International Journal of Cardiology 172 (2014) 173–178

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

# Association of interleukin 8 with myocardial infarction: Results from the Stockholm Heart Epidemiology Program☆☆

Ilais Moreno Velásquez [a,*], Paolo Frumento [b], Katarina Johansson [c], Anita Berglund [a], Ulf de Faire [a,d], Karin Leander [a], Bruna Gigante [a,e]

[a] Unit of Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[b] Unit of Biostatistics, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[c] Division of Biochemistry, Department of Medical Biochemistry and Biophysics all at Karolinska Institutet, Stockholm, Sweden
[d] Dept of Cardiology, Karolinska University Hospital, Stockholm, Sweden
[e] Division of Cardiovascular Medicine, Dept of Clinical Sciences, Karolinska Institutet, Danderyd Hospital, Stockholm, Sweden

ARTICLE INFO

*Article history:*
Received 16 July 2013
Accepted 31 December 2013
Available online 9 January 2014

*Keywords:*
Interleukin 8
Myocardial infarction
Atherosclerosis
Biomarker
Genetic variants

ABSTRACT

*Background:* Interleukin 8 (IL8) has been contradictorily associated with the risk of myocardial infarction (MI).
*Aim:* To investigate the association of IL8 serum levels with the risk of MI and the association of the IL8 (*IL8*) and IL8 receptors (*CXCR1* and *CXCR2*) genetic variants with IL8 levels and MI risk in a large case control study, the Stockholm Heart Epidemiology Program.
*Methods and results:* IL8 levels (pg/mL) were divided into quartiles and the MI risk was calculated by logistic regression and expressed as odds ratio (OR) and 95% CI. Two *IL8* SNPs (rs4073A/T, rs2227306C/T) and three SNPs tagging *CXCR1* and *CXCR2* (rs4674258C/T, rs1008563C/T, rs6723449T/C) were analyzed for association with IL8 levels and with MI risk.
Multivariate adjusted ORs for MI risk by IL8 levels in the highest quartiles indicated reduced point estimates in both women (OR 0.37; 95% CI 0.2–0.8) and men when compared to the lowest quartile. In female cases, IL8 levels decreased progressively in the six months after MI ($p = 0.03$). *IL8, CXCR1* and *CXCR2* genetic variants were not associated with IL8 levels. In men, the T allele at the *IL8* SNP rs4073 was associated with a slight increase in the MI risk under an additive and a recessive model of inheritance.
*Conclusions:* IL8 serum levels were associated with a reduced occurrence of MI among women, whereas *IL8* was associated with a slightly increased risk among men, possibly through different mechanisms. These data suggest that the biological effects of IL8 on MI risk may vary over time and warrant further cohort studies with repetitive IL8 measurements.



## 1. Introduction

Since its discovery during the late 80s, interleukin 8 (IL8) has been described as a cytokine participating in all stages of atherosclerosis from vascular inflammation to cardiac remodeling after myocardial infarction (MI) [1]. Two IL8 receptors have been reported: CXCR1 that binds IL8 and granulocyte chemoattractant protein 2 (CXCL6) and CXCR2 that binds with a high affinity multiple chemokines [2–4].

IL8 promotes and sustains the shift from an acute to a chronic inflammatory reaction in the vessel wall by mediating the release of the monocyte chemo-attractant protein 1 (MCP-1) and the shedding of soluble receptor of the interleukin 6 (IL6) from the neutrophils in the inflammatory infiltrate [5]. Hours immediately after an MI event, IL8 induces homing of neutrophils that contribute to myocardial injury [6]. However, in the long term IL8 exerts a more protective effect on cardiac remodeling by promoting new vessel generation through the mobilization and homing of circulating endothelial progenitor cells (EPCs) from the bone marrow to the injured myocardium, a mechanism that may facilitate wound healing and myocardial tissue repair [7,8].

The association of IL8 serum levels and of IL8 gene (*IL8*) with the risk of coronary heart disease (CHD) has been investigated in a few small studies with contradictory findings. In hospital based studies, circulating IL8 levels were higher in patients with acute MI or unstable angina as compared to controls [6,9,10]. The predictive value of circulating IL8 levels as a risk marker for CHD in healthy subjects seems uncertain with one study performed in the MONICA/KORA cohort, showing that

☆☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

* Corresponding author at: Unit of Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Nobelsväg 13, 17177 Stockholm, Sweden. Tel.: +46 8 52487082.
*E-mail address:* ilais.moreno.velasquez@ki.se (I.M. Velásquez).

0167-5273/$ – see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ijcard.2013.12.170

high IL8 serum levels did not predict CHD independently of other risk factors, and another study, conducted in the EPIC-Norfolk cohort, indicating that elevated IL8 plasma levels predicted CHD in men and women [11,12].

A genetic variant in the *IL8* promoter region has been associated with increased IL8 plasma levels as well as with an increased risk of acute coronary syndrome (ACS) in a Chinese Han population [13] and with a reduced risk of MI among CHD patients in a Caucasian population [14].

Finally, although widely studied in other inflammatory diseases [15], the association of variants in the IL8 receptor genes (*CXCR1* and *CXCR2*) with the risk of CHD and IL8 serum levels has not yet been investigated.

The aim of this study was to investigate the association between IL8 serum levels and the genetic variants of the *IL8, CXCR1* and *CXCR2* with the risk of MI in the Stockholm Heart Epidemiology Program (SHEEP), a large population based case control study. We also aim to explore if the *IL8*, *CXCR1* and *CXCR2* regulate the IL8 serum levels in the same population.

## 2. Methods

### 2.1. Study design

The SHEEP study base comprises all Swedish citizens from 1992 to 1994 resident in the Stockholm County who were 45 to 70 years of age and free of previous clinically diagnosed MI. Controls were randomly sampled from the study base, continuously over time as the cases occurred matched on age (5-year interval), sex and hospital catchment area. A detailed description of the study is given elsewhere [16,17]. Briefly, in the present sub-study only non-fatal cases (defined as survival at least 28 days after MI, $n = 1213$) and their matched controls ($n = 1561$) are included. Clinical investigations and blood sampling were performed at least three months after the MI. Biochemical measurements were done as previously reported [16,18–20]. Other exposure information was collected through postal questionnaires distributed to non-fatal cases and to their controls at the time of inclusion in the study. SHEEP has been approved by the Regional Ethical Review Board at Karolinska Institutet, Stockholm, Sweden. All the study participants gave their informed oral consent to be enrolled in the study.

### 2.2. IL8 serum measurement

IL8 was measured in 1847 serum samples (1133 controls and 714 cases) using electrochemiluminescence immunoassay plates produced from Meso Scale Discovery's Multi-Array ® (MSD). Our samples were analyzed in 96 Well plates using MSD® 6000 instrument, following the manufacturer's instructions. Serum concentrations were derived from the standard curve and expressed as picograms per milliliter (pg/mL). To calculate the intra and inter assay variability, $n = 648$ samples were run in duplicate and $n = 112$ samples were run in independent experiments, respectively.

### 2.3. IL8, CXCR1 and CXCR2 single nucleotide polymorphisms (SNPs) selection and genotyping

#### 2.3.1. IL8

HapMap (www.hapmap.org) identifies one tag SNP (rs2227306C/T) in *IL8* gene in sequence **NM_000584.3**. In addition we have genotyped the promoter variant rs4073A/T, in position −251 in respect to the *IL8* sequence, previously associated with IL8 serum levels and the risk of CHD [13].

#### 2.3.2. CXCR1 and CXCR2

The receptors for IL8 are coded by two genes forming a cluster on chromosome 2 (2q33–q36) in sequence **NM_000634** and **NM_001557**, respectively. Two tagSNPs were identified: rs4674258C/T, rs6723449T/C, for *CXCR2* and one tagSNP rs1008563C/T for *CXCR1*.

All tag SNPs were genotyped by MALDI-TOF (Matrix Adsorbed Laser Desorption-Ionisation-Time Of Flight) [21] on Massarray Analyzer platform (Sequenom, Mutation Analysis Facility at Karolinska Institutet). The promoter variant in *IL8* was genotyped with a custom made assay purchased from Applied Biosystems (ABI) and amplified with the TaqMan Universal PCR protocol.

### 2.4. Statistical analyses

Binary variables are expressed as percentages, quantitative variables as median and IQTR. Differences between cases and controls were determined using the *Chi* test for binary variables and the Kruskal–Wallis test for continuous variables.

IL8 serum levels had a right skewed distribution (skewness = 2.3). Serum levels were divided into quartiles (Q1 ≤ 8.3, Q2 > 8.3 ≤ 13.6, Q3 > 13.6 ≤ 20.9, Q4 > 20.9) based upon the serum values of the combined group of cases and controls. Additionally, the log2 of the IL8 serum levels was calculated to analyze the risk of MI associated with doubling of the IL8 serum levels. Unconditional logistic regression analysis was used to estimate odds ratio (ORs) and corresponding 95% confidence intervals (CIs) for the association of IL8 serum levels and the risk of MI. Crude estimates were adjusted for age, gender and hospital catchment area because these were matching factors in the original design of SHEEP. For the multivariate analyses, we consecutively adjusted for body mass index (BMI), C-reactive protein (CRP mg/L) and insulin (μU/mL) (model 1) that represent reported confounders for IL8 [22] and were thus considered potential confounders in the present study; model 1 + the conventional cardiovascular risk factors: hypertension (individuals on antihypertensive drug therapy or with a blood pressure ≥140/90 mm Hg), diabetes (subjects with a B-glucose value > 6.7 or controlling diabetes with insulin and/or other drug treatment), hypercholesterolemia (total cholesterol ≥ 6.46 mmol/L or receiving any lipid lowering medication) and current smoking (defined as actively smoking in the two years preceding MI) (model 2); model 2 + drug therapy with statins, β-blocker and hormone replacement therapy (HRT) for women (model 3) [23–25].

Serum levels of IL8, IL6, CRP and TNF-α were compared among men and women cases, sampled at different times after the MI event. The cases sampled after 6 months (180 days) ($n = 76$) from the first MI and cases with an ascertained second MI ($n = 143$) were not included in the present analysis. Eighty five percent of the samples were collected during the first 6 months post MI, the remaining 15% were collected over a long period, up to 3 years. Similarly, we excluded reinfarctions to minimize the possibility that differences of IL8 serum measurements occurred as a consequence of a symptomatic cardiovascular disease. It is plausible that the inflammatory biomarker in cases where reinfarction occurred may perhaps reflect different stages of atherosclerosis and overestimate the serum levels measurements. After exclusion, sampling occurred (M, IQTR) 100 (95–111) days after the MI event. Time to sampling was expressed in days as median (M), (IQTR) and divided in quartile (D1 <95 days; D2 ≥95 < 100; D3 ≥ 100 < 111; D4 > 111). Kruskal–Wallis test was used to analyze the differences in the inflammatory serum biomarkers at the different time points.

Differences in IL8 serum levels across the different genotypes for each genetic variant were tested by Kruskal–Wallis. To test the association of single SNPs with MI, a logistic regression analysis was performed and ORs (95% CI) were estimated under the assumption of an additive model of inheritance (assigning a score of 0 for the common allele homozygote, 1 for the heterozygote and 2 for the rare allele homozygote), a dominant model (assigning a score of 0 for the common allele homozygote, 1 for the combined rare allele homozygote/heterozygote) and a recessive model (assigning a score of 0 for combined common allele homozygote/heterozygote, 1 for the rare allele homozygote). Haplotypes for the *IL8* as well as the *CXCR1* and *CXCR2* were generated from the genotype data and tested for association with MI using THESIAS v.3.1 [26] Crude estimates were adjusted for age, gender and hospital catchment area. For the multivariate analyses, risk estimates were adjusted by hypertension, diabetes, smoking, hypercholesterolemia and BMI, defined as reported above.

All other analyses were performed using Stata software, version 11 (StataCrop LP, College Station, TX).

## 3. Results

Study participants characteristics are summarized in Table 1. With the exception of hypertension among men and women, the risk factors generally occurred more frequently among the cases than the controls.

### 3.1. IL8 serum levels and risk of MI

The median IL8 serum concentrations were significantly lower in cases than in controls in both men and women (Table 1). Doubling of IL8 serum levels was associated with a reduced risk of MI with an OR of 0.84 (95% CI 0.77–0.92), $p = 0.0001$, in the fully adjusted model (model 3). The crude and multivariate ORs and 95% CI for MI risk according to IL8 quartiles are reported in Table 2. When data were analyzed separately by sex, highest (Q4) IL8 serum levels were associated with a reduced risk of MI in women with an OR of 0.37 (95% CI, 0.2–0.8) as compared to the lowest quartile (Q1) after adjustment for all possible confounders (model 3). In men high serum levels of IL8 were also associated with a reduced, however non-significant, MI risk (Table 2).

Given the complex effect of IL8 on inflammation and cardiac remodeling, we have analyzed the difference in IL8 serum levels in cases according to the time of sampling, i.e. days after the MI event. IL8 serum levels were compared in cases ($n = 441$), where the serum was collected before 95 days (D1), between 95 and 100 days (D2), between 101 and 111 days (D3) and from 111 to 180 days (D4). As shown in Fig. 1 (bottom panel), in women IL8 serum levels (pg/mL) progressively decreased from 15.3 (10.4–19.8) in cases sampled within 95 days from MI to 8.6 (5.8–15.6) in cases sampled between 111 and 180 days post-MI ($p = 0.03$). This trend was not observed in men