Cross-sectional and Longitudinal Relationships between Inflammatory Biomarkers and Frailty in Community-dwelling Older Men: the Concord Health and Ageing in Men Project

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ABSTRACT

Background: Previous studies demonstrated associations between IL-6 and frailty, but associations between a wide range of cytokines, chemokines and growth factors with prevalent and incident frailty has not been studied.

Methods: Community-dwelling men aged 75+ enrolled in the 5-year and 8-year follow-up of the CHAMP study were assessed. Twenty-seven inflammatory biomarkers were measured using the Bio-Plex Pro Human Cytokine 27-plex Assay kit at 5-year follow-up. Frailty was determined using the Fried frailty phenotype (FP) and Rockwood frailty index (FI) at both time-points. Age, BMI, smoking, alcohol and comorbidity were also assessed.

Results: In cross-sectional analysis of the 5-year follow-up, the unadjusted odds ratio for frail versus robust evaluated by the FP showed significant associations for IL-6 (OR:1.56, 95%CI:1.23-1.98) and IL-8 (OR:1.28, 95%CI:1.00-1.63). IL-6 remained significantly associated in the age-adjusted (OR:1.58, 95%CI:1.21-2.05) and multivariable-adjusted model (OR:1.54, 95%CI:1.16-2.05). No associations were observed between pre-frail versus robust. In longitudinal unadjusted analysis, IL-8 (OR:1.32, 95%CI:1.03-1.70) and IP-10 (OR:1.32, 95%CI:1.03-1.70) at 5-year predicted incident frailty at 8-year follow-up. IL-8 remained longitudinally associated with incident frailty after age (OR:1.34, 95%CI:1.03-1.75) but not multivariable (OR:1.20, 95%CI:0.98-1.70) adjustment. Similar results were seen using the FI. None of the other biomarkers had significant associations with incident frailty.

Conclusions: Our findings suggest that IL-6 and IL-8 may be cross-sectionally associated with frailty and that all measured inflammatory biomarkers were not causally related to frailty. Together with previous studies, the results suggest that frailty is specifically linked to IL-6 and IL-8 rather than simply representing a non-specific pan-inflammatory condition.

Keywords: cytokines, IL-6, epidemiology

INTRODUCTION

Frailty in older adults is strongly associated with poor health outcomes and mortality (1). The pathophysiology and particularly the role of inflammatory markers in mediating the effects of frailty remains unclear. Previous studies have reported cross-sectional associations between frailty and higher circulating concentrations of inflammatory markers, particularly C-reactive protein (CRP) and interleukin-6 (IL-6) (2). Elevated CRP, IL-6 and frailty are consistently associated with older age, disability, comorbidity, obesity and other adverse health outcomes (3-5). Older frail adults may also be more vulnerable to dysfunction of their innate immune system, resulting in increased circulating levels of these inflammatory markers, termed 'inflammaging' (6, 7).

To date, studies examining the relationship between inflammatory parameters and frailty have mainly been cross-sectional. Most have only evaluated IL-6, while some also studied TNF-α and its receptor (2). Only three longitudinal studies have been done and examined; none showed any association between higher inflammatory biomarker levels and incidence of frailty (8-10). The inflammatory biomarkers investigated in these longitudinal studies were limited to IL-6 and CRP, with one study also further examining interleukin-1β (IL-1β) and interleukin-10 (IL-10). It is unclear if IL-6 and CRP are unique in their association with frailty, there are many other key pro-inflammatory and anti-inflammatory cytokines which may play an important role in frailty.

The objective of our study was to evaluate in older men the (a) cross-sectional associations between 27 circulating cytokines, chemokines and growth factors with frailty and (b) longitudinal associations between these 27 inflammatory biomarkers and incident frailty over 3-years.

METHODS

CHAMP is an epidemiological study of a wide range of health issues in Australian men aged 70 years and over (11). The recruitment enrollment selection of study participants has been described in detail elsewhere (11). Briefly, CHAMP involves men living in a defined urban geographical region (the Local Government Areas of Burwood, Canada Bay and Strathfield) near Concord Hospital in Sydney, Australia. The sampling frame was the New South Wales Electoral Roll, on which registration is compulsory in Australia. The only exclusion criterion was living in a residential aged care facility. Eligible men were sent a letter describing the study and, if they had a listed telephone number, were telephoned about one week later. Of the 2815 eligible men with whom contact was made, 1511 participated in the study (54%). An additional 194 eligible men living in the study area heard about the study from friends or the local media and asked to be recruited before receiving a letter, yielding a total cohort of 1705 subjects at the initial study wave.

The following study described in this paper includes data from the 5-year follow-up (n=901) and 8-year follow-up (n=631). At the 5-year follow-up, men completed a questionnaire at home before coming to the study clinic at Concord Hospital. The clinic visit consisted of physical performance measures, biological measures, medication inventory and neuropsychological testing. Data were collected by fully trained staff and the same equipment was used for all measurements and assessments, which were carried out in a single clinic. At the 8-year follow-up, assessments were conducted in participant's homes. Most, but not all, using the same measures

done as at the 5-year follow-up were repeated at 8-year except that biological measures were not collected.

Inflammatory biomarkers

Participants had an early morning fasting blood sample taken at the 5-year follow-up, with serum stored at -80 C until assay. Thawed serum blood was analyzed for 27 cytokines, chemokines and growth factors using the Bio-Plex Pro Human Cytokine 27-plex Assay kit (Bio-Rad) at the Australian Proteome Analysis Facility. The kit was used to detect FGF basic, Eotaxin, G-CSF, GM-CSF, IFN-γ, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, IP-10 (CXCL-10), MCP-1 (MCAF), MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α, and VEGF simultaneously from each serum sample. All samples were analyzed on 96-well plates using 50 µl of undiluted serum for each replication. The standards and samples were assayed on a robotic liquid handling workstation (epMotion 5075, Eppendorf, Germany) and the plates were read with the Bio-Plex Systems 200 (Bio-Rad, CA, USA).

Frailty Measurement

In the primary analysis, frailty was defined using Fried frailty phenotype (FP) criteria in the Cardiovascular Health Study (CHS) (12). The FP is comprised of five criteria: weight loss, exhaustion, low activity, slowness, and weakness. Adapted criteria were used for weight loss,

exhaustion, and low activity because the exact measurements used in the CHS were not available. Weight loss was indicated if a participant's current weight was at least 15% lower than lifetime maximum weight. Exhaustion/lethargy was indicated by subjects reporting having a lot of energy over the past 4 week either a little of the time or none of the time on the SF-12. Low physical activity was indicated by a subject's falling into the lowest sample quartile of activity as measured by the Physical Activity Scale for the Elderly (a score of 72 or lower among CHAMP subjects). Adapted criteria were used for weight loss, exhaustion, and low activity because the exact measurements used in the CHS were not available. For detailed descriptions of these criteria, see previous publications (13). At both study assessments, participants were classed as robust if they met none of the criteria, pre-frail if they met one or two of the criteria, and frail if they met three or more of the criteria. In sub-analysis, frailty was determined by calculating the Rockwood frailty index (FI) using the 29 items as shown in our previous CHAMP study (14) which was a modification of the FI (15).

Other Measurements

Body mass index (BMI) was calculated from clinic measurements of height and weight. Alcohol consumption and smoking status (current, ex- or never smoker) was by self-reported questionnaires. A comorbidity score was calculated as the sum of all conditions reported from the 19 disorders listed in the questionnaire: diabetes, thyroid dysfunction, osteoporosis, Paget's disease, stroke, Parkinson's disease, kidney stone, dementia, depression, epilepsy, hypertension,

heart attack, angina, congestive heart failure, intermittent claudication, chronic lung disease, liver disease, chronic kidney disease, or arthritis.

Statistical Analysis

Descriptive characteristics were generated for the analytic sample at the 5-year follow-up. Of the 27 inflammatory biomarkers measured, IL-2, IL-15 and GM-CSF were excluded for statistical analysis because more than 25% were below the limits of detection. The remaining 24 measured biomarkers were log-transformed for final analysis.

Cross-sectional analysis at the 5-year follow-up assessed associations between the 24 measured biomarkers, and FP (robust vs pre-frail and robust vs frail) using logistic regression analysis and FI (continuous variable) using multiple regression. Results were reported per 1SD increase in circulating concentrations. In the longitudinal analysis, logistic regression was also used to examine associations between inflammatory biomarkers at 5-year and incident FP at 8-year. This analysis only included men who were robust at 5-years, and compared men remaining robust to men deteriorating from robust to pre-frail or frail. The longitudinal analysis for FI examined the change in the frailty index score from 5-year to 8-year follow-up. The model building for all analyses included covariates of significance and relevance, with in which age, BMI, smoking status, alcohol and comorbidity were taken into account in the multivariable analyses. In sub-analysis, the use of NSAIDS and steroids medication was further adjusted for in the multivariable model. Models were fit using SPSS software version 20 (IBM Corp., Armonk, NY, USA) and SAS software

9.3 (SAS Institute Inc., Cary, NC, USA). Correlations were performed using Pearsons correlation coefficient. These analysis were performed on Sigmaplot v11 (Systat Software Inc, Germany). A correlogram was performed in R version 3.1.0 (The R Foundation for Statistical Computing) with the corrplot package. Factor analysis was used to reduce the 24 inflammatory markers into smaller sets of factors that account for most of the variance of the inflammatory variables.(16) The number of factors retained was based on the following rules: eigenvalues greater than or equal to 1 or factors above the break in the scree plot. A varimax rotation was used to obtain a set of independent and best interpretable factors. The factors were interpreted based on the loadings that relate the markers to the factors and loadings greater than or equal to 0.40 were used to identify the variables comprising a factor. Factor scores were calculated for each participant.

Ethics Approval

CHAMP was approved by the Concord Hospital Human Research Ethics Committee, and study participants provided written informed consent for all procedures.

RESULTS

The descriptive characteristics of the analytic sample are shown in Table 1. The mean age of the study population was 81.3 (SEM=0.2). At 5-year follow-up, 44% (n=394) of the participants' frailty status were robust, 47% (n=426) were pre-frail and 9% (n=81) were frail. Of the 394 men

who were robust at 5-year, 311 men had frailty data at 8-year. Of the 311 men at 8-year follow-up, 43% (n=133) remained robust, 52% (n=162) became pre-frail and 5% (n=16) became frail. The detection limit and coefficient of variation for each of the measured inflammatory biomarkers are shown in Supplementary Table 1.

The only inflammatory biomarker that was significantly associated with age was IP-10 (Pearsons correlation coefficient: 0.1, data not shown). There were strong positive correlation coefficients between many of the inflammatory biomarkers (Figure 1). IL-6 was shown to be strongly correlated with IL-10, IL-12 and MCP-1.

The cross-sectional associations between the inflammatory biomarkers and frailty at the 5-year follow-up are shown in Table 2. In unadjusted analysis, for each SD increase in IL-6, men have odds ratio of 1.56 (95%CI: 1.23-1.98) more likely to be frail when compared to robust men. There were no statistically significant association with IL-6 between robust and pre-frail (OR: 1.01, 95%CI: 0.87-1.17). Whereas for each SD increase in IL-8, men have odds ratio of 1.16 (95%CI: 1.00-1.33) more likely to be pre-frail and 1.28 (95%CI: 1.00-1.63) for being frail. Circulating IL-6 remained statistically significantly associated after age (OR: 1.58, 95%CI: 1.21-2.05) and multivariable (OR: 1.54, 95%CI: 1.16-2.05) adjustment when comparing robust and frail men. Further adjustment of NSAIDS and steroid medication did not attenuate the observed associations between IL-6 and frailty.

The longitudinal associations between inflammatory biomarkers and incident frailty are shown in Table 3. In unadjusted analysis, for each SD increase in IL-8, the men was associated with

increased odds (OR: 1.32, 95%CI: 1.03-1.70) for developing frailty (either pre-frail or frail). Likewise, IP-10 was associated with incident frailty (OR: 1.32, 95%CI: 1.03-1.70) in the unadjusted model. IL-8 remained associated (OR: 1.34, 95%CI: 1.03-1.75) with incident frailty when adjusted for age. However in the multivariable-adjusted analysis, the association between IL-8 and incident frailty was no longer statistically significantly associated (OR: 1.29, 95%CI: 0.98-1.70). No other inflammatory biomarkers had any significant association with frailty.

We also conducted the cross-sectional and longitudinal analysis between inflammatory biomarkers and the FI (see Supplementary Table 2). Similar findings were shown in IL-6 and IL-8 with our primary analysis using the FP. For each SD increase in IL-6 cross-sectionally, it was associated with frailty in unadjusted (β=0.009, 95%CI: 0.001, 0.02) and age-adjusted (β=0.008, 95%CI: 0.0005, 0.02) model. Likewise, for each SD increase in IL-8 cross-sectionally, it was associated with frailty in unadjusted (β=0.01, 95%CI: 0.004, 0.02) and age-adjusted (β=0.009, 95%CI: 0.002, 0.02) model. In multivariable-adjusted model, IL-8 remained statistically significantly associated with frailty (β=0.01, 95%CI: 0.003, 0.02), whereas IL-6 was shown to be borderline statistically significantly associated (β=0.006, 95%CI: -0.001, 0.01). Similar to the FP, no longitudinal associations were observed between the inflammatory biomarkers and the FI.

We further conducted factor analysis by grouping inflammatory biomarkers with similar patterns of responses (data not shown). There were 5 factor groupings of which the factor consisting of IFN-γ, IL-4, IL-1β, IL-1ra, G-CSF, IL-8, IL-6, IL-7 was shown to be associated with FP in both unadjusted (OR:1.40, 95%CI:1.06-1.87) and age-adjusted (OR:1.48, 95%CI:1.10-2.00).

However, the observed association was no longer statistically significant in the multivariable-adjusted model. There were no unadjusted or multivariable-adjusted associations between any of the 5 factor groupings and the FI.

DISCUSSION

This study provides a comprehensive examination of the relationships between a wide range of circulating serum cytokines, chemokines and growth factors with frailty in older men. Consistent with previous studies, cross-sectional associations, but not longitudinal associations, were observed between IL-6 and frailty. This indicates that IL-6 is not associated with developing frailty over time but instead it may be elevated only when frailty has developed. None of the other measured inflammatory biomarkers were associated with frailty, suggesting that frailty is specifically linked to IL-6 rather than simply being a non-specific pan-inflammatory condition. Traditionally IL-6 has been classified as a pro-inflammatory cytokine, although like most other cytokines has pleiotropic effects including its effects on inflammation.(17)

The association between IL-6 and aging in mice and humans has been recognized for decades and is proposed to contribute to the inflammation associated with a range of age-related conditions (18), an observation which prompted early studies of the relationship between elevated IL-6 and frailty (19, 20). A recent systematic review and meta-analysis of 32 cross-sectional studies found that have observed older participants with frailty and pre-frailty tended to have higher serum IL-6 and CRP levels when compared to robust participants (2). Furthermore, the

meta-regression analyses suggested that age and BMI are on the pathway between these inflammatory markers and frailty. The majority of these cross sectional studies have only examined IL-6 while a few have also examined TNF-α and its receptor, often in association with CRP as a global measure of inflammation (Supplementary Table 3). The majority of these cross-sectional studies found a positive association between IL-6 and frailty, consistent with our results.

A few studies examined other inflammatory biomarkers including IL-10, IL-1b, RANTES, MCP-1, IP-10 (8, 21-23) while the recent exploratory study from the Singapore Longitudinal Aging Study Wave 2 evaluated 89 blood markers of inflammation including those that we measured (24). Overall, our results together with these other reports indicate that IL-6 is the cytokine that is most robustly associated with frailty. We also found a weak association between IL-8 and frailty, which suggests it might also have a role in frailty. Apart from the negative cross sectional result published in the Singaporean study (24), the role of IL-8 in frailty has not been previously reported. Like IL-6, IL-8 is a pro-inflammatory cytokine, but in our study was not correlated closely with IL-6 levels. Future studies on frailty and aging will be required to confirm this interesting finding. We also found a weak association between IP-10 on unadjusted longitudinal analysis while IP-10 was the only cytokine associated with statistically with chronological age over the age of 70 years. Previous study have found an association between monocyte gene expression of IP-10 with frailty and noted that this was closely correlated with circulating IL-6 levels (23). By contrast we did not find any association between IP-10 and IL-6 levels.

In contrast to the robust finding in cross-sectional studies, analyses from longitudinal reveal no association between IL-6 and incident frailty. (Supplementary Table 3, (2)). Our findings were consistent with the three previous longitudinal studies to have examined this relationship in older adults, the Hertfordshire Ageing Study (n=254) of 10 years, the Longitudinal Aging Study Amsterdam (n=885) of 3 years and a nested case-control study within the Women's Health Initiative Study (n=1800) of 3 years (3, 25, 26). Only IL-6 and CRP were examined in all three studies with the most recent Hertfordshire study examining in addition, IL-1β and IL-10.

The novelty of our study was to examine not just the conventional inflammatory biomarkers (IL-6 and TNF-α), but also to examine a range of other biomarkers, a total of 27 cytokines, chemokines and growth factors. Therefore, we were able to identify a strong relationship with frailty only in IL-6 but not the other remaining 26 measured inflammatory biomarkers. It is plausible to suggest that frailty is not simply an inflammatory condition, instead may be specifically related to the IL-6 inflammatory biomarker. Nonetheless, further longitudinal research is warranted to verify and replicate our findings.

The reason for the association between source of elevated levels of IL-6 and other inflammatory biomarkers in frailty remains unclear. It has been proposed that cellular senescence and the senescent associated secretory phenotype (SASP) might provide one mechanism (25). Cellular senescence is known to contribute and promote age-related diseases, frailty and dysfunction (27). Senescence inducers causes DNA damage, oncogenic mutations, reactive metabolites, high-mitogen signals and proteotoxic stress which initiates the senescence response

288 through activating tumor suppressor genes (25). Once the senescence is activated, the process is
289 reinforced by DNA damage-induced reactive oxygen species, transforming growth factors and
290 cytokines such as IL-6 (26, 28, 29), ultimately developing senescence-associated secretory
291 phenotype (SASP) (30). Chronic inflammation and tissue dysfunction is then derived from those
292 senescent cells by either infiltrating immune cells or provoking phenotypic changes of the immune
293 system. Studies have shown that SASP cytokines, IL-6 and IL-8, may stimulate angiogenesis, disrupt
294 cell-cell communication, impede macrophage function, induce innate immune responses, and
295 promote epithelial and endothelial cell migration and invasion (31, 32). Furthermore, these SASP
296 cytokines reinforces the senescence growth arrest and causes epithelial to mesenchymal
297 transitions (33). Hence, since IL-6 and IL-8 are associated with the SASP, our findings are consistent
298 with a role for cellular senescence and SASP in the elevated levels of cytokines in frailty, and
299 perhaps even in the pathogenesis of frailty.

300 The strengths of this study include the use of longitudinal data to investigate a
301 comprehensive profile of 27 cytokines, chemokines and growth factors in conjunction with frailty
302 over time. A further strength of CHAMP is that it includes a large and representative group of older
303 Australian men, as demonstrated by similar socio-demographic and health characteristics
304 compared to older men in the nationally representative MATeS study (34).

305 Since this study was based on the 5th and the 8th year follow-up of the CHAMP study, a
306 significant limitation is the impact of survivor bias. This applies both to men who remained in the
307 study whereas the less healthy may have already died or became frail before reaching the follow-

up assessment. In addition, survivor bias may also apply to the men who are lost to follow-up in the study whether due to death or other reasons. We also had a relatively short follow-up of 3-years, hence, the results may change with longer follow-up periods. Our study was limited to community-dwelling men and so may not apply to women, similarly it may not extrapolate to non-European populations which were not strongly represented in the CHAMP study. Finally, we were unable to measure CRP, a widely used marker of inflammation, since the blood sample analyzed were from stored frozen blood analysis.

In conclusion, we have shown that elevated IL-6 levels may be cross-sectionally associated, but not casually related, with frailty in older men. No other measured inflammatory biomarkers were associated with prevalent or incident frailty. Future work is required to better understand if these cytokines could be used as potential biomarkers of frailty.

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Conflict of interest disclosures: B.H., V.H., R.G.C., V.N., F.M.B., F.C.W., L.M.W., M.J.S., D.J.H. and D.G.L.C. have nothing to declare.

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Table 1. Characteristics of the analytic sample (n=901)

	Mean (SEM) or N (%)
Age (year)	81.3 (0.2)
BMI (kg/m^2)	27.5 (0.1)
No. of Comorbidity	2.5 (0.1)
Alcohol per week	8.3 (0.3)
Smoking Status	
Non-smoker	366 (41%)
Ex-smoker	515 (56%)
Current smoker	30 (4%)
IL-1β	1.1 (0.1)
IL-1ra	29.8 (1.4)
IL-4	1.6 (0.1)
IL-5	3.6 (0.2)
IL-6	6.6 (0.7)
IL-7	5.4 (0.4)
IL-8	9.8 (0.2)
IL-9	9.3 (0.6)
IL-10	17.7 (3.2)
IL-12	7.8 (1.0)
IL-13	2.0 (0.2)
IL-17	13.7 (0.3)
Eotaxin	77.7 (1.4)
FGF-basic	9.1 (0.7)
G-CSF	25.2 (1.2)
IFN-γ	41.6 (2.5)
IP-10	365.9 (9.2)
MCP-1 (MCAF)	24.3 (1.3)
MIP-1α	0.8 (0.01)
PDGF-BB	272.3 (7.5)
MIP-Iβ	6.9 (0.2)
RANTES	51.0 (1.0)
TNF-α	13.8 (1.0)
VEGF	22.2 (0.8)
Frailty phenotype (FP)	
Robust	394 (44%)
Pre-frail	426 (47%)
Frail	81 (9%)
Frailty index (FI)	0.21 (0.1)

Table 2. Cross-sectional associations between inflammatory biomarkers and frailty phenotype

	Unadjusted		Age-adjusted		Multivariable-adjusted*	
	Prefrail	Frail	Prefrail	Frail	Prefrail	Frail
IL-1β	1.04 (0.91-1.20)	0.95 (0.75-1.21)	1.06 (0.91-1.22)	0.97 (0.75-1.24)	1.06 (0.91-1.23)	0.97 (0.74-1.27)
IL-1ra	1.01 (0.88-1.16)	1.15 (0.91-1.45)	1.04 (0.90-1.21)	1.16 (0.90-1.48)	1.05 (0.90-1.22)	1.23 (0.95-1.59)
IL-4	0.97 (0.84-1.11)	0.95 (0.75-1.21)	0.99 (0.86-1.15)	0.97 (0.74-1.27)	1.00 (0.86-1.17)	1.06 (0.79-1.43)
IL-5	0.95 (0.81-1.11)	1.11 (0.84-1.46)	0.95 (0.81-1.12)	1.09 (0.82-1.46)	0.80 (0.83-1.15)	1.10 (0.80-1.51)
IL-6	1.01 (0.87-1.17)	**1.56 (1.23-1.98)**	1.01 (0.87-1.17)	**1.58 (1.21-2.05)**	1.00 (0.86-1.16)	**1.54 (1.16-2.05)**
IL-7	0.91 (0.79-1.05)	0.94 (0.73-1.19)	0.96 (0.82-1.11)	1.01 (0.78-1.29)	0.97 (0.83-1.13)	1.00 (0.76-1.32)
IL-8	**1.16 (1.00-1.33)**	**1.28 (1.00-1.63)**	1.15 (0.99-1.33)	1.25 (0.96-1.62)	1.13 (0.97-1.32)	1.20 (0.91-1.58)
IL-9	0.97 (0.84-1.11)	0.99 (0.78-1.25)	0.98 (0.85-1.13)	1.02 (0.79-1.30)	0.97 (0.84-1.12)	1.02 (0.78-1.32)
IL-10	0.94 (0.82-1.09)	0.82 (0.64-1.06)	0.95 (0.82-1.10)	0.81 (0.61-1.06)	0.97 (0.83-1.13)	0.86 (0.64-1.15)
IL-12	0.98 (0.85-1.13)	1.06 (0.82-1.37)	0.98 (0.85-1.14)	1.03 (0.78-1.36)	0.98 (0.85-1.14)	1.05 (0.77-1.43)
IL-13	1.00 (0.86-1.17)	1.11 (0.85-1.45)	1.04 (0.89-1.21)	1.21 (0.89-1.63)	1.05 (0.90-1.24)	1.28 (0.92-1.78)
IL-17	0.96 (0.83-1.11)	1.00 (0.78-1.29)	0.99 (0.85-1.14)	1.06 (0.81-1.39)	0.98 (0.85-1.15)	1.06 (0.80-1.40)
Eotaxin	1.14 (0.99-1.31)	1.33 (0.99-1.76)	1.14 (0.99-1.31)	1.32 (0.98-1.78)	1.13 (0.98-1.31)	1.36 (0.99-1.87)
FGF-basic	0.96 (0.81-1.13)	1.24 (0.91-1.69)	0.97 (0.81-1.15)	1.23 (0.88-1.72)	0.97 (0.81-1.16)	1.24 (0.87-1.78)
G-CSF	0.97 (0.84-1.11)	0.97 (0.76-1.24)	0.97 (0.84-1.12)	0.95 (0.73-1.22)	0.98 (0.84-1.13)	1.07 (0.79-1.45)
IFN-γ	1.04 (0.90-1.19)	1.14 (0.90-1.44)	1.05 (0.91-1.21)	1.11 (0.87-1.42)	1.07 (0.92-1.24)	1.20 (0.92-1.56)
IP-10	1.11 (0.97-1.27)	0.98 (0.78-1.24)	1.06 (0.92-1.23)	0.92 (0.73-1.17)	1.06 (0.91-1.22)	0.93 (0.72-1.20)
MCP-1 (MCAF)	1.04 (0.89-1.22)	1.10 (0.84-1.45)	1.08 (0.92-1.27)	1.12 (0.84-1.49)	1.12 (0.95-1.32)	1.24 (0.90-1.70)
MIP-1α	1.01 (0.88-1.16)	1.01 (0.79-1.29)	1.01 (0.88-1.17)	0.99 (0.76-1.30)	1.00 (0.87-1.16)	0.96 (0.73-1.27)
PDGF-BB	1.05 (0.91-1.21)	0.91 (0.73-1.15)	1.07 (0.93-1.24)	0.95 (0.74-1.22)	1.07 (0.92-1.24)	0.96 (0.74-1.26)
MIP-Iβ	1.02 (0.88-1.18)	1.09 (0.84-1.43)	1.02 (0.88-1.18)	1.12 (0.84-1.50)	1.03 (0.88-1.19)	1.12 (0.82-1.52)
RANTES	1.01 (0.88-1.15)	0.94 (0.74-1.20)	1.01 (0.87-1.16)	0.96 (0.74-1.23)	1.02 (0.88-1.18)	0.95 (0.73-1.25)
TNF-α	1.01 (0.88-1.16)	1.09 (0.85-1.40)	1.02 (0.88-1.17)	1.07 (0.82-1.39)	1.02 (0.88-1.18)	1.10 (0.82-1.47)
VEGF	0.96 (0.83-1.10)	1.10 (0.84-1.45)	0.95 (0.82-1.09)	1.06 (0.80-1.42)	0.92 (0.79-1.07)	1.02 (0.75-1.38)

*Multivariable model adjusted for age, BMI, smoking, alcohol and comorbidity

†For per one-SD increase in inflammatory biomarkers.

Table 3. Longitudinal associations between inflammatory biomarkers at baseline and incident frailty over 3 year follow-up

	Unadjusted	Age-adjusted	Multivariable-adjusted*
IL-1β	1.12 (0.89-1.41)	1.10 (0.87-1.39)	1.12 (0.89-1.42)
IL-1ra	1.20 (0.93-1.55)	1.26 (0.97-1.64)	1.32 (1.00-1.73)
IL-4	1.19 (0.94-1.49)	1.21 (0.95-1.52)	1.20 (0.95-1.52)
IL-5	1.15 (0.88-1.49)	1.14 (0.87-1.48)	1.14 (0.87-1.49)
IL-6	1.03 (0.83-1.30)	1.05 (0.84-1.32)	1.06 (0.84-1.34)
IL-7	1.03 (0.81-1.32)	1.05 (0.82-1.35)	1.06 (0.82-1.36)
IL-8	**1.32 (1.03-1.70)**	**1.34 (1.03-1.75)**	1.29 (0.98-1.70)
IL-9	1.21 (0.96-1.52)	1.23 (0.97-1.56)	1.25 (0.98-1.60)
IL-10	0.93 (0.75-1.17)	0.92 (0.73-1.16)	0.93 (0.74-1.17)
IL-12	1.03 (0.82-1.28)	1.05 (0.84-1.32)	1.05 (0.83-1.32)
IL-13	1.13 (0.88-1.44)	1.14 (0.89-1.47)	1.16 (0.90-1.50)
IL-17	1.14 (0.89-1.46)	1.15 (0.89-1.47)	1.15 (0.89-1.48)
Eotaxin	0.94 (0.74-1.17)	0.94 (0.75-1.19)	0.90 (0.71-1.14)
FGF-basic	1.32 (0.98-1.77)	1.32 (0.98-1.79)	1.36 (0.99-1.89)
G-CSF	1.13 (0.89-1.43)	1.12 (0.89-1.42)	1.10 (0.87-1.40)
IFN-γ	1.12 (0.89-1.42)	1.12 (0.89-1.43)	1.16 (0.91-1.47)
IP-10	**1.32 (1.03-1.70)**	1.26 (0.98-1.64)	1.26 (0.97-1.63)
MCP-1 (MCAF)	1.11 (0.87-1.42)	1.20 (0.93-1.54)	1.26 (0.97-1.64)
MIP-1α	1.11 (0.88-1.39)	1.12 (0.89-1.42)	1.09 (0.86-1.38)
PDGF-BB	1.09 (0.88-1.36)	1.10 (0.88-1.38)	1.08 (0.86-1.35)
MIP-Iβ	1.07 (0.84-1.36)	1.05 (0.83-1.35)	1.05 (0.82-1.35)
RANTES	1.10 (0.86-1.40)	1.10 (0.86-1.41)	1.11 (0.86-1.43)
TNF-α	0.95 (0.76-1.18)	0.97 (0.77-1.21)	0.96 (0.77-1.21)
VEGF	0.99 (0.79-1.24)	0.99 (0.78-1.24)	0.97 (0.77-1.24)

*Multivariable model adjusted for age, BMI, smoking, alcohol and comorbidity

†For per one-SD increase in inflammatory biomarkers.

Figure 1. Correlogram of the correlation coefficients between cytokines, chemokines and growth factors. The strength of correlation is represented by the intensity of shading.