Published in final edited form as:

Immunol Lett. 2014 November ; 162(1 0 0): 316–322. doi:10.1016/j.imlet.2014.09.011.

Gender differences and age-specific associations between body mass index and other cardiovascular risk factors in CMV infected and uninfected people

Nadia Terrazzini^{a,b,*}, Martha Bajwa^a, David Thomas^a, Helen Smith^b, and Florian Kern^c

^aDivision of Medicine, Brighton and Sussex Medical School, Brighton, United Kingdom

^bSchool of Pharmacy and Biomolecular Sciences, Brighton, United Kingdom

^cDivision of Primary Care and Public Health, Brighton and Sussex Medical School, Brighton, United Kingdom

Abstract

Body mass index (BMI) is a known risk factor for cardiovascular disease and cancer. It is also related to white blood count (WBC) and inflammation. The effects of age and gender on these associations have not been explored. Here we have examined the relationships between BMI and inflammatory parameters/cardiovascular risk factors including WBC/neutrophil count (NC), CRP and mean arterial blood pressure (MAP), in young (20–35 years) and older (60–85 years) healthy donors with respect to gender and CMV IgG serology. In young but not older people significant associations between BMI and WBC were observed, however, with opposite directions in the two genders. Only in CMV+ older women a positive trend was preserved. Across the population, there was no significant association between NC and MAP; however, among older men we saw a positive correlation between the two parameters. Linear regression confirmed that across the whole population, age group (young versus older) and also the interaction between gender and age group but not gender alone had significant effects on this association. When analysing CMV+ older people separately we established that both NC and its interaction with gender had a significant effect on MAP. This study reveals that the correlations between common inflammatory markers/cardiovascular risk factors depend on age, gender, and CMV 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.

Keywords

Body mass index; Cardiovascular risk factors; Inflammation; Infection; CMV; Immunology

*Corresponding author at: Division of Medicine, Brighton and Sussex Medical School, Brighton, United Kingdom. n.terrazzini@bsms.ac.uk (N. Terrazzini).

1. 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/m² and 'obese' if above 30 kg/m². Obesity is generally accompanied by increased systemic inflammation, due to the release of inflammatory factors (such as TNF, 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 appears to induce the kind of immune dysregulation that is linked to immune ageing (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 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 was found to be 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 about the effect of gender and CMV infection on the expression of risk factors for cardiovascular disease at different ages.

A recent study conducted in our lab suggested that the effects of CMV on immunity and inflammation might depend on gender and age [17] and provided us with the opportunity to revisit some of these concepts with respect to BMI, inflammation and vascular parameters. Here, we have explored associations between BMI, WBC, neutrophil count (NC), and Mean Arterial Pressure (MAP), a surrogate marker for vascular stiffness [18,19] in young and older healthy volunteers with and without CMV infection. Our analysis suggests that age, gender and CMV infection have a role in governing the complex interdependency between inflammatory parameters/cardiovascular risk factors and call for more attention to these parameters in this area of research. 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.

2. Methods

2.1. 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 $\text{weight (kg)}/(\text{height (metres)})^2$. A trained research nurse recorded body temperature, heart rate, and took a standardized brachial blood pressure measurement after 10–15 min rest in a sitting position using an Omron 705CP automated blood pressure monitor (Omron Healthcare, Milton Keynes, UK). Blood was drawn from a cubital vein afterwards. The study had UK National Research Ethics Service (NRES) approval. All participants gave written informed consent.

2.2. Routine laboratory

The full WBC was obtained from the pathology department (Brighton and Sussex University Hospital, BSUH, Brighton) using the Sysmex XE-2100 Blood Analyser. C-reactive protein was obtained from the BSUH pathology laboratories (Cobas Tina-quant, CRP HS, Gen. 3, Roche Diagnostics, Mannheim, Germany) (Table 1).

2.3. Statistical analysis

SPSS-22 was used for analysis. Parametric and non-parametric bivariate correlations were calculated as appropriate. For analyses including CRP, non-parametric procedures were employed, for use in linear regression analysis CRP values were log-transformed. A two-sided $p < 0.05$ was considered statistically significant. Linear regression models were analysed using the General Linear Model in SPSS 22. Smoking history was coded as 'smoker' in the past/presence (definite "yes") versus non-smoker (definite "no"). A more detailed smoking history was not available for the majority of individuals in this study. Antihypertensive drug use was defined as being on at least one medication reducing blood pressure.

3. Results

3.1. WBC and NC are associated with body mass index and CRP in an age and sex dependent manner

Whereas in young women there was a significant positive correlation between BMI and both NC and WBC ($R = 0.492$, $p = 0.004$ and $R = 0.515$, $p = 0.002$, respectively), in young men the same correlations were negative ($R = -0.489$, $p = 0.055$ and $R = -0.496$, $p = 0.051$, respectively). When splitting in CMV+ and CMV-, the correlations remained significant only in CMV+ young women (Fig. 1). Because white blood cell subsets other than neutrophils (the most dominant subset) were not correlated with BMI, we limited further analyses to neutrophils. Linear regression analysis confirmed a significant effect of BMI ($p = 0.001$) on NC, but gender and the interaction of gender and BMI also had a significant influence ($p = 0.007$ for gender, and 0.003 for the interaction), confirming effects of opposite direction in the two genders (even when not including CMV status in the model). While a positive association (trend) between BMI and NC/WBC was also present in CMV+ older women (Fig. 1), linear regression did not show the same significant associations as in young people. These differences may reflect an effect of sex hormones levels (in particular oestrogen) on these associations. However, sex hormones had not been measured in the present study, so that this question could not be addressed.

More than other blood cells, increased neutrophils are associated with inflammation. Indeed, irrespective of CMV status, there was a significant correlation between CRP and NC in older people (Fig. 2). CRP values were not available for younger people. When older women and men were analysed separately, a positive correlation appeared to be restricted to the former ($R = 0.366$, $p = 0.004$ and $R = 0.455$, $p = 0.000$, respectively). Linear regression analysis including gender as covariate confirmed a significant effect of NC on CRP ($p = 0.006$) but neither gender nor the interaction of gender with NC had a significant effect on CRP. No effect of CMV infection on this association was observed.

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

With respect to the entire population sample, no significant link between NC and MAP was observed. However, when stratifying by gender and age, a positive correlation was found in older men, pointing to increased inflammation as a risk factor for cardiovascular complications. This would be in agreement with the literature albeit that often studies are 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 including NC, gender and age group (young versus older), confirmed significant effects of age group ($p = 0.000$) but not gender ($p = 0.674$) on the association of NC and MAP. However, the interaction of gender and age group had a highly significant effect on MAP (0.009), in agreement with the results from the stratified correlations above. Since our previous work [17] suggested a significant gender-specific effect of CMV infection on the immune system in older people we decided to explore the association between NC and MAP by linear regression in CMV+ older people as a separate group. Our linear regression model thus included NC and gender but, in addition, we included the use of antihypertensive drugs, BMI and smoking. A smoking history was

not available for everyone, slightly reducing the number of individuals included in the analysis (see Table 2), however, we continued to see a significant effect of gender, NC, and, importantly, the interaction between gender and neutrophil count on MAP (Table 2). Further study of this phenomenon is clearly required; however, our analysis indicates that specific and highly significant associations between inflammatory parameters and cardiovascular risk factors may be overlooked when age, gender and CMV status are not accounted for.

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.

4. 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 [20]. In fact, visceral adiposity and waist circumference as alternative marker of obesity, rather than subcutaneous fat, is more closely associated with metabolic syndrome 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 [21], and recent data [22] demonstrated a significant correlation between BMI and waist circumference in metabolic syndrome, especially in women. A closer association between BMI and waist circumference 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. Health organizations, including WHO, recommend measuring both BMI and waist circumference to assess obesity-related health risks and a recent survey on a Canadian cohort [21] 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 (CHD), with leucocytosis being an independent prognostic factor for cardiovascular risk [23,24]. 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.

In agreement with a recent study demonstrating increased WBC and NC associated with bigger waist circumference [25], we observed positive associations between BMI and both WBC and NC across our population sample. Since higher body mass has been associated with greater 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.

We observed a strong positive association of BMI and WBC in young women, which was accounted for by the NC (Fig. 1). In young men the effect was of the opposite direction,

whereas no significant association was seen in older people. Interestingly, the correlation between BMI and NC was strongest in the CMV+ young women, which might suggest that CMV infection somehow drives the production of pro-inflammatory factors associated with more body fat in these women; however, this is speculative and needs to be explored more systematically in a larger study. The fact that fewer young men than women in our study were CMV+ is a disadvantage but we are hopeful that we can further explore CMV related differences in young men in a larger study in future. The differences between young women and young men as well as young women and older women points to a role of sex steroids, in particular oestrogens, 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 oestrogens [26]. Women have a higher risk of CVD in postmenopausal years and a lower risk in premenopausal years, when oestrogen has an anti-inflammatory effect on the cardiovascular system [27] protecting it from metabolic injury [28]. Women also express higher levels of oestrogen receptors on neutrophils [29] 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. [30]. In this study neutrophil activation was not measured. We merely observed significant correlations between BMI and NC in younger women. The reversal of this association in young men might be explained by a protective role of testosterone in younger age. In fact, recent work has shown that androgens also exert protective vascular effects; older men with lower testosterone levels have a higher risk of CVD and endothelial dysfunction [31]. Hence, the fact that NC appear to be stronger predictors of CHD risk in older CMV+ men (i.e. an increase of MAP in our study) may be explained by an increased effect of inflammation at an age when protective androgen effects are reduced.

A large population study led by 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 correlation between NC and MAP in CMV+ (but not in CMV–) older men. 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 [32]. 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, for example as a result of hormonal influences, 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 aromatization of androgens to form oestrogens at the endothelial cell level [33], 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 [34], in this regard, body fat may serve as a reservoir for neutrophils in younger women (possibly under the influence of oestrogen).

Not all factors used for stratification were necessarily always found to be significant factors in linear regression analysis and larger studies will be required to elucidate the interesting phenomena identified here. The analysis of multiple correlations in different subgroups carries the risk of over-interpretation. However, the finding that among older CMV+ people, gender has a significant effect on the relationship between NC and MAP is potentially so important that it must be further investigated.

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 determinant 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. 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.

Acknowledgements

This work was supported by the Dunhill Medical Trust (grant number R107/0209).

References

- [1]. Makki K, Froguel P, Wolowczuk I. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. *ISRN Inflamm.* 2013; 2013:139239. [PubMed: 24455420]
- [2]. Spielmann G, Johnston CA, O'Connor DP, Foreyt JP, Simpson RJ. Excess body mass is associated with T cell differentiation indicative of immune ageing in children. *Clin Exp Immunol.* 2014; 176:246–54. [PubMed: 24401077]
- [3]. Hotamisligil GS, Erbay E. Nutrient sensing and inflammation in metabolic diseases. *Nat Rev Immunol.* 2008; 8:923–34. [PubMed: 19029988]
- [4]. Desai MY, Dalal D, Santos RD, Carvalho JA, Nasir K, Blumenthal RS. Association of body mass index, metabolic syndrome, and leukocyte count. *Am J Cardiol.* 2006; 97:835–8. [PubMed: 16516585]
- [5]. Rana JS, Boekholdt SM, Ridker PM, Jukema JW, Luben R, Bingham SA, et al. Differential leucocyte count and the risk of future coronary artery disease in healthy men and women: the EPIC-Norfolk Prospective Population Study. *J Intern Med.* 2007; 262:678–89. [PubMed: 17908163]
- [6]. Wheeler JG, Mussolino ME, Gillum RF, Danesh J. Associations between differential leucocyte count and incident coronary heart disease: 1764 incident cases from seven prospective studies of 30,374 individuals. *Eur Heart J.* 2004; 25:1287–92. [PubMed: 15288155]
- [7]. Szkandera J, Absenger G, Liegl-Atzwanger B, Pichler M, Stotz M, Samonigg H, et al. Elevated preoperative neutrophil/lymphocyte ratio is associated with poor prognosis in soft-tissue sarcoma patients. *Br J Cancer.* 2013; 108:1677–83. [PubMed: 23558897]

- [8]. Jung MR, Park YK, Jeong O, Seon JW, Ryu SY, Kim DY, et al. Elevated preoperative neutrophil to lymphocyte ratio predicts poor survival following resection in late stage gastric cancer. *J Surg Oncol.* 2011; 104:504–10. [PubMed: 21618251]
- [9]. Streblow DN, Soderberg-Naucler C, Vieira J, Smith P, Wakabayashi E, Ruchti F, et al. The human cytomegalovirus chemokine receptor US28 mediates vascular smooth muscle cell migration. *Cell.* 1999; 99:511–20. [PubMed: 10589679]
- [10]. Jarvis MA, Nelson JA. Human cytomegalovirus persistence and latency in endothelial cells and macrophages. *Curr Opin Microbiol.* 2002; 5:403–7. [PubMed: 12160860]
- [11]. Simanek AM, Dowd JB, Pawelec G, Melzer D, Dutta A, Aiello AE. Seropositivity to cytomegalovirus, inflammation, all-cause and cardiovascular disease-related mortality in the United States. *PLoS ONE.* 2011; 6:e16103. [PubMed: 21379581]
- [12]. Betjes MG, Litjens NH, Zietse R. Seropositivity for cytomegalovirus in patients with end-stage renal disease is strongly associated with atherosclerotic disease. *Nephrol Dial Transplant.* 2007; 22:3298–303. [PubMed: 17597084]
- [13]. Popovic M, Smiljanic K, Dobutovic B, Syrovets T, Simmet T, Isenovic ER. Human cytomegalovirus infection and atherothrombosis. *J Thromb Thrombolysis.* 2012; 33:160–72. [PubMed: 22161772]
- [14]. Savva GM, Pachnio A, Kaul B, Morgan K, Huppert FA, Brayne C, et al. Cytomegalovirus infection is associated with increased mortality in the older population. *Aging Cell.* 2013; 12:381–7. [PubMed: 23442093]
- [15]. Wall NA, Chue CD, Edwards NC, Pankhurst T, Harper L, Steeds RP, et al. Cytomegalovirus seropositivity is associated with increased arterial stiffness in patients with chronic kidney disease. *PLoS ONE.* 2013; 8:e55686. [PubMed: 23451030]
- [16]. Isles CG, Hole DJ, Hawthorne VM, Lever AF. Relation between coronary risk and coronary mortality in women of the Renfrew and Paisley survey: comparison with men. *Lancet.* 1992; 339:702–6. [PubMed: 1347584]
- [17]. Terrazzini N, Bajwa M, Vita S, Cheek E, Thomas D, Seddiki N, et al. A novel cytomegalovirus-induced regulatory-type T-cell subset increases in size during older life and links virus-specific immunity to vascular pathology. *J Infect Dis.* 2014; 209:1382–92. [PubMed: 24203779]
- [18]. Benetos A, Laurent S, Asmar RG, Lacolley P. Large artery stiffness in hypertension. *J Hypertens Suppl.* 1997; 15:S89–97. [PubMed: 9218205]
- [19]. Sesso HD, Stampfer MJ, Rosner B, Hennekens CH, Gaziano JM, Manson JE, et al. Systolic and diastolic blood pressure, pulse pressure, and mean arterial pressure as predictors of cardiovascular disease risk in men. *Hypertension.* 2000; 36:801–7. [PubMed: 11082146]
- [20]. Janssen I, Heymsfield SB, Allison DB, Kotler DP, Ross R. Body mass index and waist circumference independently contribute to the prediction of nonabdominal, abdominal subcutaneous, and visceral fat. *Am J Clin Nutr.* 2002; 75:683–8. [PubMed: 11916754]
- [21]. Shields M, Tremblay MS, Connor Gorber S, Janssen I. Abdominal obesity and cardiovascular disease risk factors within body mass index categories. *Health Rep.* 2012; 23:7–15. [PubMed: 22866535]
- [22]. Gierach M, Gierach J, Ewertowska M, Arndt A, Junik R. Correlation between body mass index and waist circumference in patients with metabolic syndrome. *ISRN Endocrinol.* 2014; 2014:514589. [PubMed: 24729884]
- [23]. Madjid M, Awan I, Willerson JT, Casscells SW. Leukocyte count and coronary heart disease: implications for risk assessment. *J Am Coll Cardiol.* 2004; 44:1945–56. [PubMed: 15542275]
- [24]. Margolis KL, Manson JE, Greenland P, Rodabough RJ, Bray PF, Safford M, et al. Leukocyte count as a predictor of cardiovascular events and mortality in post-menopausal women: the Women's Health Initiative Observational Study. *Arch Intern Med.* 2005; 165:500–8. [PubMed: 15767524]
- [25]. Vuong J, Qiu Y, La M, Clarke G, Swinkels DW, Cembrowski G. Reference intervals of complete blood count constituents are highly correlated to waist circumference: should obese patients have their own normal values? *Am J Hematol.* 2014
- [26]. Barrett-Connor E. Sex differences in coronary heart disease. Why are women so superior? The 1995 Ancel Keys Lecture. *Circulation.* 1997; 95:252–64. [PubMed: 8994444]

- [27]. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med.* 1999; 340:1801–11. [PubMed: 10362825]
- [28]. Ranki HJ, Crawford RM, Budas GR, Jovanovic A. Ageing is associated with a decrease in the number of sarcolemmal ATP-sensitive K⁺ channels in a gender-dependent manner. *Mech Ageing Dev.* 2002; 123:695–705. [PubMed: 11850031]
- [29]. Molero L, Garcia-Duran M, Diaz-Recasens J, Rico L, Casado S, Lopez-Farre A. Expression of estrogen receptor subtypes and neuronal nitric oxide synthase in neutrophils from women and men: regulation by estrogen. *Cardiovasc Res.* 2002; 56:43–51. [PubMed: 12237165]
- [30]. Nadkarni S, Cooper D, Brancalione V, Bena S, Perretti M. Activation of the annexin A1 pathway underlies the protective effects exerted by estrogen in polymorphonuclear leukocytes. *Arterioscler Thromb Vas.* 2011; 31:2749–59. [PubMed: 21836070]
- [31]. Akishita M, Yu J. Hormonal effects on blood vessels. *Hypertens Res.* 2012; 35:363–9. [PubMed: 22297478]
- [32]. Grundy JE, Lawson KM, MacCormac LP, Fletcher JM, Yong KL. Cytomegalovirus-infected endothelial cells recruit neutrophils by the secretion of C-X-C chemokines and transmit virus by direct neutrophil–endothelial cell contact and during neutrophil transendothelial migration. *J Infect Dis.* 1998; 177:1465–74. [PubMed: 9607821]
- [33]. Arnal JF, Fontaine C, Billon-Gales A, Favre J, Laurell H, Lenfant F, et al. Estrogen receptors and endothelium. *Arterioscler Thromb Vasc Biol.* 2010; 30:1506–12. [PubMed: 20631350]
- [34]. Talukdar S, Oh da Y, Bandyopadhyay G, Li D, Xu J, McNelis J, et al. Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. *Nat Med.* 2012; 18:1407–12. [PubMed: 22863787]

Fig. 1.

Correlations analysis between BMI and blood cell fraction counts was carried out in young and older donors, stratified by their CMV status. Empty black circles and black dotted black lines indicate WBC, filled black circles and solid black lines indicate neutrophils, grey diamonds and grey dotted grey lines indicate lymphocytes, filled grey triangles and grey solid lines indicate monocytes. Note that 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).

			CRP/ WBC	CRP/ NeuVal
CMV -	Men	R	0.122	0.145
		Sig. (2-tailed)	0.527	0.454
	Women	R	0.507**	0.450*
		Sig. (2-tailed)	0.01	0.024
CMV +	Men	R	0.103	0.044
		Sig. (2-tailed)	0.593	0.820
	Women	R	0.422*	0.408*
		Sig. (2-tailed)	0.025	0.031

Fig. 2.

Linear correlations analysis between CRP (log transformed) and WBC (empty circles) or NC (full circles) in older people. Lines correspond to Pearson correlation and relative values are reported in a summary table.

Fig. 3.

Correlations analysis between MAP and blood cell fractions counts, in young and older donors, split by CMV status. Empty black circles and black dotted black lines indicate WBC, filled black circles and solid black lines indicate neutrophils, grey diamonds and grey dotted grey lines indicate lymphocytes, filled grey triangles and grey solid lines indicate monocytes. Note that significant positive correlation is observed in older men ($R = 0.468/p = 0.005^{**}$ and $R = 0.492/p = 0.003^{**}$, for WBC and NC respectively).

Fig. 4.
Correlations analysis between BMI and MAP, in young and older donors, split by CMV status.

Table 1

Participant characteristics.

		N	Age	BMI	WBC	Neu Val	Lym Val	Mon Val	MAP	CRP	% AD	% smokers
CMV −	Men	11	25(4)	23.5(4)	5.7(1.1)	3.1(0.9)	1.9(0.3)	0.4(0.1)	98.1(9.9)	N/A	0	N/A
	Women	18	26(4)	23.9(3.5)	6(1.3)	3.7(1.1)	1.7(0.3)	0.5(0.1)	88.8(6.6)	N/A	0	N/A
	Men	34	73(8)	26.3(4.2)	6.3(1.6)	3.8(1.2)	1.7(0.5)	0.6(0.2)	96.8(9.6)	1.5(1.2)	35%	47%
	Women	28	72(8)	25.1(4.9)	6.3(1.5)	3.9(1.5)	1.7(0.4)	0.5(0.2)	96.4(8.3)	1(0.6)	25%	32%
	Men	5	28(1)	24.5(2.4)	5.2(1)	2.6(0.9)	2.1(0.4)	0.4(0.1)	96.7(8.2)	N/A	0	N/A
	Women	18	26(5)	24.1(4.1)	6.6(1.6)	3.8(1.4)	2.2(0.5)	0.4(0.1)	86(7.8)	N/A	0	N/A
	Men	35	71(8)	26.3(3.6)	6.5(1.5)	3.6(1)	2(0.7)	0.6(0.2)	100.4(9.2)	1.3(1.7)	48%	43%
	Women	34	71(8)	25.9(3.5)	5.9(1.3)	3.4(1.1)	1.9(0.4)	0.5(0.1)	98.5(10)	1.2(2.4)	38%	35%
CMV +												

Donors' age (years). BMI (body mass index calculated as (weight (kg)/(height (metres))²), WBC (white blood count per nL), Neu Val/Lym Val/Mon Val (respectively, count of neutrophils/lymphocytes/monocytes per nL), MAP (mean arterial blood pressure, mm Hg) are reported as mean and standard deviation. C-reactive protein (CRP, mg per L) is reported as median value and IQR. CRP and smoking history were not available for younger people. % AD indicates percentages of older people on antihypertensive drugs.

Table 2

Linear regression confirms significant effects gender and NC on MAP in older CMV infected people.

MAP		
Factor	Parameter estimate (95% CI)^a	p^a
Smoker	-2.27 (-8.83 to -3.85)	0.459
Gender (male sex shown)	-29.20 (-47.85 to -10.55)	0.003
Age (years)	0.23 (-0.19 to 0.63)	0.274
BMI	0.15 (-0.74 to 1.05)	0.732
Not on antihypertensive treatment	-0.29 (-6.27 to 5.70)	0.924
Neutrophil count	-3.86 (-7.26 to -0.45)	0.027
Neutrophil count × gender	8.871 (3.93–13.83)	0.001

^aParameter effects and statistical significance (*p*) as determined by linear regression analysis are shown.