Gender differences and age-specific associations between Body Mass Index and other cardiovascular risk factors in CMV infected and uninfected people

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Abstract

Recent studies have highlighted Body Mass Index (BMI) as an important parameter associated with cardiovascular risk and cancer.

Here we have explored the relationship between BMI and other cardiovascular risk factors such as white blood count (WBC) and mean arterial blood pressure (MAP) in young (20-35 years) and older (60-85 years) healthy donors stratified by gender and CMV IgG serostatus.

We found a positive correlation between BMI and WBC in young women, which was significant in CMV+ women. Interestingly, there was a non-significant opposite trend in young men. In older women the positive trend was preserved in the presence of CMV-infection, but no clear trend was seen in older men. Gender differences were also observed by opposite trends regarding an association between MAP and WBC (positive in young women, negative in young men). These associations were not observed at older ages. However, in CMV+ older men, there was a significant association between MAP and WBC as well as neutrophil count (NC). CRP values were only available in older participants, and interestingly, correlated with WBC and NC only in women, and more closely in CMV-women.

This study reveals that the correlations between common inflammatory markers/cardiovascular risk factors depend on age, gender, and CMV infection status in a complex fashion. Our findings support the need to evaluate risk factors independently in men and women and to take into account CMV infection status. More focused studies will be required to shed light on these novel findings.

Key-words: Body Mass Index; cardiovascular risk factors; inflammation; infection; CMV; immunology.

Introduction

Increasing evidence shows that inflammation is a key feature of atherosclerosis and a number of inflammatory markers have been described as cardiovascular disease (CVD) risk factors, including body fat, white blood cells count (WBC) and Cytomegalovirus (CMV) infection.

Body Mass index (BMI) measures individuals' body fat, with standard charts considering people 'overweight' if BMI is 25-29.9 kg/m^2 and 'obese' if above 30 kg/m^2**.** Obesity is generally accompanied by increased systemic inflammation, due to release of inflammatory factors (such as TNF alpha, IL6, IL10, adiponectin, leptin) collectively called 'adipokines', secreted by the adipose tissue itself and by fat-infiltrating lymphocytes, macrophages and mast cells [1]. Obesity increases in older age, but inflammation caused by adipose tissue induces the immune dysregulation linked to immune aging (or immunosenescence) even in children [2]. Indeed, excess body fat is a known risk factor for a number of metabolic conditions from type 2 diabetes to CVD and cancer [3].

The white blood cell count (WBC) is also known to be linked to increased cardiovascular morbidity and mortality [4-6]. Among white blood cells, neutrophils have been described as the strongest predictors of coronary heart disease (CHD) [5] and have been implicated in the initiation and progression of many human cancers, where the neutrophil/lymphocyte ratio is believed to be associated with poorer prognosis [7] [8].

Cytomegalovirus (CMV) infection targets endothelial and smooth muscle cells [9, 10] resulting in chronic infection that has been associated with cardiovascular disease [11-13] and increased mortality in older age [14]. In a recent study on patients with chronic kidney disease, CMV seropositivity has been found associated with increased arterial stiffness, a predictor of cardiovascular mortality [15].

Gender differences in cardiovascular complications have long been described [16], but little is known on the association between the inflammatory risk factor BMI and CMV and gender different cardiovascular risks in younger or older age.

In this study, we have analysed the correlations between BMI, WBC, neutrophil count (NC), and Mean Arterial Pressure (MAP), a surrogate marker for vascular stiffness [17] [18] in young and older healthy volunteers stratified by CMV serostatus.

Our analysis suggests that age, gender and CMV infection have a role in governing the complex interdependency between inflammatory parameters/cardiovascular risk factors.

While the impact of increased BMI on pro-inflammatory markers (WBC and neutrophils counts) was more noticeable in young women and CMV positive older women, in the latter this was not linked to MAP and probably not to higher CHD/CVD risk factors.

Methods:

Study Participants

A total of 131 Healthy older donors (60-80 years old) were recruited from general practices in Surrey and Sussex, UK, through the Primary Care Research Network (PCRN). Healthy young volunteers (20-35 years old) were recruited from university students/staff. Over 90% of volunteers were white Caucasian, the remainder black African or Asian. Exclusion criteria were known immunodeficiency (including HIV-infection), organ transplantation, use of immunosuppressive/immunomodulation drugs within the last year, cancer or treatment for cancer within the previous 5 years, insulin dependent diabetes, moderate or advanced renal failure, liver disease, endocrine disorders other than corrected thyroid dysfunction, autoimmune disease, dementia/mental incompetence, alcohol/other drug abuse, acute infection or illness in the last 4 weeks, and raised body temperature.

Demographic parameters (age, sex), lifestyle factors (smoking habits and alcohol use) and measurements of height and weight were recorded in all subjects. BMI was calculated as weight(kg)/(height(meters))^2. A trained research nurse recorded body temperature and heart rate and drew 30 ml of blood from a cubital vein. A standardized brachial blood pressure measurement was taken after 10 – 15 minutes rest in a sitting position using an Omron 705CP automated blood pressure monitor (Omron Healthcare, Milton Keynes, UK). The study had UK National Research Ethics Service (NRES) approval. All participants gave written informed consent.

Routine laboratory

Routine blood analysis was obtained from the pathology department (Brighton and Sussex University Hospital, BSUH, Brighton) using the Sysmex XE-2100 Blood Analyser. C-reactive protein (Cobas Tina-quant, CRP HS, Gen. 3, Roche Diagnostics, Mannheim, Germany) was obtained from the BSUH pathology laboratories.

Statistical Analysis

SPSS-20 was used for analysis. Parametric and non-parametric bivariate correlations were calculated as appropriate. For correlations including CRP the non-parametric procedure was used, for linear regression analysis CRP was log-transformed. A two-sided $p<0.05$ was considered statistically significant. Linear regression models (UNIANOVA) were employed to evaluate associations of inflammatory parameters/risk factors and gender.

Results:

1. WBC and NC are associated with Body Mass Index and CRP in an age and sex dependent manner

Increased waist circumference (WC), an alternative marker of obesity, has recently been associated with increased WBC and NC [19]. In agreement with these findings, we observed positive associations between BMI and both WBC and NC across our population sample. Since increased body mass has been associated with increased inflammation and men and women are known to differ significantly in regards to inflammatory responses, we were wondering if the observed associations were the same in the two genders. Also, since CMV infection is thought to increase inflammation in general, and most importantly in the context of ageing, we wanted to explore the effect of CMV on these associations.

Whereas in young women there was a significant positive correlation between BMI and both Neutrophil counts and WBC (0.492, p=0.004 and .515, p=0.002, respectively), in young men the same correlations were negative (-.489, p=0.055 and -.496, p=0.051, respectively) (**FIG. 1**). When splitting in CMV+ and CMV-, the correlations remained significant only in CMV+ young women. Linear regression analysis (UNIANOVA) confirmed a significant effect of gender on the correlation between BMI and NC (0.007 for gender, and .003 for the interaction). If NC is an accepted marker of inflammation, this result suggests that in young women but not young men, increased body mass is related to increased inflammation. Interestingly, none of these associations were observed in older people to the same extent; however, a positive association (trend) between BMI and NC/WBC was still present in CMV+ older women. The difference between older and young women might suggest an effect of sex hormones, in particular estrogen levels on this association, however, these were not measured in the present study.

More than any other blood cells, increased neutrophils have been associated with inflammation. Indeed, irrespective of CMV status, there was a significant non-linear correlation (Spearman rank) between CRP and both WBC and NC in older people (**Fig. 2**). When the genders were studied separately, the positive correlation was again restricted to older women (r=0.366, p=0.004 and r=0.455, p=0.000, respectively). Linear regression (UNIANOVA) accounting for gender and CMV-status indicated a significant effect of CRP on Neutrophil and WBC, a significant effect of the interaction gender/CRP on Neutrophils (p=0.048) and an almost significant effect on WBC (p=0.064). This would suggest that in older women there is a positive association of WBC/NC and inflammation (CRP) that is not found in older men. CRP levels were not available for young people in this study, and it remains unanswered if the same associations are present.

2. White blood cell and NC correlate with higher MAP in CMV+ older men

In the whole population sample, no association between WBC, NC and and MAP were observed. However, when stratifying by gender and age, a positive correlation was found between both WBC, NC and MAP in older men, pointing to inflammation as a risk factor for cardiovascular complications, which is in agreement with the literature, albeit that most studies were not stratified by gender. When additionally stratifying by CMV status, a highly significant positive correlation between NC and MAP was observed in CMV+ older men but no other group **(Fig 3)**. Linear regression analysis confirmed a significant association between NC and MAP in older CMV+ people, the association between gender and NC was highly significant (**see Table 1**). This analysis shows that specific and highly significant associations between inflammatory parameters and cardiovascular risk factors may be overlooked when gender and CMV status are not accounted for.

Table 1: NC and MAP in older CMV infected people

MAP		
Factor	***Parameter Estimate (95% CI)***	***P***
Smoker	*3.17 (-1.78 – 8.11)*	*0.205*
Male sex	*-29.296 (-44.70 - -13.89)*	*0.000*
Age (years)	*0.25 (-0.08 – 0.57)*	*0.134*
BMI	*0.08 (-0.56 – 0.72)*	*0.797*
Not on antihypertensive treatment	*-0.28 (-5.06 – 4.49*	*0.906*
Neutrophil count	*-3.37 (-6.28 - -0.463)*	*0.024*
*Neutrophil count*gender*	*8.530 (4.40 – 12.66)*	*0.000*

It is of interest that among older men and women in this study, there were no significant differences between CRP levels, NC, WBC and MAP **(Fig 4)**. However, stratifying the group by gender and CMV status revealed differential associations between these factors in the two genders in the presence and absence of CMV infection.

Discussion

Elevated BMI is associated with an increased risk of cardiovascular disease It has been noted that since BMI estimates body fat based on total body weight, it is a reflection of the more abundant subcutaneous fat rather than visceral fat [Reference]. In fact, visceral adiposity and waist circumference (WC), rather than subcutaneous fat, is more closely associated with metabolic syndrome (MS) including insulin resistance, high triglycerides, low HDL cholesterol, and high blood pressure [4]. Despite this, BMI remains an accepted tool to measure obesity related health risks [20], and recent data [21] demonstrated a significant correlation between BMI and WC in metabolic syndrome, especially in women. A better association between BMI and WC in women may be explained by physiological differences in body fat distribution between the sexes. Females typically have more fat around the core area and hips (below the waist, known as ‘pear shape’) including more subcutaneous fat, while in males fat concentrates predominantly in the waist area (‘apple shape’) including a higher proportion of visceral fat. Many World Health organizations, including WHO, recommend measuring both BMI and WC to assess obesity-related health risks and a recent survey on a Canadian cohort [20] demonstrated that measuring both parameters is the most useful approach to the assessment of CVD risks. Interestingly, a number of studies point to an association of WBC and coronary heart disease, with leucocytosis being an independent prognostic factor for cardiovascular risk [22] [23]. More recently, studies have identified a dominant role of the NC (rather than lymphocyte or monocyte count) in the association of WBC with CHD [5] and vascular endothelial damage.

We observed a strong positive association of BMI and WBC in young women which was accounted for mainly by the NC (fig 1). We noted that this associations were most significant in the young CMV+ group, weaker (non significant) in the old CMV+ group, and absent in the CMV- groups. In young men there was an opposite trend whereas no trend was seen in older men, irrespective of CMV status in each case. This finding suggests that CMV infection somehow drives the association between the amount of body fat (i.e. BMI, mostly subcutaneous fat) and NC in women, but not in men. The relatively small group of young CMV+ men is a disadvantage of this study, however, the fact that the observed trend had the opposite direction in young males compared to females is reassurance that there is a

clear gender effect, which may be related to fat type and distribution, and this effect of gender was confirmed by linear regression analysis. The difference between young women and young men as well as young women and older women points to a role of sex steroids, in particular estrogens, whose levels are high in pre-menopausal women but dramatically decline after menopause.

It is well-known that the incidence of CVD/CHD varies significantly with gender and age, which has indeed been attributed to sex hormone changes, in particular estrogens [24]. Women have a higher risk of CVD in postmenopausal years and a lower risk in premenopausal years, when estrogen has an anti-inflammatory effect on the cardiovascular system [25] protecting it from metabolic injury [26]. Women also express higher levels of estrogen receptors on neutrophils [27] which has been linked to a lesser activation of these cells in premenopausal women via the Annexin A1(AnxA1) mechanism as described by Nadkarni et al. [28]. In this study, neutrophil activation was not measured, we merely observed significant correlations between BMI and WBC/NC in younger women. That this association was reversed in young men could be explained by the protective role of testosterone in younger age. Recent evidence in fact suggest that androgens also exert a protective vascular effects and older men with low testosterone levels are more at risk of CVD and endothelial dysfunction [29]. Hence, the fact that NC appear to be stronger predictors of CHD risk in CMV+ men may be explained by an increased effect of inflammation at the age when protective androgen effects are reduced.

A large population study led by JS Rana [5] on people aged 45-79 years showed associations between NC and the risk of CVD/CHD to be more consistent in men than women, and completely absent in postmenopausal women. This is in agreement with our finding of a significant positive correlations between NC and MAP in CMV+ (but not in CMV-) older men. Interestingly, in younger donors trends were observed in males and females, but reversed in the former (all our young donors were well below the age of 45). Of interest, it was shown that CMV-infected endothelial cells produce large amounts of C-X-C cytokines, including IL-8, which is thought to play an important role in recruiting neutrophils to the site of infection [30]. This might explain increased vascular damage in CMV-infected endothelia, and if CMV-infected endothelia produced more neutrophil chemo-attractants in older men than older women this could to some extent explain differential associations between NC and MAP in the two genders despite the absence of differences in NC per se. This is clearly speculation, and while such differences are conceivable given the complex interactions of sex hormones with endothelia, including the aromatisation of androgens to form estrogens at the endothelial cell level [31], understanding these processes in more detail will require dedicated studies.

Neutrophils mediate inflammation in innate immune responses by releasing pro-inflammatory mediators such as the proteolytic enzyme elastase and cytotoxic free oxygen radicals (ROS). Neutrophils are also found increased in hypertrophic adipose tissue, possibly attracted by the inflammatory cytokine IL8 secreted by adipocytes [32], in this regard, body fat may serve as a reservoir for neutrophils in younger women (possibly under the influence of estrogen).

When analysing the relationship between WBC/NC and the inflammatory marker, C-reactive protein (CRP), in the older cohort, we found significant correlations only in women. This throws up an apparent paradox, since in older men, WBC/NC correlated with MAP but not CRP, a known risk factor for CVD/CHD. In women on the other hand, WBC/NC correlate well with CRP but not MAP. CRP did not significantly correlate with MAP. Our population was selected for generally good health and MAP is merely a surrogate marker for vascular compliance, which is thought to be the best marker of cardiovascular risk at this time. The analysis of multiple correlations carries the risk of over-interpretation, in particular on relatively small datasets. It remains though that CMV+ appears to have modulated the relationship between WBC/NC and MAP in older men, whether by direct effects on infected endothelial cells or indirectly, for example by generating strong CD8 T-cell responses [33].

Older women may be protected from negative effects of neutrophils on vascular endothelium by factors we cannot account for in this study. If indeed CMV infection of vascular endothelium leads to the recruitment of neutrophils to the site of infection, the absence of a protective factor in men may translate into a positive association of NC and MAP (where CMV is not the cause of the rise in neutrophils but the cause of their recruitment to vascular endothelium – note that neutrophil counts in CMV+ and CMV- older people were not significantly different). We recently described an increased frequency of CMV-specific iTregs in older CMV+ women and discussed their possible role in protecting them from potential vascular damage [33]. It is not known if Tregs can directly downregulate the effects leading to increased recruitment of neutrophils.

In conclusion, it is important to keep in mind that gender differences in this study do not relate to simple differences in the measured risk factors, since WBC/NC, BMI, CRP, and MAP by themselves were not significantly different between men and women. The gender differences described here relate to the specific *associations* between these different factors. The situation is complicated by the effect of CMV infection, and even more complicated by the fact that a major determinator of sex differences, namely sex hormone levels, change considerably between young and older people as a result of the menopause in women and the andropause in men. We observed an association of WBC/NC with BMI in CMV+ young women, and an association of WBC/NC and increased MAP in CMV+ older men. Neither of

these associations was significant in CMV- people. Large studies of cardiovascular risk factors do generally not account for CMV status, but depending on the age of the studied populations, CMV infection (which is often not accounted for) may have significant effects on study results, as it appears to change the relationships between well-established risk factors in gender-specific ways.

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Highlights

- We examine the relationship between Body Mass Index (BMI) and white blood count (WBC), neutrophil counts (NC), mean arterial blood pressure (MAP).
- WBC and NC correlate with BMI in an age and sex dependent manner, with opposite trends observed in younger men and women, lost in older age except for CMV+ women.
- A significant association is between MAP and WBC or NC is seen in CMV+ older men.
- Correlations between cardiovascular risk factors depend on age, gender and CMV infection status.

Figure 1

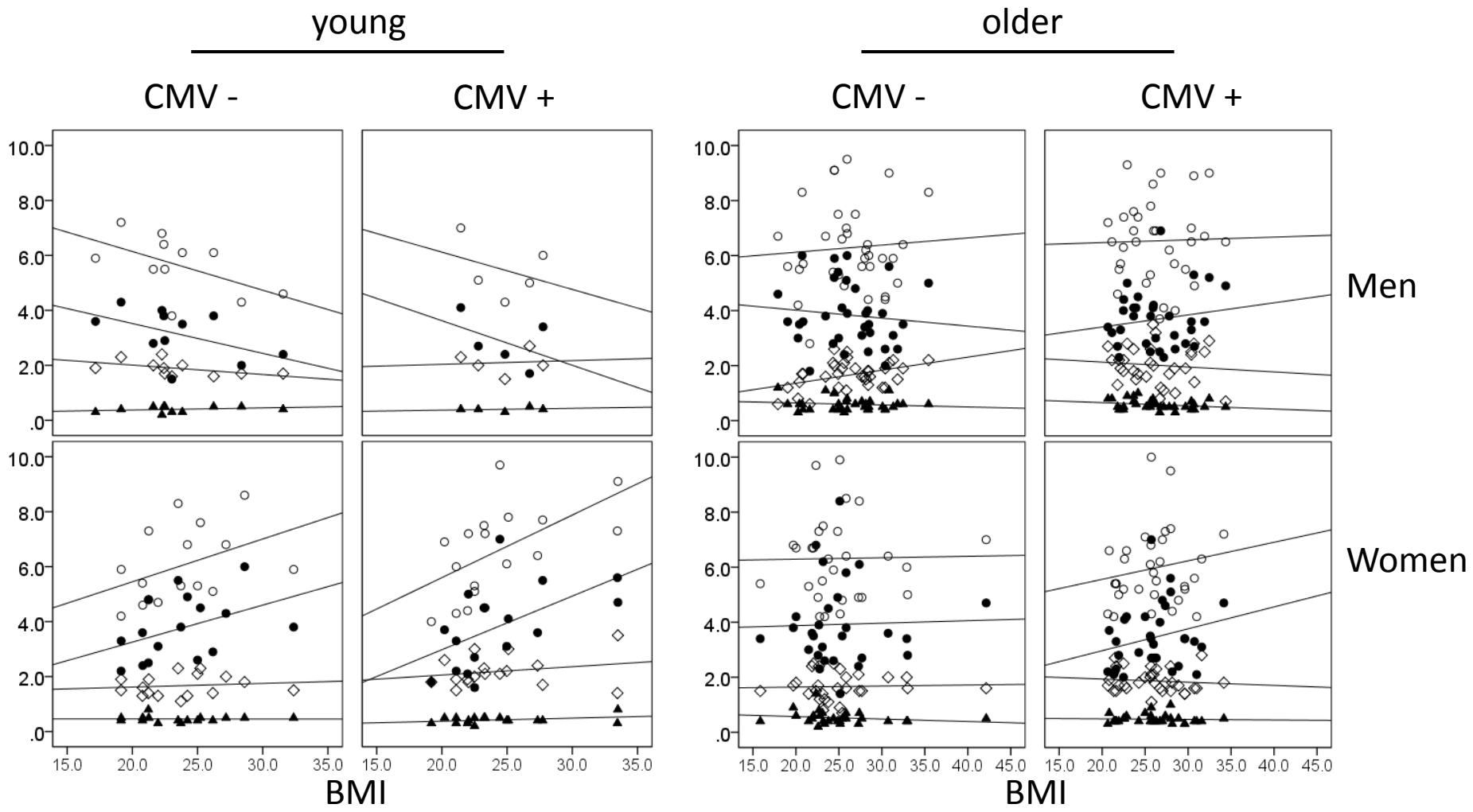
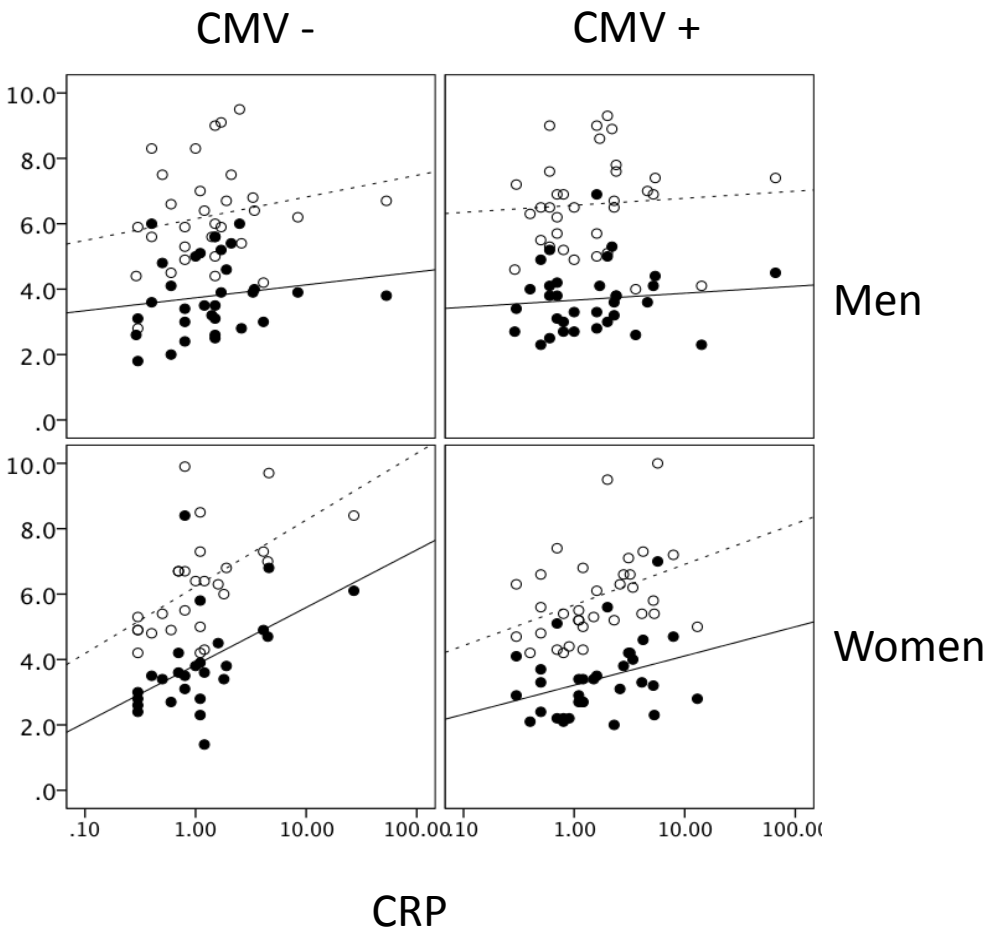


Fig 1: Correlations analysis between MBI and blood cell fraction counts (empty circles indicate total white blood cells WBC; filled circles neutrophils, diamonds lymphocytes, filled triangles monocytes) was carried out in young (males n = 16; females n= 33) and older (males n = 16; females n= 33) donors. Gender specific correlations are observed, especially at young age. Significant correlations are observed in young CMV + women (R=0.580/P=0.015* and R=0.538/P=0.026*, for WBC and NC respectively). Increased total leucocytes are accounted for by increased neutrophil counts.

Figure 2



			CRP/ WBC	CRP/ NeuVal
CMV -	**MEN**	Spearman's rho	0.242	0.251
		Sig. (2-tailed)	0.190	0.174
		N	31	31
	WOMEN	Spearman's rho	0.561**	0.546**
		Sig. (2-tailed)	0.003	0.004
		N	26	26
CMV +	**MEN**	Spearman's rho	0.176	0.105
		Sig. (2-tailed)	0.328	0.560
		N	33	33
	WOMEN	Spearman's rho	0.415*	0.345*
		Sig. (2-tailed)	0.016	0.049
		N	33	33

Fig 2. Linear correlations analysis between CRP (log transformed) and WBC (empty) or neutrophils (full) in older people. Lines correspond to Pearson correlation (but Spearman was used in analysis).
Positive correlations are observed in all groups and significant correlations are observed in women

Figure 3

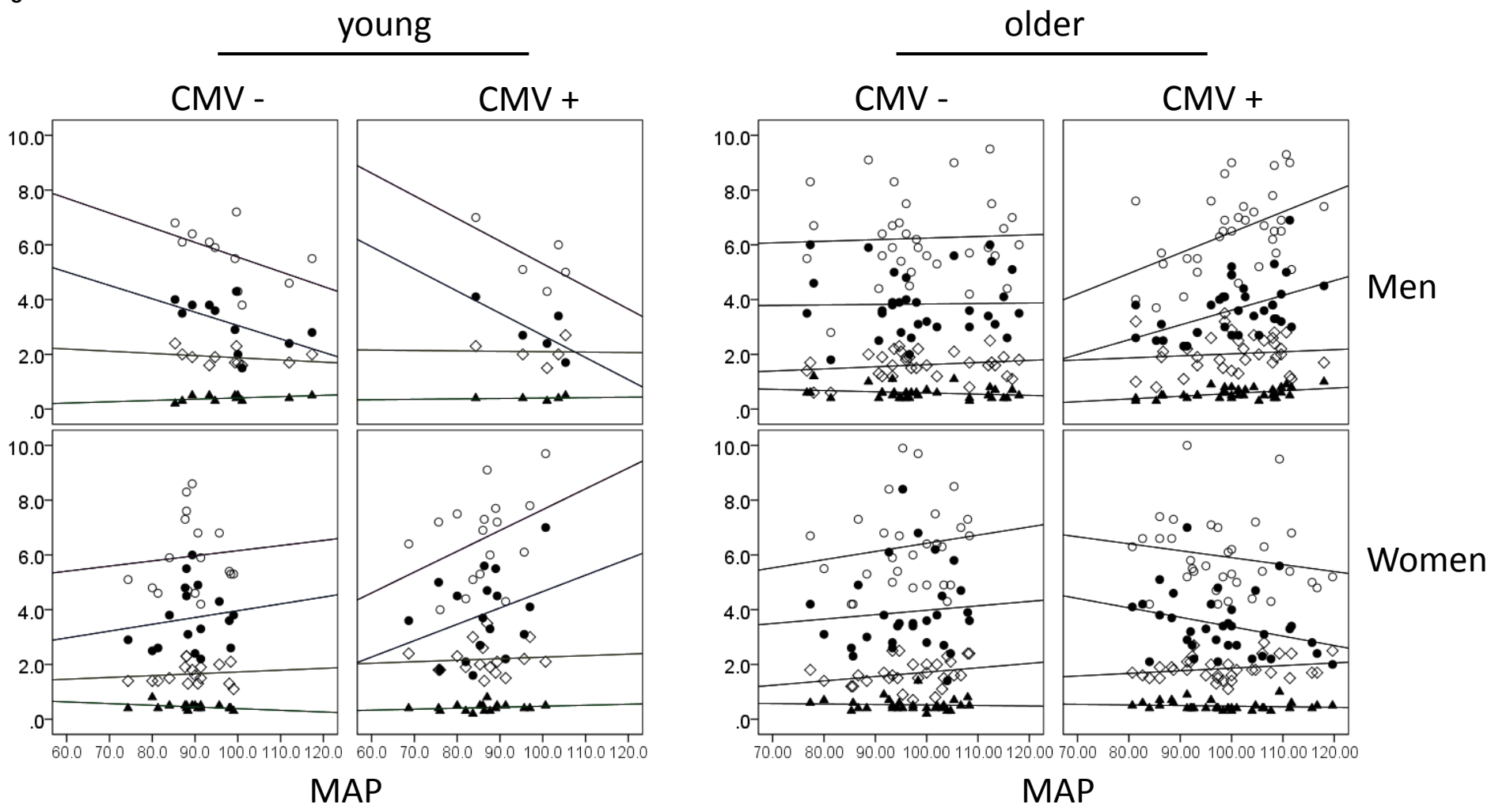


Fig 3: Correlations analysis between MAP and blood cell fractions counts, in young and older donors, split by CMV status (empty circles indicate total white blood cells, filled circles neutrophils, diamonds lymphocytes, filled triangles monocytes). Gender specific correlations observed between MAP and Neutrophils are inverted in older seropositive people, noticeably with significant positive correlation in men (R=0.468/P=0.005** and R=0.492/P=0.003**, for WBC and NC respectively).

Figure 4

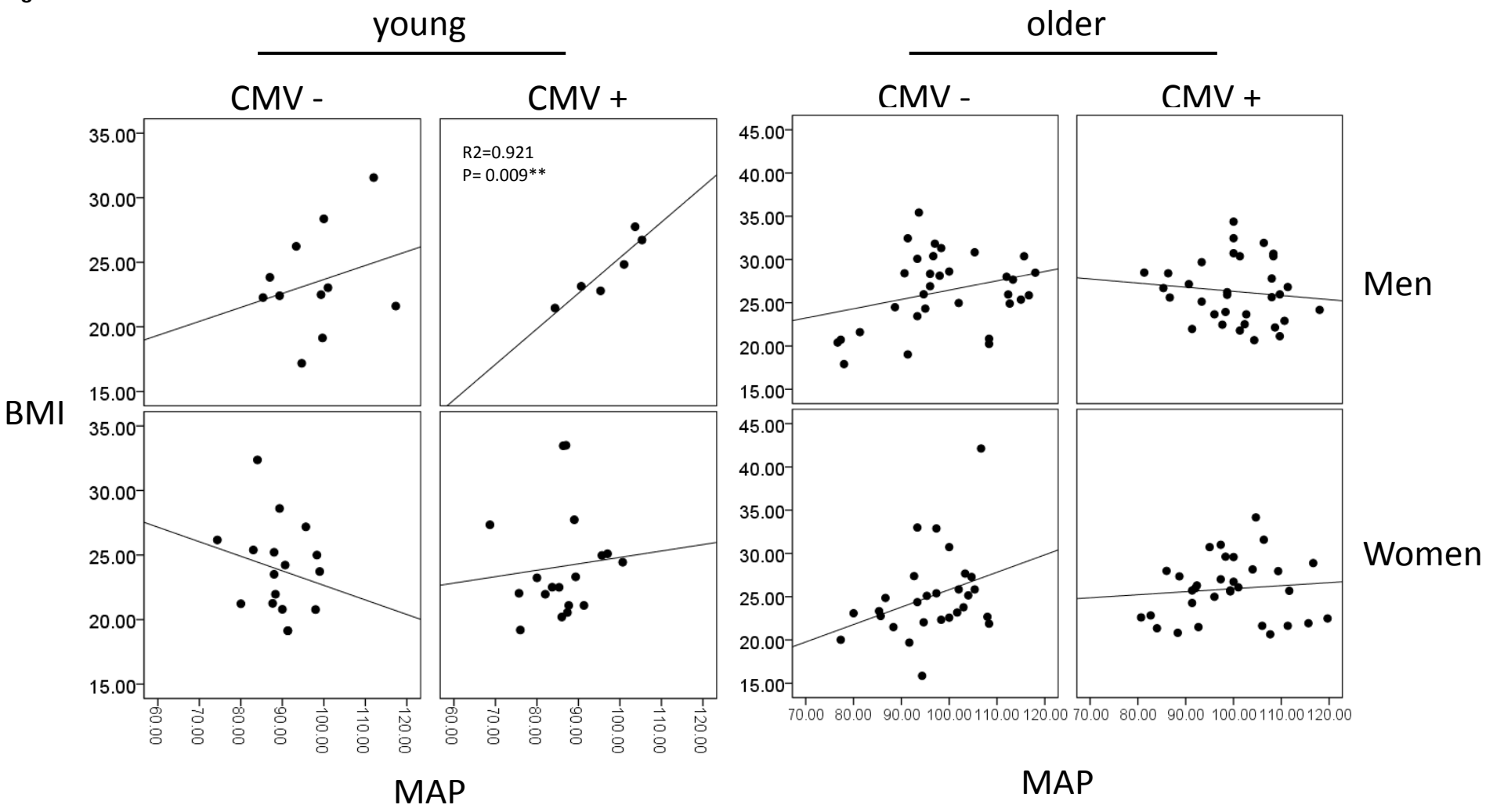


Fig 4: Correlations analysis between BMI and MAP, in young and older donors, split by CMV status. Positive trends are observed in all groups, with significant correlations CMV+ young men. Negative trends are noticeable in young women.