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MRP 8/14 as a predictive biomarker in elderly survivors of acute coronary syndrome

MRP 8/14, en prediktiv biomarkör hos äldre överlevare av akut koronärt syndrom

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1. Abstract

Background and aims: Myeloid related protein 8/14 (MRP 8/14) is constitutively expressed in human neutrophils and monocytes and is secreted upon activation. In the acute coronary syndrome (ACS) MRP 8/14 is elevated at the site of the coronary occlusion and high levels have been associated with an increased risk of subsequent coronary events (CE) such as unstable angina (UA) or myocardial infarction (MI).

Our aim was to examine the value of MRP 8/14 as a cardiovascular (CV) risk predictor in a population of older survivors of ACS.

Methods: We measured MRP 8/14 levels in 119 elderly survivors of ACS in plasma samples collected at the acute event and at 6 week follow-up. The median age of the population was 79 years and 35% were female. The correlation between MRP 8/14 and the incidence of CE, stroke and all-cause mortality over a median follow-up period of 1.8 years (2.7 for mortality) was studied using Cox regression models corrected for age and sex (Model A); age, sex and traditional CV risk factors [hypertension, smoking, body mass index (BMI), diabetes mellitus, high density lipoprotein (HDL) and low density lipoprotein (LDL)] (Model B) and all of the above plus c-reactive protein (CRP) (Model C).

Results: We found that MRP 8/14 at 6 weeks after the acute event and the difference between the values at 6 weeks follow-up and at the acute event, but not values at the acute event were associated with the risk of new CE.

Conclusion: MRP 8/14 may prove to be a useful follow-up biomarker to assess prognosis in ACS survivors.

2. Populärvetenskaplig sammanfattning

Hjärtinfarkt är en av de vanligaste dödsorsakerna i världen och beror vanligtvis på att ett åderförkalkningsplack i hjärtats blodkärl brister. Placket består av en ansamling av fett, skumceller och rester av döda celler som ligger under ett lager av bindväv i kärlväggen. När placket brister aktiveras blodets koagulationssystem, då kärlväggen ej längre är intakt. Blodet levrar sig inuti kärlet och bildar en blodpropp, en så kallad tromb. Tromben täpper till blodflödet, och orsakar syrebrist i det område kärlet försörjer vilket kan leda till att hjärtmuskelceller dör.

Man har på senare år funnit att immunförsvarets celler, de vita blodkropparna, har en viktig roll både vid uppkomsten av åderförkalkning och i processerna som leder till att ett plack försvagas så att det riskerar att brista. MRP 8/14 är ett protein som finns i vissa typer av vita blodkroppar som kallas neutrofiler och monocyter. Man har upptäckt att MRP 8/14 är förhöjt lokalt vid platsen för tromben vid en hjärtinfarkt och att höga nivåer i blodet kan höja risken för att man skall få ytterligare en hjärtinfarkt i ett senare skede. Ingen av forskningen på MRP 8/14 som vi kunnat ta del av är gjord på äldre människor över 75 år, vilka utgör en stor del av alla som får hjärtinfarkt. I vår studie mätte vi nivåerna av MRP 8/14 på 119 personer över 75 år som haft en plackruptur i hjärtats kärl, i prover tagna strax efter att de kom till sjukhuset och vid ett återbesök efter sex veckor. Vi fann att förhöjda värden av MRP 8/14 vid det akuta insjuknandet inte i sig indikerade någon ökad risk för att åter drabbas. Däremot personer med förhöjda värden av MRP 8/14 vid återbesöket sex veckor efter insjuknandet och de som hade ökat i värde vid återbesöket jämfört med det akuta insjuknandet hade förhöjd risk för nya plackrupturer. MRP 8/14 skulle således kunna tänkas fungera som ett hjälpmedel för läkare att bedöma risken för att en patient skall återinsjukna i hjärtsjukdom.

3. Introduction

3.1 MPR 8/14

The myeloid related proteins (MRP) 8 and 14 are members of the S100-protein family and are also titled S100A8 and S100A9 as well as calgranulin A and calgranulin B[1]. MRP 8 and 14 are mainly secreted as the heterodimer MRP 8/14. MRP 8/14 is expressed in cells of myeloid origin, such as neutrophils and monocytes. MRP 8/14 expression is markedly downregulated when monocytes differentiate into macrophages[2]. MRP 8/14 is an endogenous activator of toll like receptor 4 (TLR4) and the receptor for advanced glycation endproducts (RAGE). The intracellular function of MRP 8/14 is not fully mapped but its calcium dependent interactions with cytoskeletal components are established[3]. MRP 8/14 is released into the extracellular space via active secretion through a not yet elucidated route, as well as from necrotic cells after tissue damage[1]. Toll-like receptors (TLRs) play important roles in the innate immune responses and function as rapid pathogen sensors. Carriership of certain subtypes of TLR4 allele in combination with a subtype of its co-receptor CD14 is an independent predictor of atherosclerotic disease[4]. RAGE is an activator of signaling mechanisms that cause cell stress. RAGE is highly expressed in human atherosclerotic plaques[5] and studies suggest that RAGE expression in macrophages, smooth muscle cells (SMC) and endothelial cells contributes to the pathogenesis of atherosclerosis[6].

MRP 8/14 stimulates recruitment and activation of neutrophils and monocytes and plays a pivotal role as innate immune amplifier in various autoimmune and inflammatory diseases, such as rheumatoid arthritis, juvenile idiopathic artritis, inflammatory bowel disease, psoriasis, vasculitis, sepsis, cancer and transplant rejection. MRP 8/14 levels are increased in these patients and are currently used as inflammatory biomarkers for disease severity and progression[1, 7-10].

3.2 Atherosclerosis

3.2.1 Atherogenesis- Fatty streak

The atheroma or athersosclerotic lesion is an asymmetric focal thickening of the tunica intima of the arterial wall. The precursor to the atheroma is the fatty streak, characterized

by the accumulation of lipid-loaded macrophages and a small amount of T cells under the endothelium. The fatty streak does not cause symptoms, can evolve to an atheroma or revert and disappear[11]. Local shear stress and hyperlipidemia enable LDL to infiltrate and accumulate in the intima[12]. The LDL particles are then liable to oxidative modification. The oxidized LDL (oxLDL) is recognized as non-self by the immune system, which will be activated in order to remove it. The oxLDL and compounds thereof activate endothelial cells to express adhesion molecules and chemokines. This causes the migration of monocytes into the intima[13]. In the intima monocytes differentiate into macrophages and upregulate scavenger receptors, which enables them to phagocyte oxLDL[14]. The cholesterol that cannot be utilized from the macrophages accumulates in cytosolic droplets. This causes an exhaustion of the macrophage and transforms it into a foam cell[15].

Hyperlipidemia also stimulates granulopoesis in the bone marrow, leading to neutrophilia. Neutrophils have been detected in human atheroma and the quantity of neutrophils in the blood is correlated to the amount of atherosclerosis[12, 16].Recent studies suggest that neutrophils are recruited early into the vessel wall and that they send out inflammatory signals that trigger the recruitment of monocytes[17]. Ox-LDL may induce transmigration and degranulation of neutrophils. Neutrophils are short lived cells that quickly become apoptotic and send out “find me” and “eat me” signals that attract macrophages for scavenging[17]. Thus neutrophils might have a key role in the initiation and development of atherosclerosis. MRP 8/14 is abundantly found inside the neutrophils, constituting approximately 40% of all cytosolic proteins[2]. Extracellular MRP 8/14 promotes phagocytosis and has proinflamatory effects on neutrophils and monocytes/macrophages, probably via activation of TLR4 and RAGE[18, 19]. Intracellular MRP 8/14 promotes adhesion and migration of neutrophils and macrophages through calcium and microtubule regulation[3].

3.2.2 Atherogenesis – The advanced fibrotic lesion

The ubiquitously present pathogen-like molecular patterns bind to TLRs on macrophages, triggering a signal cascade leading to cell activation[20]. Activated macrophages produce inflammatory cytokines, chemokines, proteases and cytotoxic

oxygen radical molecules. This in turn can causes tissue damage and further recruitment of inflammatory cells such as T lymphocytes[12].

T helper type 1 lymphocytes (Th1) and their signature cytokines interferon-γ (IFN- γ), interleukin-12 (IL-12) and interleukin-18 (IL-18) are present in the human plaque and are thought to contribute to the atherosclerotic process[21]. Experiments in mice have shown that blocking the generation of Th1 cells as well as blocking the production or signaling ability of IFN- γ decreases plaque burden and that elevated levels of IFN- γ or IL-12 lead to increased atherosclerosis[12].

The activated macrophages and endothelial cells secrete growth factors that cause the recruitment and proliferation of SMC from the tunica media of the arterial wall. These cells secrete matrix protein, including interstitial forms of collagen. In time, the SMC and their secreted fibers form a fibrous cap that covers the foam cells and separates the atheroma from the lumen[12]. Under the fibrotic cap the foam cells are trapped and gradually become apoptotic and die, releasing their inner lipids into the extracellular space. This process gradually leads to the formation of the necrotic core, which is mainly made up by cell debris and extracellular lipids.

3.2.2 Atherogenesis – plaque stability and rupture

As the plaque grows it can occlude the arterial lumen, lowering the blood flow to such an extent that it causes symptoms from its irrigated territory. This is the case in stable arterial disease such as angina pectoris or claudicatio intermittens. It is known that tight coronary stenoses are often stable and do not precipitate an acute MI[22].

In histological studies it has been shown that the stable plaque usually has a relatively thick fibrous cap and a small lipid core. The rupture-prone plaque, termed vulnerable plaque, has a large lipid core and a thin fibrous cap[23] and is often characterized by an accumulation of activated macrophages and T cells into the rupture-prone shoulder region of the lesion[24]. The integrity of the fibrous cap can be compromised by several inflammatory processes. Macrophages, activated by Th1 cytokines and other factors, can release matrix metalloproteinases (MMPs) that degrade the collagen within the cap[25]. Collagen production in the SMCs of the plaque can also be inhibited by Th1 cells[26]. Smaller studies of human atheroma show that vulnerable plaques are rich in

inflammatory cell that all show a robust expression of MRP 8/14 and that rupture prone lesions have a higher percentage of MRP 8/14 positive macrophages than stable ones[27].

3.3 Coronary Artery Disease

When atherosclerotic lesions develop in the coronary arteries they give rise to coronary artery disease (CAD). Worldwide more than 7 million people die from ischemic heart disease each year.[28] CAD can be divided into stable and unstable disease. Stable CAD develops when a stable atherosclerotic lesion has grown to such extent that it restricts the blood flow to the myocardium. In a situation with increased demand this restricted blood flow will not suffice and a relative ischemia will develop, causing discomfort and pain (angina pectoris).

3.3.1 Acute Coronary Syndrome

The unstable CAD or the ACS is commonly caused by a thrombus that forms in a coronary artery, following the rupture of an atherosclerotic plaque[29].

Based on the ECG aspect and the release of markers of myocardial necrosis, ACS can be divided in to the subcategories: ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (non-STEMI) and UA[29], where the coronary oclusion causes an relative ischemia leading to discomfort but not myocardial necrosis.

The serum levels of MRP 8/14 in ACS patients have been shown to be elevated compared to controls with stable CAD[30] and were shown to peak between days 3-5 after the onset of symptoms [31].

Comparison of MRP 8/14 levels in samples taken from the site of coronary occlusion with samples from the femoral artery showed that MRP 8/14 is significantly elevated at the site of the thrombi[30]. It has been shown that inflammation plays a significant role in the reparation of the injured heart[32] and that the inflammatory process can continue beyond the initial repair phase, spreading from the infarcted area into the surrounding myocardium and playing a role in the harmful remodeling leading to chronic heart failure[33]. In mice, sustained MRP 8/14 activation critically contributes to the development of post ischemic heart failure via activation of RAGE[34]. Pathological studies in humans showed a significantly larger area stained positive for MRP 8/14 in an infarcted myocardium compared to controls. Initially, 6h to 2 days after the onset of MI, the vast

majority of MRP 8/14 positive cells in this area were neutrophils, but shifted towards macrophage domination in the sub acute phase, day 7-9 after MI[31].

Roughly 33% of all ACS episodes occur in patients above the age of 75 years and account for approximately 60% of the mortality cases due to ACS[35]. The elderly represent a special challenge in the diagnosis of ACS as well as in managing the disease. They present more often with atypical symptoms and many have abnormal resting ECG[36, 37]. The risk for adverse events is also higher in elderly ACS patients. Complication rates for reperfusion, anticoagulation and antiplatelet therapies are higher than that of younger patients, but at the same time the elderly have a higher chance to benefit from these therapies[38, 39]. Still elderly patients are at greater risk of getting suboptimal treatment for ACS and there is a need for developing improved methods for risk stratification[40].

3.4 Biomarkers for cardiovascular risk and secondary prevention

It has been shown that smoking and dyslipidemia are associated with approximately 60% of the risk to develop an acute MI. Taken together with hypertension, diabetes, abdominal obesity, psychosocial factors, low consumption of fruits and vegetables and reduced regular physical activity, the above mentioned factors account for more than 90% of the CV risk [41].

There is an abundance of biomarkers of less importance than the above mentioned, but with the potential to identify patients with a more specified risk. Some of these are readily available and well linked to CV disease, such as high sensitivity-CRP (hsCRP), homocystein, natriuretic peptides and neutrophil count[42-44]. Other inflammatory biomarkers which have been thought to signal the presence of unstable plaques and plaque rupture, such as MMP-9, myeloperoxidase, vascular cell adhesion molecule (VCAM) are less available and lack reliable evidence[42]. A meta analysis assessing the relation between early CRP levels after ACS and the risk of adverse outcomes has found a moderately dose-dependent positive association between early blood CRP values and long term risk of recurrent CV events or death in patients with ACS (CRP >10mg/L is associated with 2.18-fold higher risk)[45].

3.4.1 MRP 8/14 as a biomarker in primary and secondary prevention

In a primary prevention population study on healthy postmenopausal women, elevated MRP 8/14 levels (>3.36 mg/L) at baseline predicted a relative risk of 2.3 for a CV event in the next 2.9 years, when controlling for both traditional risk factors and CRP[46].

A later study on patients presenting with chest pain showed that high concentrations of MRP 8/14 measured at the acute event were associated with increased risk for CV death[47]. MRP 8/14 was also associated with all cause mortality but when controlling for traditional clinical risk factors and CRP in a Cox proportional hazard analysis MRP 8/14 lost significance and CRP emerged as a far stronger predictor[47].

3.5 Objective

Our aim was to examine the value of MRP 8/14 as a CV risk predictor in a population of older survivors of ACS (MI and UA).

4. Methods

4.1 Study population

The study population was part of the "Elderly project", a prospective study including patients being treated for ACS at the Cardiology Clinic SUS Malmö from 2008 and onwards. All participants provided informed consent and the study was approved by the ethical committee at Lund University, Sweden.

Participants in this study were chosen on the criteria that they were above 75 years of age when they were enrolled in the study and that they participated in the 6 week follow-up.

4.2 Blood sampling

Samples of venous blood were obtained from all patients in tubes containing EDTA, at inclusion on the day after the acute event and at the 6 week follow-up. Plasma was extracted from the samples and stored at -80^{o}C. Plasma levels of MRP 8/14 were

measured using an enzyme-linked immunoassay (ELISA) (BMA Biomedicals, Augst Swizerland,).

4.3 Clinical data

Information on the presence of CV risk factors was gathered from the Swedeheart national register. For patients that had not been registered in the Swedeheart national register, medical information was gathered from local journals. Biochemical laboratory measurement data at the time of the acute event and at follow-up were gathered from the journals of study participants.

Measurement of the marker for myocardium necrosis troponin was changed from the I subtype to the T subtype at SUS Malmö in may 2011. To enable the use of these separate markers as a measurement of myocardial necrosis in our analysis, the recorded values were divided by the maximum value of their individual normal range, thus forming a troponin quota that was included in our statistical analysis. Blood pressure was measured with a mercury-column sphygmomanometer after resting for 10 min in the supine position. Hypertension was defined as systolic blood pressure (SBP) 140 mmHg, diastolic blood pressure (DBP) 90 mmHg or use of antihypertensive medication. Diabetes mellitus was defined as a fasting whole-blood glucose level greater than 6.0 mmol/L, a self-reported physician diagnosis of diabetes or use of antidiabetic medication.

4.4 Study end-points

The study end-points were CE (MI, UA or CV death), stroke, heart failure and all-cause mortality during follow-up. Events were identified through linkage of the 10-digit personal identification number of each Swedish citizen with the Swedish hospital discharge register and the Swedish Cause of Death Register. CE was defined as main diagnose being MI ICD10 codes I21 and I22 or unstable or undefined angina pectoris codes I200, I208 or I209. Stroke was defined as codes I60-I64, heart failure as I50. Follow-up for outcomes from the Swedish hospital discharge register continued up until 1 January 2012, outcomes from the Swedish Cause of Death Register continued until 1 November 2012.

4.5 Statistical analysis

SPSS software (version 21; SPSS Inc, Chicago, IL, USA) was used for all statistical calculations. The degree of co-variation between MRP 8/14 levels and other CV risk factors was studied in a bivariate Spearman correlation including the factors shown in Table 2. To assess the importance of increasing, respectively decreasing MRP values at the 6 weeks follow-up compared to the acute event, a Pearson chi-square analysis was performed (Table 3). We performed a multivariate Cox regression (time to event) analysis to assess whether increasing tertiles of MRP 8/14 were related to increased risk of coronary events, stroke or all-cause mortality. Separate analyses were performed for inclusion and follow-up values as well as for the differential between the two (ΔMRP 8/14). We used three different models adjusted for: age and sex (Model A), age, sex, hypertension, smoking, BMI, diabetes mellitus, HDL and LDL (Model B) and age, sex, hypertension, smoking, BMI, diabetes mellitus, HDL, LDL and CRP (Model C). The values for MRP 8/14 and CRP were logarithmically transformed before being included in the analysis as continuous variables. Data were expressed as hazard ratio (HR) and 95% confidence interval (CI). A two sided value of $P< 0,05$ was considered statistically significant. Kaplan Meyer curves were rendered to illustrate the association between MRP 8/14 and the risk for CE.

5. Results

5.1 Characteristics of the study group

Of the 572 patients included in the "Elderly project" at the time, 119 fulfilled the inclusion criteria. Follow-up data for coronary events, stroke, heart failure and CV death were available for 113 subjects, and for all-cause mortality for 119 individuals. The median age of the participants was 79 years and 35% were women (Table 1). At the time of inclusion 23% of the patients had diabetes, 65% had hypertension and 8% were active smokers. During a median follow-up of 1.8 years (interquartile range (IQR), 0.7-2.7), 35 individuals suffered another CE, 8 were diagnosed with stroke, 14 with heart failure (data not shown) and 6 died of CVD. During a median follow-up of 2.7 years (IQR 1.5-3.6) we recorded a total of 16 deaths (Table 1).

5.2 MRP 8/14 and the presence of traditional risk factors for CVD

In a Spearman correlation analysis (Table 2), MRP 8/14 values at the acute event and at 6 weeks follow-up were both significantly correlated with CRP. The MRP 8/14 concentration at the acute event was strongly correlated with the white blood cell count (WBC) (R=0.342 p<0.000 The 6 week follow-up MRP 8/14 similarly correlated with follow-up WBC (R= 0.259 p= 0.005).

At inclusion there was a strong correlation between MRP 8/14 and hypertension (R= 0.31 p=0.001), but this correlation lost power at follow-up (R=0.18 p=0.052), becoming borderline significant. The troponin quota correlated with MRP 8/14 at inclusion (R= 0.254 p=0.006), but not with the MRP 8/14 concentration at 6 week follow-up. No other significant correlations could be shown between MRP 8/4 and the traditional risk factors.

5.3 MRP 8/14 and CV risk

A chi-square analysis (Table 3) investigating if increase or decrease of MRP 8/14 values from the acute ACS event to the 6 week follow-up were connected to morbidity or mortality did not yield any significant results. Nevertheless, a trend could be recognized, with a higher probability for CE (P=0.081) and CV death (P= 0.094) for patients with increasing MRP 8/14 values.

The study participants were categorized according to tertiles of plasma MRP 8/14 concentration at inclusion and at the 6 week follow-up as well as of the differential between the two (MRP). In Cox regression models adjusted for age and sex (Model A); age, sex and traditional risk factors (Model B) and age, sex, traditional risk factors and CRP (Model C) the inclusion values did not account for an increased risk in any of the considered endpoints (Table 4.1). The 6 week follow-up values (table 4.2) showed an association with the risk of a CE, association that remained significant through the three different analysis models. When controlling for age, sex, traditional risk factors and CRP (Model C), the 3rd tertile yielded a HR of 4.09 (1.29- 12.96) when compared to the 1st tertile. No significant associations could be found for stroke and all cause mortality. The Cox analysis of the ΔMRP 8/14 (table 4.3) also rendered significant results for CE, 2.88 (1.00- 8.32) for the 3rd tertile in Model C. To visualize the incidence of CE during the

follow-up period by MRP 8/14 tertiles, three Kaplan-Meyer graphs were rendered (Figure 1).

6. Discussion

In recent years an abundance of novel inflammatory biomarkers for prediction of CVD have emerged[42]. Of these, MRP 8/14 has shown promising results in a number of studies[30, 31, 46, 48]. MRP 8/14 correlates strongly with leukocyte count, implying a connection to the systemic inflammatory activity. In a previous study on 664 individuals from the Malmö Diet and Cancer (MDC) cohort we found that MRP 8/14 concentrations correlate strongly with the numbers of circulating white blood cells and neutrophils, but not circulating monocytes or monocyte sub-population (unpublished results)., Increased neutrophil counts have been associated with the incidence of CE in a healthy population[43, 44]. Since MRP 8/14 correlates to neutrophil counts and both high neutrophil counts and high MRP 8/14 are associated with increased risk for CE, MRP 8/14 might be a marker for neutrophil activation, leading to plaque destabilization and CE. The strong correlation between MRP 8/14 and the leukocyte counts might be a sign the increased neutrophil activation and MRP 8/14 secretion during the acute phase of the ACS[31].

The strong and significant correlation between MRP 8/14 at the acute event and the troponin quota implies that the release of MRP 8/14 is related to the grade of myocardium necrosis in the ACS. It has been shown that MRP 8/14-positive neutrophils and monocytes infiltrate the infarcted myocardium[31] and that the blood levels of MRP 8/14 are elevated at the site of the occlusion compared to the systemic circulation[30]. Furthermore, MRP 8/14 has an active role in the thrombus responsible for the ACS where they stimulate monocytes via TLR4[49] and contribute to platelet activation[46, 50]. Puzzling is the correlation between hypertension and MRP 8/14, which has not been mentioned in any previous study. Rather than reflecting on a physiological mechanism this might be a result of the relatively small size of the study and to the broad definition of hypertension that we have used.

To our knowledge, no studies have been performed on the value of MRP 8/14 as a biomarker in an elderly population. We set out to elucidate the potential of MRP 8/14 as a predictor of recurrent CV events in a population of ACS survivors over 75 years of age. We found that MRP 8/14 sampled 6 weeks after the acute event was an independent

predictor of major adverse CE, were patients with MRP 8/14 values within the 3rd tertile ran a 4 times higher risk to develop subsequent CE compared to the first tertile. This is in line with the results of Morrow et al.[48] comparing ACS patients that suffered a recurrent ACS in the first month after the initial episode with those that did not have a second episode during the same period. MRP 8/14 were measured 30 days after the initial event, and was found to be significantly elevated in the group with recurrent ACS. Patients with a MRP 8/14 of >11mg/L had relative odds of 2.0 of CV death or another MI, even when controlling for both clinical variables/traditional risk factors and hsCRP. Notable similarities between these studies are the predictive value of MRP 8/14 in a time well after the acute event (30 days/6 weeks) and an association with increased risk that withstands control for traditional risk factors and CRP.

Patients with increasing MRP 8/14 at the 6 weeks follow-up had a higher risk for CE and CV death. Our interpretation of these results is that sustained or amplified neutrophil activation during the period following ACS is a potential trigger for subsequent CV events.

A previous study on patients presenting with chest pain has shown that the concentration of MRP 8/14 at the acute event has predictive value for the risk of CV death [47, 48]. We could not show any predictive value of MRP 8/14 measured at the acute event for either CV death, all cause mortality, CE or stroke. This could be due to differences in sampling since the samples in our study were collected not at admittance to the hospital but at inclusion on the day after admittance. Another reason for discrepancy in results could be the difference in the characteristics of the study populations. Our population of elderly ACS survivors (median age 79 years) that were healthy enough to attend the 6 week follow-up visit is significantly older than the population included in previous studies (median age 61 and 64 years) and might also differ in other risk factors.

Limitations to this study merit consideration. Firstly, our results regarding CV death and stroke have to be interpreted with due caution due to the low numbers of events recorded during follow-up. The study excluded ACS sufferers that could not attend the 6 week follow-up, a group which could be assumed to be in lesser health than the study population. Since MRP 8/14 is elevated in a row of oncological and immunological diseases[1, 7], these diseases could vey well have a higher prevalence in the excluded group.

In conclusion, the results of our prospective study suggest that MRP 8/14 measured during follow-up in elderly ACS survivors is able to predict the risk of a new CE independently of age, sex, traditional risk factors and CRP. If these results can be confirmed in larger studies, MRP 8/14 might be employed as a useful inflammatory biomarker for guiding risk stratification in secondary prevention of acute CE.

7. References

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8. Tables

Table 1. Baseline characteristics of the study population

Characteristic	Population	Non-Cases	Coronary Event	Stroke	Cardiovascular Death	All Cause Death
Number of participants	119	74	35	8	6	16
Age, median (interquartile range) years	79 (77-91)	80 (77-84)	79 (75-83)	80 (78-83)	84 (83-86)	83 (80-85)
Female gender (%)	41 (35)	26 (35)	12 (34)	5 (63)	2 (33)	4 (25)
BMI (SD), kg/m2	26.5 (4)	26.8 (4.4)	26.3 (3.5)	26.2 (3.7)	25.8 (4.0)	25.6 (3.6)
Diabetes (%)	27 (23)	18 (24)	8 (23)	0 (0)	1 (17)	2 (13)
Current Smoking (%)	9 (8)	3 (4)	2 (6)	1 (13)	0(0)	3 (19)
Hypertension (%)	67 (65)	44 (60)	26 (74)	6 (75)	6 (100)	13 (82)
Blood pressure, mean (SD), mm Hg						
Systolic	150 (25)	153 (23)	142 (28)	180 (*)	170 (36)	150 (38)
Diastolic	80 (13)	82 (12)	73 (12)	80 (*)	78 (3)	80 (11)
Laboratory values						
CRP at acute event (IQR), mg/L	3 (>2-9)	3.8 (2.0-13.8)	3 (>2-8)	2.5 (>2-6.8)	3 (2.0-3.5)	3 (>2-6.9)
CRP at 6 weeks follow-up median (IQR), mg/L, mg/L	3 (1-9)	3.7 (1.1- 6.55)	3 (1-7.4)	4.5 (1-9)	7 (1-36.3)	1 (1-8.4)
MRP 8/14 at acute event, median (IQR), mg/L	2.56 (2.01-3.56)	2.35 (1.96-3.75)	2.65 (2.13-3.48)	2.54 (1.80-3.27)	2.58 (2.32- 3.20)	2.58 (2.18-3.33)
MRP 8/14 at 6 weeks follow-up median (IQR), mg/L	2.16 (1.66-3.09)	2.46 (1.56- 2.83)	2.63 (2.06-3.39)	2.64 (2.13- 3.30)	3.21 (2.04-4.45)	2.39 (1.89-3.55)
Leucocytes at the acute event (SD), x 10^9 /L	9.2 (3.3)	9.2 (3.4)	8.8 (3.4)	7.9 (1.9)	9.8 (5.3)	10.9 (4.2)
Leucocytes at 6 week follow-up(SD), x 10^9 /L	7.2 (2.0)	7.2 (1.9)	7.1 (2.2)	7.2 (2.3)	7.3 (2.7)	7.7 (2.4)
HDL-C at the acute event (SD), mmol/L	1.2 (0.4)	1.2 (0.4)	1.2 (0.3)	1.4 (0.4)	1.1 (0.2)	1.1 (0.3)
LDL-C at the acute event (SD), mmol/L	3.0 (1.0)	3.2 (1.2)	2.8 (0.9)	3.2 (0.6)	2.7 (0.8)	2.9 (0.94)
TG at the acute event(SD), mmol/L	1.1 (0.5)	1.1 (0.6)	1.0 (0.3)	0.9 (0.29)	0.97 (0.42)	1.1 (0.31)

BMI, body mass index; CRP, C-reactive protein; MRP 8/14 Myeloid Related Protein 8/14; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, Triglycerides. *BP info only available for 1 patient in the stroke group.

Table 2. Associations between MRP 8/14 levels, CV risk factors and other inflammatory biomarkers

	MRP 8/14 at the acute event		**MRP 8/14 at 6 weeks follow up**	
	Correlation coefficient	***P****	**Correlation Coefficient**	***P****
Age	0.082	0.38	-0.009	0.92
Gender	-0.022	0.82	-0.019	0.84
BMI	0.077	0.41	0.029	0.76
Diabetes	0.166	0.07	0.040	0.67
Smoking	0.105	0.26	0.018	0.85
Hypertension	0.311	0.00	0.180	0.05
Blood pressure, mm Hg				
Systolic	0.112	0.34	-0.127	0.28
Diastolic	0.057	0.63	-0.211	0.07
Laboratory values				
CRP	0.432	0.00	0.481	0.00
Leucocytes	0.342	0.00	0.259	0.01
HDL-C	-0.102	0.3	0.022	0.82
LDL-C	-0.083	0.41	0.015	0.88
TG	0.171	0.08	0.000	0.98
Troponin quota[#]	0.254	0.01	0.123	0.18

* Bivariate Spearman correlation test. [#] Recorded values of troponin I and troponin T, divided by the maximum value of their individual normal range.

Table 3. MRP 8/14 development following the acute event and the risk of subsequent CVdisease

	Increasing (n=39)	Decreasing (n=78)	*P**
Coronary event, (%)	15 (38.5)	18 (23.1)	0.081
Stroke, (%)	4 (10.3)	4 (5.1)	0.30
Cardiovascular death (%)	4 (10.3)	2 (2.6)	0.094
All-cause death (%)	7 (17.9)	9 (11.5)	0.341
Heart failure (%)	3 (7.7)	10 (12.8)	0.40

* Pearson Chi-square test.

Table 4.1 MRP 8/14 at the acute event and the risk for subsequent CVD and all-cause mortality

	MRP 8/14 tertile Hazard ratio (95% CI)		Linear trend	
Event	**T2 vs. T1**	**T3 vs. T1**	**Hazard ratio (95% CI)**	***P***
Coronary event				
A	1.08 (0.46- 2.55)	1.15 (0.501-2.64)	1.07 (0.71 – 1.625)	0.74
B	1.27 (0.49- 3.29)	0.75 (0.28-2.03)	0.86 (0.54 – 1.38)	0.54
C	1.50 (0.54 – 4.2)	1.16 (0.37 – 3.6)	1.09 (0.63 – 1.87)	0.76
Stroke				
A	1.96 (0.35-**10**.83)	0.80 (0.11-5.85)	0.90 (0.38- 2.09)	0.80
B	3.15 (0.43- 23.4)	0.84 (0.09-7.56)	0.89 (0.36- 2.20)	0.80
C	3.42 (0.43 -27.48)	0.75 (0.06- 9.52)	0.92 (0.315-2.70)	0.88
All cause mortality				
A	3.38 (0.93- 12.33)	0.92 (0.22-3.8)	0.94 (0.53 -1.68)	0.84
B	4.05 (0.71-23.18)	0.59 (0.81-4.34)	0.72 (0.32-1.60)	0.46
C	15.11 (1.5 – 152.27)	0.69 (0.06-7.64)	0.84 (0.34-2.24)	0.78

Model A adjusted for age and sex.
Model B adjusted for age, sex, hypertension, smoking, BMI, diabetes, HDL and LDL.
Model C adjusted for age, sex, hypertension, smoking, BMI, diabetes, HDL, LDL and CRP.

Table 4.2 MRP 8/14 at 6 week follow-up and and the risk for subsequent CVD and all-cause mortality..

	MRP 8/14 tertiles Hazard ratio (95% CI)		Linear trend	
Event	**T2 vs. T1**	**T3 vs. T1**	**Hazard ratio (95% CI)**	***P***
Coronary Event				
A	2.26 (0.78-6.5)	3.90 (1.43-10.66)	1.91 (1.21- 3.03)	0.005
B	2.18 (0.68- 6.98)	3.41 (1.20-9.70)	1.80 (1.10-2.92)	0.018
C	2.38 (0.72- 7.93)	4.09 (1.29- 12.96)	1.97 (1.14-3.37)	0.014
Stroke				
A	2.31 (0.24- 22.56)	3.99 (0.43-36.73)	1.92 (0.72- 5.17)	0.19
B	0.85 (0.54-13.45)	2.74 (0.28-26.72)	1.98 (0.63- 6.17)	0.24
C	0.87 (0.05-15.90)	2.83 (0.23-34.52)	2.05 (0.61- 6.86)	0.24
All cause Mortality				
A	1.35 (0.38- 4.84)	2.47 (0.66- 9.16)	1.58 (0.81- 3.09)	0.18
B	0.98 (0.23- 4.18)	1.51 (0.35- 6.65)	1.24 (0.58- 2.65)	0.58
C	1.04 (0.23- 4.77)	1.66 (0.311-8.93)	1.31 (0.56- 3.08)	0.54

Model A adjusted for age and sex.
Model B adjusted for age, sex, hypertension, smoking, BMI, diabetes, HDL and LDL.
Model C adjusted for age, sex, hypertension, smoking, BMI, diabetes, HDL, LDL and CRP

Table 4.3 ΔMRP 8/14* and and the risk for subsequent CVD and all-cause mortality.

	MRP 8/14 tertile (T) Hazard ratio (95% CI)		Linear trend	
Event	**T2 vs. T1**	**T3 vs. T1**	**Hazard ratio (95% CI)**	***P***
Coronary Event				
A	1.63 (0.61- 4.33)	2.48 (0.99- 6.2)	1.56 (1.00-2.44)	0.046
B	1.84 (0.63- 5.39)	2.74 (0.99- 7.59)	1.64 (1.00- 2.67)	0.049
C	1.84 (0.62- 5.4)	2.88 (1.00- 8.32)	1.68 (1.00- 2.83)	0.049
Stroke				
A	4.09 (0.39-42.69)	5.08 (0.55- 47.11)	1.95 (0.78-4.86)	0.151
B	3.33 (0.27- 41.40)	4.84 (0.49- 47.40)	1.99 (0.75- 5.28)	0.164
C	3.41 (0.27- 43.00)	5.02 (0.49- 51.94)	2.03 (0.74- 5.52)	0.167
All cause mortality				
A	1.25 (0.29- 5.37)	2.98 (0.85-10.50)	1.77 (0.93-3.36)	0.082
B	1.78 (0.33-9.65)	3.67 (0.81- 16.64)	1.93 (0.91-4.08)	0.087
C	2.10 (0.36- 12.14)	5.20 (9.96-28.14)	2.30 (0.99- 5.32)	0.052

Model A adjusted for age and sex.
Model B adjusted for age, sex, hypertension, smoking, BMI, diabetes, HDL and LDL.
Model C adjusted for age, sex, hypertension, smoking, BMI, diabetes, HDL, LDL and CRP.
*ΔMRP 8/14 is the differential between MRP 8/14 at the acute event and at 6 week follow-up.

Figure 1. Kaplan-Meyer graphs depicting one minus coronary event free survival by MRP8/14 tertile