Influence of CMV/EBV serostatus on respiratory infection incidence during 4 months of winter training in a student cohort of endurance athletes

Cheng-Shiun He[1], Michal Handzlik[2], Ayu Muhamad[1] and Michael Gleeson[1]

[1]School of Sport, Exercise and Health Sciences, Loughborough University, [2]School of Biomedical Sciences, Nottingham University

Running title: CMV/EBV serostatus and infection incidence in athletes

Address for correspondence:

Prof Michael Gleeson

School of Sport, Exercise and Health Sciences

Loughborough University

Loughborough

Leucestershire

LE11 3TU

United Kingdom

Tel. +44(0)1509 226345

Fax +44(0)1509 226301

Email: m.gleeson@lboro.ac.uk

Abstract

Aim: The purpose of this study was to examine the influence of previous infection with cytomegalovirus (CMV) or Epstein Barr virus (EBV) on the incidence, severity and duration of upper respiratory tract infections (URTIs) in endurance athletes during a 4-month winter training period. **Methods:** Blood samples were obtained from 236 subjects (186 males and 70 females, aged 18-35 years) at the start of the study period. Plasma samples were analysed for CMV and EBV serostatus. Weekly training and daily illness logs were kept for the next 16 weeks. **Results:** With regard to CMV/EBV serostatus, the results indicated that athletes with previous CMV infection (n=58, 25% of cohort) had significantly fewer URTI symptom days (median 2 vs 4 days, p=0.033) during the study period than those with no previous infection (n=178, 75%) whereas positive EBV serostatus (n=197, 84%) had no influence on URTI episode incidence, severity or duration. Moreover, we found that athletes with prior infection of both CMV and EBV (n=50, 21%) had 50% fewer URTI episodes (p=0.04) and symptom days (median 2 vs 8 days, p=0.01) than athletes who were seronegative for both CMV and EBV (n=31, 13%). **Conclusions:** Previous coinfection with CMV and EBV might promote protective immune surveillance to lower the risk of URTI. Further research is required to clarify why previous CMV and EBV infection reduces the incidence of URTI.

Key words: exercise training, herpesvirus, T lymphocytes, common cold

Introduction

Upper respiratory tract infections (URTI) are the most common infectious illness in athletes undertaking prolonged intense exercise and may decrease the quality of training and hinder performance during competition. The relationship between exercise and susceptibility to infection has been modelled in the form of a 'J' shaped curve (Nieman 1994). This model suggests that while engaging in moderate activity may enhance immune function above sedentary levels, excessive amounts of prolonged high-intensity exercise may depress immune function, resulting in an elevated risk of URTI. Several epidemiological studies have showed that there is an increased incidence of URTI among highly trained athletes, compared with low or moderate exercising groups (Gleeson et al. 2013; Nieman 1994; Peters et al. 1993).

Several risk factors have been identified for URTI in athletes including low salivary IgA secretion rate (Fahlman and Engels 2005; Gleeson et al. 2012), high anti-inflammatory cytokine response to antigen challenge (Gleeson et al. 2012) and low vitamin D status (He et al. 2013). Another possible risk factor that might predispose highly trained athletes to more frequent infection is previous infection with cytomegalovirus (CMV) and/or Epstein Barr virus (EBV). Both CMV and EBV are the members of the human herpes virus group and they persist in the body in latent form for a long time after primary infection and can be become reactivated when immune function is depressed. It has been shown that the chronic latency of both viruses has an influence on the immune system (Chang and Barry 2010; Liu et al. 1997). Positive CMV serostatus (identified by the presence of IgG antibodies in plasma) is common among adults and although CMV does not cause overt symptoms of illness in itself, it can cause a general suppression of immune function via its production of a viral homologue of human interleukin-10 which is a potent inhibitory cytokine (Chang and Barry 2010). Thus, when immunity is depressed (e.g. during hard training) the latent CMV can become reactivated and may produce a more severe immunodepression making the individual more susceptible to infection. This possibility has been suggested but not yet confirmed in athletes. Likewise, EBV infection, which

in 50-70% of cases produces symptoms of infectious mononucleosis (glandular fever) (Niederman et al. 1970) also produces a viral homologue of human interleukin-10 (Liu et al. 1997) and could have similar effects to CMV in increasing susceptibility to other infections such as infuenza and the common cold.

The aim of the present study was to determine the influence of previous infection with CMV or EBV on the incidence, severity and duration of URTIs in endurance athletes during a winter training period. To this end, during a 4-month winter training period we conducted a study on a large cohort of endurance athletes who completed daily URTI symptom diaries and reported weekly training loads using validated questionnaires. We also collected blood samples from these athletes at the start of the study and analysed plasma for the presence of antibodies against CMV and EBV as an indicator of prior infection with one or both of these viruses.

Methods

Subjects

Two hundred and sixty seven subjects who were engaged in regular sports training (predominantly endurance-based activities such as running, cycling, swimming, triathlon, team games and racquet sports) volunteered to participate in the study. Subjects ranged from recreationally active to Olympic triathletes and their self-reported training loads averaged 10 h/week. Subjects were required to complete a comprehensive health-screening questionnaire prior to starting the study and had not taken any regular medication or antibiotics in the 3 months prior to the study. All subjects were fully informed about the rationale for the study and of all experimental procedures to be undertaken. Subjects provided written consent to participate in the study, which had earlier received the approval of Loughborough University ethical advisory committee. Subjects were enrolled after having fulfilled all inclusion criteria, and presenting none of the exclusion criteria (determined by both questionnaire and interview). Subjects could be included if they were currently healthy, had been involved in endurance

training for at least 2 years, engaged in at least 3 sessions and at least 3 h of total moderate/high-intensity training time per week and were between 18-40 years of age. Subjects representing one or more of the following criteria were excluded from participation: smoking or use of any medication, suffered from or had a history of cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, haematological or psychiatric illness.

A total of 267 healthy individuals (83 females and 184 males) were recruited as subjects from Loughborough University, UK during November 2011 with the mean age of the study cohort at recruitment being 21 ± 3 years (mean ± SD). For the first visit to the laboratory, subjects arrived in the morning at 08:30-10:30 following an overnight fast of approximately 12 h and their body mass and height were recorded. Information about the study was given to them and they then signed an informed consent form. Subjects then sat quietly for 10 min and completed a health-screening questionnaire and inclusion/exclusion criteria questionnaire. A resting venous blood sample (5 ml) was obtained by venepuncture from an antecubital forearm vein into a Vacutainer tube (Becton Dickinson, Oxford, UK) containing K_3EDTA. Haematological analysis was immediately carried out on the this sample (including haemoglobin, haematocrit and total and differential leukocyte count) using an automated cell-counter (A^c.T™5diff haematology analyser, Beckman Coulter, High Wycombe, UK). Subjects had to have normal haematology to be included in the study. The remaining blood was centifuged for 10 min at 1500 *g* and 4°C and the plasma stored at -80°C prior to analysis.

Study protocol

During the 4-month study period subjects were requested to continue with their normal training programs. Subjects completed a validated health (URTI symptoms) questionnaire (Jackson et al. 1958) on a daily basis. Subjects were not required to abstain from medication when they were suffering from illness symptoms but they were required, on a weekly basis, to report any unprescribed medications taken, visits to the doctor or any prescribed medications.

The illness symptoms listed on the questionnaire were: sneezing, headache, malaise, nasal discharge, nasal obstruction, sore throat, cough, ear ache, hoarseness, fever, chilliness and joint aches and pains. The non-numerical severity ratings of mild, moderate and severe of severity of symptoms were scored as 1, 2 or 3, respectively to provide a quantitative means of data analysis and the total symptom score for every subject each day was calculated as a sum of multiplied numbers of symptoms experienced by the numerical severity ratings. An URTI episode was deemed present when (i) total symptom score was ≥15 on any two consecutive days and (ii) when a subject positively indicated suffering a common cold on ≥ 3 days according to Jackson et al. (1958). Subjects were also asked to rate the impact of illness symptoms on their ability to train (above normal, at the same level, below normal or training stopped). The total number of URTI symptom days was also determined as the number of days with a symptom score of ≥5 according to Predy et al. (2005).

Subjects were also asked to fill in a standard short form of International Physical Activity Questionnaire (IPAQ; http://www.ipaq.ki.se/downloads.htm) at weekly intervals, thus providing a quantitative information on training loads in metabolic equivalents (MET)-h/week (Craig et al. 2003). Venous blood samples were collected only at the start and end of the study period. A total of 236 subjects completed the study and provided sufficient blood for routine haematology and determination of CMV and EBV serostatus. The intra-assay coefficient of variation for all blood variables was less than 3.0%.

Plasma analysis

EDTA plasma was assayed for IgG antibodies to CMV and EBV using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Biocheck Inc., CA, USA and NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany, respectively) according to the manufacturers' instructions.

Statistical analysis

The Shapiro-Wilk test was used to determine if data sets were normally distributed. The difference in proportion of subjects who presented with symptoms of infection during the trial between CMV (and EBV) positive and negative groups was compared by chi-squared test. Differences in mean training loads between CMV (and EBV) positive and negative groups were compared by independent t-test. Differences in the total number of URTI episodes and symptom days, the mean symptom-severity score and the mean duration of subjects with infection symptoms between CMV (and EBV) positive and negative serostatus were assessed with Mann-Whitney test. A similar analysis was applied for CMV-EBV combined serostatus. Mann-Whitney test was also used to examine differences in the blood leukocyte counts between CMV-EBV combined positive and negative serostatus. Data are presented as mean (±SD); for data sets that were not normally distributed, the 95% confidence intervals (CI) are also shown. The accepted level of significance was $p<0.05$.

Results

Adherence to the study

Of the 267 subjects, 239 subjects (189 males, 70 females) completed the full 16 weeks of the study. Reasons for dropout included overseas travel, injury or persistent non-respiratory illness (preventing them from performing training) or due to undisclosed reasons. Blood samples were obtained from 236 subjects (186 males, 70 females) with sufficient volume for both CMV and EBV serostatus analysis.

Baseline characteristics and physical activity levels

Baseline characteristics of the 236 subjects who completed the study and for whom CMV and EBV serostatus was established were (mean ± SD) age: 21 ± 2 years, body mass: 73.5 ± 11.2 kg, height: 176.5 ± 9.3 cm, body mass index 23.6 ± 2.2 kg/m^2 and self-reported weekly training duration: 9.6 ± 5.2 h/week. Analysis of the IPAQ questionnaires indicated that the training loads

were fairly consistent over the 16 weeks of the study. Mean training loads were 67.5 ± 31.2 MET-h/week which is equivalent to about 11 ± 5 hours of moderate-vigorous activity per week.

Infection symptom incidence and its impact on training loads

Analysis of the URTI symptom questionnaires indicated that 4.0 ± 1.6% of the cohort experienced an URTI episode each week (Figure 1); 133 subjects did not experience a single URTI episode during the study period whereas 103 subjects experienced at least one URTI episode during the study period. The proportion of subjects whose training was negatively affected when URTI was present was 0.70 and when URTI was present subjects reduced their weekly training load by an average of 24%.

CMV serostatus and infection incidence, severity and duration

Twenty five percent of the subject cohort were CMV positive with a similar proportion in males (24%) and females (26%). There was no difference in the mean training load between CMV positive and negative groups (positive: 64.5 ± 29.9 MET-h/week, negative: 67.6 ± 31.1 MET-h/week; p=0.505). There was no difference in the proportion of subjects who presented with symptoms of infection between CMV positive and negative groups (positive 0.38, negative 0.44; p=0.349). The total number of URTI symptom days in the CMV negative group was significantly higher than the CMV positive group (Table 1). There was no significant difference for the total number of URTI episodes and there were no significant differences in symptom-severity score or the duration of episodes between subjects with CMV positive and negative serostatus.

EBV serostatus and infection incidence, severity and duration

Eighty four percent of the subject cohort were EBV positive with a similar proportion in males (84%) and females (83%). There was no difference in the mean training load between EBV positive and negative groups (positive: 68.4 ± 31.1 MET-h/week, negative: 59.1 ± 28.3 MET-h/week; p=0.085). There was no difference in the proportion of subjects who presented with

symptoms of infection between EBV positive and negative groups (positive 0.41, negative 0.51; p = 0.266). There were no significant differences for the number of URTI episodes, number of symptom days, the symptom-severity score or the duration of episodes between subjects with EBV positive and negative serostatus (Table 2).

CMV-EBV combined serostatus and infection incidence, severity and duration

Twenty one percent of the subject cohort were both CMV and EBV positive (CMV+EBV+) whereas 13% of the subject cohort had no prior CMV or EBV infection (CMV-EBV-). There was no difference in the mean training load between the CMV+EBV+ and CMV-EBV- groups (positive: 61.9 ± 27.8 MET-h/week, negative: 53.5 ± 22.5 MET-h/week; p=0.161). There was no difference in the proportion of subjects who presented with symptoms of infection between the CMV+EBV+ and CMV-EBV- groups (positives 0.38, negatives 0.55; p=0.138). However, subjects with prior infection with both CMV and EBV had fewer URTI episodes and fewer URTI symptom days than subjects with no prior history of CMV and EBV infection (Table 3). The symptom-severity score and the duration of episodes were not significantly different between subjects in the CMV+EBV+ and CMV-EBV- groups.

Blood total and differential leukocyte counts in CMV+EBV+ and CMV-EBV- subjects

The circulating number of lymphocytes in the CMV+EBV+ group was significantly higher than the CMV-EBV- group. However, there was no difference in the circulating numbers of total leukocytes, neutrophils or monocytes between the CMV+EBV+ and CMV-EBV- groups (Table 4).

Discussion

This research is of direct relevance to the on-going study of the factors that determine illness susceptibility in athletes. The main findings of the present study were as follows: (1) There was no difference in the proportion of subjects who presented with symptoms of infection between CMV/EBV positive and negative groups; (2) The total number of URTI symptom days in the CMV negative group was significantly higher than in the CMV positive group; (3) EBV serostatus had no influence on URTI episode incidence, severity or duration; (4) Athletes with prior infection of both CMV and EBV had fewer URTI episodes and symptom days and higher numbers of circulating lymphocytes than athletes who were seronegative for both CMV and EBV.

Contrary to our original hypothesis athletes who had previous infection with CMV or EBV were not predisposed to increased susceptibility to URTI. The data from our study show that there was no difference in the proportion of subjects who presented with symptoms of infection during the 4-month study period between CMV/EBV positive and negative groups. Although CMV or EBV can cause a general suppression of immune function via the production of a viral homologue of human interleukin-10 which is a potent inhibitory cytokine (Chang and Barry 2010; Liu et al. 1997), this does not seem to be an important influence on URTI risk. However, a previous study has reported a significant association between prior infection with EBV and upper respiratory symptoms (Gleeson et al. 2002) in elite swimmers. Gleeson et al. (2002) indicated that while 9 of 11 EBV seropositive athletes had upper respiratory symptoms during a 30-day intensive training period, there were no reported upper respiratory symptoms in 3 EBV seronegative athletes. The reason for the different result from our study may be due to the relatively small numbers of athletes in their study. More investigations are warranted to clarify the relationship between previous CMV and EBV infection and the incidence of URTI.

In fact, we found that athletes with previous CMV infection had fewer URTI symptom days during the 4-month study period than those with no previous infection. Furthermore, athletes who had experienced prior infection with both CMV and EBV had fewer URTI episodes and fewer symptom days than those with negative serostatus for both CMV and EBV despite having similar mean training loads. The reasons for this are still unclear but could be related to the altered T cytotoxic cell response to exercise in individuals with positive CMV serostatus (Turner et al. 2010). Latent cytomegalovirus infection appears to amplify memory CD8 T-lymphocyte mobilisation and egress in response to exercise. Memory CD8 T cells possessing the ability to rapidly produce cytokines, such as interferon gamma and tumor necrosis factor alpha, are an important component of protective immunity against viral infections (Wherry and Ahmed 2004). The amplification of this response may promote protective immune surveillance, and thereby reduce risk of infection. It could be especially protective against infection in the post-exercise period when several other aspects of immune function such as neutrophil oxidative burst, macrophage antigen presentation, T lymphocyte cytokine production and proliferation are depressed (Gleeson and Walsh 2012). In the present study, it was also shown that the circulating numbers of lymphocytes at rest are significantly higher in athletes with CMV-EBV combined positive serostatus than those with negative serostatus which might also provide a higher level of immunosurveillance. Further research is still needed to understand the influence of previous CMV or EBV infection on the immune system.

On the basis of the present data, we found that 25% of athletes had a previous CMV infection, 84% of athletes had a previous EBV infection and 21% of athletes were both CMV and EBV positive. The proportion of subjects with previous CMV infection in the present study is lower than previously reported in the general population; Bate et al. (2010) found that approximately 50% individuals aged 6-49 years in the general population of the USA were CMV positive and that CMV seropositivity was independently associated with older age, female sex, foreign birthplace and high household crowding. Nevertheless, the high proportion of previous EBV

infection in our study is similar to other studies on young adults (Gleeson et al. 2002; Pottgiesser et al. 2006). Given that large proportion of athletes appear to have had a previous infection with CMV or EBV, it was important to clarify whether previous CMV or EBV infection (or both) has an important influence on the risk of URTI.

In conclusion, our study indicated that athletes who had experienced prior infection with both CMV and EBV had fewer URTI episodes and fewer symptom days than those with negative serostatus for both CMV and EBV. Previous coinfection with CMV and EBV might promote protective immune surveillance to lower the risk of URTI. Further research is required to clarify why previous CMV and EBV infection reduces the incidence of URTI.

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Figure legends

Figure 1. Percentage of the cohort reporting an URTI episode for each week of the study period.

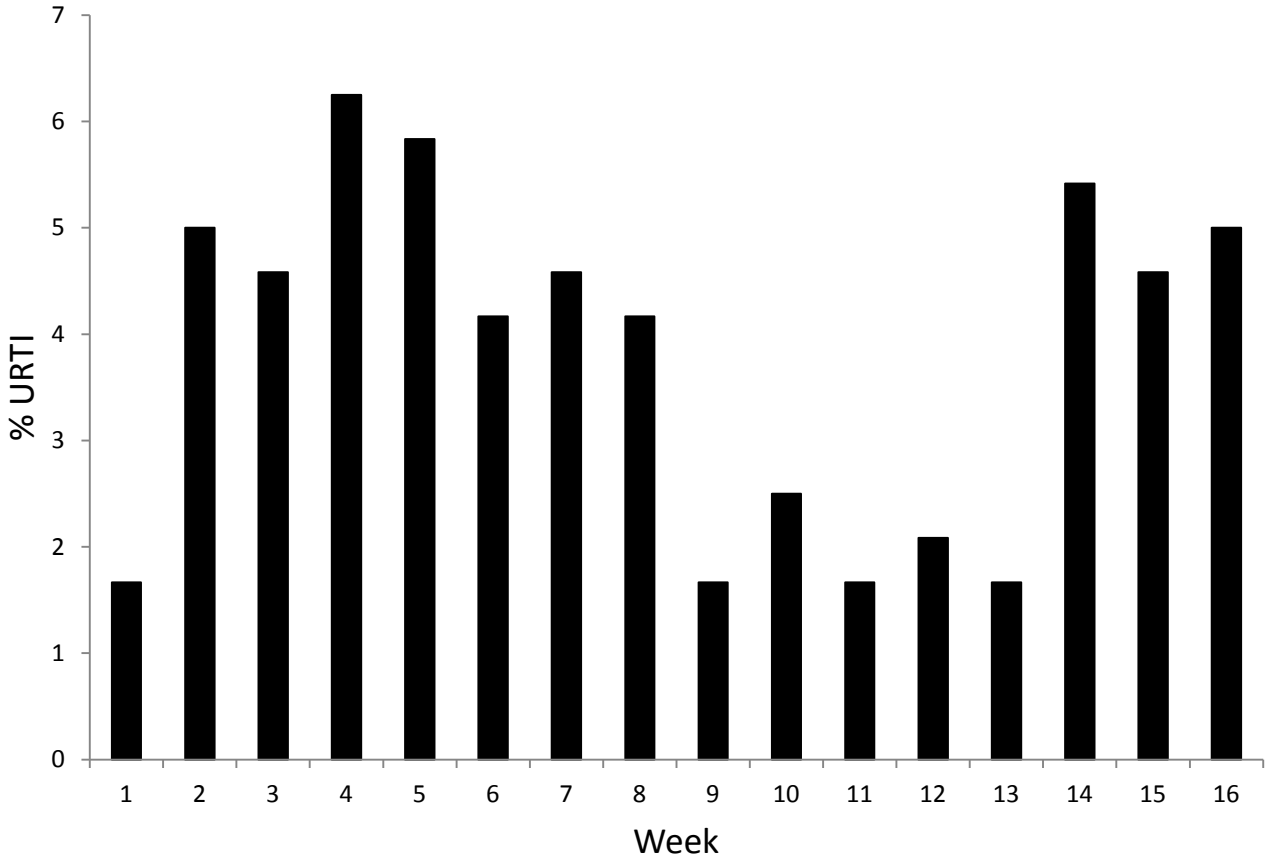

Table 1. Infection symptom incidence in CMV positive and negative subjects. Also shown are the severity score and duration of URTI episodes. Data are shown as mean ± SD and also as 95 % confidence intervals (CI). The *p* values refer to outcomes of Mann-Whitney U tests on nonparametric data.

	CMV-positive	CMV-negative	*p*
	n = 58	n = 178	
Number of episodes			
Mean ± SD	0.5 ± 0.7	0.7 ± 0.9	
95 % CI	0.3-0.7	0.6-0.8	0.107
Number of symptom days			
Mean ± SD	4.1 ± 5.4	5.8 ± 6.2	
95 % CI	2.7-5.6	4.9-6.8	0.033
	n = 22	n = 80	
Symptom-severity score			
Mean ± SD	66.3 ± 46.0	65.2 ± 36.9	
95 % CI	45.9-86.7	56.9-73.4	0.817
Episode duration (days)			
Mean ± SD	10.6 ± 5.7	8.6 ± 4.5	
95 % CI	8.1-13.1	7.6-9.6	0.082

Table 2. Infection symptom incidence in EBV positive and negative subjects. Also shown are the mean severity score and duration of URTI episodes. Data are shown as mean ± SD and also as 95 % confidence intervals (CI). The *p* values refer to outcomes of Mann-Whitney U tests on nonparametric data.

	EBV-positive	EBV-negative	*p*
	n = 197	n = 39	
Number of episodes			
Mean ± SD	0.6 ± 0.8	0.9 ± 1.0	
95 % CI	0.5-0.7	0.5-1.2	0.132
Number of symptom days			
Mean ± SD	5.1 ± 5.9	6.9 ± 6.8	
95 % CI	4.3-5.9	4.7-9.1	0.133
	n = 82	n = 20	
Symptom-severity score			
Mean ± SD	65.0 ± 40.3	67.3 ± 32.9	
95 % CI	56.1-73.8	51.9-82.7	0.404
Episode duration (days)			
Mean ± SD	9.0 ± 5.0	9.1 ± 4.4	
95 % CI	7.9-10.1	7.1-11.2	0.859

Table 3. Infection symptom incidence in CMV+EBV+ and CMV-EBV- subjects. Also shown are the severity score and duration of URTI episodes. Data are shown as mean ± SD and also as 95 % confidence intervals (CI). The *p* values refer to outcomes of Mann-Whitney U tests on nonparametric data.

	CMV+EBV+	CMV-EBV-	*p*
	n = 50	n = 31	
Number of episodes			
Mean ± SD	0.5 ± 0.8	1.0 ± 1.1	
95 % CI	0.3-0.7	0.6-1.4	0.040
Number of symptom days			
Mean ± SD	4.3 ± 5.6	7.9 ± 7.0	
95 % CI	2.7-5.9	5.3-10.4	0.010
	n = 19	n = 17	
Symptom-severity score			
Mean ± SD	66.8 ± 49.4	68.0 ± 35.3	
95 % CI	43.0-90.6	49.8-86.1	0.763
Episode duration (days)			
Mean ± SD	10.0 ± 5.9	8.2 ± 4.0	
95 % CI	7.2-12.9	6.2-10.3	0.299

Table 4. Blood total and differential leukocyte counts in CMV+EBV+ and CMV-EBV- subjects. Data are shown as mean ± SD and also as 95 % confidence intervals (CI). The *p* values refer to outcomes of Mann-Whitney U tests on nonparametric data.

	CMV+EBV+	CMV-EBV-	*p*
	n = 50	n = 31	
Leukocytes ($x10^9$ cells/L)			
Mean ± SD	6.4 ± 1.5	5.9 ± 1.3	
95 % CI	5.9-6.8	5.4-6.3	0.593
Neutrophils ($x10^9$ cells/L)			
Mean ± SD	3.1 ± 1.2	3.0 ± 1.0	
95 % CI	2.8-3.5	2.6-3.3	0.888
Lymphocytes ($x10^9$ cells/L)			
Mean ± SD	2.4 ± 0.7	2.0 ± 0.5	
95 % CI	2.2-2.5	1.8-2.2	0.029
Monocytes ($x10^9$ cells/L)			
Mean ± SD	0.6 ± 0.2	0.6 ± 0.1	
95 % CI	0.6-0.7	0.6-0.7	0.574