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Abstract

Background: Subtypes of white blood cell counts are known biomarkers of systemic inflammation and a high neutrophil-to-lymphocyte ratio (NLR) has been associated with several autoimmune diseases. Few studies have investigated the NLR in multiple sclerosis (MS).

Objective: To examine the association between NLR, MS and disability measured by the MS severity score (MSSS).

Methods: Patients were included from the Danish Multiple Sclerosis Biobank. Information on patient NLR was obtained just before their first treatment and clinical information was provided by the Danish Multiple Sclerosis Treatment Register. Information on NLR from controls was collected from the Danish Blood Donor Study. Patients and controls were 1:2 propensity score matched by baseline confounders.

Results: Propensity score matching left 740 of 743 MS patients and 1420 of 4691 controls for further analyses. Odds-ratio (OR) was 3.64 (95% confidence interval 2.87–4.60, $p < 0.001$) for MS disease per unit increase of logarithmically transformed NLR (ln-NLR), corresponding to an OR of 2.68 for each doubling of NLR. Mean NLR was 2.12 for patients and 1.72 for controls ($p < 0.001$). Ln-NLR correlated weakly with patient MSSS ($R^2 = 0.019$, $p = 0.008$).

Conclusion: Patients with early MS had increased levels of NLR compared to healthy controls and NLR was weakly correlated with MSSS.

Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease in the central nervous system (CNS), where inflammation, demyelination and axonal loss lead to neurological symptoms and varying levels of clinical disability.[1] The aetiology is regarded as multifactorial with genetic as well as environmental risk factors.[2]

Systemic inflammation might cause chronic neurodegeneration and is hypothesized to play an important role in the pathogenesis of MS via production of pro-inflammatory cytokines and the activation of both innate and adaptive immune cells, leading to an inflammatory response within the CNS.[3,4]

In the search of an easy-accessible biomarker useful for diagnosing MS disease and predicting disease course, several different inflammatory blood biomarkers, e.g. tumour necrosis factor-alpha and interleukin-6, have been investigated, but none have proven to be clinically useful.[1,5]

The differential count of white blood cells is a widely-used biomarker of systemic inflammation. Findings of neutrophilia and lymphopenia in

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response to for example bacterial infections or cancer, has led to the investigation of the neutrophil-to-lymphocyte ratio (NLR) as a biomarker that might reflect systemic inflammation better than the neutrophil or lymphocyte count alone.[6]

NLR has been linked to autoimmune diseases such as rheumatoid arthritis[7] and chronic inflammatory bowel disease.[8] An association between increased NLR and MS was found in some previous studies, whereas the results regarding a possible association between MS disability and NLR are inconsistent.[4,9,10]

C-reactive protein (CRP) is a more thoroughly investigated biomarker of systemic inflammation than NLR. CRP is an acute-phase protein synthetized in hepatocytes and released to the blood in response to infection, inflammation and tissue injury.[11]

The aim of this large Danish case-control study was to investigate whether a high NLR is associated with MS and whether NLR is useful as a biomarker predicting MS. Furthermore, to examine if NLR or CRP correlates with MS disability, as measured by MS severity score (MSSS) at the start of the very first disease modifying treatment (DMT), and to investigate the association between NLR and CRP in MS patients.

Methods

Design
The study is a retrospective case-control study comparing MS patients with healthy controls.

Patients
We targeted all patients from the Danish Multiple Sclerosis Center at Copenhagen University Hospital included in the Danish Multiple Sclerosis Biobank, who fulfilled the McDonald criteria 2005 and 2010[12,13] or the Poser criteria for relapsing-remitting MS (RRMS) and had started their very first DMT with interferon-beta (IFN-beta) within the period June 1996 to January 2013. Among the about 12,500 MS cases in Denmark in 2013, 6791 had been treated with IFN-beta as their first DMT. Of these 29.9% were enrolled in the Danish Multiple Sclerosis Biobank.

Controls
The control group consisted of healthy Caucasian Danish blood donors living in Greater Copenhagen, who were recruited through the Danish Blood Donor Study[14] in the period October 2011 to May 2014 from five major blood donor locations. This recruitment has been more thoroughly described elsewhere.[15]

Blood tests
We obtained cell counts of neutrophils, lymphocytes and CRP for the patients from the Department of Clinical Biochemistry at Copenhagen University Hospital, Rigshospitalet. We used the last blood test values from just before commencement of their first DMT, excluding blood tests taken within 40 days after start of short-term steroid treatment for relapses. Counts of neutrophils and lymphocytes from the blood donor controls were collected from the Danish Blood Donor Study as a standard procedure in connection with the bloodletting.

We only collected CRP values from the MS patients for comparisons within this group because the Danish Blood Donor Study used a different analytic protocol. All CRP test results had the unit mg/l.

Clinical data
Clinical information regarding MSSS[16] and date of the commencement of first DMT as well as relapse treatment was obtained from the Danish Multiple Sclerosis Treatment Register.[17]

BMI and smoking
Information on body mass index (BMI) and smoking came from a comprehensive questionnaire, developed at Karolinska Institute in Stockholm and with permission translated into Danish.[18] The patients filled in the questionnaire from 2009 to 2014. The donor controls filled in exactly the same questionnaire as the MS patients on the same day as collection of their blood sample from 2011 to 2014. BMI was calculated by self-reported height and weight both at 20 years of age and at the time of filling in the questionnaire, in this paper referred to as current BMI. For patients we chose either BMI at 20 years of age or current BMI based on which one being closest to the date of first DMT. For controls we used current BMI. BMI was treated as a continuous variable in our analyses. We dichotomized data on smoking to a dummy variable: regular smoker or not. BMI and smoking were not study parameters in the present study and we only included them as potential confounders.

Statistical methods
NLR was calculated as the quotient between the neutrophil and lymphocyte cell counts in blood. The distribution of NLR was, as expected for a quotient,

highly skewed, but after $\log_e$ transformation it was almost perfectly Gaussian distributed. We investigated the association between $\log_e$NLR (ln-NLR) and MS by binary logistic regression with MS-case-control status as dependent variable and ln-NLR as independent variable with adjustment for baseline variables sex, age at blood test and smoking (regular smoker v. not). In a complementary test, we analysed ln-NLR as a dependent continuous variable with MS-status as independent variable and sex, age at blood test and smoking as covariates, using generalized linear models (GLM).

By iteration we dichotomized NLR at the specific cut-off value resulting in peak Youdens J (sensitivity + specificity − 1) and calculated the corresponding sensitivity and specificity with 95% confidence interval (CI).[19]

Data on CRP were only available for a random subset of MS patients. Because of the distributional properties of CRP, we dichotomized the values as normal (<5 mg/l) or elevated (≥ 5 mg/l). Within the patient group we examined the correlation between ln-NLR and MSSS, between dichotomized CRP and ln-NLR, and between dichotomized CRP and MSSS, with sex, age at blood test and smoking status as covariates.

The patients and control persons constitute two different populations as to distribution on sex, age and other covariates. To make them comparable we propensity score matched individuals from the two groups by logistic regression for variables that would prove to be confounders, i.e. variables that was associated with both case-control status and NLR with p-values of less than 0.10. We used 1:2 'nearest neighbour' matching without replacement, exact match for sex with exclusion of individuals who could not be matched with individuals from the opposite group within a 'caliper' of 0.2 standard deviations of propensity score. We used SPSS v22 for data management and analyses, and for propensity score matching we used a SPSS plug-in program in the statistical software, 'R'.[20]

Ethics statement
Permission to obtain clinical data from The Danish Multiple Sclerosis Register and data on blood samples was approved by The Danish Patient Safety Authority (3-3013-1870/1/). Written informed consent regarding the questionnaire was obtained from all MS patients and approved by the local Ethics Committee (KF-01 314009). Informed consent
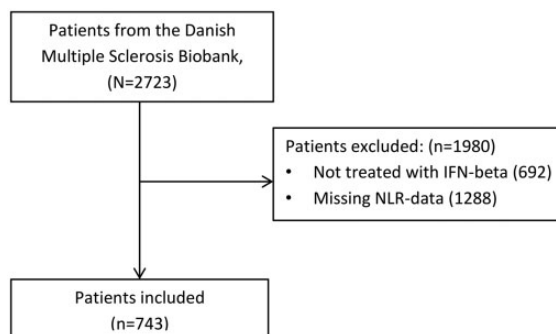

Figure 1. Flowchart showing the selection of patients. IFN: interferon; NLR: neutrophil-to-lymphocyte ratio.

from the blood donors was obtained through the 'The Danish Blood Donor Study' approved by the local Ethics Committee (M-20090237).

Results
This study included 743 MS patients and 4691 healthy blood donor controls. Patients were included from the Danish Multiple Sclerosis Biobank consisting of data on 2723 MS patients. A total of 692 patients not treated with IFN-beta, and 1288 patients without information on NLR were not included in this study (see Figure 1).

We propensity score matched for sex, age at blood test and smoking status, as these background variables were associated with both NLR and case-control status with $p < 0.10$ in binary logistic regression or t-test. BMI was not a confounder as it was not associated with ln-NLR ($p = 0.98$) and hence we did not match or adjust for it in any analyses.

We removed three MS patients and 3271 controls by the 1:2 matching because of unmatchable propensity scores leaving 740 MS patients and 1420 controls for analyses. Table 1 show the baseline variables with and without matching.

NLR
After propensity score matching the crude mean NLR was 2.44 in MS patients as opposed to 1.83 in controls. The crude mean ln-NLR was 0.75 in MS patients and 0.54 in controls (see analyses after adjustment below and in Tables 2 and 3).

Binary logistic regression analysis with ln-NLR as independent and case/control status as dependent variable with adjustment for sex, age and smoking, showed highly significant effect of ln-NLR ($p < 0.0001$) with an odds-ratio (OR) of 3.64 (95% CI: 2.87–4.60) per unit increase of ln-NLR, which

Table 1. Baseline characteristics for patients and controls before and after propensity score matching.

	Before propensity score matching		After propensity score matching	
	MS cases $N = 743$	Controls $N = 4691$	MS cases $N = 740$	Controls $N = 1420$
Females (%)	520 (70.0)	2149 (45.8)	517 (69.9)	974 (68.7)
Mean age at blood test (SD)	35.4 (10.0)	44.0 (12.1)	35.4 (9.9)	36.3 (10.4)
Regular smokers (%)	324 (43.6)	1598 (34.1)[a]	322 (43.5)	610 (43.0)
Mean BMI (SD)	24.4 (5.0)[b]	25.4 (3.9)[c]	24.4 (5.0)[d]	24.9 (4.0)[e]

SD: standard deviation.
[a]missing information in 30; [b]missing information in 214; [c]missing information in 57; [d]missing information in 213; [e]missing information in 15.

Table 2. Binary logistic regression of case/control status by ln-NLR with odds ratios (OR) for ln-NLR after stepwise entering of covariates before and after propensity score matching.

	Before propensity score matching MS cases: 743; controls: 4691			After propensity score matching MS cases: 740; controls: 1420		
Adjusted for covariates	OR per unit ln-NLR	95% CI	*p*	OR per unit ln-NLR	95% CI	*p*
None	3.40	2.80–4.12	<0.0001	3.48	2.76–4.38	<0.0001
Sex	3.27	2.68–3.99	<0.0001	3.49	2.77–4.40	<0.0001
Sex + age	3.86	3.13–4.75	<0.0001	3.63	2.87–4.60	<0.0001
Sex + age + smoking	3.89	3.16–4.80	<0.0001	**3.64**	**2.87–4.60**	**<0.0001**

ln-NLR: $\log_e$(neutrophil-to-lymphocyte ratio); CI: confidence interval. Bold numbers are the final results.

corresponds to an OR of 2.68 for each doubling of NLR. Results from the binary logistic regression analysis with adjustment for confounders before and after propensity score matching are shown in Table 2.

In the GLM analysis (Table 3) we used the opposite design with ln-NLR as dependent variable and case-control status as predictive variable with adjustment for sex, age at blood test and smoking. The estimated mean ln-NLR results and the geometric mean of NLR with adjustment for confounders before and after propensity score matching was 2.12 in patients and 1.72 in controls as shown in Table 3. The difference in ln-NLR between MS and controls was statistically significant ($p < 0.0001$).

NLR as predictor of MS
Figure 2 shows a receiver operating characteristic curve (ROC) showing sensitivity by 1 minus specificity of NLR relative to case-control status in the propensity matched cases and controls. The area

under the ROC curve was 63% of the theoretical maximum value (95% CI: 60–66). The peak value of Youdens J (sensitivity + specificity − 1) was 0.19 at a cutpoint of NLR of 2.07 at which the sensitivity was 0.49 (95% CI: 0.45-0.52) and the specificity was 0.70 (95% CI: 0.68-0.73).

NLR and MSSS
Out of the 743 patients primarily included in this study, 713 had information on MSSS. MSSS mean was 5.06 (95% CI: 4.87–5.26). Linear regression was done with MSSS as dependent variable and ln-NLR as independent variable. As sex and smoking status were associated with both MSSS and NLR, we included them as covariates in the analysis. Ln-NLR correlated weakly with the MSSS ($R^2 = 0.019$), however statistically significant ($p = 0.008$) with regression coefficient of 0.56 (95% CI: 0.15–0.97) meaning that MSSS is expected to increase with 0.56 for each unit increase in ln-NLR analogous to an expected increase of 0.41 for each doubling of NLR.

Table 3. Mean ln-NLR in patients and controls using generalized linear models before and after propensity score matching.

Adjusted for covariates	Before propensity score matching MS cases: 743; controls: 4691					After propensity score matching MS cases: 740; controls: 1420				
	Estimated marginal mean of ln-NLR[a]	95% CI	Geometric mean of NLR[b]	95% CI	P	Estimated marginal mean of ln-NLR[a]	95% CI	Geometric mean of NLR[b]	95% CI	P
None										
Cases	0.746	0.712–0.781	2.11	2.04–2.18	<0.0001	0.748	0.713–0.782	2.11	2.04–2.19	<0.0001
Controls	0.545	0.535–0.556	1.73	1.71–1.74		0.536	0.517–0.555	1.71	1.68–1.74	
Sex										
Cases	0.738	0.703–0.773	2.09	2.02–2.17	<0.0001	0.747	0.712–0.782	2.11	2.04–2.18	<0.0001
Controls	0.547	0.536–0.557	1.73	1.71–1.75		0.536	0.517–0.555	1.71	1.68–1.74	
Sex + age										
Cases	0.764	0.729–0.798	2.15	2.07–2.22	<0.0001	0.750	0.715–0.784	2.12	2.04–2.19	<0.0001
Controls	0.543	0.542–0.553	1.72	1.70–1.74		0.535	0.516–0.554	1.71	1.68–1.74	
Sex + age + smoking										
Cases	0.765	0.730–0.780	2.15	2.08–2.22	<0.0001	**0.750**	**0.715–0.784**	**2.12**	**2.05–2.19**	**<0.0001**
Controls	0.542	0.531–0.553	1.72	1.70–1.74		**0.535**	**0.516–0.554**	**1.72**	**1.67–1.74**	

[a]Estimated marginal means are mean values adjusted for sex, age and smoking status. [b]Geometric mean of NLR is the antilogarithm of the arithmetic mean of ln-NLR.
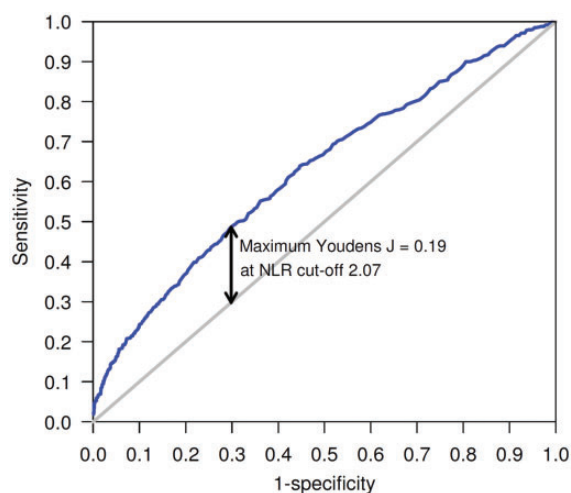Bold numbers are the final results.

Figure 2. Receiver operating characteristic curve showing sensitivity and specificity of NLR in MS patients. NLR: neutrophil-to-lymphocyte ratio; Youdens J = sensitivity + specificity − 1. The grey diagonal represents pure chance. With NLR cut-off at the peak value of Youdens J sensitivity was 0.49 and specificity was 0.70.

CRP and NLR
In 554 of the MS patients, information on both ln-NLR and CRP was available. We dichotomized CRP into normal (<5 mg/l) and elevated (≥5 mg/l). GLM adjusted for sex, age at blood test and smoking showed that elevated CRP increases NLR. The estimated mean ln-NLR was 0.849 with elevated CRP and 0.727 with normal CRP ($p = 0.038$) corresponding to geometrical mean of NLR of 2.34 and 2.07, respectively.

CRP and MSSS
In 533 of the MS patients, information on both CRP and MSSS was available. In 79 MS (14.8%) patients the CRP was elevated. With GLM, adjusted for sex, age at blood test and smoking, mean estimated MSSS was 5.32 as opposed to 5.07 among those with normal CRP. The difference was not statistically significant ($p = 0.447$).

Discussion
In this study we found that for each doubling of NLR, the odds of MS increased 2.68 times within the study population. Mean NLR was 2.12 for cases and 1.72 for controls and there was a statistically significant difference in ln-NLR between the groups. Although the association between NLR and MS was highly significant, NLR had, in our study, poor value as a predictor of MS or as an ancillary diagnostic tool, and we could not confirm the high sensitivity and specificity in another study.[4]

NLR correlated only weakly with MSSS, and there was no association between CRP and MSSS despite a statistical significant albeit weak association between CPR and NLR.

NLR is a marker of systemic inflammation based on routine analysis and thus with low cost and simple to achieve. Apart from being of importance as a prognostic marker in other diseases as cancer and autoimmune diseases, small studies have indicated importance in MS. Our study is of importance, as our results, based on the hitherto largest number of patients and controls, confirm findings from previous studies on NLR in MS, which all showed an association between a high NLR and MS.[4,10,21]

When looking for a possible association to MS disability, the results from other studies are less consistent. Two studies found NLR to correlate with Expanded Disability Status Scale (EDSS)[4,9] and one study did not find any association.[10] Giovannoni et al.[22] suggest that systemic inflammation might only result in disability after several years of MS disease, and this might explain why only a weak association between NLR and MSSS was seen in our patients with early MS. We chose MSSS in preference to EDSS because it is less dependent on disease duration. To further clarify whether NLR is associated to MS disability, studies with long follow-up time are needed, as this would enable measuring of disease progression.

Tobacco smoking and high BMI are two known risk factors for MS[2] and are associated with systemic inflammation. They have shown to be associated with increased total white blood cell counts, increased neutrophil counts,[23,24] and with high levels of CRP.[25,26] However, in our study BMI was neither associated with NLR or case-control status, and hence it was not included as a covariate.

Sex, age and smoking proved to be confounders as they were associated with both case-control status and NLR in our study. To make our patient and control group comparable, patients and controls were propensity score matched for these variables. Removal of unmatchable individuals from both groups resulted in a better balance between the groups as to the known confounders. When looking at the results of the binary logistic regression analysis before propensity score matching, sex appeared to be a positive confounder, whereas age and smoking were negative confounders. Our results showed a highly significant effect of NLR both before and after propensity score matching, with only a slight decrease in OR after matching. The difference between the estimated marginal means of ln-NLR between patients and controls was also highly statistically significant both before and after propensity score matching ($p < 0.001$).

NLR has been linked to different autoimmune diseases[7,8] and neurological diseases including Alzheimer's disease[27] and Parkinson's disease.[28] NLR has been shown to be correlated to the prognosis of diseases such as rheumatoid arthritis,[7] cancer[29] and stroke.[30]

Neutrophil count appears to be increased in MS patients with an elevation of NLR compared to healthy controls.[10,31] The cause of this increase and whether increased neutrophil counts contribute to the development of CNS autoimmunity remains unclear.[32]

Studies on experimental autoimmune encephalomyelitis (EAE), the animal model of MS, showed that depletion of neutrophils led to a reduction in the development of the disease, and that neutrophils might play an important role in the pathogenesis of EAE by producing cytokines, impairing the blood brain barrier, and by contributing to parenchymal brain inflammation.[32] Naegele et al.[31] demonstrated that the increase in neutrophil count in RRMS is most likely due to a decrease in apoptosis and that neutrophils had an altered expression of surface molecules, which might enhance recruitment to sites of inflammation. They also found that neutrophils had enhanced effector mechanisms, including degranulation, oxidative burst and release of neutrophil extracellular traps, which might cause tissue injury and demyelination, enhance T-cell activation and impair the blood brain barrier, thereby contributing to MS pathogenesis.[31] Neutrophils are, however, not part of the cellular infiltrate in MS.

Only a subgroup of the patients in our study had information on CRP. Elevated levels of CRP are associated with different autoimmune diseases,[11] and other studies have examined CRP and its association with MS disability, but the results are inconclusive.[4,9,22,33] Our study showed a weak association between increased CRP levels and the NLR but no association between CRP and MSSS.

The strengths of our study are the large sample size as well as the fact that patients were treatment naïve, since we sampled blood tests before their first dose

of DMT ever. Steroid treatment affects white blood cell counts, but the increase in neutrophil count, and thereby NLR, disappears before 48 hours after discontinuation of prednisolone,[34] and by ignoring blood tests closer to steroid treatment than 40 days, it is unlikely that our results are affected by medication taken in relation to MS.

Limitations of this study are the lack of information about medications, apart from steroids and DMT, and lack of information about other diseases or infections that might contribute to an elevation in systemic inflammatory markers in MS patients.

Another limitation is the inclusion of solely RRMS patients and thus no evaluation of other MS forms. Furthermore the use of blood donors as control group can be considered a limitation, as blood donors constitute a healthy subset of the general population with better than average health, probably due to the strict selection criteria.[35] Also the fact that blood donation is not allowed during infections might reduce systemic inflammation in blood donors compared to the general population and might contribute to the difference in NLR seen between our patients and controls.

Conclusions

We found that patients with early MS, just before their first DMT, had increased systemic inflammation, as measured by NLR, compared to controls, and that NLR was weakly correlated with MSSS. To further clarify the association between NLR and MS disease and disability, more studies preferably of large size and with long follow up time are needed. The results from our and other studies suggest that an elevated NLR is associated with MS, and therefore investigating the role of neutrophils in MS patients might provide new insights into the pathogenesis of MS.

Conflict of Interests

Funding

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References

1. Reich DS, Lucchinetti CF and Calabresi PA. Multiple Sclerosis. *New Engl J Med* 2018; 378: 169–180.
2. Olsson T, Barcellos LF and Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. *Nat Rev Neurol* 2017; 13: 25–36.
3. Perry VH, Cunningham C and Holmes C. Systemic infections and inflammation affect chronic neurodegeneration. *Nat Rev Immunol* 2007; 7: 161–167.
4. Demirci S, Demirci S, Kutluhan S, et al. The clinical significance of the neutrophil-to-lymphocyte ratio in multiple sclerosis. *Int J Neurosci* 2015: 1–7.
5. Vladic A, Horvat G, Vukadin S, et al. Cerebrospinal fluid and serum protein levels of tumour necrosis factor-alpha (TNF-alpha) interleukin-6 (IL-6) and soluble interleukin-6 receptor (sIL-6R gp80) in multiple sclerosis patients. *Cytokine* 2002; 20: 86–89.
6. Yoon NB, Son C and Um SJ. Role of the neutrophil–lymphocyte count ratio in the differential diagnosis between pulmonary tuberculosis and bacterial community-acquired pneumonia. *Ann Lab Med* 2013; 33: 105–110.
7. Fu H, Qin B, Hu Z, et al. Neutrophil- and platelet-to-lymphocyte ratios are correlated with disease activity in rheumatoid arthritis. *Clin Lab* 2015; 61: 269–273.
8. Acarturk G, Acay A, Demir K, et al. Neutrophil-to-lymphocyte ratio in inflammatory bowel disease – as a new predictor of disease severity. *Bratisl Lek Listy* 2015; 116: 213–217.

9. Guzel I, Mungan S, Oztekin ZN, et al. Is there an association between the Expanded Disability Status Scale and inflammatory markers in multiple sclerosis? *J Chin Med Assoc* 2016; 79: 54–57.

10. Bisgaard AK, Pihl-Jensen G and Frederiksen JL. The neutrophil-to-lymphocyte ratio as disease activity marker in multiple sclerosis and optic neuritis. *Multi Scler Relat Dis* 2017; 18: 213–217.

11. Pepys MB and Hirschfield GM. C-reactive protein: a critical update. *J Clin Investig* 2003; 111: 1805–1812.

12. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the 'McDonald Criteria'. *Ann Neurol* 2005; 58: 840–846

13. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011; 69: 292–302.

14. Pedersen OB, Erikstrup C, Kotze SR, et al. The Danish Blood Donor Study: A large, prospective cohort and biobank for medical research. *Vox Sang* 2012; 102: 271.

15. Gustavsen S, Sondergaard HB, Oturai DB, et al. Shift work at young age is associated with increased risk of multiple sclerosis in a Danish population. *Multi Scler Relat Dis* 2016; 9: 104–109.

16. Roxburgh RH, Seaman SR, Masterman T, et al. Multiple Sclerosis Severity Score: Using disability and disease duration to rate disease severity. *Neurology* 2005; 64: 1144–1151.

17. Magyari M, Koch-Henriksen N and Sorensen PS. The Danish Multiple Sclerosis Treatment Register. *Clin Epidemiol* 2016; 8: 549–552.

18. Hedstrom AK, Akerstedt T, Hillert J, et al. Shift work at young age is associated with increased risk for multiple sclerosis. *Ann Neurol* 2011; 70: 733–741.

19. Schisterman EF, Perkins NJ, Liu A, et al. Optimal cut-point and its corresponding Youden Index to discriminate individuals using pooled blood samples. *Epidemiology* 2005; 16: 73–81.

20. Thoemmes F. Propensity score matching in SPSS, https://arxiv.org/ftp/arxiv/papers/1201/1201.6385.pdf (2012).

21. Al-Hussain F, Alfallaj MM, Alahmari AN, et al. Relationship between neutrophil-to-lymphocyte ratio and stress in multiple sclerosis patients. *J Clin Diag Res* 2017; 11: cc01–cc04.

22. Giovannoni G, Miller DH, Losseff NA, et al. Serum inflammatory markers and clinical/MRI markers of disease progression in multiple sclerosis. *J Neurol* 2001; 248: 487–495.

23. Higuchi T, Omata F, Tsuchihashi K, et al. Current cigarette smoking is a reversible cause of elevated white blood cell count: Cross-sectional and longitudinal studies. *Prev Med Rep* 2016; 4: 417–422.

24. Herishanu Y, Rogowski O, Polliack A, et al. Leukocytosis in obese individuals: Possible link in patients with unexplained persistent neutrophilia. *Europ J Haematol* 2006; 76: 516–520.

25. Bermudez EA, Rifai N, Buring JE, et al. Relation between markers of systemic vascular inflammation and smoking in women. *Am J Cardiol* 2002; 89: 1117–1119.

26. Choi J, Joseph L and Pilote L. Obesity and C-reactive protein in various populations: A systematic review and meta-analysis. *Obes Rev* 2013; 14: 232–244

27. Kuyumcu ME, Yesil Y, Ozturk ZA, et al. The evaluation of neutrophil-lymphocyte ratio in Alzheimer's disease. *Dement Geriatr Cogn Disord* 2012; 34: 69–74.

28. Akil E, Bulut A, Kaplan I, et al. The increase of carcinoembryonic antigen (CEA), high-sensitivity C-reactive protein, and neutrophil/lymphocyte ratio in Parkinson's disease. *Neurol Sci* 2015; 36: 423–428.

29. Wei B, Yao M, Xing C, et al. The neutrophil lymphocyte ratio is associated with breast cancer prognosis: An updated systematic review and meta-analysis. *OncoTargets Ther* 2016; 9: 5567–5575.

30. Tokgoz S, Kayrak M, Akpinar Z, et al. Neutrophil lymphocyte ratio as a predictor of stroke. *J Stroke Cerebrovasc Dis* 2013; 22: 1169–1174.

31. Naegele M, Tillack K, Reinhardt S, et al. Neutrophils in multiple sclerosis are characterized by a primed phenotype. *J Neuroimmunol* 2012; 242: 60–71.

32. Pierson ER, Wagner CA and Goverman JM. The contribution of neutrophils to CNS autoimmunity. *Clin Immunol* 2018; 189: 23–28.

33. Soilu-Hänninen M, Koskinen JO, Laaksonen M, et al. High sensitivity measurement of CRP and disease progression in multiple sclerosis. *Neurol* 2005; 65: 153–155.

34. Crockard AD, Boylan MT, Droogan AG, et al. Methylprednisolone-induced neutrophil leukocytosis–down-modulation of neutrophil L-selectin and Mac-1 expression and induction of granulocyte-colony stimulating factor. *Int J Clin Lab Res* 1998; 28: 110–115.

35. Edgren G, Tran TN, Hjalgrim H, et al. Improving health profile of blood donors as a consequence of transfusion safety efforts. *Transfusion* 2007; 47: 2017–2024.