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Interferon gamma (IFN-γ)-mediated inflammation and the kynurenine pathway in relation to risk of hip fractures: the Hordaland Health Study

E. M. Apalset · C. G. Gjesdal · P. M. Ueland · J. Øyen · K. Meyer · Ø. Midttun · G. E. Eide · G. S. Tell

Abstract

Summary The cytokine interferon gamma (IFN-γ) stimulates neopterin release and tryptophan degradation into kynurenines through the kynurenine pathway. High levels of neopterin were associated with increased hip fracture risk, as were some of the kynurenines, suggesting a role of IFN-γ-mediated inflammation in the processes leading to hip fracture.

Introduction Low-grade systemic inflammation has been associated with bone loss and risk of fractures. Interferon gamma (IFN-γ) initiates macrophage release of neopterin and also stimulates degradation of tryptophan along the kynurenine pathway as part of cell-mediated immune activation. Plasma neopterin and the kynurenine/tryptophan ratio (KTR) are thus markers of IFN-γ-mediated inflammation. Risk of hip fracture was investigated in relation to markers of inflammation and metabolites in the kynurenine pathway (kynurenines).

Methods Participants (71 to74 years, $N=3,311$) in the community-based Hordaland Health Study (HUSK) were followed for hip fractures from enrolment (1998–2000) until 31 December 2009. Plasma C-reactive protein (CRP), neopterin, KTR, and six kynurenines were investigated as predictors of hip fracture, using Cox proportional hazards regression analyses.

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Results A hazard ratio (HR) of 1.9 (95 % confidence interval (CI) 1.3–2.7) for hip fracture was found in the highest compared to the lowest quartile of neopterin (*p* trend across quartiles <0.001). CRP and KTR were not related to hip fracture risk. Among the kynurenines, a higher risk of fracture was found in the highest compared to the lowest quartiles of anthranilic acid and 3-hydroxykynurenine. For subjects in the highest quartiles of neopterin, CRP, and KTR compared to those in no top quartiles, HR was 2.5 (95 % CI 1.6–4.0). *Conclusions* This may indicate a role for low-grade immune activation in the pathogenic processes leading to hip fracture.

Keywords Hip fracture · Inflammation · Kynurenine pathway · Neopterin · Osteoimmunology · Osteoporosis

Introduction

Patients with inflammatory diseases are known to have higher risk of osteoporosis and fractures [1], and even low-grade subclinical systemic inflammation has been associated with low bone mineral density (BMD) [2]. High serum levels of proinflammatory cytokines, cytokine-soluble receptors [3, 4], and C-reactive protein (CRP) [5–9] have been associated with increased risk of fractures, although the findings are ambiguous for some of the inflammatory markers [3, 4].

The immune system influences differentiation and activity of bone cells within the bone microenvironment [1]. CRP is an acute phase reactant produced in response to cytokine release during inflammation, and high-sensitivity (hs) CRP allows assessment of low-grade inflammation. Interferon gamma (IFN-γ) is a major stimulator of cell-mediated immune activation [10] and differentiation of T cells and macrophages. In vitro and animal studies indicate a dual effect of IFN-γ on bone. In normal bone remodeling, IFN-γ may improve bone mass [11] and decrease bone degradation by blunting osteoclast formation [1]. In mouse models of inflammatory states, IFN-γ enhances bone degradation e.g., via stimulation of T cell secretion of the receptor activator of NF-κB ligand (RANKL) and tumor necrosis factor α [12]. In humans, IFN-γ has been used to increase bone resorption in pathologically dense bone tissue (osteopetrosis) [1]. Results from a few [13–15], but not all [16] studies have indicated an association between IFN-γ or gene variants influencing IFN-γ level, with osteoporosis and fracture risk.

In a state of immune activation, IFN-γ stimulates macrophages to release neopterin [17], which due to its stability in body fluids is used as an index of IFN-γ-mediated inflammation [10] and for the assessment of disease activity in several conditions [10]. Further, IFN-γ stimulates the indoleamine dioxygenase (IDO) enzyme [18], initiating degradation of the essential amino acid tryptophan through the kynurenine pathway into metabolically active compounds known as kynurenines (Fig. 1). IDO and the kynurenines are increasingly recognized as important regulators of the immune system, e.g., by modulating the function of regulatory T cells [18, 19]. Some kynurenines are powerful regulators of oxidative stress as well, having both pro- and anti-oxidant abilities [20].

Kynurenine is the initial degradation product of tryptophan through the kynurenine pathway, and the kynurenine/tryptophan ratio (KTR) is a marker of IDO activity and cell-mediated inflammation [18]. Increased KTR has been related to neurological and mental conditions [21], as well as to cardiovascular [22] and renal disease [23]. A possible relation between neopterin or the kynurenines and risk of hip fractures has not previously been reported.

We have earlier reported inverse associations between BMD and IFN-γ-mediated inflammation and positive associations with some metabolites of the kynurenine pathway [24]. The aim of the present study was to extend our previous findings by further examining IFN-γ-associated inflammation markers and kynurenines, in addition to hs-CRP, as predictors of hip fracture.

## Materials and methods

Subjects

The community-based Hordaland Health Study (HUSK) was conducted in the city of Bergen and three neighboring municipalities, and cohort inclusion took place from April 1998 to June 1999. Subjects born during 1925–27 who had previously participated in the Hordaland Homocysteine Study 1992–93 were invited (*N*=4,338). Of these, 3,341 met for the study and answered questionnaires. Blood samples were analyzed from 3,311 participants (76.3 % of those invited). The Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate approved the study protocol, and all participants signed an informed consent. The study was performed according to the principles of the Declaration of Helsinki of the World Medical Association.

Blood samples

Non-fasting blood samples were drawn and stored in EDTA-containing tubes, which were chilled within 15–30 min and then centrifuged. Unfrozen plasma samples were transported to the laboratory, usually arriving within 1 day, always within 4 days. The samples were frozen at −80 °C and stored for up to 13 years before analysis [25]. Plasma analyses of tryptophan and the kynurenines (kynurenine, kynurenic acid, anthranilic acid, 3-hydroxykynurenine, xanthurenic acid, and 3-hydroxyanthranilic acid), neopterin, and cotinine (a nicotine metabolite used as a biomarker of recent nicotine exposure) were performed using liquid chromatography-tandem mass

Fig. 1 The kynurenine pathway of tryptophan metabolism. Degradation of the essential amino acid tryptophan through the kynurenine pathway is initiated by either indoleamine 2,3-dioxygenase or by tryptophan dioxygenase (TDO). TDO is primarily found in the liver and is induced by tryptophan and corticosteroids, whereas IDO is an ubiquitous enzyme induced by cytokines. *AMO* anthranilate monooxygenase, *KAT* kynurenine aminotransferase, *KMO* kynurenine monooxygenase, *KYNU* kynureninase
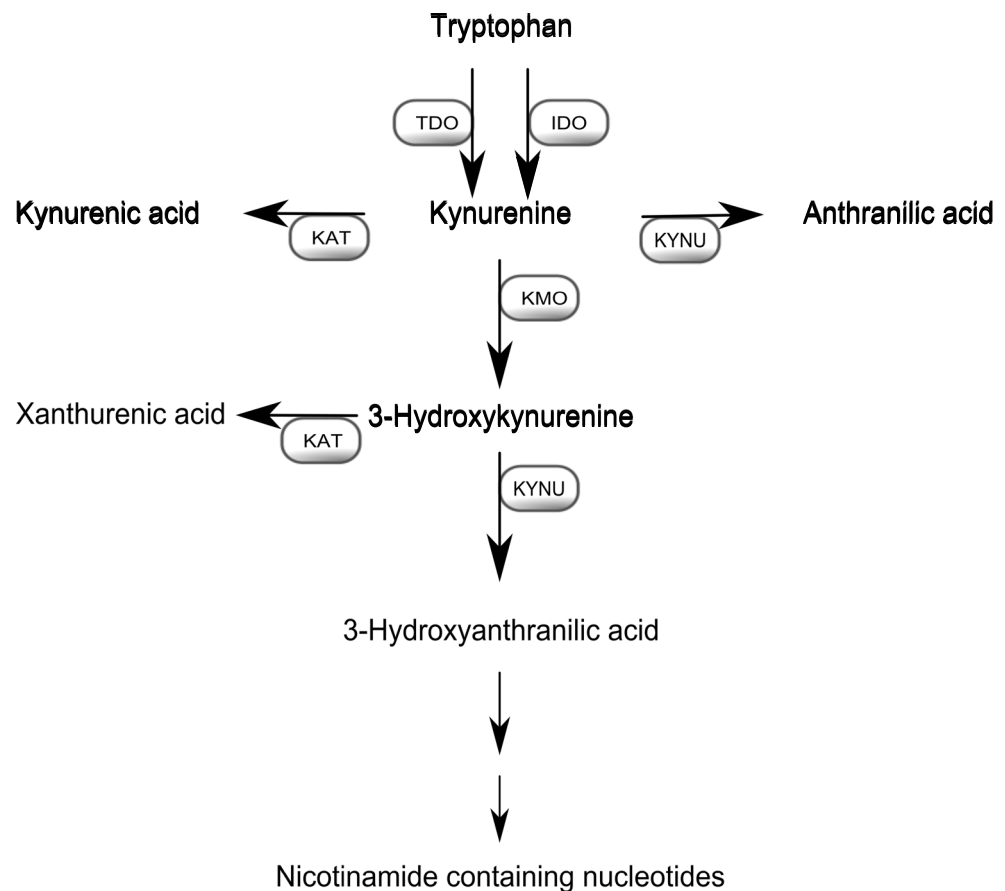
spectrometry (LC/MS/MS) at Bevital A/S (www.bevital.no/), Bergen, Norway [26]. Studies of stability and deterioration kinetics indicate that the studied biomarkers are stable under these conditions [27, 28].

Sensitive C-reactive protein EDTA plasma levels were determined at Bevital A/S by a novel immuno-assay based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Hip fractures

A hip fracture was defined as the first fracture of the proximal femur occurring during the observation period between baseline and 31 December 2009. Information on hip fractures was obtained from the hospitals serving Hordaland County, using a computerized search of discharge diagnosis records. Only hip fractures confirmed by a concurrent code of an adequate surgical procedure code were included, as previously described [29] and validated [30]. The discharge diagnoses used to classify a hip fracture were according to the International Classification of Diseases, Ninth Revision (ICD-9): 820–820.9 and Tenth Revision (ICD-10): S720-S722. Surgical treatments for each hospital stay were coded according to the Norwegian Classification of Surgical Procedures (version 2, 1989 and 3, 1995) and later the Nordic Medico-Statistical

Committee (NOMESCO) Classification of Surgical Procedures (version 1, 1999- version 3, 2004). The Norwegian Population Register provided information on time of death. Follow-up time was from enrolment until first hip fracture, death or 31 December 2009.

Additional data

Self-administered questionnaires provided information on health status, use of medication, sociodemographic data, and lifestyle factors. Use of disease-modifying anti-rheumatic drugs (DMARDs) was included in some of the analyses as a marker of inflammatory rheumatic disease. Reported use of DMARDSs included methotrexate, sulfasalazine, hydroxychloroquine, cyclosporine, auranofin, and penicillamine. Use of non-steroidal anti-inflammatory drugs (NSAIDs) was included in supplementary analyses to study any effect of these medications on the relation between inflammatory markers and hip fractures.

Height and weight were measured in light clothing, and body mass index (BMI; kg/m^2) calculated. BMD was measured by dual X-ray densitometer (EXPERT-XL; Lunar Company Inc, Madison, WI, USA, software version 1.72 and 1.90) in a subgroup of 2,221 participants as described earlier [24].

Statistical analyses

Distribution of continuous variables is reported as means and standard deviations (SD) for normally distributed variables, or medians and interquartile range for skewed distributions. Counts (percentages) are reported for categorical variables. Subjects with and without later hip fractures were compared within each sex, using the independent t tests for continuous variables and the exact chi-square test for categorical outcomes.

Nicotine exposure (cotinine) was categorized into no, light, or heavy exposure for the baseline characteristics table, but cotinine was used as a continuous variable in the analyses. Use of corticosteroids, DMARDs, and NSAIDs was dichotomized into present use or no use. Renal function was estimated by glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) formula [31] and was dichotomized according to normal (60 mL/min or above) or reduced (below 60 mL/min) renal function.

Correlations between CRP, neopterin and KTR in the whole cohort were assessed by Spearman's rho (rs).

Cox proportional hazards model was used to estimate the associations between serum neopterin, KTR, tryptophan, and kynurenines (continuous and categorized into quartiles) and risk of subsequent hip fracture. Analyses using quartiles of the inflammatory markers and kynurenines are reported, as well as p for trend across quartiles. Multicollinearity between covariates and interactions between covariates and time were tested. The multivariable model includes sex, BMI, cotinine, and eGFR.

A composite measure was constructed by combining those being in the highest quartile of all the three inflammatory markers (CRP, neopterin, and KTR), the highest quartile of two (CRP and neopterin, or CRP and KTR), one or none, and the risk of hip fracture was examined using Cox regression analyses.

Significance level of 0.05 was chosen for all statistical tests. The statistical package SPSS (version 18 for Windows, Chicago, IL, USA) was used. Venn diagram was created using Google (https://developers.google.com/chart/image/docs/chart_wizard).

Results

Follow-up data and baseline characteristics

Median follow-up time was 10.8 years (range 0.01–11.7), and a hip fracture during follow-up was recorded among 277 (8.4 %) of the participants. Baseline characteristics are shown and compared according to hip fracture status within each sex in Table 1. No differences in factors related to health or lifestyle were found among men, but those later suffering a hip fracture had higher neopterin and anthranilic acid levels.

Women with a hip fracture during follow-up had lower baseline BMI and a higher exposure to nicotine. No significant differences in inflammation markers or kynurenines were found among women with and without a later hip fracture.

Correlations

A positive correlation was found between CRP and neopterin (r_s=0.19, $p<0.001$), CRP and KTR (r_s=0.22, $p<0.001$), and neopterin and KTR (r_s=0.52, $p<0.001$).

Inflammation markers, kynurenines, and risk of hip fracture

Cox proportional hazards regression analyses of risk of hip fractures were performed according to quartiles of CRP, neopterin, KTR, tryptophan, and kynurenines as shown in Table 2. A statistically significant prediction of hip fracture was found for neopterin and tryptophan after adjustment for sex. In the fully adjusted model (adjustment for sex, BMI, cotinine, and eGFR), a significant higher risk of hip fracture was found in the highest compared to the lowest quartiles of neopterin, anthranilic acid, and 3-hydroxykynurenine, with a significant trend across quartiles. The estimates were mostly unchanged after additional adjustment for use of corticosteroids and DMARDs, and there were no major changes after adjusting for use of NSAIDs. Levels of CRP and KTR were not associated with risk of hip fracture.

Additional analyses for anthranilic acid and 3-hydroxykynurenine as continuous variables in relation to risk of hip fracture were done. Anthranilic acid was not significantly associated with hip fracture in analysis adjusting for sex only ($p=0.060$), but significantly associated in the model adjusted for sex, BMI, cotinine, and eGFR ($p=0.003$). A statistically significant association was also found between 3-hydroxykynurenine and risk of hip fracture ($p=0.033$ adjusted for sex, $p=0.001$ in the multiple adjusted model).

Cox proportional hazards regression analyses were also performed for risk of hip fracture in relation to the inflammatory markers and the kynurenines with additional adjustment for BMD among the 2,221 participants with BMD measurement, but this did not materially change the estimates.

Cox regression analyses were performed for men and women combined. However, an interaction between sex and neopterin was found for risk of hip fracture (p interaction=0.021), with hazard ratio (HR) 3.23 (95 % confidence interval (CI) 1.69–6.19) for men in the highest compared to the lowest neopterin quartile (p trend across quartiles <0.001), and HR 1.48 (CI 0.99–2.22) among women (p trend=0.069). No interaction was found between sex and CRP, KTR, or kynurenines in relation to risk of hip fracture.

Subjects in the highest quartile (Q4) of all three inflammatory markers (CRP, neopterin, and KTR) ($n=171$, constituting 5 % of the cohort) had a higher risk of hip fracture compared

Table 1 Baseline characteristics 1998–99 in 3,311 women and men (71–74 years old), stratified by hip fracture during follow-up. The Hordaland Health Study

	Men			Women		
	No hip fracture (n =1,369)[a]	Hip fracture (n=86)[a]	p value[b]	No hip fracture (n =1,665)[a]	Hip fracture (n =191)[a]	p value[b]
BMI, mean (SD)	26.0 (3.2)	25.8 (3.5)	0.556	26.4 (4.4)	24.8 (4.2)	0.001
eGFR below 60 mL/min, n (%)	273 (20.0)	19 (22.1)	0.677	928 (55.7)	92 (48.2)	0.055
Current use of corticosteroids, n (%)	22 (1.6)	3 (3.5)	0.180	34 (2.0)	7 (3.7)	0.185
Current use of DMARD, n (%)	11 (0.8)	2 (2.3)	0.177	21 (1.3)	5 (2.6)	0.179
Current use of NSAID, n (%)	35 (2.6)	2 (2.3)	0.999	111 (6.7)	15 (7.9)	0.543
Current use of estrogen, n (%)	NA	NA		176 (14.5)	21 (15.4)	0.798
Cotinine, n (%)[c]			0.149			0.001
<85 nmol/L (light nicotine exposure)	1,100 (80.4)	62 (72.1)		1,409 (84.6)	144 (75.4)	
85–1,000 nmol/L (moderate nicotine exposure)	89 (6.5)	8 (9.3)		131 (7.9)	16 (8.4)	
>1,000 nmol/L (heavy nicotine exposure)	180 (13.1)	16 (18.6)		125 (7.5)	31 (16.2)	
Cotinine (nmol/L), median (IQR)[d]	0.9 (9.9)	1.3 (586.3)	0.081	0.4 (3.4)	0.9 (25.9)	0.001
Inflammation markers						
CRP (μg/mL), median (IQR)	2.2 (3.4)	2.3 (4.9)	0.441	2.1 (3.4)	2.2 (3.7)	0.605
Neopterin (nmol/L), median (IQR)	8.6 (3.3)	9.6 (5.2)	0.003	8.7 (3.3)	8.8 (4.1)	0.809
Kynurenine/tryptophan ratio, median (IQR)	26.1 (10.1)	27.5 (11.1)	0.172	26.3 (10.1)	26.1 (11.4)	0.863
Kynurenines						
Tryptophan (μmol/L), median (IQR)	68.2 (18.3)	64.3 (19.6)	0.117	62.6 (17.8)	61.2 (17.1)	0.102
Kynurenine (μmol/L), median (IQR)	1.8 (0.6)	1.8 (0.7)	0.913	1.7 (0.6)	1.6 (0.6)	0.148
Kynurenic acid (nmol/L), median (IQR)	54.6 (27.3)	53.5 (38.8)	0.452	48.2 (24.9)	46.9 (27.5)	0.466
Anthranilic acid (nmol/L), median (IQR)	16.7 (7.3)	19.0 (9.3)	0.028	15.9 (6.8)	16.1 (7.2)	0.549
3-Hydroxykynurenine (nmol/L), median (IQR)	36.2 (15.3)	37.0 (19.7)	0.480	35.3 (15.8)	35.1 (17.1)	0.386
Xanthurenic acid (nmol/L), median (IQR)	16.6 (11.0)	16.3 (10.3)	0.515	14.1 (9.1)	13.4 (8.4)	0.054
3-Hydroxyanthranilic acid (nmol/L), median (IQR)	36.0 (18.0)	33.1 (22.7)	0.949	32.8 (16.4)	32.1 (16.4)	0.522

BMI body mass index, *CRP* C-reactive protein, *DMARD* disease-modifying anti-rheumatic drug, *eGFR* estimated glomerular filtration rate, *IQR* interquartile range, *NSAID* non-steroidal anti-inflammatory drug, *NA* not applicable, *SD* standard deviation

[a] Total numbers may vary between variables due to missing data

[b] Groups with and without hip fracture compared within each sex by independent samples t test (continuous data) or the exact chi-square test (categorical data)

[c] Cotinine compared as categorical variable

[d] Cotinine compared as continuous variable

to those not being in Q4 of any of the inflammatory markers (n=1,638) (Fig. 2), with HR 2.51 (CI 1.59–3.98) adjusted for sex, cotinine, BMI, and eGFR (p trend<0.001 according to being in the highest quartile of one, two, or three markers). The distribution of participants according to any combination of the highest quartile of one, two, or all three inflammatory markers is shown in Fig. 3.

Discussion

In this study, we investigated CRP and the IFN-γ-induced inflammation markers neopterin and KTR, as well as kynurenines, in relation to risk of hip fracture. A higher risk of hip fracture among subjects in the highest compared to the lowest quartiles of neopterin, anthranilic acid, and 3-

hydroxykynurenine was found, after adjustment for sex, BMI, cotinine, and renal function. These findings persisted after additional adjustment for BMD in a subgroup analysis. Further, we found that participants in the top quartiles of all three inflammatory markers (CRP, neopterin, and KTR) had a 2.5-fold higher risk of hip fracture compared to those with no inflammatory marker in the highest quartile.

Plasma concentrations of all the kynurenines were similar to those obtained in fresh samples indicating acceptable conditions for blood sample collection and storage [27]. Levels of neopterin, tryptophan, and kynurenine were comparable to those found in other studies [22, 32], as were correlations between the inflammatory markers CRP, neopterin, and KTR [21, 22, 33].

Strengths of the study include the large size of the cohort, the long follow-up time, as well as the comprehensive

Table 2 Risk of hip fracture by Cox proportional hazard model according to quartiles (Q1 lowest–Q4 highest) of inflammation markers and metabolites of the kynurenine pathway in 71–74 years old women and men. The Hordaland Health Study

	n	Model	Q1	Q2 HR (CI)	Q3 HR (CI)	Q4 HR (CI)	p trend[a]
CRP	3,198	Model 1	1.00 (ref)	0.91 (0.65, 1.29)	0.95 (0.68, 1.34)	1.08 (0.77, 1.50)	0.638
		Model 2	1.00 (ref)	1.00 (0.71, 1.42)	1.06 (0.75, 1.51)	1.28 (0.91, 1.81)	0.160
Neopterin	3,311	Model 1	1.00 (ref)	1.06 (0.74–1.52)	1.17 (0.83–1.66)	1.70 (1.22–2.36)	0.001
		Model 2	1.00 (ref)	1.08 (0.76–1.55)	1.23 (0.86–1.75)	1.88 (1.34–2.65)	0.001
KTR	3,289	Model 1	1.00 (ref)	0.80 (0.57–1.12)	0.85 (0.61–1.19)	1.10 (0.80–1.51)	0.515
		Model 2	1.00 (ref)	0.85 (0.60–1.19)	1.00 (0.71–1.41)	1.31 (0.94–1.82)	0.087
Tryptophan	3,311	Model 1	1.00 (ref)	0.95 (0.70–1.30)	0.64 (0.46–0.91)	0.73 (0.53–1.02)	0.013
		Model 2	1.00 (ref)	1.04 (0.76–1.42)	0.71 (0.50–1.01)	0.81 (0.58–1.13)	0.065
Kynurenine	3,289	Model 1	1.00 (ref)	0.78 (0.56–1.09)	0.87 (0.62–1.20)	0.94 (0.68–1.31)	0.863
		Model 2	1.00 (ref)	0.88 (0.63–1.23)	1.05 (0.75–1.47)	1.25 (0.89–1.77)	0.146
Kynurenic acid	3,311	Model 1	1.00 (ref)	0.72 (0.52–1.01)	0.70 (0.50–0.97)	0.91 (0.66–1.25)	0.500
		Model 2	1.00 (ref)	0.80 (0.58–1.12)	0.83 (0.59–1.17)	1.19 (0.85–1.66)	0.398
Anthranilic acid	3,289	Model 1	1.00 (ref)	0.85 (0.60–1.20)	1.00 (0.72–1.40)	1.20 (0.87–1.66)	0.170
		Model 2	1.00 (ref)	0.96 (0.67–1.36)	1.24 (0.87–1.75)	1.48 (1.06–2.08)	0.009
3-Hydroxykynurenine	3,310	Model 1	1.00 (ref)	1.14 (0.82–1.59)	0.90 (0.63–1.27)	1.29 (0.93–1.79)	0.30
		Model 2	1.00 (ref)	1.26 (0.90–1.76)	1.04 (0.73–1.49)	1.63 (1.15–2.30)	0.022
Xanthurenic acid	3,310	Model 1	1.00 (ref)	1.09 (0.79–1.50)	0.88 (0.63–1.24)	0.82 (0.59–1.16)	0.15
		Model 2	1.00 (ref)	1.17 (0.85–1.62)	1.08 (0.73–1.43)	1.00 (0.70–1.42)	0.80
3-Hydroxyanthranilic acid	3,310	Model 1	1.00 (ref)	0.82 (0.59–1.14)	0.73 (0.52–1.02)	0.87 (0.63–1.20)	0.31
		Model 2	1.00 (ref)	0.94 (0.68–1.31)	0.87 (0.62–1.22)	1.10 (0.79–1.53)	0.73

Model 1: adjusted for sex

Model 2: adjusted for sex, body mass index, cotinine, eGFR (estimated glomerular filtration rate over/below 60 mL/min)

CI 95 % confidence interval, *CRP* C-reactive protein, *HR* hazard ratio, *KTR* kynurenine/tryptophan ratio, *Q1–Q4* quartiles 1 to 4

[a] Across quartiles

laboratory analyses performed at the same laboratory. Data on medication may have been insufficient as participants were only asked about medications taken the previous day. Concentrations of biomarkers and data on potential confounders were obtained at baseline and not repeated during follow-up. However, the biomarkers have sufficient reproducibility (intraclass correlation coefficient over 2–3.5 years in the range 0.5–0.7) to allow one-exposure assessment of biomarker status [28].

KTR and the kynurenines are earlier found to be associated with BMI [34], smoking [34, 35], and renal function [23, 34]. These factors are confounders in the relation between kynurenines and osteoporosis/fracture risk [36–38] and were therefore adjusted for.

CRP levels were not significantly associated with risk of hip fractures in the present study. This was somewhat unexpected, as an association between CRP and low-trauma fractures has previously been reported [5–7, 9]. To our knowledge, risk of hip fractures separately in relation to CRP has only been investigated in one study [39], demonstrating no significant association.

We found that the IFN-γ-mediated inflammation marker neopterin was associated with an increased risk of hip fracture.

A higher IFN-γ production in peripheral blood mononuclear cells has been reported for postmenopausal women with vertebral fractures, compared to controls [13], and experimental studies have indicated a role of IFN-γ in postmenopausal bone loss [40]. However, we are aware of no previous studies on plasma neopterin and fracture risk. Due to the complexity of the immunologic pathways involved in inflammation, combining different markers may better reflect overall activation of the immune system than one single inflammatory marker. An enhanced prediction of fracture risk by combining inflammatory markers has been reported for other studies as well [3, 4].

The observed association between inflammation assessed by neopterin and risk of fracture was independent of BMD. This is in agreement with earlier studies [4–7, 9], suggesting an impact of inflammation on bone microarchitecture and bone strength [5, 8], or the finding could possibly be explained by other factors such as reduced muscle strength, impaired balance, or impaired nutritional status.

In the present study, anthranilic acid and 3-hydroxykynurenine were associated with an increased risk of hip fracture. We are not aware of previous studies on kynurenines in relation to fracture risk, but the finding is in accordance with one other study describing increased levels

Fig. 2 Kaplan-Meier survival curves for time to hip fracture in 3,178 men and women (71–74 years at inclusion), stratified on subjects in the highest quartiles of none ($n=1,638$), one ($n=879$), two ($n=490$), or three ($n=171$) inflammation markers (CRP, neopterin, kynurenine/tryptophan ratio). The Hordaland Health study
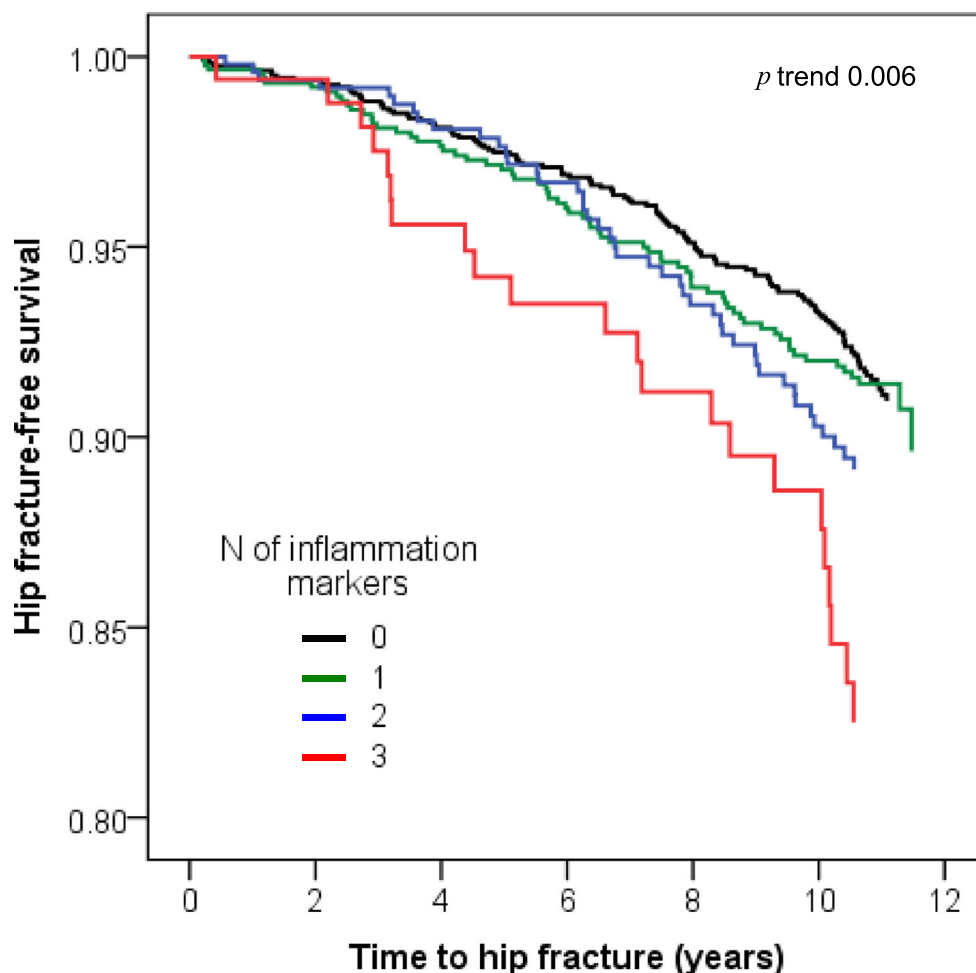
of anthranilic acid in osteoporotic patients compared to controls [41].

Our knowledge of the immune system has evolved from the idea of a mere protector against invading organisms to an

Fig. 3 Venn diagram showing the distribution of participants in the Hordaland Health Study according to inflammatory status assessed as any combination of inflammatory markers in the upper quartile of each marker. Of the 3,178 participants with measurements of all three inflammatory markers, 1,638 (51.5 %) persons were not in the highest quartile of any of the inflammatory markers. The correct proportional overlap between the inflammatory markers is shown, and *numbers* show percentages of the total cohort
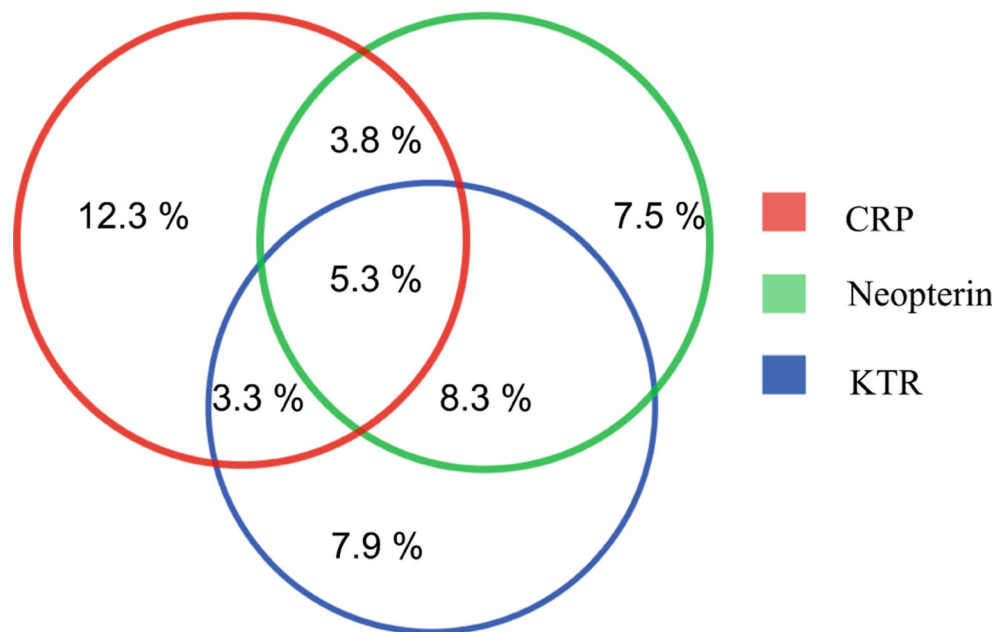
increasing understanding of its cruciate role in physiological processes, including differentiation and stimulation of bone cells [1]. CRP release is mainly elicited by interleukin-6 (IL-6) [42], one of several cytokines inducing release of RANKL [1], the main stimulator of bone degradation. IFN-γ has been found to stimulate osteoclast formation in states of immune activation through T cell RANK signaling [12]. T cell RANKL expression has, however, not been found as an important stimulator of bone resorption in non-inflammatory conditions [43]. In contrast, murine studies have shown that IFN-γ is essential for development of normal bone structure under physiological conditions [11]. IFN-γ also reduces bone resorption in vitro by inhibiting the ability of 1,25-dihydroxyvitamin D3, parathyroid hormone, and IL-1 to stimulate the formation of osteoclast-like cells [1]. Higher expression of IFN-γ-related genes in human hip bone samples from osteoarthritic compared with osteoporotic patients has been reported [16], which is not in agreement with our earlier and present findings indicating an association between IFN-γ-stimulated inflammation and increased risk of low BMD and hip fracture [24]. This may illustrate a difference in local and systemic effects.

3-Hydroxykynurenine has both pro- and anti-oxidative properties [20, 44] and was associated with risk of hip fracture in the present study. An increase in reactive oxygen species has been linked to higher bone resorption [45]. The association between 3-hydroxykynurenine and an increased risk of hip fracture in the present study may also be related to a positive association between 3-hydroxykynurenine and inflammatory markers [33].

In this study, we found that the inflammation marker neopterin was positively associated with risk of hip fracture, while CRP and KTR were not. This may possibly reflect a greater impact of IFN-γ-mediated inflammation on fracture risk than components of the innate immune system as measured by CRP. Further, high levels of anthranilic acid and 3-hydroxykynurenine were also associated with increased risk of hip fracture. Although these findings may suggest an association between INF-γ-mediated inflammation and risk of hip fractures, additional studies are required to explore the role of IDO and kynurenines in bone metabolism.

Conflicts of interest None.

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