45	11186
	Shift worker	695	5740	338	-	4086		

Table 5 Statistics from experimental studies

Study	Group	Sample size	Leucocyte Mean/ cell*µl^{-1}	Standard error of mean	Standard deviation of population mean	Variance of mean	Difference of mean	Variance of the difference
Born *et al.* 1997, German[11]	Control	10	6772	338	-	114244	549	10
	Sleep deprivation	10	7321	370	-	136900		
Kerkhofs *et al.* 2006, Belgium[26]	Before sleep restriction	10	5070	-	850	72250	1240	10
	After 3 nights sleep restriction	10	6310	-	800	64000		
Boudjetia *et al.* 2008, Belgium[18]	Control baseline	9	6710	-	-			
	Control 3rd night	9	6800	-	1310	190678		
	Study group baseline	8	5790	-	430	23112.5	1010[a]	8
	Study group 3rd restriction night	8	6800	-	420	22050	0[b]	8
Costa Ricardo *et al.* 2008, UK[27]	Control 30h	11	5600	-	600	32727	400	11
	Sleep deprivation 30h	11	6000	-	500	22727		
Liu *et al.* 2009, China[28]	Baseline day 1 and day 2	10	5607	-	97495	337745	743	10
	Sleep restriction day 3	10	6350	1550	240250			
Ruiz *et al.* 2010, Brazil[19]	Control day 1 and day 2	11	6172	-	-	5236	420[b]	11
	Sleep deprivation baseline	11	5800	400	-	16000		
	Sleep deprivation day 1 and day 2	11	6592	-		5236	792[a]	11
	Sleep deprivation day 1 and day 2	11	6700	-	300	8182		
Christoffersson *et al.* 2014, Sweden[29]	Baseline	16	4500	200	-	40000	700	16
	Sleep deprivation	16	5200	200	-	40000		

a Within-subject difference.
b Between-subject difference.

Results

Summary of demographics

120 non-duplicate literatures through search logics in databases (PubMed, Web of Science, EMBASE) and 366 non-duplicate literatures through hand search were screened. Out of total 486 references, twelve studies were determined eligible for this study. The most common reason for a study to be excluded was that the desired exposure was neither shift work during 24:00 am – 5:00 am nor sleep restriction/deprivation, or the anticipated outcome did not report total leucocyte count in cells per volume.

Among the twelve eligible studies, five were cross-sectional studies on occupational populations[21-25]. The other seven studies were experimental—five of them were within-subjects designs[11, 18, 26-29] and two studies report both within- and between-subjects designs[18, 19]. Characters and statistics of each eligible study were summarized in Table 3—5. Overall, study population, including male and, aged between 21-65 years. The sample size of cross-sectional studies was generally ten times higher than that of experimental studies, except for Okamoto *et al.*[23] observational study, in which only 35 subjects were recruited. Among all eligible studies, only 30% studies included female as their study subjects[23, 25, 26, 28]. Kerkhofs *et al.*[26] exclusively recruited female subjects aged between 55-65 years old[26]. Study subjects among cross-sectional studies were mostly recruited from the same companies with different career positions, while subjects among experimental studies were all healthy volunteers. All eligible studies shared some similarities in their inclusion and exclusion criteria in recruiting study subjects—excluded individuals on infection status (except for Puttonen *et al.*[25]), age, history of disease,

sleep disorder and mediation. Experimental studies, as what had been mentioned before, had further control requirements. All eligible studies also demonstrated the blood samples were drawn approximately at the sample time for every study subject. This performance minimized the variation effect of circadian rhythm on leucocyte count[7]. Either standard errors of means or standard deviations of population means were presented along with mean total leucocyte counts in cells per volume in every eligible study.

Night shift work, sleep deprivation/restriction and total leucocyte counts

Cross-sectional observational studies

Definition of night shift work

There were five observational studies which were included in the meta-analysis. In these studies workers with night shift work were compared to workers in the day work group. Workers in day work group usually started to work between 6:00 to 8:00 in the morning and finished after a 9- to 12-hour duty. The schedules of night shift work were customized to workers' actual career and positions. The synthetic fiber workers from Nishitani *et al.*[21] had night shift 22:00—7:00 (no further detailed demonstration on how many night shift in a 28-day cycle). Factory workers from Sookoian *et al.*[22] and Burgueno *et al.*[24] carried out a 2-shfit scheme that consists of ten night shifts and six day shifts in every 28 days. Japanese physicians, Okamoto *et al*.[23], did their jobs with a more complicated shift schedule: evening duty 17:00—24:30, midnight duty 00:30—8:30, or night duty 17:00—8:30. And airline company workers, Puttonen *et al*.[25], had 2-shift work and 3-shift work between 23:00 and 06:00 next morning (no further detailed

demonstration on how many night shift in a 28-day cycle). Night shift work commonly lasted for 8 to 12 hours.

Four shift work schemes from the eligible cross-sectional studies were excluded in this meta-analysis. Okamoto *et al.*[23] also reported a traditional work group called "*Tochoku*"—a unique Japanese day-and-night work style. This traditional work group was excluded because it was conspicuously different from other night duties in Japan's Labor Standard Law (article 41) and thus including it into the meta-analysis might obscure the fine line between day work and night shift work and caused bias in evaluating the effect of night shift work on total leucocyte count. Former shift work group and in-flight work group in Puttonen *et al.*[25] were excluded because of unclear definition of night shift hours.

Association between night shift work and total leucocyte count in the observational studies

The five cross-sectional studies used univariate analysis (i.e. ANOVA with Boferroni's test, t-test, Mann-Whitey test) with or without adjustment to other risk factors. Poor sleep, BMI, age, smoking habit, HOMA index, triglycerides and hypertension, alcohol consumption, physical activities, education levels, obesity (BMI>30), sleep habits and stress levels were considered as covariates which significantly associated with total leucocyte count through either logistic regression or multiple regression modeling.

After adjusting for sleep quality, age, BMI, and smoking habit, significant difference in total leucocyte count between day workers and night shift workers in Nishitani *et al.*[21]

became insignificant. Okamoto *et al*.[23] generated insignificant conclusion without any adjustment. Sookoian *et al*.[22] and Burgueno *et al*.[24] both concluded that there was a significant elevation in total leucocyte count among rotating shift workers independent of other leucocyte-increasing factors (i.e. elevated BMI, waist-hip ratio, diastolic arterial blood pressure (DABP), fasting insulin, HOMA index, triglycerides, and uric acid).

In the study of Puttonen *et al*.[25], significantly higher total leucocyte count was shown in 2-shift male workers and 3-shift female workers, independent of age, infection, smoking status, alcohol consumption, physical activity, education level, obesity (BMI>30), sleep habits and stress levels. Also, total leucocyte count from male subjects was positively associated with stress. Obesity was suggested to be singling associated with higher total leucocyte count but did not work as a confounder in multiple adjustments among 2-shift or 3-shift workers. Puttonen *et al*.[25] was the only cross-sectional study that considered the healthy worker effect in the results. It discussed this effect might bias the total leucocyte difference between day workers and shift workers [25, 30]. It also mentioned the selection bias from assigning subjects into different shift groups (e.g. in-flight work group and former shift work group). For covariates smoking and obesity, Puttoene *et al*.[25] questioned whether they also worked as mediators in the relationship between night shift work and leucocyte count as shift work might increase smoking and body weight[25, 31]. And thus including smoking and obesity as confounders might bias the effect of night shift work on total leucocyte count to the null.

Experimental studies

As noted in Methods, experimental studies used the same individuals in both the control and the sleep restriction group, and could not be included in the meta-analysis of the observational studies due to their different design and lack of data on variance of effect measures to use for inverse-variance weighting (i.e. the variance of the mean paired difference). However, two experimental studies, Boudjetia *et al*.[18] and Ruiz *et al*.[19], recruited different individuals in their control and study group, while also providing within-subjects comparison data, and these studies were included in supplementary analysis of the observational studies.

Meta-analysis calculation and results

For observational studies, the mean difference in total leucocyte count was first calculated between day worker group and night shift worker group/rotating shift group among cross-sectional observational studies, or between control and sleep restriction group among the two experimental studies with control groups. Then, based on the standard error of the mean (SEM) or standard deviation of the population (SD), the variances together for the shift workers and the day workers were calculated as well as the variance of the effect measure (difference in two total leucocyte counts), which was used as the inversed variance weight for each effect measure. Variances and weights were presented in Tables 4&5. Lastly, average effect measure (mean difference of total leucocyte count) across studies was derived in a meta-analysis, to produce an overall effect measure with 95% CI. For the two experimental studies with non-sleep deprived control group, a similar produce was used. Meta-analysis results were presented in Table 6&7, with and without the two experimental studies.

In the meta-analysis of the five cross-sectional studies, the overall effect of night shift work was greater than 0, which suggested that there is a significant elevation in total leucocyte count (357.16 cells*μl^{-1}, 95% CI 233.45—480.87 cells*μl^{-1}) (Table 6, Figure 2). The forest plot also showed every cross-sectional study yielded significant results before synthesizing. Sookoian *et al*.[22] contributed mostly to the overall effect measure while Okamoto *et al*.[23] contributed the least. The maximum and minimum contributions were determined by sample size and variance of individual effect measure (mean difference). Among the extracted data, only mean difference from Puttonen *et al*.[25] was adjusted for risk factors (e.g. age, BMI, infection, alcohol consumption). Also, considering Puttonen *et al*.[25] was the only cross-sectional study that provides separate male and female data, combined total leucocyte counts from both genders were synthesized before being included into this meta-analysis. Fixed effect model was used in synthesizing data and heterogeneity test supported no significant heterogeneity existed across the five cross-sectional studies (Q=8.08, df=4, p=0.82).

After adding the two experimental studies (Boudjetia *et al.* and *Ruiz et al.*)[18, 19] to the observational cross-sectional studies, the effect of short-term night shift work (equivalent) on total leucocyte count was intensified. The mean difference in total leucocyte count increased from 357.16 to 369.47 cells*μl^{-1} with a more precise 95% CI 316.11—422.83 cell*μl^{-1} (Table 7, Figure 3). The overall effect measure did not change quite substantially. Fixed effect model was also used in this result with justification from heterogeneity test (Q(Chi-squre)=9.06, df=6, p=0.38).

This study also estimated meta-analysis results for the seven experimental studies with paired comparisons, weighting by study size. The estimated average difference between subjects before and after sleep deprivation was 759.37 cell*μl^{-1}. This effect could be underestimated or overestimated, due to the lack of inversed variance weighting as used in the meta-analysis of the observational studies. The only appropriate conclusion drawn form the seven experimental studies with paired comparisons was that elevated total leucocyte count was observed consistently across each experimental study and that sleep deprivation or sleep restriction increased the total leucocyte count without knowing the exact effect magnitude. However, it is likely that the leucocyte count elevation within study subjects from short-term sleep deprivation in the experimental studies is greater than the corresponding leucocyte count elevation in the observational studies.

Table 6 Fixed effect computations for cross-sectional studies

Study	Effect measure	Variance of effect measure	Weight	Effect measure× Weight
Nishitani *et al.* 2006, Japan[21]	700	49287	2.02895E-05	0.01420
Sookoian *et al.* 2007, Argentina[22]	300	10420	9.59693E-05	0.02879
Okamoto *et al.* 2008, Japan[23]	1215	164510	6.07866E-06	0.00739
Burgueno *et al.* 2009, Argentina[24]	404.8	25453	3.92886E-05	0.01590
Puttonen, *et a.l* 2011, Finland[25]	261.45	11186	8.93975E-05	0.02337
Sum			0.00025	0.08966
Meta analysis calculation result	Overall effect measure	Variance of overall effect measure	Upper CI of overall effect measure	Lower CI of overall effect measure
	357.16	3983.69	480.87	233.45

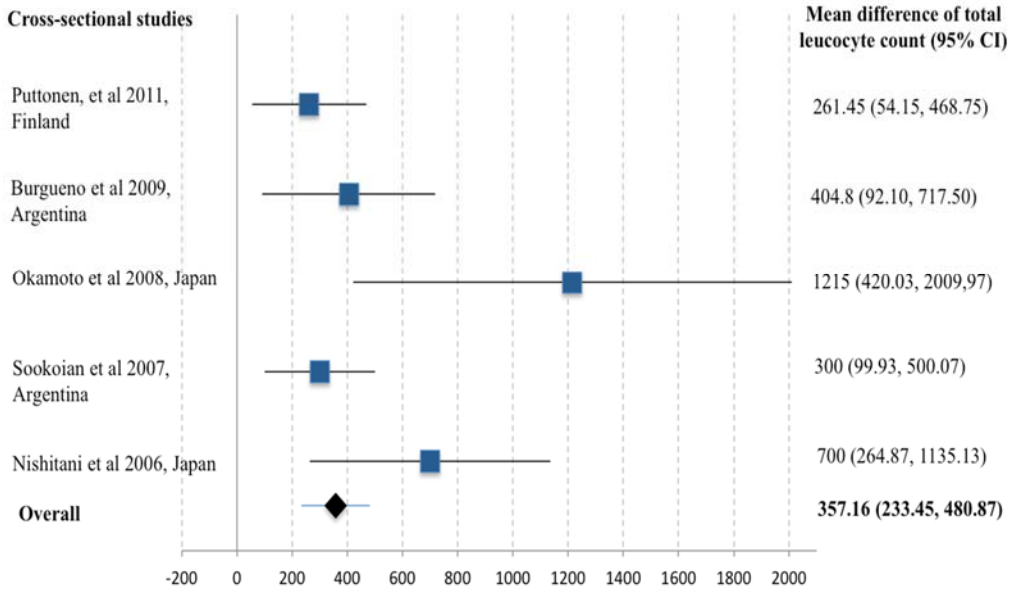

Figure 2 Effect measure from cross-sectional studies

Table 7 Fixed effect computations for cross-sectional studies and experimental studies

Study	Effect measure	Variance of effect measure	Weight	Effect measure× Weight
Cross sectional studies				
Nishitani *et al.* 2006, Japan[21]	700	49287	2.02895E-05	0.01420
Sookoian *et al.* 2007, Argentina[22]	300	10420	9.59693E-05	0.02879
Okamoto *et al.* 2008, Japan[23]	1215	164510	6.07866E-06	0.00739
Burgueno *et al.* 2009, Argentina[24]	404.8	25453	3.92886E-05	0.01590
Puttonen, *et al.* 2011, Finland[25]	261.45	11186	8.93975E-05	0.02337
Experimental studies				
Boudjetia *et al.* 2008,Belgium[18]	0	212728	4.70084E-06	0
Ruiz *et al.* 2010, Brazil[19]	420	10473	9.54861E-05	0.04010
Sum			0.00035	0.12976
Meta analysis calculation result	Overall effect measure	Variance of overall effect measure	Upper CI of overall effect measure	Lower CI of overall effect measure
	369.47	2847.30	422.83	316.11

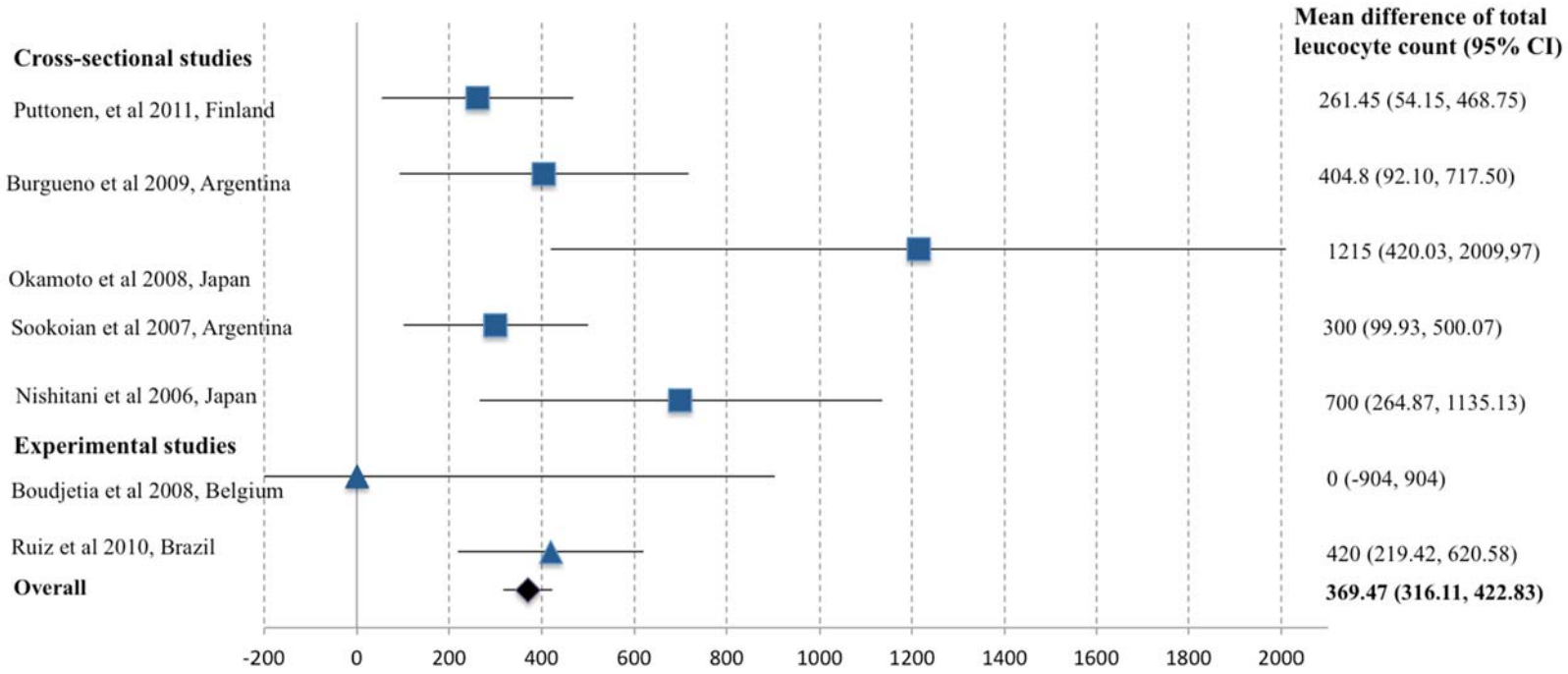

Figure 3 Overall effect measure from five cross-sectional studies and two experimental studies.

Discussion

This meta-analysis firstly summarized the alteration in total leucocyte count level among occupational subjects who undertake chronic nigh shift work. And the results indicated that chronic sleep disturbance could activate the systemic immunization and elevate peripheral total leucocyte count. This effect might be intensified when sleep disturbance became from chronic long-term to acute short-term.

There might be more experimental studies that reported between-subject comparison data but were published in languages other than English. Also, for future reference, there might be alternatives to derive paired variance other than using sample size as in this study.

Further, no investigation in gender difference was achieved in this meta-analysis. Out of the twelve eligible studies, two cross-sectional studies (Okamoto *et al*. and Puttonen *et al*.)[23, 25] and two experimental studies (Kerkhofs *et al*. and Liu *et al*.)[26, 28] included female as their study subjects. And only Okamoto *et al*.[23] investigated gender difference in the association between night shift work and total leucocyte count. No significant results were yielded. Conclusions from other studies on how menstrual cycle affects immunological risk factors were various. Some studies reported menstrual cycle has no significant effect on NK cells[32], and others reported significant results[33]. So far, there has yet to be any conclusive analysis on whether and how menstrual cycle influences total leucocyte count either on occupational or volunteer healthy subjects.

Future study may work on how subgroups of total leucocyte (e.g. granulocytes, neutrophils, monocytes) contribute to the alteration in total leucocyte count from sleep disturbance while independent of natural circadian rhythm. Studies have provided different observations to this question without a systematic conclusion. Further, in observational studies with occupational subjects, the medical and biochemical evaluation were usually carried out on-site, which suggest unconstrained factors (e.g. exposure to hazardous) may also contribute to leucocyte count change without identification. Investigations on whether on-site exposures interact with shift work schedule and how they desynchronize immune system are also necessary.

Conflict of interest statement

No conflict of interest was declared.

Acknowledgements

The author was grateful to professor Kyle Steenland (Emory University) for his assistance and supervision in this meta-analysis study and to the Health Science Library (Emory University) for providing the interlibrary loan service.

Appendix

Appendix A

Table 8 Database and searching logics

Appendix B

Table 9 Quality assessment scores for cross-sectional studies (total score=19)

Table 10 Quality assessment scores for experimental studies (total score=30)

Appendix A

Table 8 Database and searching logics

Database		Search logics	Number of non-duplicated studies searched
PubMed (1982-2016)		(night work*[Title/Abstract] OR shift work*[Title/Abstract] OR night shift work*[Title/Abstract] OR rotating shift work*[Title/Abstract] OR overtime work*[Title/Abstract] OR sleep deprivation[Title/Abstract] OR sleep restriction[Title/Abstract]) AND (leucocyte* OR leukocyte*) AND adult;	50
Web of Science (1956-2016)	#1	TS=(night work* OR shift work* OR night shift work* OR rotating shift work* OR overtime work*) AND TS=(leucocyte* OR leukocyte*) AND TS=(adult);	19
	#2	TS=(sleep deprivation OR sleep restriction) AND TS=(leucocyte* OR leukocyte*) AND TS=(adult);	
	#3	TS=(overtime work* OR rotating shift work*) AND TS=(leucocyte* OR leukocyte*) AND TS=(adult);	
	#1 OR #2 OR #3	Combine searching strings above with function OR.	
EMBASE (1966-2016)	#1	'night work*':ab,ti OR 'shift work*':ab,ti OR 'night shift work*':ab,ti OR 'rotating shift work*':ab,ti OR 'overtime work*':ab,ti AND (leucocyte* OR leukocyte*) AND ('adult'/exp OR adult);	51
	#2	'sleep restriction':ab,ti OR 'sleep deprivation':ab,ti AND (leucocyte* OR leukocyte*) AND adult;	
	#3	'night work*':ab,ti OR 'shift work*':ab,ti OR 'night shift work*':ab,ti OR 'overtime work*':ab,ti OR 'rotating shift work*':ab,ti OR 'sleep deprivation':ab,ti OR 'sleep restriction':ab,ti AND (leucocyte* OR leukocyte*) AND ('adult'/exp OR adult) AND [<1966-2016]/py;	
	#1 OR #2 OR #3	Combine searching strings above with function OR.	

TS=search on topic, :ab,ti=search in abstract or title, /exp=search for related narrower or child term, /py=publication year.

Appendix B

Table 9 Quality assessment scores for cross-sectional studies (total score=19)

Study	Nishitani et al 2006, Japan	Sookoian et al 2007, Argentina	Okamoto et al 2008, Japan	Burgueno et al 2009, Argentina	Puttonen, et al 2011, Finland
Sub-scale I: Reporting quality[a]					
1	1	1	1	1	1
2	1	1	1	1	1
3	1	1	1	1	1
4	-	-	-	-	-
5[d]	1	1	2	1	2
6	0	0	0	0	0
7	1	1	1	1	1
8	-	-	-	-	-
9	-	-	-	-	-
10	1	1	1	1	1
Sub-scale II: External validity[b]					
11	0	1	0	1	1
12	0	1	0	1	1
13	-	-	-	-	-
14	-	-	-	-	-
15	-	-	-	-	-
16	1	1	1	1	1
17	-	-	-	-	-
18	1	1	1	1	1
19	-	-	-	-	-
20	1	1	1	1	1
Sub-scale III: Internal validity - confounding (selection bias)[b]					
21	1	1	0	1	1
22	1	1	1	0	1
23	-	-	-	-	-
24	-	-	-	-	-
25	1	0	0	0	1
26	-	-	-	-	-
Sub-scale IV: Power[c]					
27	0	0	0	1	0
Sum	12	13	11	13	15

a. Item 1-10: yes=1, no=0.

b. Item 11-26: yes=1, no=0, unable to determine=0.

c. Recoded as not mention-0, power calculated=1, differences between groups calculated=2.

d. Item 5: yes=2, partially=1, no=0.

Table 10 Quality assessment scores for experimental studies (total score=30)

Study	Born et al 1997, German	Kerkhofs et al 2006, Belgium	Boudjetia et al 2008, Belgium	Costa Ricardo et al 2008,	Liu et al 2009, China
Sub-scale I: Reporting quality[a]					
1	1	1	1	1	1
2	1	1	1	1	1
3	1	1	1	1	1
4	1	1	1	1	1
5[d]	2	2	2	1	2
6	0	0	0	0	0
7	1	1	1	1	1
8	0	0	0	0	0
9	0	0	0	0	0
10	0	1	1	0	1
Sub-scale II: External validity[b]					
11	0	0	0	0	0
12	0	0	0	0	0
13	1	1	1	1	1
14	0	0	0	0	0
15	0	0	0	0	0
16	1	1	1	1	1
17	1	1	1	1	1
18	1	1	1	1	1
19	1	1	1	1	1
20	1	1	1	1	1
Sub-scale III: Internal validity - confounding (selection bias)[b]					
21	0	0	0	0	0
22	0	0	0	0	0
23	0	0	0	0	0
24	0	0	0	0	0
25	1	1	1	1	1
26	0	0	0	0	0
Sub-scale IV: Power[c]					
27	0	0	0	0	0
Sum	14	15	15	13	15

a. Item 1-10: yes=1, no=0.
b. Item 11-26: yes=1, no=0, unable to determine=0.
c. Recoded as not mention-0, power calculated=1, differences between groups calculated=2.
d. Item 5: yes=2, partially=1, no=0.

Table 10 *Continued*

Study	Ruiz et al 2010, Brazil	Christoffersson et al 2014, Sweden
Sub-scale I: Reporting quality[a]		
1	1	1
2	1	1
3	1	1
4	1	1
5[d]	2	2
6	0	0
7	1	1
8	0	0
9	0	0
10	0	0
Sub-scale II: External validity[b0]		
11	0	0
12	0	0
13	1	1
14	0	0
15	0	0
16	1	1
17	1	1
18	1	1
19	1	1
20	1	1
Sub-scale III: Internal validity - confounding (selection bias)[b]		
21	0	0
22	0	0
23	1	0
24	0	1
25	1	1
26	0	0
Sub-scale IV: Power[c]		
27	0	0
Sum	15	15

a. Item 1-10: yes=1, no=0.
b. Item 11-26: yes=1, no=0, unable to determine=0.
c. Recoded as not mention-0, power calculated=1, differences between groups calculated=2.
d. Item 5: yes=2, partially=1, no=0.

Reference

1. DerSimonian, R. and N. Laird, *Meta-analysis in clinical trials.* Controlled Clinical Trials, 1986. **7**(3): p. 177-188.
2. Canuto, R., M.T.A. Garcez As Fau - Olinto, and M.T. Olinto, *Metabolic syndrome and shift work: a systematic review.* Sleep Medicine Review, 2013. **17**(6): p. 425-431.
3. Lee, S., D. McCann, and J.C. Messenger, *Working Time Around the World*. 1 ed. 2007: International Labour Office.
4. Price, M., *The risks of night work.* Monitor on Psychology, 2011. **42**(1): p. 38.
5. Costa, G., *Shift work and health: current problems and preventive actions.* Safety Health Work, 2010. **1**(2): p. 112-123.
6. Neil-Sztramko, S.E., et al., *Health-related interventions among night shift workers: a critical review of the literature.* Scand J Work Environ Health, 2014. **40**(6): p. 543-56.
7. Touitou, Y. and E. Haus, *Biologic rhythms in clinical and laboratory medicine*. 2012: Springer Science & Business Media.
8. Roitman, B., et al., *Circadian acrophases for total blood leukocytes counted on different continents.* Chronobiologia, 1975. **2**(Suppl 1): p. 58.
9. Halberg, F., et al. *Agreement of circadian characteristics for total leucocyte counts in different geographic locations*. in *Proc XII Intl Conf Intl Soc Chronobiology*. 1977.

10. Reinberg, A., et al., *Rythmes circadiens et circannuels des leucocytes, proteines totales, immunoglobulines a, g, et m, etude chez 9 adultes jeunes et sains.* Nouv Presse Med, 1977. **6**(41): p. 3819-3823.

11. Born, J., et al., *Effects of sleep and circadian rhythm on human circulating immune cells.* Journal of Immunology, 1997. **158**(9): p. 4454-4464.

12. Coventry, B.J., et al., *Immune profiling in human breast cancer using high-sensitivity detection and analysis techniques.* JRSM open, 2015. **6**(9): p. 2054270415603909.

13. Genel, S., et al., *The relevance of inflammatory markers in metabolic syndrome.* Maedica, 2014. **9**(1): p. 15.

14. Zewinger, S., et al., *Innate immunity in CKD-associated vascular diseases.* Nephrology Dialysis Transplantation, 2015: p. gfv358.

15. Springer, T.A., *Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm.* Cell, 1994. **76**(2): p. 301-314.

16. Stahle, L., et al., *Effects of food or sleep deprivation during civilian survival training on clinical chemistry variables.* Wilderness Environ Med, 2013. **24**(2): p. 146-52.

17. Gomez-Merino, D., et al., *Effects of combined stress during intense training on cellular immunity, hormones and respiratory infections.* Neuroimmunomodulation, 2005. **12**(3): p. 164-72.

18. Boudjeltia, K.Z., et al., *Sleep restriction increases white blood cells, mainly neutrophil count, in young healthy men: A pilot study.* Vascular Health and Risk Management, 2008. **4**(6): p. 1467-1470.

19. Ruiz, F.S., et al., *Immune alterations after selective rapid eye movement or total sleep deprivation in healthy male volunteers.* Innate Immun, 2012. **18**(1): p. 44-54.

20. Downs, S.H. and N. Black, *The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions.* Journal of Epidemiology and Community Health, 1998. **52**(6): p. 377-384.

21. Nishitani, N. and H. Sakakibara, *Subjective poor sleep and white blood cell count in male Japanese workers.* Ind Health, 2007. **45**(2): p. 296-300.

22. Sookoian, S., et al., *Effects of rotating shift work on biomarkers of metabolic syndrome and inflammation.* J Intern Med, 2007. **261**(3): p. 285-92.

23. Okamoto, H., et al., *An occupational health study of emergency physicians in Japan: health assessment by immune variables (CD4, CD8, CD56, and NK cell activity) at the beginning of work.* J Occup Health, 2008. **50**(2): p. 136-46.

24. Burgueno, A., et al., *Increased levels of resistin in rotating shift workers: a potential mediator of cardiovascular risk associated with circadian misalignment.* Atherosclerosis, 2009. **210**(2): p. 625-9.

25. Puttonen, S., K. Viitasalo, and M. Harma, *Effect of shiftwork on systemic markers of inflammation.* Chronobiol Int, 2011. **28**(6): p. 528-35.

26. Kerkhofs, M., et al., *Sleep restriction increases blood neutrophils, total cholesterol and low density lipoprotein cholesterol in postmenopausal women: A preliminary study.* Maturitas, 2007. **56**(2): p. 212-215.

27. Ricardo, J.S., et al., *No effect of a 30-h period of sleep deprivation on leukocyte trafficking, neutrophil degranulation and saliva IgA responses to exercise.* Eur J Appl Physiol, 2009. **105**(3): p. 499-504.

28. Liu, H., et al., *Effects of sleep and sleep deprivation on blood cell count and hemostasis parameters in healthy humans.* J Thromb Thrombolysis, 2009. **28**(1): p. 46-9.

29. Christoffersson, G., et al., *Acute sleep deprivation in healthy young men: impact on population diversity and function of circulating neutrophils.* Brain Behav Immun, 2014. **41**: p. 162-72.

30. Knutsson, A., N. Hammar, and B. Karlsson, *Shift workers' mortality scrutinized.* Chronobiology international, 2004. **21**(6): p. 1049-1053.

31. Nabe-Nielsen, K., et al., *Cardiovascular risk factors and primary selection into shift work.* Scandinavian journal of work, environment & health, 2008: p. 206-212.

32. Kirschbaum, C., et al., *Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis.* Psychosomatic medicine, 1999. **61**(2): p. 154-162.

33. Souza, S., et al., *Influence of menstrual cycle on NK activity.* Journal of reproductive immunology, 2001. **50**(2): p. 151-159.