Association of markers of inflammation, the kynurenine pathway and B vitamins with age and mortality, and a signature of inflammaging

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ABSTRACT

Background: Inflammation is a key feature of aging. We aimed to i) investigate the association of 34 blood markers potentially involved in inflammatory processes with age and mortality, ii) develop a signature of 'inflammaging'.

Methods: Thirty-four blood markers relating to inflammation, B vitamin status and the kynurenine pathway were measured in 976 participants in the Melbourne Collaborative Cohort Study at baseline (median age=59 years) and follow-up (median age=70 years). Associations with age and mortality were assessed using linear and Cox regression, respectively. A parsimonious signature of inflammaging was developed and its association with mortality was compared with two marker scores calculated across all markers associated with age and mortality, respectively.

Results: The majority of markers (30/34) were associated with age, with stronger associations observed for neopterin, cystatin C, IL-6, TNF-α, several markers of the kynurenine pathway and derived indices KTR (kynurenine/tryptophan ratio), PAr index (ratio of 4-pyridoxic acid and the sum of pyridoxal 5´-phosphate and pyridoxal), and HK:XA (3-hydroxykynurenine/xanthurenic acid ratio). Many markers (17/34) showed an association with mortality, in particular IL-6, neopterin, CRP, quinolinic acid, PAr index, and KTR. The inflammaging signature included ten markers and was strongly associated of mortality (HR per SD=1.40, 95%CI:1.24-1.57, $P=2x10^{-8}$), similar to scores based on all age-associated (HR=1.38, 95%CI:1.23-1.55, $P=4x10^{-8}$) and mortality-associated markers (HR=1.43, 95%CI:1.28-1.60, $P=1x10^{-10}$), respectively. Strong evidence of replication of the inflammaging signature association with mortality was found in the Hordaland Health Study.

Conclusion: Our study highlights the key role of the kynurenine pathway and vitamin B6 catabolism in aging, along with other well-established inflammation-related markers. A signature of inflammaging based on ten markers was strongly associated with mortality.

Keywords: biological aging; kynurenines; vitamin status; inflammaging; biomarkers

INTRODUCTION

Aging is characterised by "a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death" **(1)**. One pathophysiological change that underlies aging is inflammation, which is thought to cause or underlie many chronic diseases such as cancer, atherosclerosis, diabetes and Alzheimer's disease **(2, 3).** The term "inflammaging" has been used to describe the chronic, sterile, low-grade inflammation occurring during aging **(3)**.

Many blood markers of inflammatory processes have been proposed and assessed for their association with disease risk. More recently, Franceschi et al. identified circulating tryptophan concentration as being involved in inflammaging **(3)**. In liver, the bulk of ingested tryptophan is converted via a group of intermediates, called kynurenines, to acetyl coenzyme A by the substrate-induced tryptophan dioxygenase with small but important fractions directed towards the synthesis of nicotinamide adenine dinucleotide (NAD+) and picolinic acid, a cation chelator **(4)**. In other tissues, notably hematopoietic and endothelial, the first step in this pathway is catalysed by indoleamine 2,3-dioxygenase, activated by a combination of oxidative stress and pro-inflammatory stimuli, including interferon-γ (IFN-γ). IFN-γ also induces macrophage production of neopterin. The kynurenine/tryptophan ratio and neopterin have been proposed as markers of IFN-γ activity and all three are considered as inflammatory biomarkers **(5)**. The metabolites downstream of kynurenine, many of which are considered immunomodulatory, are formed by vitamin B6 and B2-dependent enzymes and include kynurenic acid (KA), 3-hydroxykynurenine (HK), xanthurenic acid (XA), anthranilic acid (AA) and 3-hydroxyanthranilic acid (HAA). The ratios HK:XA **(6)** and HKr (HK/(KA+AA+XA+HAA), **(7)**) reflect functional vitamin B6 status **(6)**. Plasma concentrations of vitamin B6 in particular but also B2 appear to be determinants of plasma concentrations of several kynurenines in a healthy population, consistent with their role as co-factors for rate limiting enzymes prompting

the recommendation that measures of B6 and B2 should be included in epidemiological studies assessing the association of the kynurenine pathway with health and disease **(8)**.

Using a total of 34 markers related to inflammation, B vitamin status and the kynurenine pathway measured at two time points approximately ten years apart, we aimed to: i) describe the overall pattern of correlation between markers, ii) assess their individual associations with age, iii) assess their individual associations with mortality, and iv) develop a signature of 'inflammaging' and assess its association with mortality.

METHOD

The Melbourne Collaborative Cohort Study (MCCS)

The MCCS is a prospective cohort study of 17,044 men and 24,469 women aged 27-88 years at recruitment, 99.3% of whom were aged 40-69 years. Recruitment occurred between 1990 and 1994 **(9, 10)**. Southern European migrants to Australia (including 5,411 Italians and 4,525 Greeks) were over-sampled to extend the range of lifestyle exposures. Subjects were recruited via the Electoral Roll (registration to vote is compulsory for adults in Australia), advertisements, and community announcements in local media. At recruitment participants' height and weight were measured, blood samples collected and questionnaires covering lifestyle (diet, smoking, physical activity and alcohol consumption), demographics and medical history completed. A follow-up survey conducted between 2003 and 2007 (wave 2) repeated most baseline measures, including blood sample measurements. The majority of participants (90%) were fasting when blood was collected. Demographic variables were obtained by questionnaires at baseline and wave 2, and included age, sex, country of birth and highest level of education attained. Study participants provided informed consent in accordance with the Declaration of Helsinki. The study was approved by Cancer Council Victoria's Human

Research Ethics Committee and performed in accordance with the institution's ethical guidelines. A total of 976 participants were included in this sub-study. A flowchart of the study is presented in **Supplementary Figure 1**.

Biochemical analyses

Plasma samples were stored in liquid nitrogen at -180°C from the time of collection until shipment to the Bevital laboratory (www.bevital.no) for biochemical analyses. Markers related to vitamin status included: plasma concentrations of thiamine (vitamin B1), riboflavin and flavin mononucleotide (vitamin B2), nicotinamide, N1-methylnicotinamide (vitamin B3), and pyridoxal 5'-phosphate, pyridoxal, and 4-pyridoxic acid (vitamin B6). As part of the tryptophan-kynurenine pathway, we measured plasma concentrations of kynurenine, tryptophan and downstream metabolites, 3-hydroxykynurenine, kynurenic acid, xanthurenic acid, anthranilic acid, 3-hydroxyanthranilic acid, picolinic acid, and quinolinic acid. These markers in addition to cystathionine, neopterin and trigonelline were measured with liquid chromatography-tandem mass spectrometry **(11)**. C-reactive protein, serum amyloid A, calprotectin, cystatin C and were measured using matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry **(12)**. A plasma sample was included as a quality control in all batches. Resources detailing the platforms used for marker measurement and their reliability are provided in the **Online Supplementary Material**. All markers were found suitable for reliable measurement in large-scale epidemiological studies **(13-15)**.

Separate aliquots were shipped to the laboratories of IARC in Lyon France for measurement of other markers of inflammation including interleukin 6, 8, 10, and 13 (IL-6, IL-8, IL-10, and IL-13, respectively, in pg/mL), interleukin 8 (IL-8, in pg/mL), interleukin 10 (IL-10, in pg/mL), interleukin 13 (IL-13, in pg/mL), interferon-γ (IFN-γ ,in pg/mL) and tumour necrosis factor alpha (TNF-α, in pg/mL), using Meso Scale Discovery 6-Plex kit. Marker values below their limit of quantification (LOQ) were assigned the value LOQ/2 (IL-6: N=19, 1%; IL-8: N=1,

0.1%; IL-10: N=94, 5%; IL-13: N=1,366, 70% (not included in the analyses); IFN-γ: N=2, 0.1%; TNF-α: N=2, 0.1%).

We also calculated the following derived markers: KTR (kynurenine-to-tryptophan ratio), as an indicator of cell-mediate immune activation **(16)**; the PAr index, calculated as the ratio of 4-pyridoxic acid and (pyridoxal + PLP), as a marker of vitamin B6 catabolism during inflammation **(17, 18)**; HK:XA (ratio of 3-hydroxykynurenine and xanthurenic acid) and HKr (HK/(KA+AA+XA+HAA) as indicators of functional vitamin B6 status **(19)**.

Vital status

Vital status up to 31 December 2019 was ascertained through record linkage to the Victorian Registry of Births, Deaths and Marriages (via the Victorian Cancer Registry) and the National Death Index (via the Australian Institute of Health and Welfare). These registries are considered to be virtually complete up to 31 October 2019 and 31 August 2015 (cause of death), respectively. Causes of death were classified as follows, using the International Classification of Disease version 10: cancer: ICD-10 C00 to D49; cardiovascular disease (CVD): ICD-10 I00 to I99; other-cause: all other ICD codes, and missing (N=2).

Statistical analysis

Marker values were sequentially, and separately for baseline and follow-up measures: i) log-transformed to obtain distributions closer to Gaussian, ii) winsorized at 3 standard deviations from the mean to minimise the potential influence of outliers, and iii) re-scaled to Z-scores for easier comparison of effect size estimates between markers. Median and interquartile range (IQR) and Spearman rank order correlations were used as descriptive statistics.

Association between markers and age

We used linear regression models to assess associations between blood markers and age. This was done separately for baseline measures, follow-up measures, and measures from both time points combined (to obtain estimates calculated across a wider age range); for the latter, we used robust standard errors from a mixed model to account for the correlations between participants' repeated measures at baseline and follow-up. These models were adjusted for sex and country of birth (Australia/New-Zealand/other, UK, Italy, Greece).

Association between markers and mortality

We used Cox models to estimate hazard ratios for the association between markers and mortality; these analyses were done separately for baseline and follow-up markers. Age was used as the underlying time scale **(20)**. Time at risk was calculated from the date of follow-up visit to the date of death, date of departure from Australia or end of follow-up (31 October 2019); these models were also adjusted for sex and country of birth. Associations with mortality were also assessed for the first five and ten years of follow-up. In cause-specific analyses, deaths from other causes than that of interest were censored, which is a way to model the cause-specific hazard function, appropriately taking the competing risk of other-cause death into account.

We further evaluated the association of changes in marker levels from baseline to follow-up with overall mortality. The same regression models were used, without and with additional adjustment for marker levels at the start of follow-up.

Inflammaging signature and marker scores

To obtain a signature of inflammaging using a parsimonious set of markers, we proceeded as follows: i) we excluded markers for which the association with age had $P>0.05$; ii) we applied Lasso regression to predict age using the remaining markers. Participants with missing values for any of the markers were excluded (N=39), leaving 937 participants for the analysis. Lasso

regression was conducted using the R package *glmnet*, and the regularization parameter *lambda* was obtained by 5-fold cross-validation, using the one-standard error rule to reduce overfitting **(21, 22)**; iii) we ran 1,000 iterations of the Lasso and markers selected in >80% of the models were retained to build the final model, using their mean coefficient.

In addition, we calculated, using the same sample, two weighted scores representing the combined effect of blood markers: i) the weighted average of marker values for all markers showing an association with age at P<0.05, using as weights the regression coefficients of their association with age; ii) the weighted average of marker values for all markers showing an association with mortality at P<0.05, using as weights the log(HR) of their association with mortality. All three scores were calculated using follow-up data and further re-scaled as Z-scores to facilitate the comparison with individual markers.

Replication analysis

We attempted replication of the lasso-based signature of inflammaging using data from the Hordaland Health Study (HUSK) in which baseline examinations were conducted in Hordaland County, western Norway, in 1998–1999 (https://husk.b.uib.no). Details of the cohort have been described previously **(23)**. The study population consisted of 7,051 men and women born during 1925–1927 and 1950–1951 who had previously (1992–1993) participated in the Hordaland Homocysteine Study **(24)**. Associations with mortality were estimated using the same models as in the MCCS, and also stratified by sex, length of follow-up and cause of death. To calculate the signature, we used the weights obtained in the MCCS for all markers available in the HUSK study.

All analyses were carried out using R version 3.6.1.

RESULTS

Overall correlation pattern:

The distribution of marker levels is shown in **Table 1** and **Supplementary Figure 2**. Spearman correlations between baseline and follow-up marker values were generally moderate (median: 0.41, IQR: 0.33-0.51), **Table 1**. Spearman correlations between blood marker values are shown in **Supplementary Figure 3.** Correlations were stronger for markers within the same group (kynurenine pathway, vitamin status, and inflammatory markers). All correlations were positive within the kynurenine pathway, ranging between 0.03 and 0.72 at baseline, and between 0 and 0.74 at follow-up, the majority of them being between 0.2 and 0.6. Kynurenine pathway markers also showed moderate correlations with inflammatory markers, in particular neopterin, and with cystathionine and cystatin-C (a marker of kidney function). Positive correlations were also observed within markers of B1, B2, B3 and B6 status, and within other inflammatory markers. Although vitamin status markers showed generally weak and positive correlations with metabolites of the kynurenine pathway, correlations were weak and negative with other inflammatory markers.

Associations of individual markers with sex and country of birth

The associations between levels of blood markers (baseline and follow-up measures combined) and sex and country of birth are shown in **Supplementary Table 1**. Compared with men, women had higher levels of thiamine and TMP, SAA, IL-8, IFN-γ, KTR and HK:XA and HKr, and lower levels of tryptophan, kynurenine, kynurenic acid, xanthurenic acid, 3-hdroxyanthranilic acid, picolinic acid, anthranilic acid, neopterin, cystathionine, cystatin C, serum amyloid A and IFN-γ. Associations were also observed comparing Italy- and Greece-born with Australia-born participants, in particular for plasma concentrations of nicotinamide, N1-methylnicotinamide, tryptophan, anthranilic acid, trigonelline and CRP.

Associations with age

The pattern of associations between markers and age was similar at baseline and follow-up **Figure 1** and **Supplementary Table 1 and 2**. After pooling together baseline and follow-up data and adjusting for sex and country of birth, we observed: i) 30 of 34 markers associated with age (P ranging from 0.04 to 10^{-38}); ii) relatively weak evidence of association for markers of vitamin status; these were negative for TMP, FMN, nicotinamide, N1-methylnicotinamide, and pyridoxal 5-phosphate (PLP), and positive for 4-pyridoxic acid; iii) strong and positive associations for several metabolites in the kynurenine pathway: kynurenine, 3-hydroxykynurenine, kynurenic acid, anthranilic acid, and quinolinic acid, and negative associations for tryptophan and xanthurenic acid; iv) all other markers were positively associated with age, very strong associations being observed for neopterin and cystatin C, and strong for cystathionine, CRP, calprotectin, SAA, IL-6, IL-8, IFN-γ and TNF-α; v) the derived ratios PAr, KTR and HK:XA showed very strong associations with age; the association for HKr was strong.

Associations of individual markers with mortality

The median and interquartile range (IQR) of follow-up time was 14.3 years (12.7 to 15.3 years). Results for the associations between blood markers and all-cause mortality are presented in **Figure 2** and **Supplementary Table 3**. For baseline measures, there was evidence, albeit quite weak, of association with mortality for IL-6, CRP, and PAr index (HR per SD~1.15). For follow-up measures, associations with mortality were observed for several markers: HR per SD>1.25 for IL-6, neopterin, quinolinic acid, KTR, and CRP, and HR per SD>1.10 for cystatin C, HK:XA, calprotectin, PAr index, serum amyloid A, thiamine, TNF-α, anthranilic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and 4-pyridoxic acid Most other markers of inflammation or in the kynurenine pathway had HR>1, but associations were weaker. Inverse associations with mortality were observed for tryptophan and nicotinamide.

Results for the associations between change in marker levels from baseline to follow-up and mortality are shown in **Supplementary Table 4**. Significant associations (P<0.05) were observed for 8 markers (thiamine, anthranilic acid, quinolinic acid, neopterin, cystatin C, TNF-α, PAr index and HK:XAr) but these were substantially attenuated after adjustment for marker levels at the start of follow-up (2003-2007). Only the association observed for thiamine remained similar (HR=1.18, P=0.01).

Associations of inflammaging signature and marker scores with mortality

The Lasso-prediction of age selected 10 markers: nicotinamide, tryptophan, anthranilic acid, quinolinic acid, neopterin, cystatin C, IL-6, IL-8, PAr index, and HK:XA (mean coefficients over 1,000 iterations are provided in **Supplementary Table 5**). The scores based on associations of markers with age and mortality included 29 (**Supplementary Table 2**) and 17 markers (**Supplementary Table 3**), respectively. All three scores showed substantially stronger associations with all-cause mortality than individual markers: score based on 10 markers from lasso regression: HR=1.40, 95%CI: 1.24-1.57, score based on 29 markers associated with age at follow-up: HR=1.38, 95%CI: 1.23-1.55, score based on 17 markers associated with mortality at follow-up: HR=1.43, 95%CI: 1.28-1.60. These associations were similar in men and women (**Table 2**). For all three scores, the associations appeared larger in the first five or ten years of follow-up (HR>1.5) than for the total follow-up, and larger for cardiovascular and other-cause mortality (HR>1.5) than for cancer mortality (HR≤1.3) (**Table 3**).

Replication of the association between inflammaging signature and mortality

After exclusions, 6,742 participants in the HUSK study were available for the analysis, among which 1,425 died during follow-up. As IL-6, IL-8 and cystatin C were not measured, the signature was calculated using seven of the ten markers identified in the MCCS. We observed

a strong association with mortality (HR per SD= 1.33, 95% CI: 1.26-1.41, $P=2x10^{-22}$) with no evidence of heterogeneity by sex (P=0.38). Similar to the MCCS, associations appeared larger in the first years of follow-up (within 5 years: HR=1.62, 95% CI: 1.41-1.86; within 10 years: 1.39, 95% CI: 1.28-1.51) and larger for cardiovascular compared with cancer mortality (HR=1.42, 95% CI: 1.27-1.58 and HR=1.20, 95% CI: 1.09-1.33, respectively), **Table 4**.

DISCUSSION

We evaluated associations with age and mortality for 34 blood markers of the tryptophan-kynurenine pathway, vitamin status, and other markers associated with kidney function, inflammation, including well-established markers such as CRP and interleukins. Most of these were strongly associated with age, and associations were of similar strength at baseline (median age 59) and follow-up (median age: 70). There was also evidence for associations between many markers and mortality; these appeared substantially stronger for the follow-up measures. Interestingly, several of the markers that showed strong associations with age were also strongly associated with mortality, in particular for neopterin, IL-6, quinolinic acid, the derived indices, PAr index, kynurenine-tryptophan ratio, and the HK:XA ratio. The majority of well-established markers of inflammation and related processes such as CRP, IL-6, IL-8, IL-10, cystatin-C (a marker of renal function), calprotectin, TNF-α, IFN-γ were associated with age and mortality, but generally not more strongly than were markers of the kynurenine pathway. Associations with age and mortality for markers of vitamin status (B2, B3, B6) were in comparison weaker, except for the derived marker PAr index, known to reflect vitamin B6 catabolism during inflammation **(17)**, for which associations were strong.

As most markers measured in our study are thought to reflect inflammatory and related processes, we sought to combine them to more fully characterise inflammaging. A

parsimonious set of ten markers appeared to provide better prediction of mortality than each marker individually (HR per SD=1.40 for the ten-marker score, compared with 1.33, 1.28, and 1.27 for IL-6, neopterin, and quinolinic acid, respectively), and is likely to be more robust to study sample variability. Using a score for the combined effect of all 29 markers associated with age resulted in similar prediction of mortality (HR per SD=1.38). A slightly stronger association was observed for a score based on 17 markers that were associated with mortality (HR per SD=1.43); this score was based on observed associations with mortality, so while providing an 'upper bound' for the combined effect of the markers measured in our study, it might be less specific to the aging process and more prone to overfitting. To our knowledge, few prospective cohorts have measured a similar comprehensive set of markers related to inflammation. We used data from the Hordaland Health Study to replicate our findings; despite using only seven of the ten markers included in our inflammaging signature, we observed a strong association of this seven-marker score with mortality (HR=1.33, 95% CI: 1.26-1.41 compared with HR=1.40, 95% CI: 1.24-1.57). We also observed, similar to the MCCS: 1) stronger associations within the first years of follow-up, 2) larger associations for CVD compared with cancer mortality, and 3) similar associations in men and women. These findings require further replication.

Associations with mortality observed in our study were generally similar to those observed in the study by Zuo and colleagues **(25)** (neopterin: HR=1.28 vs 1.27, KTR: HR=1.26 vs 1.22; CRP: HR=1.26 vs 1.22; tryptophan: HR=0.86 vs 0.85; kynurenine: HR=1.07 vs 1.09; anthranilic acid: HR=1.15 vs 1.05, kynurenic acid: HR=1.11 vs 1.00; 3-hydroxykynurenine: HR=1.14 vs 1.18, 3-hydroxyanthranilic acid: HR=1.13 vs 0.98; xanthurenic acid: HR=0.98 vs 0.89). Although it is not possible to strictly compare our findings with other studies because analyses were performed in different populations, our signature based on ten markers appeared to perform well, e.g. showing greater risk estimates than recently developed measures of

epigenetic aging **(26, 27)** (per 1-year HR=1.11 in our study, compared with HR=1.02, 1.02 and 1.04 for various measures of 'epigenetic age acceleration' reported in the meta-analysis by Chen et al. **(28)**, and HR=1.09 for 'PhenoAge' epigenetic aging **(29)**). Similarly, a meta-analysis reported the association between telomere length and all-cause mortality to be HR=1.09 per SD increment **(30)**, compared with HR=1.40 for our ten-marker score of aging. Additional studies assessing biological aging measures and our inflammaging signature in a similar population would be useful to confirm this finding. Other signatures of inflammaging have been developed, but these included different sets of markers or were based on small samples **(31, 32)** and did not appear to be more predictive of mortality than that developed in our study. Although it was not our aim to provide a thorough comparison of our signature with other markers of aging linked to inflammation (whether epigenetic / metabolic etc **(1, 3, 33-35)**) this indicates that the associations we observed were strong, and suggests that the markers measured in our study play an important role among the multi-faceted processes involved in inflammation **(3)**.

Previous studies of the role of inflammatory markers in aging have tended to focus on CRP and IL-6, showing patterns of association with age consistent with inflammaging **(36)**. Parker and colleagues examined a range of metabolic and immune markers in nearly 1,000 adults covering an age range from 30 to over 80 years. Age was positively correlated with TNF-α, TNF receptor I and II, IL-6, IL-2 and other biomarkers increasing linearly from age 30 **(37)**. Forsey and colleagues observed higher concentrations of IL-6, but not the anti-inflammatory IL-10, with age in Swedish men and women aged 86-94 years **(38)**. Alvarez-Rodriguez and colleagues included pro-inflammatory interleukins, TNF-α, interferon-γ, and the anti-inflammatory IL-10 in their cross-sectional study. A positive association between pro-inflammatory cytokines and age between 20 and 80 years was observed and proposed as a cause for age-related conditions **(39)**. These trends are consistent with our results and with the

concept of inflammaging. It was also reported that indoleamine 2,3-dioxygenase (IDO) activity, as reflected by KTR, was increased in nonagenarians compared with healthy blood donors of median age of 45 years. Among the nonagenarians, lower tryptophan, higher kynurenine and higher KTR were associated with mortality, the latter association being robust to adjustment of other inflammatory biomarkers (CRP, IL-6 and IgA) **(40).** Alley and colleagues measured CRP and IL-6 on two occasions 3 years apart in 736 elderly Italians. No associations were seen between baseline levels and mortality but increases in both were associated with subsequent mortality. After further adjustment, including for levels of CRP and IL-6 at the second measure, CRP was associated with increased mortality, consistent with our findings and with the concept of inflammaging **(41)**. In another study with similar design, IL-6 and CRP were measured twice 8.9 years apart, and the concentrations at the second time-point and change from baseline were associated with subsequent mortality **(42)**.

The markers included in our study represent a wide variety of interrelated inflammatory processes, although not all are considered specific to inflammation. Calprotectin is potentially inflammatory; it sequesters zinc thus inhibiting zinc-dependent enzymes, which are important in angiogenesis, wound healing, inflammation, cancer and tissue repair; and its expression is regulated by proinflammatory cytokines **(43)**. Serum amyloid A proteins increase rapidly in parallel with CRP in the acute phase response to infection and is also associated with chronic inflammation **(44)**. The pro-inflammatory effect of serum amyloid A is mediated by induction of cytokines and chemokines but itself is induced by cytokines IL-6, IL-1β and TNF-α **(45)**. Cystathionine, while generally not regarded as an inflammatory biomarker, appears to inhibit inflammation in response to oxidised low-density lipoprotein, suggesting a potential biological significance in protecting against the inflammatory response by inhibiting DNA binding of NF-κβ p65 **(46)**; this marker was strongly positively associated with age in our data. It was proposed that L-cystathionine could be a target for prevention and treatment of inflammation

related diseases such as atherosclerosis which suggests its value as a marker of inflammaging. Cystatin C is a marker of kidney function that is indirectly involved in inflammatory processes and showed strong association with age and mortality in our study. Trigonelline is a plant alkaloid found in coffee and fenugreek seeds that is used in traditional medicine, and has possible anti-inflammatory properties **(47)**; circulating concentration of trigonelline was found to be an accurate objective marker of coffee consumption **(48)**. Our study also included components of the one-carbon metabolism pathway (vitamins B2 and B6), several of which were associated with age and mortality in our data. Other studies have previously established links between B vitamins and inflammation, in particular vitamin B6 **(49)**. Although most studies on vitamin B6 focused on PLP (pyridoxal 5-phosphate) **(7, 49, 50),** our study found stronger associations for 4-pyridoxic acid. Interestingly, we observed that the PAr index, which is indicative of impaired vitamin B6 metabolism during inflammation **(51)**, was one of the markers most strongly associated with age and mortality.

The key strength of our study was that many markers were measured, so it was possible to examine the overall correlation pattern and compare their association with age and mortality in a same setting, which to our knowledge has not been previously reported. We also assessed KTR, HK:XA, HKr and the PAr index for association with mortality; these derived markers were previously identified as associated with risk of cancer **(19),** most notably lung cancer **(52-54),** and risk of stroke **(5)** and cardiovascular mortality **(55)** but overall relatively little evidence exist to date on their association with health outcomes. Several other derived markers using ratios in the kynurenine pathways have been proposed such as QA/KA, 3-HAA/AA, 3-HK/KA, Kyn/KA, 3HAA/Kyn, 3HAA/3HK, 3HAA/AA; these may provide additional information about the inflammaging process and should be evaluated in future studies.

Limitations of our study included the relatively small sample size to investigate cause-specific mortality risk with precision. In the study by Zuo and colleagues, associations appeared

stronger with cardiovascular disease mortality, consistent with our study **(5)**. We also acknowledge that many more markers would be useful to studying inflammaging: only a few cytokines were measured, we had no measures of white blood cell counts and also had no information on prostaglandin levels or COX2 expression, NF-Kb or other relevant transcription factors **(56)**. Another important missing marker is glycoprotein acetylation (GlycA) which was shown to be a good marker of inflammation **(57)** and is highly predictive of mortality **(58)**. Our study did not include technical replicates to assess the reliability of the measurements, but the platforms we used were validated in previous methodological studies and all markers have previously been measured in large-scale studies.

Finally, the rate of aging / inflammaging is determined by the interplay of many genetic, environmental and lifestyle factors, and these factors may help in identifying specific marker patterns associated with less healthy aging, or modulate the role of markers in inflammaging. We only evaluated the main demographic variables (sex, country of birth) for their association with blood markers. Future studies should examine the effects of genetics, nutrition, lifestyle, obesity and other health risk factors on marker levels as well as the determinants of change in markers over time. While the associations observed in our study were strong, the usefulness of our inflammaging signature as a predictor of mortality and its potential utility in the clinical setting should be further investigated.

CONCLUSION

We have assessed the association with age and mortality for 34 blood markers potentially involved in inflammation processes and attempted to better characterise inflammaging. Our findings highlight the prominent role played by the kynurenine pathway and vitamin B6 catabolism along with other well-established markers of inflammation in the aging process. A

ten-marker signature of inflammaging was developed and found to be strongly associated with mortality. Our findings require further replication in additional prospective cohorts.

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Disclaimer: Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/ World Health Organization.

This study used data from the Norwegian Cause of Death Registry. The interpretation and reporting of these data are the sole responsibility of the authors, and no endorsement by the Norwegian Cause of Death Registry is intended, nor should be inferred.

CONFLICTS OF INTEREST

None to declare.

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Table 1. Study sample characteristics, baseline and follow-up; Melbourne Collaborative Cohort Study

	Baseline (1990-1994)	Follow-up (2003-2007)	Complete at baseline / F-up	Spearman correlation
Demographics	**976**	**976**		
Women	307 (31.5)	307 (31.5)		
Country of birth Aust/NZ/Other	753 (77.2)	753 (77.2)		
Country of birth UK/Malta	106 (10.9)	106 (10.9)		
Country of birth Italy	80 (8.2)	80 (8.2)		
Country of birth Greece	37 (3.8)	37 (3.8)		
Age	59 [51, 64]	70 [63, 76]		
One-carbon metabolism pathway				
Thiamine (B1) (nmol/L)	12.7 [10.0, 16.9]	3.80 [2.73, 5.67]	970/972	0.39
Thiamine monophosphate (B1) (nmol/L)	0.68 [0.00, 1.00]	7.96 [6.41, 9.80]	970/972	0.08
Riboflavin (B2) (nmol/L)	23.6 [17.3, 34.2]	15.5 [10.1, 24.3]	970/972	0.60
Flavin mononucleotide (B2) (nmol/L)	5.62 [4.28, 7.79]	13.7 [10.9, 17.4]	970/972	0.32
Nicotinamide (B3) (nmol/L)	540 [409, 695]	445 [340, 566]	918/948	0.08
N1-methylnicotinamide (B3) (nmol/L)	155 [106, 220]	152 [114, 201]	970/972	0.25
Pyridoxal 5'-phosphate (B6) (nmol/L)	37.6 [26.4, 56.8]	47.5 [34.3, 70.0]	970/972	0.53
Pyridoxal (B6) (nmol/L)	21.6 [16.1, 29.6]	12.2 [8.7, 19.1]	970/972	0.37
4-Pyridoxic acid (B6) (nmol/L)	22.1 [17.4, 30.3]	25.5 [19.2, 35.8]	970/972	0.41
Kynurenine pathway				
Tryptophan (µmol/L)	65.9 [59.3, 73.6]	59.4 [51.6, 66.8]	970/972	0.34
Kynurenine (µmol/L)	1.52 [1.31, 1.78]	1.63 [1.40, 1.93]	970/972	0.51
3-Hydroxykynurenine (nmol/L)	38.7 [31.2, 46.7]	39.3 [31.8, 49.3]	910/948	0.53
Kynurenic acid (nmol/L)	48.1 [38.4, 61.0]	53.1 [43.1, 69.5]	970/972	0.50
Xanthurenic acid (nmol/L)	15.0 [11.4, 20.4]	15.3 [10.9, 20.4]	970/972	0.44
Anthranilic acid (nmol/L)	20.3 [16.3, 26.7]	16.7 [13.9, 20.6]	918/948	0.39
3-Hydroxyanthranilic acid (nmol/L)	30.1 [23.0, 38.3]	32.2 [26.0, 41.5]	918/948	0.38
Picolinic acid (nmol/L)	30.0 [23.7, 39.1]	33.8 [26.4, 44.5]	970/972	0.41
Quinolinic acid (nmol/L)	378 [315, 468]	447 [360, 589]	970/972	0.61
Other markers				
Trigonelline (µmol/L)	0.68 [0.33, 1.32]	0.68 [0.39, 1.28]	970/972	0.41
Neopterin (nmol/L)	10.5 [8.2, 13.6]	10.0 [8.1, 12.6]	918/948	0.46
Cystathionine (µmol/L)	0.19 [0.14, 0.25]	0.24 [0.17, 0.36]	970/972	0.33
C-reactive protein (µg/ml)	0.97 [0.41, 2.25]	1.02 [0.43, 2.20]	936/963	0.57
Cystatin C (µg/ml)	0.87 [0.77, 1.01]	0.97 [0.85, 1.14]	948/970	0.46
Calprotectin (µg/ml)	1.83 [1.27, 3.15]	0.82 [0.69, 1.07]	947/970	0.26
Serum amyloid A (µg/ml)	2.33 [1.58, 3.70]	2.36 [1.47, 4.00]	947/970	0.53
Interleukin 6 (pg/mL)	0.60 [0.43, 0.87]	0.79 [0.56, 1.15]	971/972	0.42
Interleukin 8 (pg/mL)	12.3 [7.6, 25.3]	4.60 [3.61, 6.25]	971/972	0.09
Interleukin 10 (pg/mL)	0.24 [0.16, 0.32]	0.24 [0.18, 0.34]	971/972	0.36
Interleukin 13 (pg/mL)	0.24 [0.24, 0.75]	0.24 [0.00, 0.24]	971/972	0.21
Interferon gamma (pg/mL)	5.15 [3.76, 7.62]	5.52 [3.81, 8.44]	971/972	0.47
Tumour necrosis factor alpha (pg/mL)	2.16 [1.53, 2.74]	2.00 [1.42, 2.68]	971/972	0.24
Derived indices				
PAr index = PA / (PL+PLP)	0.38 [0.30, 0.49]	0.42 [0.32, 0.56]	970/972	0.55
KTR= Kyn / Trp x100	2.30 [1.99, 2.71]	2.76 [2.39, 3.36]	970/972	0.58
HK:XA = HK / XA	2.52 [1.95, 3.31]	2.59 [1.96, 3.44]	910/948	0.48
HKr= HK / (KA+AA+XA+HAA) x 100	32.8 [27.3, 39.3]	32.5 [26.5, 39.0]	910/948	0.51

Abbreviations: Kyn: kynurenine; Trp: tryptophan; PA: 4-pyridoxic acid; PL: pyridoxal; PLP: pyridoxal 5'-phosphate; HK: 3-hydroxykynurenine; XA: xanthurenic acid; KA: kynurenic acid; AA: Anthranilic acid; HAA: 3-Hydroxyanthranilic acid

Table 2. Associations between marker scores and all-cause mortality (follow-up levels of blood markers)

	Total sample N=937, N deaths= 307			**Men** N=643, N deaths=228		**Women** N=294, N deaths=79		
	HR[4]	**95%CI**	**P**	**HR[4]**	**95%CI**	**HR[4]**	**95%CI**	**P-heterogeneity[5]**
Score 1[1] Ten-marker 'inflammaging' signature	1.40	1.24-1.57	$2x10^{-8}$	1.44	1.26-1.64	1.28	1.01-1.62	0.39
Score 2[2] 29 markers associated with age	1.38	1.23-1.55	$4x10^{-8}$	1.41	1.24-1.60	1.29	1.02-1.63	0.51
Score 3[3] 17 markers associated with mortality	1.43	1.28-1.60	$1x10^{-10}$	1.43	1.27-1.62	1.41	1.11-1.80	0.91

[1] This score was calculated using Lasso regression for all markers showing an association with age. The 10 variables retained in the model were nicotinamide, tryptophan, anthranilic acid, quinolinic acid, neopterin, cystatin C, IL-6, IL-8, PAr index, and HK:XA.
[2] This score was calculated as the weighted average of marker values for all markers showing an association with age (N=29), using as weights the regression coefficients of their association with age (Supplementary Table 2).
[3] This score was calculated as the weighted average of marker values for all markers showing an association with age (N=17), using as weights the coefficients of their association with mortality (log HR) (Supplementary Table 3).
[4] Hazard ratios and 95% confidence intervals (HR, 95%CI) were calculated using Cox proportional hazard models adjusting for age, sex (except in sex-stratified analyses), and country of birth.
[5] Effect modification by sex was assessed using a likelihood ratio test comparing models with and without inclusion of interaction terms between sex and each marker scores

Table 3. Associations between marker scores and mortality (follow-up levels of blood markers), by length of follow-up and cause of death

	5-year follow-up N=937 N deaths=62		**10-year follow-up** N=937 N deaths=151		**Cancer mortality[5]** Up to 31/08/2015 N=937 N deaths=51		**CVD mortality[5]** Up to 31/08/2015 N=937 N deaths=56		**Other-cause mortality[5]** Up to 31/08/2015 N=937 N deaths=67	
	HR[4]	**95%CI**	**HR[4]**	**95%CI**	**HR[4]**	**95%CI**	**HR[4]**	**95%CI**	**HR[4]**	**95%CI**
Score 1[1] 10-marker 'inflammaging' signature	1.58	1.23-2.03	1.57	1.33-1.85	1.30	0.97-1.75	1.55	1.19-2.03	1.51	1.19-1.93
Score 2[2] 29 markers associated with age	1.54	1.21-1.97	1.54	1.32-1.81	1.18	0.88-1.59	1.52	1.17-1.98	1.61	1.27-2.03
Score 3[3] 17 markers associated with mortality	1.54	1.22-1.93	1.60	1.38-1.86	1.27	0.96-1.68	1.60	1.25-2.05	1.62	1.30-2.03

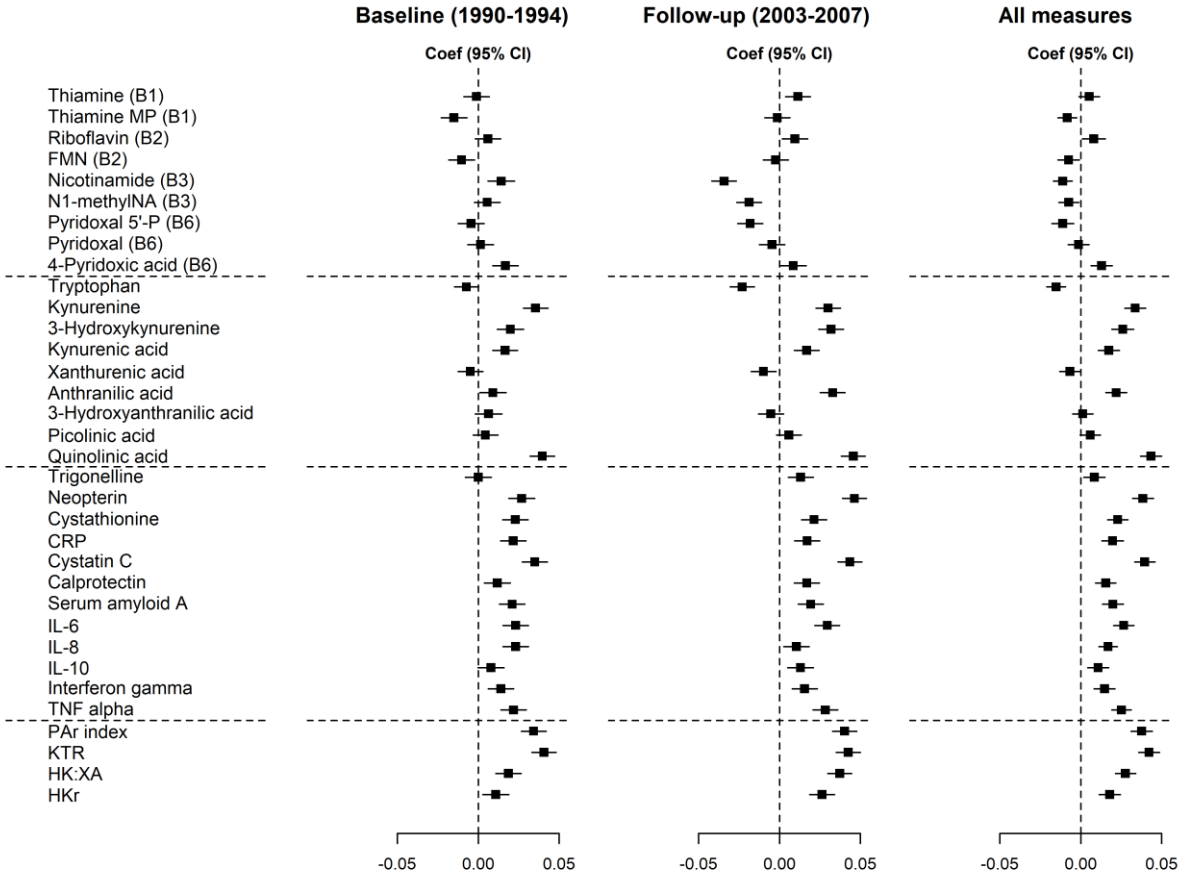
[1] This score was calculated using Lasso regression for all markers showing an association with age. The 10 variables retained in the model were nicotinamide, tryptophan, anthranilic acid, quinolinic acid, neopterin, cystatin C, IL-6, IL-8, PAr index, and HK:XA.
[2] This score was calculated as the weighted average of marker values for all markers showing an association with age (N=29), using as weights the regression coefficients of their association with age (Supplementary Table 2).
[3] This score was calculated as the weighted average of marker values for all markers showing an association with age (N=17), using as weights the coefficients of their association with mortality (log HR) (Supplementary Table 3).
[4] Hazard ratios and 95% confidence intervals (HR, 95%CI) were calculated using Cox proportional hazard models adjusting for age, sex, and country of birth.
[5] Causes of death were classified as follows, using the International Classification of Disease version 10: Cancer: ICD-10 from C00 to D49; Cardiovascular disease (CVD): ICD-10 from I00 to I99; Other-cause: all other ICD codes, and missing (N=2)

Table 4. Replication of the association between inflammaging signature (seven of ten markers[1]) and mortality in the Hordaland Health Study

	N	N deaths	HR[2]	95%CI
All participants	6,742	1,425	1.33	1.26-1.41
Men	2,964	766	1.36	1.25-1.48
Women	3,778	659	1.30	1.20-1.42
Follow-up ≤ 5 years	6,742	212	1.62	1.41-1.86
Follow-up ≤ 10 years	6,742	695	1.39	1.28-1.51
Cancer mortality[3]	6,742	499	1.20	1.09-1.33
CVD mortality[3]	6,742	405	1.42	1.27-1.58
Other-cause mortality[3]	6,742	522	1.40	1.27-1.54

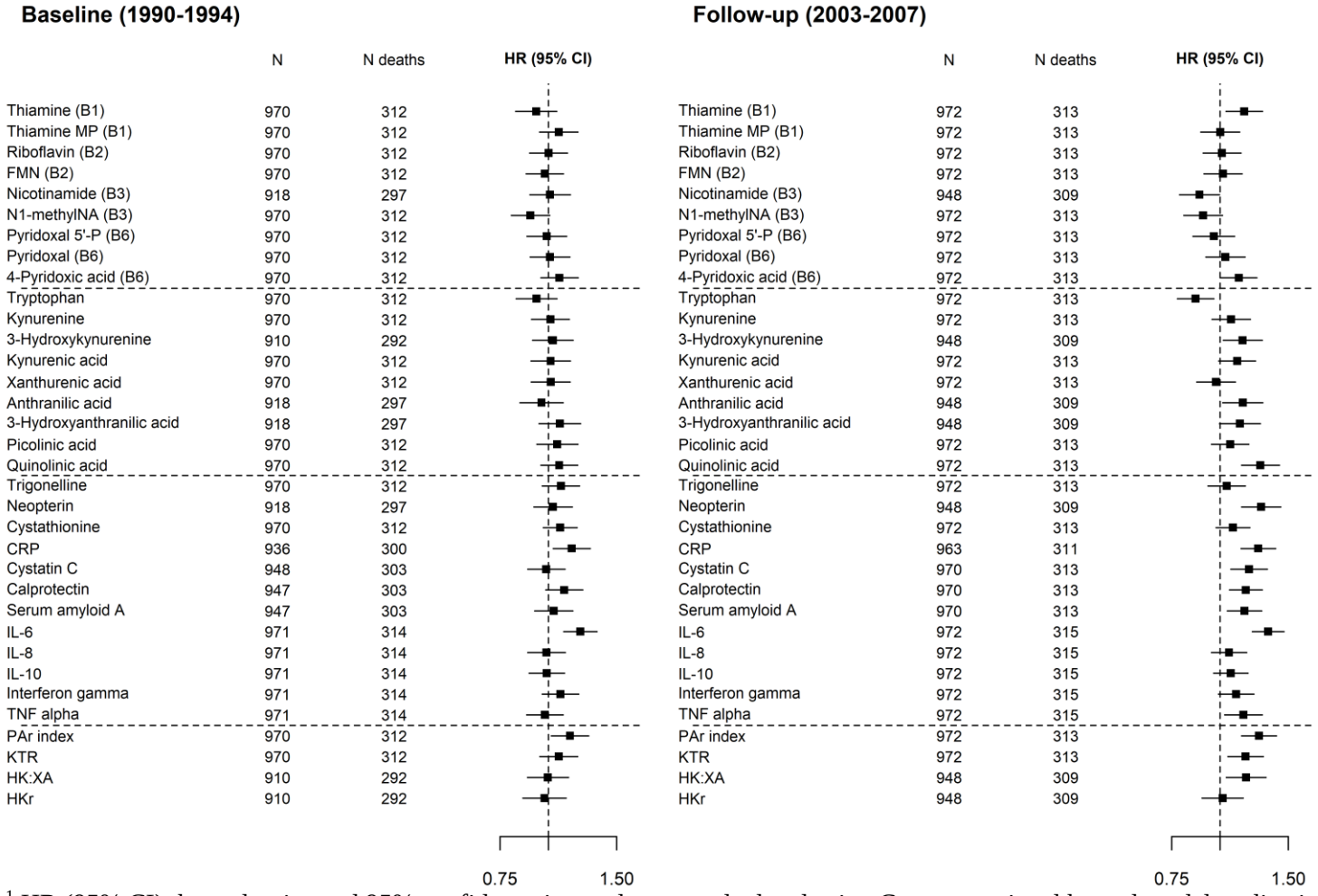
1 cystatin C, IL-6, IL-8 were not measured in the Hordaland Health Study. The same weights as obtained in the MCCS were used to calculate the seven-marker signature.
[2] Hazard ratios and 95% confidence intervals (HR, 95%CI) were calculated using Cox proportional hazard models adjusting for age, sex, and country of birth.
[3] Causes of death were classified as follows, using the International Classification of Disease version 10: Cancer: ICD-10 from C00 to D49; Cardiovascular disease (CVD): ICD-10 from I00 to I99; Other-cause: all other ICD codes, and missing

Figure 1. Associations (regression coefficients, 95% confidence intervals[1]) between 35 blood markers and age in the Melbourne Collaborative Cohort Study (N=976)



Legend:
[1] Regression coefficients and 95% confidence intervals were calculated using linear regression models, adjusting for sex, and country of birth (N=976)

Figure 2. Prospective associations (hazard ratio, 95% confidence interval[1]) between 35 blood markers and all-cause mortality in the Melbourne Collaborative Cohort Study (N=976)



Legend:[1] HR (95% CI): hazard ratios and 95% confidence intervals were calculated using Cox proportional hazard models, adjusting for age, sex, and country of birth (N=976)

Supplementary Material for: "Association of markers of inflammation, the kynurenine pathway and B vitamins and with age and mortality, and a signature of inflammaging"

Pierre-Antoine Dugué, Allison M Hodge, Per M Ueland, Øivind Midttun, Arve Ulvik, Sabina Rinaldi, Robert J Macinnis, Sherly X Li, Klaus Meyer, Anne-Sophie Navionis, Leon Flicker, Gianluca Severi, Dallas R English, Paolo Vineis, Melissa C Southey, Roger L Milne, Graham G. Giles

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Supplementary Methods: Methodological papers detailing the platforms used for marker measurement and their reliability

1) Platform used for measurement of riboflavin, PLP, PA, pyridoxal, pyridoxamine, pyridoxine, neopterin, cystathionine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, xanthurenic acid, kynurenic acid, kynurenine, tryptophan, anthranilic acid:

Reference: Midttun Ø, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/ tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*. 2009;23:1371-1379. doi: 10.1002/rcm.4013

2) Platform used for measurement of C-reactive protein, serum amyloid A, calprotectin and cystatin C:

Reference: Gao J, Meyer K, Borucki K, Ueland PM. Multiplex immuno-MALDI-TOF MS for targeted quantification of protein biomarkers and their proteoforms related to inflammation and renal dysfunction. *Anal Chem*. 2018;90:3366-3373. doi: 10.1021/acs.analchem.7b04975

3) Platform used for measurement of trigonelline:

Reference: Midttun O, Ulvik A, Nygard O, Ueland PM. Performance of plasma trigonelline as a marker of coffee consumption in an epidemiologic setting. *The American journal of clinical nutrition*. 2018;107:941-947.

4) Performance of the platforms for large-scale marker measurement:

Reference: Midttun et al. Most Blood Biomarkers Related to Vitamin Status, One-Carbon Metabolism, and the Kynurenine Pathway Show Adequate Preanalytical Stability and Within-Person Reproducibility to Allow Assessment of Exposure or Nutritional Status in Healthy Women and Cardiovascular Patients, *J Nutr*, 2014

Vitamin B1: Reference: McCann et al. Comparable Performance Characteristics of Plasma Thiamine and Erythrocyte Thiamine Diphosphate in Response to Thiamine Fortification in Rural Cambodian Women, *Nutrients* 2017, 9, 676; doi:10.3390/nu9070676

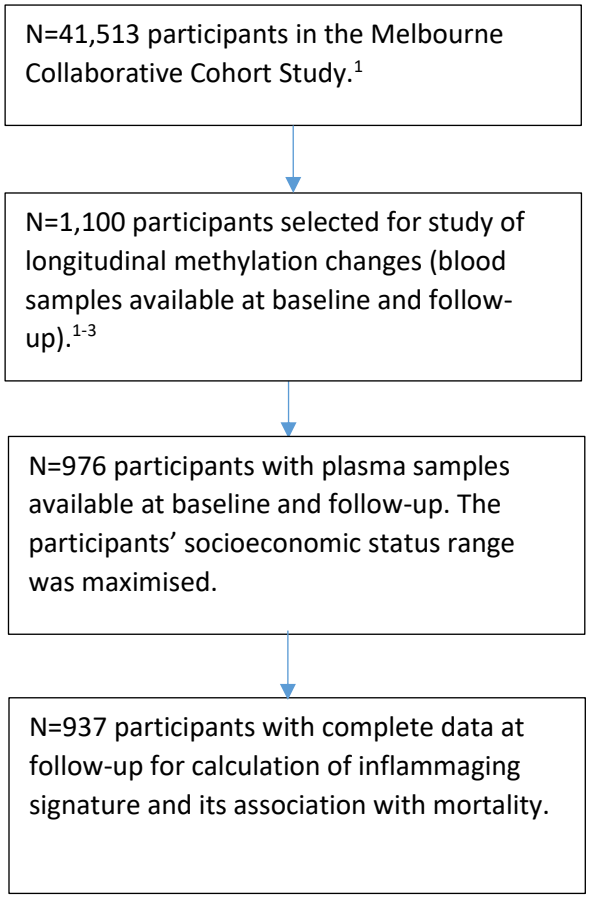
5) Additional details:

https://folk.uib.no/mfapu/Pages/BV/BVSite/summary.html#

Supplementary Table 5. 1000 iterations of the Lasso of age on 29 markers associated with age at follow-up

Marker	Selection frequency	Mean coefficient	Final Lasso model
(Intercept)	**1000**	**68.86**	**68.86**
Thiamine (B1)	8	0.10	
Riboflavin (B2)	19	0.07	
Nicotinamide (B3)	**1000**	**-1.33**	**-1.33**
N1-methylnicotinamide (B3)	286	-0.06	
Pyridoxal 5'-phosphate	19	-0.33	
4-Pyridoxic acid (B6)	5	1.10	
Tryptophan	**904**	**-0.22**	**-0.22**
Kynurenine	0		
3-Hydroxykynurenine	4	-0.58	
Kynurenic acid	5	0.56	
Xanthurenic acid	46	-0.42	
Anthranilic acid	**983**	**0.28**	**0.28**
Quinolinic acid	**1000**	**0.90**	**0.90**
Trigonelline	0		
Neopterin	**1000**	**0.76**	**0.76**
Cystathionine	701	0.12	
C-reactive protein	8	-0.18	
Cystatin C	**1000**	**0.65**	**0.65**
Calprotectin	36	-0.15	
Serum amyloid A	4	0.07	
Interleukin 6	**961**	**0.09**	**0.09**
Interleukin 8	**813**	**0.19**	**0.19**
Interleukin 10	5	0.10	
Interferon gamma	14	-0.15	
Tumour necrosis factor alpha	66	0.05	
PAr	**998**	**0.63**	**0.63**
KTR	192	0.05	
HKXAr	**994**	**0.76**	**0.76**
HKr	40	0.35	

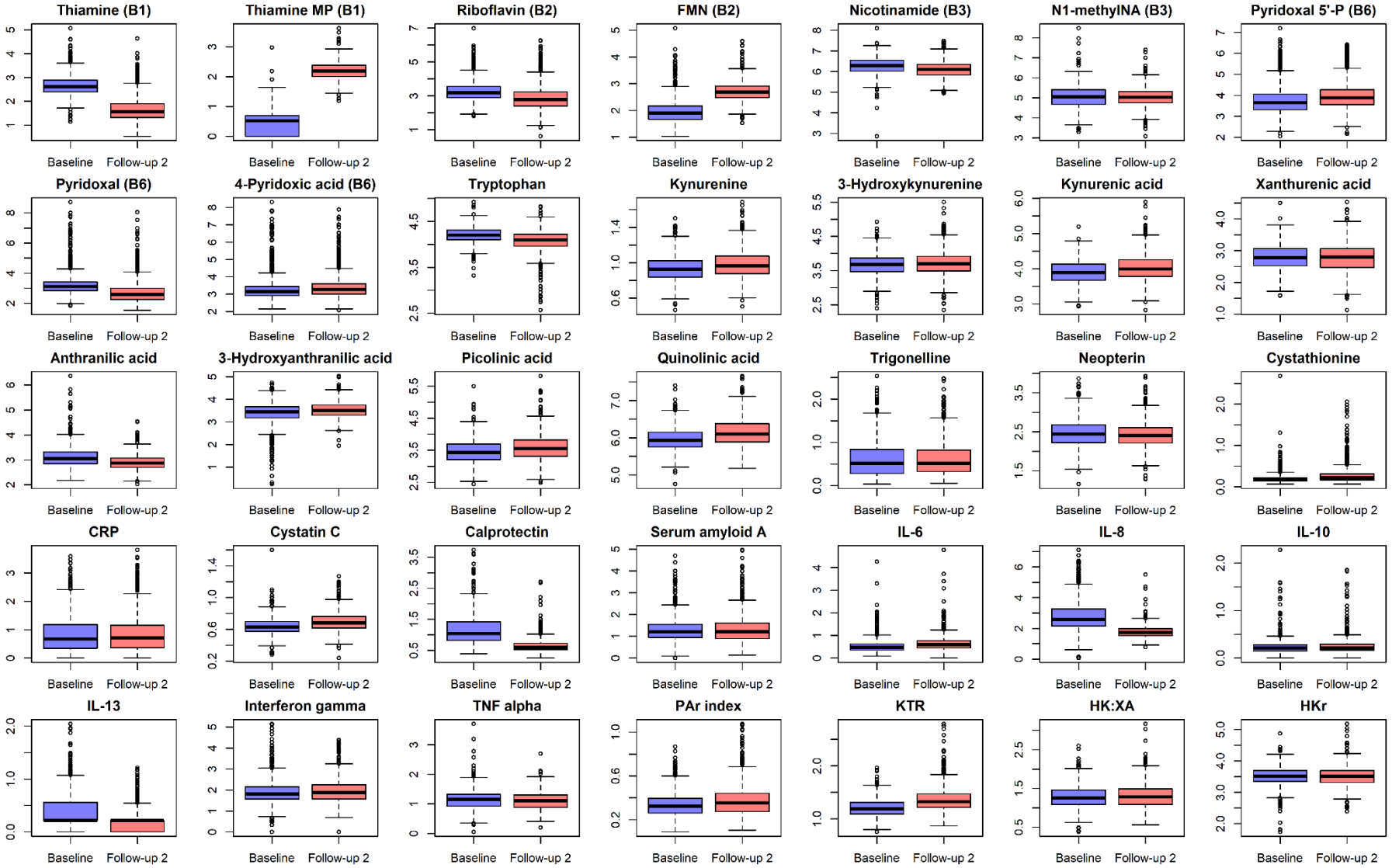
Supplementary Figure 1. Study flowchart. N=976 participants with plasma sample available at baseline (1990-1994) and follow-up (2003-2007).



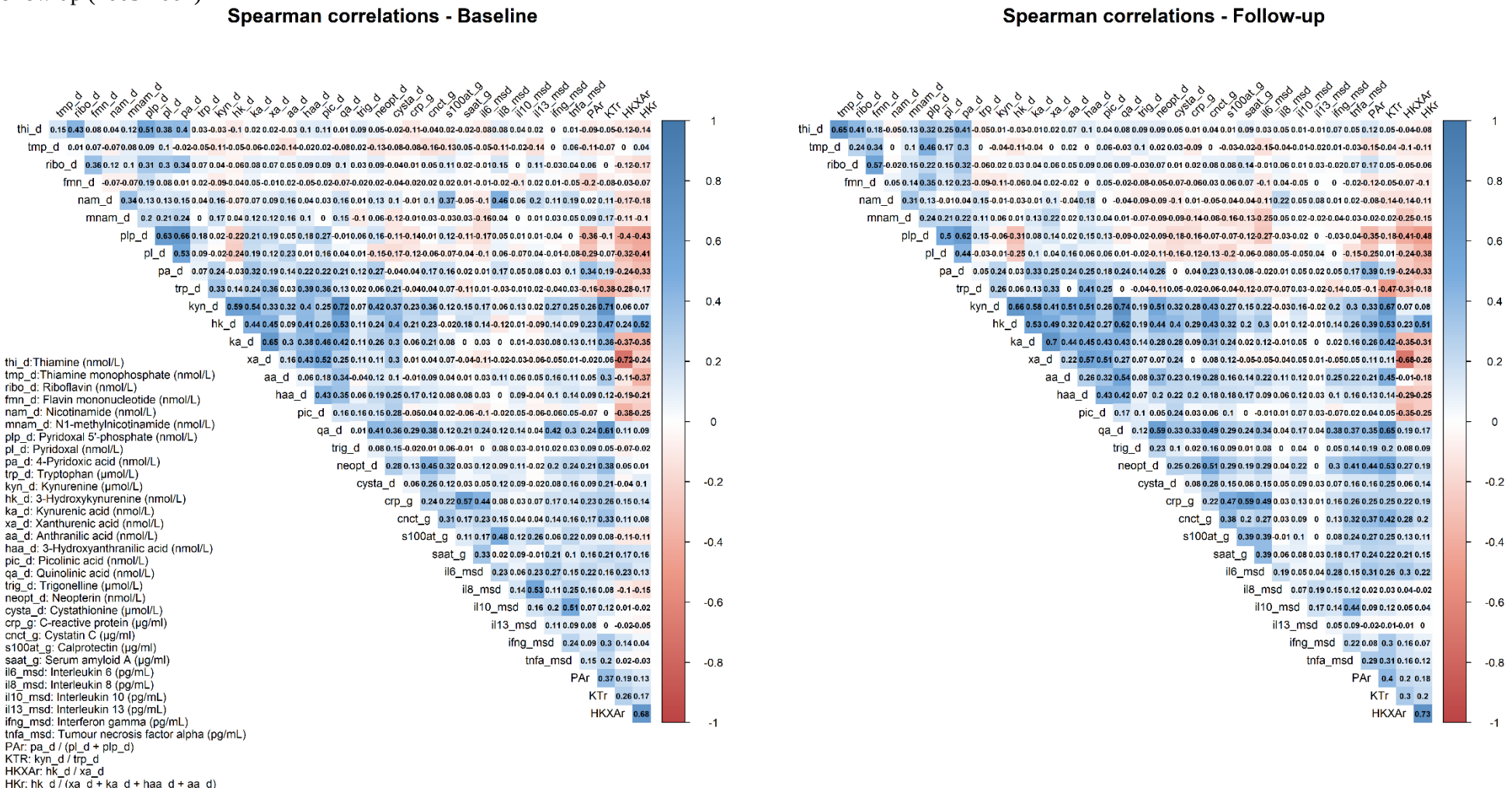
References:

1. Milne RL, Fletcher AS, MacInnis RJ, et al. Cohort Profile: The Melbourne Collaborative Cohort Study (Health 2020). Int J Epidemiol 2017; 46(6): 1757-1757i; doi 10.1093/ije/dyx085.
2. Geurts YM, Dugué PA, Joo JE, et al. Novel associations between blood DNA methylation and body mass index in middle-aged and older adults. Int J Obes (Lond) 2018; 42(4): 887-896; e-pub ahead of print 2017/12/27; doi 10.1038/ijo.2017.269.
3. Dugué PA, Wilson R, Lehne B, et al. Alcohol consumption is associated with widespread changes in blood DNA methylation: Analysis of cross-sectional and longitudinal data. Addiction biology 2019: e12855; doi 10.1111/adb.12855.

Supplementary Figure 2. Distribution of log-transformed values of 35 blood markers in the Melbourne Collaborative Cohort Study at baseline (1990-1994) and follow-up (2003-2007)



Supplementary Figure 3. Spearman rank correlations between 35 blood markers in the Melbourne Collaborative Cohort Study at baseline (1990-1994) and follow-up (2003-2007)



Supplementary Table 1. Individual associations of measures and derived blood markers (both time points combined) with age, sex, and country of birth in the Melbourne Collaborative Cohort Study

	Age			Sex			UK vs Aus born			Italy vs Aus born			Greece vs Aus born		
One-carbon metabolism pathway	**Coef**	**95% CI**	**P**	**Coef**	**95% CI**	**P**	**Coef**	**95% CI**	**P**	**Coef**	**95% CI**	**P**	**Coef**	**95% CI**	**P**
Thiamine (B1) (nmol/L)	0.005	001; 0.012	9.9E-02	0.432	323; 0.541	8.7E-15	-0.042	206; 0.122	6.1E-01	-0.285	0.471; -0.1	2.6E-03	-0.511	776; -0.245	1.6E-04
Thiamine monophosphate (B1) (nmol/L)	-0.008	)14; -0.003	4.5E-03	0.356	258; 0.455	1.4E-12	-0.023	171; 0.125	7.6E-01	-0.045	212; 0.123	6.0E-01	-0.110	).349; 0.13	3.7E-01
Riboflavin (B2) (nmol/L)	0.008	001; 0.015	2.4E-02	0.103	019; 0.226	9.8E-02	0.188	004; 0.371	4.5E-02	-0.415	623; -0.207	9.1E-05	-0.255	553; 0.042	9.3E-02
Flavin mononucleotide (B2) (nmol/L)	-0.008	)14; -0.001	2.6E-02	0.079	035; 0.192	1.8E-01	0.195	024; 0.366	2.6E-02	-0.494	0.688; -0.3	5.8E-07	-0.121	398; 0.157	3.9E-01
Nicotinamide (B3) (nmol/L)	-0.011	)17; -0.005	1.8E-04	-0.082	181; 0.017	1.0E-01	-0.016	164; 0.131	8.3E-01	-0.401	569; -0.234	2.6E-06	-0.281	635; -0.026	3.1E-02
N1-methylnicotinamide (B3) (nmol/L)	-0.007	)14; -0.001	2.0E-02	0.032	075; 0.138	5.6E-01	-0.117	278; 0.043	1.5E-01	-0.194	375; -0.012	3.6E-02	-0.596	856; -0.337	6.6E-06
Pyridoxal 5'-phosphate (B6) (nmol/L)	-0.011	)18; -0.004	1.2E-03	-0.012	128; 0.104	8.4E-01	-0.044	218; 0.131	6.2E-01	-0.200	398; -0.003	4.7E-02	-0.428	711; -0.145	3.0E-03
Pyridoxal (B6) (nmol/L)	-0.001	008; 0.005	6.9E-01	-0.019	).13; 0.092	7.3E-01	-0.108	275; 0.058	2.0E-01	-0.346	635; -0.157	3.3E-04	-0.363	633; -0.093	8.5E-03
4-Pyridoxic acid (B6) (nmol/L)	0.013	006; 0.019	8.9E-05	-0.002	113; 0.109	9.7E-01	-0.079	246; 0.088	3.6E-01	-0.327	617; -0.138	7.1E-04	-0.564	835; -0.292	4.6E-05
Kynurenine pathway															
Tryptophan (µmol/L)	-0.015	)21; -0.009	3.2E-07	-0.613	713; -0.513	2.8E-33	0.067	083; 0.217	3.8E-01	0.479	308; 0.649	3.5E-08	0.131	113; 0.374	2.9E-01
Kynurenine (µmol/L)	0.034	).027; 0.04	6.3E-25	-0.299	.41; -0.189	1.1E-07	0.035	).13; 0.201	6.8E-01	0.245	057; 0.433	1.1E-02	-0.200	469; 0.068	1.4E-01
3-Hydroxykynurenine (nmol/L)	0.026	019; 0.033	3.4E-14	-0.037	153; 0.079	5.3E-01	0.041	133; 0.215	6.4E-01	0.329	133; 0.525	1.0E-03	-0.134	429; 0.161	3.7E-01
Kynurenic acid (nmol/L)	0.017	011; 0.024	1.3E-07	-0.514	626; -0.403	1.9E-19	0.040	127; 0.208	6.4E-01	-0.149	339; 0.041	1.2E-01	-0.452	.724; -0.18	1.1E-03
Xanthurenic acid (nmol/L)	-0.007	-0.013; 0	4.3E-02	-0.525	635; -0.415	7.3E-21	0.206	041; 0.371	1.4E-02	0.122	065; 0.308	2.0E-01	-0.529	796; -0.261	1.1E-04
Anthranilic acid (nmol/L)	0.022	016; 0.028	1.7E-11	-0.196	306; -0.086	4.9E-04	-0.073	238; 0.091	3.8E-01	-0.376	.562; -0.19	7.5E-05	-0.404	684; -0.123	4.8E-03
3-Hydroxyanthranilic acid (nmol/L)	0.001	005; 0.008	6.9E-01	-0.425	533; -0.316	1.5E-14	0.032	).13; 0.194	7.0E-01	0.266	083; 0.449	4.5E-03	-0.097	).373; 0.18	4.9E-01
Picolinic acid (nmol/L)	0.006	0; 0.012	6.3E-02	-0.600	707; -0.493	6.7E-28	0.074	087; 0.236	3.7E-01	-0.004	.187; 0.179	9.6E-01	-0.197	459; 0.064	1.4E-01
Quinolinic acid (nmol/L)	0.044	).037; 0.05	1.3E-38	0.025	088; 0.138	6.7E-01	-0.001	).171; 0.17	9.9E-01	-0.013	).206; 0.18	9.0E-01	-0.344	.62; -0.068	1.4E-02
Other markers															
Trigonelline (µmol/L)	0.008	002; 0.015	1.0E-02	0.116	005; 0.227	4.1E-02	0.084	083; 0.251	3.2E-01	0.456	267; 0.645	2.2E-06	0.225	045; 0.496	1.0E-01
Neopterin (nmol/L)	0.039	032; 0.045	1.4E-31	-0.165	276; -0.054	3.7E-03	-0.045	211; 0.122	6.0E-01	0.095	093; 0.283	3.2E-01	-0.158	441; 0.124	2.7E-01
Cystathionine (µmol/L)	0.023	017; 0.029	4.3E-13	-0.130	237; -0.024	1.7E-02	0.103	057; 0.263	2.1E-01	-0.060	242; 0.121	5.1E-01	-0.159	-0.419; 0.1	2.3E-01
C-reactive protein (µg/ml)	0.020	013; 0.026	6.3E-09	0.105	-0.01; 0.22	7.4E-02	-0.002	175; 0.171	9.8E-01	0.518	322; 0.713	2.1E-07	0.324	045; 0.604	2.3E-02
Cystatin C (µg/ml)	0.040	033; 0.046	7.0E-36	-0.196	303; -0.089	3.3E-04	-0.093	254; 0.068	2.6E-01	-0.109	292; 0.073	2.4E-01	-0.143	403; 0.118	2.8E-01
Calprotectin (µg/ml)	0.016	009; 0.022	9.8E-07	0.026	081; 0.132	6.4E-01	0.023	138; 0.183	7.8E-01	0.072	).11; 0.254	4.4E-01	0.010	-0.25; 0.27	9.4E-01
Serum amyloid A (µg/ml)	0.020	014; 0.026	1.2E-09	0.327	216; 0.438	7.4E-09	0.018	148; 0.184	8.3E-01	0.404	215; 0.593	2.8E-05	0.278	008; 0.548	4.4E-02
Interleukin 6 (pg/mL)	0.027	).02; 0.033	1.3E-16	0.009	-0.1; 0.118	8.7E-01	-0.082	245; 0.081	3.3E-01	0.166	019; 0.352	7.9E-02	0.297	032; 0.561	2.8E-02
Interleukin 8 (pg/mL)	0.017	011; 0.023	3.5E-09	0.117	).02; 0.213	1.8E-02	0.084	062; 0.229	2.6E-01	-0.352	518; -0.187	2.9E-05	-0.266	502; -0.031	2.7E-02
Interleukin 10 (pg/mL)	0.011	004; 0.017	1.0E-03	-0.052	162; 0.059	3.6E-01	-0.005	171; 0.161	9.5E-01	0.033	156; 0.222	7.3E-01	-0.032	301; 0.237	8.2E-01
Interferon gamma (pg/mL)	0.015	008; 0.021	6.4E-06	0.263	152; 0.373	3.3E-06	0.089	077; 0.255	2.9E-01	0.128	).06; 0.317	1.8E-01	-0.187	456; 0.082	1.7E-01
Tumour necrosis factor alpha (pg/mL)	0.025	019; 0.031	2.0E-16	-0.102	205; 0.001	5.3E-02	-0.014	169; 0.141	8.6E-01	-0.038	214; 0.138	6.7E-01	0.008	243; 0.259	9.5E-01
Derived markers															
KTr = Kyn / Trp	0.038	031; 0.044	6.3E-30	0.000	112; 0.112	1.0E+00	0.002	).167; 0.17	9.8E-01	-0.149	).34; 0.042	1.3E-01	-0.234	507; 0.039	9.3E-02
PAr index = PA / (PL+PLP)	0.042	036; 0.049	4.6E-38	0.180	).07; 0.291	1.4E-03	-0.005	172; 0.161	9.5E-01	-0.140	328; 0.049	1.5E-01	-0.286	556; -0.016	3.8E-02
HK:XA = HK / XA	0.028	022; 0.034	2.6E-18	0.551	444; 0.658	5.1E-24	-0.189	349; -0.028	2.1E-02	0.150	031; 0.331	1.0E-01	0.538	264; 0.811	1.2E-04
HKr= HK / (KA+AA+XA+HAA)	0.018	012; 0.024	5.2E-08	0.502	).39; 0.613	1.0E-18	-0.032	199; 0.135	7.1E-01	0.388	0.2; 0.576	5.4E-05	0.271	013; 0.555	6.2E-02

Baseline and follow-up measures were included together in same model, using robust standard errors to account for correlations between participants' repeated measures.
These models were mutually adjusted for age, sex and country of birth (Australia/New-Zealand/other, UK, Italy, Greece).
In blue: 29 markers associated with age at P<0.05, used to calculate a marker score (weighted average using coefficients as weights)

Supplementary Table 2. Individual associations of measures and derived blood markers (at follow-up) with age, sex, and country of birth in the Melbourne Collaborative Cohort Study

	Age			Sex			UK vs Aus born			Italy vs Aus born			Greece vs Aus born		
One-carbon metabolism pathway	**Coef**	**95% CI**	**P**	**Coef**	**95% CI**	**P**	**Coef**	**95% CI**	**P**	**Coef**	**95% CI**	**P**	**Coef**	**95% CI**	**P**
Thiamine (B1) (nmol/L)	0.011	0.004; 0.019	3.5E-03	0.404	0.273; 0.536	2.6E-09	-0.052	-0.25; 0.146	6.0E-01	-0.294	0.518; -0.07	1.0E-02	-0.630	-0.951; -0.309	1.3E-04
Thiamine monophosphate (B1) (nmol/L)	-0.001	0.009; 0.006	7.4E-01	0.482	0.35; 0.614	1.8E-12	-0.067	0.266; 0.132	5.1E-01	-0.154	0.379; 0.071	1.8E-01	-0.350	-0.672; -0.028	3.4E-02
Riboflavin (B2) (nmol/L)	0.010	0.002; 0.017	1.7E-02	0.102	0.033; 0.237	1.4E-01	0.199	0.004; 0.401	5.4E-02	-0.350	-0.579; -0.121	2.8E-03	-0.286	-0.614; 0.042	8.8E-02
Flavin mononucleotide (B2) (nmol/L)	-0.002	-0.01; 0.005	5.6E-01	0.136	0.003; 0.269	4.5E-02	0.211	0.011; 0.411	3.9E-02	-0.614	-0.84; -0.387	1.3E-07	-0.482	-0.806; -0.158	3.7E-03
Nicotinamide (B3) (nmol/L)	-0.034	-0.042; -0.027	3.6E-18	-0.098	-0.229; 0.033	1.4E-01	-0.019	-0.216; 0.178	8.5E-01	-0.376	-0.596; -0.156	8.5E-04	-0.614	-0.951; -0.276	3.8E-04
N1-methylnicotinamide (B3) (nmol/L)	-0.019	-0.026; -0.011	2.2E-06	-0.059	-0.191; 0.074	3.9E-01	-0.070	-0.269; 0.13	4.9E-01	-0.253	-0.479; -0.028	2.8E-02	-0.831	-1.154; -0.508	5.7E-07
Pyridoxal 5'-phosphate (B6) (nmol/L)	-0.018	-0.026; -0.01	5.8E-06	-0.003	-0.137; 0.131	9.7E-01	-0.089	-0.29; 0.113	3.9E-01	-0.205	-0.433; 0.023	7.8E-02	-0.571	-0.897; -0.245	6.2E-04
Pyridoxal (B6) (nmol/L)	-0.005	-0.012; 0.003	2.6E-01	-0.083	-0.218; 0.052	2.3E-01	-0.111	-0.314; 0.092	2.8E-01	-0.328	-0.558; -0.098	5.4E-03	-0.409	-0.739; -0.08	1.5E-02
4-Pyridoxic acid (B6) (nmol/L)	0.009	0.001; 0.016	3.1E-02	-0.080	-0.214; 0.055	2.5E-01	-0.075	-0.278; 0.127	4.7E-01	-0.275	-0.504; -0.046	1.9E-02	-0.661	-0.99; -0.333	8.4E-05
Kynurenine pathway															
Tryptophan (µmol/L)	-0.023	-0.03; -0.016	2.3E-09	-0.521	-0.65; -0.392	6.3E-15	0.003	-0.191; 0.196	9.8E-01	0.466	0.247; 0.685	3.3E-05	0.124	-0.19; 0.437	4.4E-01
Kynurenine (µmol/L)	0.030	0.023; 0.038	1.5E-14	-0.269	-0.399; -0.139	5.6E-05	0.022	-0.173; 0.218	8.2E-01	0.348	0.126; 0.57	2.1E-03	-0.044	-0.362; 0.273	7.9E-01
3-Hydroxykynurenine (nmol/L)	0.032	0.024; 0.04	1.3E-15	-0.027	-0.16; 0.106	6.9E-01	0.039	-0.161; 0.239	7.0E-01	0.258	-0.034; 0.482	2.4E-02	-0.097	-0.44; 0.246	5.8E-01
Kynurenic acid (nmol/L)	0.017	0.009; 0.025	1.8E-05	-0.439	-0.57; -0.307	1.1E-10	0.081	-0.117; 0.279	4.2E-01	-0.090	-0.314; 0.134	4.3E-01	-0.477	-0.799; -0.156	3.6E-03
Xanthurenic acid (nmol/L)	-0.010	-0.018; -0.002	1.1E-02	-0.488	-0.62; -0.356	7.8E-13	0.192	-0.005; 0.39	5.7E-02	0.149	-0.075; 0.373	1.9E-01	-0.445	-0.766; -0.124	6.7E-03
Anthranilic acid (nmol/L)	0.033	0.025; 0.041	1.0E-16	-0.164	-0.296; -0.032	1.5E-02	-0.079	-0.278; 0.119	4.3E-01	-0.339	-0.561; -0.117	2.8E-03	-0.463	-0.803; -0.122	8.0E-03
3-Hydroxyanthranilic acid (nmol/L)	-0.005	-0.013; 0.002	1.8E-01	-0.482	-0.616; -0.348	3.5E-12	0.077	-0.124; 0.279	4.5E-01	0.314	-0.089; 0.539	6.3E-03	-0.116	-0.461; 0.229	5.1E-01
Picolinic acid (nmol/L)	0.006	-0.002; 0.014	1.3E-01	-0.565	-0.696; -0.433	1.3E-16	0.046	-0.152; 0.243	6.5E-01	-0.038	-0.262; 0.185	7.4E-01	-0.103	-0.423; 0.217	5.3E-01
Quinolinic acid (nmol/L)	0.046	0.038; 0.053	7.5E-32	0.042	-0.084; 0.169	5.1E-01	-0.017	-0.207; 0.174	8.6E-01	0.044	-0.172; 0.259	6.9E-01	-0.322	-0.63; -0.013	4.1E-02
Other markers															
Trigonelline (µmol/L)	0.013	0.005; 0.021	8.2E-04	0.110	-0.023; 0.243	1.0E-01	0.015	-0.184; 0.214	8.8E-01	0.668	0.442; 0.893	9.0E-09	0.479	0.156; 0.802	3.7E-03
Neopterin (nmol/L)	0.046	0.039; 0.054	2.1E-32	-0.068	-0.196; 0.06	3.0E-01	-0.046	-0.238; 0.147	6.4E-01	0.161	-0.054; 0.376	1.4E-01	-0.008	-0.337; 0.322	9.6E-01
Cystathionine (µmol/L)	0.021	0.014; 0.029	9.1E-08	-0.022	-0.156; 0.113	7.5E-01	0.091	-0.111; 0.292	3.8E-01	0.004	-0.225; 0.232	9.7E-01	-0.025	-0.352; 0.302	8.8E-01
C-reactive protein (µg/ml)	0.017	0.009; 0.025	1.5E-05	0.096	-0.038; 0.229	1.6E-01	-0.042	-0.242; 0.158	6.8E-01	0.502	0.275; 0.73	1.7E-05	0.520	0.196; 0.844	1.7E-03
Cystatin C (µg/ml)	0.044	0.036; 0.051	3.4E-29	-0.196	-0.323; -0.068	2.7E-03	-0.089	-0.28; 0.102	3.6E-01	0.073	-0.143; 0.289	5.1E-01	-0.168	-0.478; 0.141	2.9E-01
Calprotectin (µg/ml)	0.017	0.009; 0.025	2.2E-05	0.111	-0.023; 0.246	1.0E-01	0.066	-0.136; 0.268	5.2E-01	0.373	-0.145; 0.602	1.4E-03	-0.004	-0.331; 0.323	9.8E-01
Serum amyloid A (µg/ml)	0.019	0.012; 0.027	8.6E-07	0.355	0.223; 0.488	1.9E-07	0.051	-0.148; 0.25	6.2E-01	0.307	-0.082; 0.532	7.7E-03	0.316	-0.006; 0.639	5.5E-02
Interleukin 6 (pg/mL)	0.030	0.022; 0.037	7.8E-14	0.021	-0.111; 0.153	7.5E-01	-0.123	-0.321; 0.075	2.2E-01	0.181	-0.044; 0.407	1.2E-01	0.334	-0.012; 0.655	4.2E-02
Interleukin 8 (pg/mL)	0.011	0.003; 0.018	8.7E-03	0.072	-0.063; 0.208	3.0E-01	0.073	-0.13; 0.277	4.8E-01	-0.200	-0.432; 0.032	9.1E-02	-0.229	-0.559; 0.101	1.7E-01
Interleukin 10 (pg/mL)	0.013	0.005; 0.021	1.2E-03	-0.103	-0.239; 0.033	1.4E-01	-0.078	-0.282; 0.125	4.5E-01	0.029	-0.203; 0.26	8.1E-01	-0.046	-0.376; 0.284	7.9E-01
Interferon gamma (pg/mL)	0.016	0.008; 0.023	8.6E-05	0.323	0.19; 0.457	2.5E-06	-0.026	-0.227; 0.175	8.0E-01	0.077	-0.152; 0.305	5.1E-01	-0.255	-0.58; 0.071	1.3E-01
Tumour necrosis factor alpha (pg/mL)	0.028	0.021; 0.036	9.1E-13	-0.147	-0.28; -0.015	3.0E-02	-0.030	-0.229; 0.169	7.7E-01	-0.058	-0.285; 0.168	6.1E-01	-0.087	-0.41; 0.236	6.0E-01
Derived markers															
KTr = Kyn / Trp	0.040	0.033; 0.048	7.9E-25	-0.138	-0.267; -0.009	3.6E-02	0.071	-0.123; 0.264	4.7E-01	-0.069	-0.288; 0.15	5.4E-01	-0.188	-0.501; 0.126	2.4E-01
PAr index = PA / (PL+PLP)	0.043	0.035; 0.05	1.1E-27	0.179	0.051; 0.307	6.1E-03	0.018	-0.174; 0.21	8.5E-01	-0.064	-0.282; 0.153	5.6E-01	-0.122	-0.433; 0.19	4.4E-01
HK:XA = HK / XA	0.037	0.03; 0.045	2.1E-22	0.539	0.412; 0.666	2.8E-16	-0.159	-0.35; 0.031	1.0E-01	0.082	-0.131; 0.295	4.5E-01	0.460	0.133; 0.787	5.9E-03
HKr= HK / (KA+AA+XA+HAA)	0.026	0.019; 0.034	1.5E-11	0.488	0.357; 0.619	5.7E-13	-0.054	-0.251; 0.143	5.9E-01	0.282	-0.062; 0.502	1.2E-02	0.332	-0.005; 0.67	5.4E-02

Supplementary Table 3. Prospective associations between 35 blood markers measured at baseline and follow-up and all-cause mortality in the Melbourne Collaborative Cohort Study

	Baseline (1990-1994)					Follow-up (2003-2007)				
One-carbon metabolism pathway	**N**	**Ndeaths**	**HR**	**95% CI**	**P**	**N**	**Ndeaths**	**HR**	**95% CI**	**P**
Thiamine (B1) (nmol/L)	970	312	0.93	0.82; 1.05	2.5E-01	972	313	1.15	1.04; 1.29	9.2E-03
Thiamine monophosphate (B1) (nmol/L)	970	312	1.06	0.95; 1.19	2.8E-01	972	313	1.00	0.89; 1.12	9.8E-01
Riboflavin (B2) (nmol/L)	970	312	1.00	0.9; 1.12	9.8E-01	972	313	1.01	0.9; 1.13	8.3E-01
Flavin mononucleotide (B2) (nmol/L)	970	312	0.98	0.88; 1.09	7.2E-01	972	313	1.02	0.91; 1.14	7.5E-01
Nicotinamide (B3) (nmol/L)	918	297	1.01	0.9; 1.14	8.6E-01	948	309	0.89	0.79; 1	4.5E-02
N1-methylnicotinamide (B3) (nmol/L)	970	312	0.90	0.8; 1.01	6.8E-02	972	313	0.90	0.81; 1.01	8.6E-02
Pyridoxal 5'-phosphate (B6) (nmol/L)	970	312	0.99	0.88; 1.12	8.8E-01	972	313	0.96	0.85; 1.09	5.6E-01
Pyridoxal (B6) (nmol/L)	970	312	1.01	0.9; 1.14	8.6E-01	972	313	1.03	0.92; 1.16	5.9E-01
4-Pyridoxic acid (B6) (nmol/L)	970	312	1.07	0.96; 1.19	2.4E-01	972	313	1.12	1; 1.25	4.3E-02
Kynurenine pathway										
Tryptophan (µmol/L)	970	312	0.93	0.83; 1.05	2.6E-01	972	313	0.86	0.78; 0.96	8.7E-03
Kynurenine (µmol/L)	970	312	1.01	0.9; 1.13	8.3E-01	972	313	1.07	0.95; 1.2	2.5E-01
3-Hydroxykynurenine (nmol/L)	910	292	1.03	0.91; 1.16	6.6E-01	948	309	1.14	1.02; 1.28	2.3E-02
Kynurenic acid (nmol/L)	970	312	1.01	0.9; 1.14	8.2E-01	972	313	1.11	0.99; 1.24	6.7E-02
Xanthurenic acid (nmol/L)	970	312	1.01	0.9; 1.14	8.0E-01	972	313	0.98	0.87; 1.1	6.9E-01
Anthranilic acid (nmol/L)	918	297	0.96	0.84; 1.09	5.4E-01	948	309	1.15	1.02; 1.29	2.5E-02
3-Hydroxyanthranilic acid (nmol/L)	918	297	1.07	0.95; 1.21	2.8E-01	948	309	1.13	1; 1.27	5.5E-02
Picolinic acid (nmol/L)	970	312	1.06	0.93; 1.19	3.9E-01	972	313	1.06	0.95; 1.19	2.9E-01
Quinolinic acid (nmol/L)	970	312	1.07	0.96; 1.19	2.5E-01	972	313	1.27	1.14; 1.42	2.8E-05
Other markers										
Trigonelline (µmol/L)	970	312	1.08	0.96; 1.2	1.8E-01	972	313	1.04	0.93; 1.16	4.8E-01
Neopterin (nmol/L)	918	297	1.03	0.92; 1.15	6.3E-01	948	309	1.28	1.14; 1.44	3.6E-05
Cystathionine (µmol/L)	970	312	1.07	0.97; 1.19	1.7E-01	972	313	1.08	0.98; 1.19	1.4E-01
C-reactive protein (µg/ml)	936	300	1.15	1.03; 1.28	1.4E-02	963	311	1.26	1.13; 1.39	1.5E-05
Cystatin C (µg/ml)	948	303	0.99	0.88; 1.1	8.3E-01	970	313	1.19	1.07; 1.33	1.7E-03
Calprotectin (µg/ml)	947	303	1.10	0.98; 1.23	9.1E-02	970	313	1.17	1.06; 1.29	2.1E-03
Serum amyloid A (µg/ml)	947	303	1.03	0.92; 1.16	5.7E-01	970	313	1.16	1.04; 1.28	5.2E-03
Interleukin 6 (pg/mL)	971	314	1.21	1.1; 1.33	1.7E-04	972	315	1.33	1.21; 1.46	2.8E-09
Interleukin 8 (pg/mL)	971	314	0.99	0.88; 1.11	8.6E-01	972	315	1.06	0.95; 1.17	3.1E-01
Interleukin 10 (pg/mL)	971	314	0.99	0.89; 1.1	8.7E-01	972	315	1.07	0.96; 1.18	2.2E-01
Interferon gamma (pg/mL)	971	314	1.08	0.97; 1.2	1.9E-01	972	315	1.10	0.99; 1.22	7.2E-02
Tumour necrosis factor alpha (pg/mL)	971	314	0.98	0.88; 1.09	7.2E-01	972	315	1.15	1.03; 1.29	1.4E-02
Derived markers										
PAr index = PA / (PL+PLP)	970	312	1.14	1.02; 1.27	2.2E-02	972	313	1.26	1.14; 1.4	1.2E-05
KTr = Kyn / Trp	970	312	1.06	0.95; 1.19	2.8E-01	972	313	1.16	1.05; 1.29	4.9E-03
HK:XA = HK / XA	910	292	1.00	0.88; 1.13	9.7E-01	948	309	1.17	1.04; 1.31	1.0E-02
HKr= HK / (KA+AA+XA+HAA)	910	292	0.98	0.86; 1.11	7.3E-01	948	309	1.02	0.9; 1.15	8.0E-01

Cox proportional hazards regression models were adjusted for age, sex and country of birth (Australia/New-Zealand/other, UK, Italy, Greece).
In blue: 13 markers associated with mortality at P<0.05, used to calculate a marker score (weighted average using coefficients as weights)
In green: IL-13 was not selected due to many values below the limit of quantification

Supplementary Table 4. Prospective associations between change in 35 blood markers from baseline and follow-up and all-cause mortality in the Melbourne Collaborative Cohort Study

	Model 1					Model 2				
One-carbon metabolism pathway	**N**	**Ndeaths**	**HR**	**95% CI**	**P**	**N**	**Ndeaths**	**HR**	**95% CI**	**P**
Thiamine (B1) (nmol/L)	970	312	**1.21**	**1.09; 1.35**	**4.6E-04**	972	313	1.18	1.03; 1.36	1.4E-02
Thiamine monophosphate (B1) (nmol/L)	970	312	0.97	0.89; 1.06	4.6E-01	972	313	0.94	0.84; 1.05	2.9E-01
Riboflavin (B2) (nmol/L)	970	312	1.01	0.89; 1.16	8.6E-01	972	313	1.01	0.87; 1.17	9.1E-01
Flavin mononucleotide (B2) (nmol/L)	970	312	1.04	0.93; 1.16	5.2E-01	972	313	1.04	0.91; 1.18	5.8E-01
Nicotinamide (B3) (nmol/L)	918	297	0.94	0.86; 1.02	1.5E-01	948	309	0.99	0.87; 1.11	8.2E-01
N1-methylnicotinamide (B3) (nmol/L)	970	312	1.00	0.91; 1.10	9.4E-01	972	313	1.09	0.97; 1.23	1.5E-01
Pyridoxal 5'-phosphate (B6) (nmol/L)	970	312	0.98	0.87; 1.10	6.8E-01	972	313	0.99	0.87; 1.13	8.8E-01
Pyridoxal (B6) (nmol/L)	970	312	1.02	0.91; 1.13	7.5E-01	972	313	1.00	0.88; 1.14	9.8E-01
4-Pyridoxic acid (B6) (nmol/L)	970	312	1.04	0.94; 1.16	4.4E-01	972	313	0.97	0.86; 1.10	6.8E-01
Kynurenine pathway										
Tryptophan (µmol/L)	970	312	0.94	0.85; 1.03	1.9E-01	972	313	1.03	0.91; 1.17	6.1E-01
Kynurenine (µmol/L)	970	312	1.06	0.94; 1.19	3.2E-01	972	313	1.04	0.91; 1.18	6.1E-01
3-Hydroxykynurenine (nmol/L)	910	292	1.10	0.97; 1.25	1.3E-01	948	309	1.04	0.90; 1.21	5.6E-01
Kynurenic acid (nmol/L)	970	312	1.10	0.99; 1.24	8.6E-02	972	313	1.06	0.92; 1.21	4.1E-01
Xanthurenic acid (nmol/L)	970	312	0.97	0.87; 1.08	5.7E-01	972	313	0.97	0.85; 1.11	6.7E-01
Anthranilic acid (nmol/L)	918	297	**1.16**	**1.04; 1.31**	**9.7E-03**	948	309	1.13	0.98; 1.30	9.5E-02
3-Hydroxyanthranilic acid (nmol/L)	918	297	1.03	0.92; 1.14	6.4E-01	948	309	0.96	0.85; 1.10	5.6E-01
Picolinic acid (nmol/L)	970	312	1.02	0.91; 1.13	7.7E-01	972	313	0.97	0.85; 1.11	6.4E-01
Quinolinic acid (nmol/L)	970	312	**1.25**	**1.10; 1.43**	**7.9E-04**	972	313	1.14	0.99; 1.32	6.3E-02
Other markers										
Trigonelline (µmol/L)	970	312	0.98	0.88; 1.08	6.6E-01	972	313	0.94	0.83; 1.05	2.8E-01
Neopterin (nmol/L)	918	297	**1.21**	**1.08; 1.35**	**1.4E-03**	948	309	1.10	0.97; 1.25	1.5E-01
Cystathionine (µmol/L)	970	312	1.01	0.92; 1.10	9.0E-01	972	313	0.95	0.85; 1.06	3.4E-01
C-reactive protein (µg/ml)	936	300	1.11	0.99; 1.23	7.0E-02	963	311	0.97	0.86; 1.09	6.0E-01
Cystatin C (µg/ml)	948	303	**1.16**	**1.05; 1.29**	**3.0E-03**	970	313	1.10	0.98; 1.24	1.1E-01
Calprotectin (µg/ml)	947	303	1.06	0.97; 1.17	1.9E-01	970	313	0.94	0.84; 1.06	3.2E-01
Serum amyloid A (µg/ml)	947	303	1.10	0.99; 1.21	7.8E-02	970	313	1.02	0.91; 1.16	7.0E-01
Interleukin 6 (pg/mL)	971	314	1.09	0.99; 1.20	8.1E-02	972	315	0.90	0.81; 1.00	4.4E-02
Interleukin 8 (pg/mL)	971	314	1.04	0.96; 1.12	3.8E-01	972	315	1.01	0.90; 1.13	8.6E-01
Interleukin 10 (pg/mL)	971	314	1.07	0.97; 1.17	2.0E-01	972	315	1.04	0.93; 1.17	4.8E-01
Interferon gamma (pg/mL)	971	314	1.02	0.93; 1.13	6.3E-01	972	315	0.96	0.85; 1.08	4.8E-01
Tumour necrosis factor alpha (pg/mL)	971	314	**1.11**	**1.01; 1.21**	**2.3E-02**	972	315	1.06	0.95; 1.18	3.3E-01
Derived markers										
PAr index = PA / (PL+PLP)	970	312	**1.12**	**1.00; 1.26**	**4.5E-02**	972	313	0.98	0.86; 1.11	7.4E-01
KTr = Kyn / Trp	970	312	1.12	1.00; 1.26	5.9E-02	972	313	1.04	0.91; 1.19	6.0E-01
HK:XA = HK / XA	910	292	**1.14**	**1.02; 1.28**	**2.4E-02**	948	309	1.08	0.94; 1.23	2.8E-01
HKr= HK / (KA+AA+XA+HAA)	910	292	1.03	0.91; 1.16	6.4E-01	948	309	1.03	0.89; 1.19	6.7E-01

Model 1: Cox proportional hazards regression models were adjusted for age, sex and country of birth (Australia/New-Zealand/other, UK, Italy, Greece).

Model 2: Model 1 + additional adjustment for follow-up levels of markers