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REVIEW ARTICLE

Critical appraisal of inflammatory markers in cardiovascular risk stratification

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Abstract

Despite great progress in prevention strategies, pharmacotherapy and interventional treatment of coronary artery disease (CAD), cardiovascular events still constitute the leading cause of mortality and morbidity in the modern world. Traditional risk factors, including hypertension, diabetes mellitus, smoking, obesity, dyslipidemia, and positive family history account for the occurrence of the majority of these events, but not all of them. Adequate risk assessment remains the most challenging in individuals classified into low or intermediate risk categories. Inflammation plays a key role in the initiation and promotion of atherosclerosis and may lead to acute coronary syndrome (ACS) by the induction of plaque instability. For this reason, numerous inflammatory markers have been extensively investigated as potential candidates for the enhancement of cardiovascular risk assessment. This review aims to critically assess the clinical utility of well-established (C-reactive protein [CRP] and fibrinogen), newer (lipoprotein-associated phospholipase A2 [Lp-PLA2] and myeloperoxidase [MPO]) and novel (growth differentiation factor-15 [GDF-15]) inflammatory markers which, reflect different pathophysiological pathways underlying CAD. Although according to the traditional approach all discussed inflammatory markers were shown to be associated with the risk of future cardiovascular events in individuals with and without CAD, their clear clinical utility remains not fully elucidated. Current recommendations of numerous scientific societies predominantly advocate routine assessment of CRP in healthy people with intermediate cardiovascular risk. However, these recommendations substantially vary in their strength among particular societies. These discrepancies have a multifactorial background, including: (i) the strong prognostic value of CRP supported by solid scientific evidence and proven to be comparable in magnitude with that of total and high-density lipoprotein cholesterol, or hypertension, (ii) favourable analytical characteristics of commercially available CRP assays, (iii) lack of CRP specificity and causal relationship between CRP concentration and cardiovascular risk, and (iv) CRP dependence on other classical risk factors. Of major importance, CRP measurement in healthy men $\geq$50 years of age or healthy women $\geq$60 years of age with low-density lipoprotein cholesterol $<$130 mg/dL may be helpful in the selection of patients for statin therapy. Additionally, evaluation of CRP and fibrinogen or Lp-PLA2 may be considered to facilitate risk stratification in ACS patients and in healthy individuals with intermediate cardiovascular risk, respectively. Nevertheless, the clinical utility of CRP requires further investigation in a broad spectrum of CAD patients, while other promising inflammatory markers, particularly GDF-15 and Lp-PLA2, should be tested in individuals both with and without established CAD. Further studies should also focus on novel performance metrics such as measures of discrimination, calibration and reclassification, in order to better address the clinical utility of investigated biomarkers and to avoid misleadingly optimistic results. It also has to be emphasized that, due to the multifactorial pathogenesis of CAD, detailed risk stratification remains a complex process also involving, beyond assessment of inflammatory biomarkers, the patient's clinical characteristics, results of imaging examinations, electrocardiographic findings and other laboratory parameters (e.g. lipid profile, indices of renal function, markers of left ventricular overload and fibrosis, and biomarkers of myocardial necrosis, preferably cardiac troponins).

Keywords

Acute coronary syndrome, coronary artery disease, CRP, fibrinogen, GDF-15, inflammation, Lp-PLA2, MPO, risk prediction

History

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Abbreviations: AACE: American Association of Clinical Endocrinologists; **ACCF**: American College of Cardiology Foundation; **AHA**: American Heart Association; **ACS**: acute coronary syndrome; **apoB**: apolipoprotein B; **ARIC**: Atherosclerosis Risk in Communities; **ATP III**: Adult Treatment Panel III; **AUC**: area under the receiver operating characteristic curve; **BNP**: brain natriuretic peptide; **CAC**: coronary artery calcium; **CAD**: coronary artery disease; **CANTOS**: Canakinumab Anti-inflammatory Thrombosis Outcomes Study; **CAPTURE**: Chimeric c7E3 AntiPlatelet Therapy in Unstable angina REfractory to standard treatment; **CI**: confidence interval; **CRP**: C-reactive protein; **cTn**: cardiac troponin; **CVD**: cardiovascular disease; **ED**: emergency department; **EDTA**: ethylenediaminetetraacetic acid; **EPIC**: European Prospective Investigation into Cancer and Nutrition; **ERFC**: Emerging Risk Factors Collaboration; **ESC**: European Society of Cardiology; **FRISC**: Fragmin and Fast Revascularization During Instability in Coronary Artery Disease; **FRS**: Framingham Risk Score; **FSC**: Fibrinogen Studies Collaboration; **GDF-15**: growth differentiation factor-15; **GRACE**: Global Registry of Acute Coronary Events; **HDL**: high-density lipoprotein; **HDL-C**: high-density lipoprotein cholesterol; **HF**: heart failure; **HFPEF**: heart failure with preserved ejection fraction; **HPS**: Heart Protection Study; **HR**: hazard ratio; **hs**: high sensitivity; **IL-1**: interleukin-1; **IL-6**: interleukin-6; **JUPITER**: Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin; **LDL**: low-density lipoprotein; **LDL-C**: low-density lipoprotein cholesterol; **LIPID**: Long-term Intervention with Provastatin in Ischemic Disease; **Lp-PLA2**: Lipoprotein-associated phospholipase A2; **MACE**: major adverse cardiovascular event; **MERLIN-TIMI 36**: Metabolic Efficiency With Ranolazine for Less Ischaemia in Non-ST Elevation Acute Coronary-Thrombolysis In Myocardial Infarction 36; **MI**: myocardial infarction; **MIC-1**: macrophage inhibitory cytokine-1; **MIDAS**: Motivational Interventions for Drugs & Alcohol misuse in Schizophrenia; **MPO**: myeloperoxidase; **NACB**: National Academy of Clinical Biochemistry; **NNT**: number needed to treat; **non-HDL-C**: non- high-density lipoprotein cholesterol; **NRI**: net reclassification improvement; **NSTE-ACS**: non-ST-elevation acute coronary syndrome; **NT-proBNP**: N-terminal pro B-type natriuretic peptide; **OR**: odds ratio; **PAFA**: platelet-activating factor acetylhydrolase; **PEACE**: Prevention of Events with Angiotensin-Converting Enzyme Inhibition; **PHS**: Physicians' Health Study; **PIVUS**: Prospective Investigation of the Vasculature in Uppsala Seniors; **RR**: relative risk; **SCORE**: Systematic COronary Risk Evaluation; **SOLID-TIMI-52**: The Stabilization Of pLaques usIng Darapladib-Thrombolysis In Myocardial Infarction 52; **sST2**: soluble suppression of tumorigenicity 2; **STABILITY**: STabilisation of Atherosclerotic plaque By Initiation of darapLadIb TherapY; **TIMI**: Thrombolysis in Myocardial Infarction; **TNF-α**: tumor necrosis factor alpha; **VLDL**: very low-density lipoprotein; **WOSCPS**: West of Scotland Coronary Prevention Study

Introduction

Despite great progress in prevention strategies, pharmacotherapy and interventional treatment of coronary artery disease (CAD), cardiovascular events still constitute the leading cause of mortality and morbidity in the modern world[1]. Traditional risk factors, including hypertension, diabetes mellitus, smoking, obesity, dyslipidaemia, and positive family history of CAD, account for the occurrence of the majority of these events, but not all of them[2]. It is estimated that up to 20% of CAD patients have no traditional risk factors occur, whereas 40% demonstrate only one[3,4]. For this reason, numerous novel risk markers have been extensively investigated as potential candidates for the enhancement of cardiovascular risk assessment. However, only a few of them showed the ability to improve risk stratification beyond the one provided by traditional risk factors and risk scores[5].

Inflammation plays a key role in the initiation and promotion of atherosclerotic lesions and may lead to acute coronary syndrome (ACS) by the induction of plaque instability[6,7]. In addition, inflammation is involved in the phenomenon of restenosis, i.e. vessel re-narrowing after initially successful balloon angioplasty or coronary stenting[8,9]. Better understanding of pathophysiological processes involved in CAD has led to the identification of biomarkers with potentially important clinical value. Therefore, circulating inflammatory markers have rapidly become a subject of interest for many researchers. The basic assumption of many studies was the hypothesis that circulating inflammatory markers reflect the severity of subclinical inflammation and mediate all stages of atherosclerosis[10]. Therefore, inflammatory markers may be useful for the assessment of the preclinical phase of CAD that develops asymptomatically over several years before being disclosed as an overt disease[11]. Most of the initial epidemiological studies conducted both on healthy individuals and on patients with diagnosed CAD, were focused on demonstrating independent relationships between the concentration of inflammatory biomarkers and the occurrence of coronary events. Since this approach has been criticized due to its minor clinical utility, currently considerable emphasis is placed on the extent to which a particular biomarker is capable of adding an incremental value to the cardiovascular risk prediction[5,12,13]. It seems that the ability of a biomarker to accurately discriminate between cases and controls as well as an improved reclassification of individuals are currently the most important trend in clinical decision-making (Table 1)[5,12–24].

This review aims to critically assess the clinical utility of well-established (C-reactive protein [CRP] and fibrinogen), newer (lipoprotein-associated phospholipase A2 [Lp-PLA2] and myeloperoxidase [MPO]) and novel (growth differentiation factor-15 [GDF-15]) inflammatory markers, which reflect different pathophysiological pathways underlying CAD.

Search strategy

A search covering the period from January 1930 to March 2014 was conducted by two independent investigators (Magdalena Krintus and Marek Kozinski) using the MEDLINE, PUBMED CENTRAL and Google Scholar

Table 1. Clinical value of inflammatory biomarkers for primary cardiovascular risk prediction.

Biomarker	Recommendations	Discrimination (c-statistic)	Reclassification (NRI)
CRP	ACCF/AHA, NACB, ESC[16–18]	No improvement to +0.04	No improvement to ~5%[19]
Fibrinogen	ESC[18]	No improvement to +0.0015 (in women)	No improvement to 1.3–3.2% (in women)[20]
Lp-PLA2	ACCF/AHA, ESC[16,18]	+0.006 to 0.02	No improvement to ~8% (in women)[21]
MPO	Not recommended	no data available	no data available
GDF-15	Not recommended	+0.01 to 0.06	6%[22–24]

ACCF/AHA: American College of Cardiology Foundation/American Heart Association; CRP: C-reactive protein; ESC: European Society of Cardiology; GDF-15: growth differentiation factor-15; Lp-PLA2: Lipoprotein-associated phospholipase A2; MPO: myeloperoxidase; NACB: National Academy of Clinical Biochemistry; NRI: Net Reclassification Index.

databases. Proceedings from the Scientific Sessions of the American Association for Clinical Chemistry (https://www.aacc.org/), the American College of Cardiology (http://www.acc.org), American Heart Association (http://www.heart.org), the European Society of Cardiology (http://www.escardio.org), and the International Federation of Clinical Chemistry and Laboratory Medicine (http://www.ifcc.org) were also considered. The following keywords were applied: ''acute coronary syndrome'', ''C-reactive protein'', ''cardiovascular disease'', ''cardiovascular risk'', ''cardiovascular risk factor'', ''coronary artery disease'', ''CRP'', ''fibrinogen'', ''growth differentiation factor-15'', ''GDF-15'' ''inflammation'', ''inflammatory biomarker''. ''inflammatory mediator'', ''lipoprotein-associated phospholipase A2'', ''Lp-PLA2'', ''myeloperoxidase'', ''MPO'', ''risk prediction'', and ''risk stratification''. References given in the studies retrieved were searched manually for additional studies and reviews. No language restrictions were applied.

CRP

Background

CRP has been the most extensively studied inflammatory marker within the last 15 years. CRP is an acute phase protein synthesized predominantly in the liver in response to the release of proinflammatory cytokines (interleukin-6 [IL-6], and to a lesser extent, interleukin-1 [IL-1] and tumor necrosis factor alpha [(TNF-α]) mainly by macrophages and adipocytes. However, unstable atherosclerotic plaques were also demonstrated to be a source of CRP[25,26]. CRP was initially identified in the serum of patients with acute inflammation as a substance that reacted with the C-polysaccharide of *Streptococcus pneumoniae*, hence its name[27]. CRP binds to phosphocholine expressed on the surface of dead or dying host cells and some types of microbes in order to activate the complement system and to enhance macrophage phagocytosis. CRP is encoded by a gene located on the proximal long arm of the first chromosome. Due to its annular pentameric structure, CRP belongs to the family of pentraxins. Following an infectious trigger or a major tissue injury, CRP concentration raises up to 10 000-fold within 6 h and peaks at 48 h. With resolution of the inflammatory stimulus, CRP declines with a relatively short half-life of 18 h. Of importance, polymorphism of the CRP gene is responsible for 35–40% of interindividual variation in CRP concentration[28].

Beyond its primary application as a non-specific and an extremely sensitive marker of inflammation, mild elevations in CRP concentration (more than 2–3 mg/L but less than 10 mg/L) were reported to be associated with an increased cardiovascular risk. Therefore, the value of CRP for improved risk prediction was assessed in several clinical scenarios: (i) in patients at risk of developing CAD, (ii) after ACS and (iii) in patients with stable CAD[29]. Additionally, the concept of CRP-guided therapy was established relying on basic research data that suggest direct involvement of CRP in the pathogenesis of atherosclerosis; it was also based on the results of *post hoc* analyses of randomized clinical trials indicating a positive correlation between the magnitude of statin-associated benefit and baseline CRP concentration.

Evidence supporting the role of CRP in cardiovascular risk assessment

The initial observation of Ridker et al. of a link between CRP concentration and the risk of developing CAD made in the population of the Physicians' Health Study (PHS) was subsequently confirmed in numerous prospective, controlled epidemiological studies[30]. However, the strength of the association between CRP and cardiovascular outcomes, particularly after multiple adjustments for traditional risk factors, has been debated over years[31,32]. The most comprehensive data on this topic are derived from recent meta-analyses[33,34]. The Emerging Risk Factors Collaboration (ERFC) in its first individual participant-level meta-analysis of 54 long-term prospective studies, including 160 309 individuals with no history of vascular disease, demonstrated that there is a continuous association between CRP concentration and the risk of CAD, ischaemic stroke, vascular mortality, and death from several cancers and lung diseases[33]. The relative risk (RR) for CAD per 1 standard deviation increases in $\log_e$ CRP concentration after adjustment for systolic blood pressure, smoking status, history of diabetes, body mass index, triglycerides, high-density lipoprotein cholesterol (HDL-C), non-HDL-cholesterol (non-HDL-C), and alcohol intake was 1.37 (95% CI 1.27–1.48) and was even higher than the corresponding RRs for systolic blood pressure (1.33 [95% CI 1.23–1.45]) and non-HDL-C (1.28 [95% CI 1.16–1.40]). Additionally, the authors found that associations of CRP with ischemic vascular diseases depend considerably on conventional risk factors and other markers of inflammation (e.g. fibrinogen, IL-6 and leukocyte count). The same research collaboration analyzed data from 38 prospective studies that included 166 596 participants without a history of cardiovascular disease to investigate the value of adding CRP concentration to conventional risk factors for the prediction of cardiovascular risk[34]. The authors calculated measures of discrimination and reclassification during the follow-up and modeled clinical implications of initiation of

the statin therapy after the assessment of CRP. Adding information on total cholesterol concentration to a prognostic model for cardiovascular disease that originally comprises factors such as age, sex, smoking status, blood pressure, and the history of diabetes, increased the C-statistic, a measure of risk discrimination, by 0.0043. Subsequent addition of HDL-C to the latter model changed the C-statistic by 0.0050. The incremental change in the C-statistic after a further build-up of the model by incorporating information on CRP was 0.0039. The magnitude of the CRP effect was comparable with both lipid parameters. Furthermore, the addition of CRP yielded a net reclassification improvement of 1.52% for the predicted 10-year risk categories of ''low'' (<10%), ''intermediate'' (10% to <20%), and ''high'' (≥20%) ($p < 0.02$). Assuming that statin therapy would be initiated in accordance with the Adult Treatment Panel III (ATP III) guidelines (i.e. for individuals with a predicted risk of ≥20% and for those with other major risk factors, such as diabetes, irrespective of their 10-year predicted risk), the authors demonstrated that the assessment of CRP concentration in persons at intermediate risk for a cardiovascular event could help prevent one additional event over a period of 10 years for every 440 persons screened.

Similarly, the evaluation of CRP concentration in ACS patients provides prognostic information independent of the classical risk factors and enhances the value of well-established risk scores[35,36]. In a cohort study by Schiele et al.[35], with 1501 consecutive ACS patients, the multivariable analysis demonstrated that CRP is an important and independent predictor of 30-day mortality. Of note, in this study patients with the highest CRP concentrations, when compared with those with lower CRP values, presented with the highest mortality and also had other predictors of poor prognosis, including older age, co-existence of more comorbidities and worse hemodynamic conditions, as well as less frequent use of optimal treatment. This fact suggests complex interrelations between patients' characteristics, implemented therapy, CRP concentration and short-term mortality in ACS patients. Importantly, addition of CRP to the GRACE (Global Registry of Acute Coronary Events) model modestly improved the global fit, discriminatory capacity (C-statistic from 0.795 to 0.823), and calibration of the model. Patients were divided into four groups according to the GRACE risk score prediction: <1%, 1% to <5%, 5% to <10%, and ≥10%. With the addition of CRP to the model, reclassification was adequate in 12.2% of cases and inappropriate in 5%. Improvement in the mortality prediction in the ACS setting, attributed to CRP measurement, was also demonstrated in large sub-analyses of the CAPTURE (Chimeric c7E3 AntiPlatelet Therapy in Unstable angina REfractory to standard treatment), FRISC (Fragmin and Fast Revascularization During Instability in Coronary Artery Disease) and TIMI 11A (Thrombolysis in Myocardial Infarction) trials[37–39]. Finally, a meta-analysis of 20 cohort studies or secondary analyses of randomized controlled trials including 17 422 ACS patients indicated that high CRP concentration measured within 72 h of ACS onset moderately increases the long-term risk of recurrent cardiovascular events or death[40]. In detail, 13 studies containing 1364 end points identified from 9787 patients during the follow-up periods reported the risk estimates by CRP categories. Compared with the bottom CRP category (≤3 mg/L), the pooled RRs and their 95% confidence intervals (CIs) were 1.40 (1.18-1.67) for the middle (>3.0 mg/L but ≤10 mg/L) category and 2.18 (1.77-2.68) for the top (>10 mg/L) category of CRP values with a random-effects model, respectively. Another four and three studies reported the risk by unit of CRP or logarithmically transformed CRP concentration, respectively. The pooled RRs (95% CI) were 1.49 (1.06-2.08) per 5 mg/L and 1.26 (0.95-1.69) per $\log_e$ of CRP concentration (mg/L), respectively. However, as demonstrated in the CAPTURE trial CRP assessment fails to facilitate the selection of patients with non-ST-elevation acute coronary syndrome (NSTE-ACS) who benefit from an invasive treatment strategy[37]. In other studies, elevated CRP concentrations were associated with a larger infarct zone and greater risk of post-ACS left ventricular systolic dysfunction, left ventricular remodeling and heart failure (HF)[41–43]. The widespread adoption of CRP assessment in the ACS setting is hampered by the lack of a commonly accepted cut-off point associated with an unfavorable outcome and the fact that CRP concentration is influenced by the ACS type, extent of myocardial necrosis and timing of blood sampling. However, among several proposed values, 10 mg/L is the most frequently used cut-off point[44]. Additionally, it has to be stressed that CRP has no role for the diagnosis of ACS.

A large number of observational studies evaluated the prognostic value of CRP in patients with stable CAD. Despite the fact that these studies, in general, supported CRP assessment, the magnitude of any independent association between CRP concentration and prognosis in stable CAD cannot be established due to multiple types of reporting bias and publication bias[45]. Therefore, no clinical practice recommendations pertaining to CRP in this setting can be made.

CRP-guided therapy

The concept of CRP-guided therapy was prospectively tested in the JUPITER (Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial which was a randomized, double-blind, placebo-controlled trial recruiting apparently healthy men 50 years of age or older, and women 60 years of age or older, with low-density lipoprotein cholesterol (LDL-C) concentration of less than 130 mg/dL and CRP concentrations of 2.0 mg/L or higher[46]. A total of 17,802 study participants were assigned to therapy with rosuvastatin, 20 mg daily, or placebo. The combined primary endpoint included myocardial infarction (MI), stroke, arterial revascularization, hospitalization for unstable angina, or death from cardiovascular diseases. Rosuvastatin decreased LDL-C concentration by 50% and CRP concentration by 37%. After a median follow-up of 1.9 years, rosuvastatin reduced the rates of the primary endpoint (0.77 versus 1.36 per 100 person-years of follow-up; hazard ratio [HR] 0.56; 95% CI 0.46–0.69; $p < 0.00001$) when compared with placebo, MI (0.17 versus 0.37 per 100 person-years of follow-up; HR 0.46; 95% CI 0.30–0.70; $p = 0.0002$), stroke (0.18 versus 0.34 per 100 person-years of follow-up; HR 0.52; 95% CI 0.34–0.79; $p = 0.002$), arterial revascularization (0.38 versus 0.71 per 100 person-years of

follow-up; HR 0.54; 95% CI 0.41–0.72; $p < 0.0001$), and death from any cause (1.00 and 1.25 per 100 person-years of follow-up; HR 0.80; 95% CI 0.67–0.97; $p = 0.02$). The enthusiasm regarding the findings of the JUPITER trial was not commonly shared[47]. However, it should be taken into account that the benefits of statin therapy in primary prevention, including CRP-guided statin therapy, may be partially offset by a higher incidence of diabetes mellitus, which was demonstrated in the JUPITER trial[46] and in a recent network meta-analysis[48]. Furthermore, opponents of the JUPITER study emphasized low absolute benefits of rosuvastatin therapy. In contrast, the JUPITER investigators proved that the number needed to treat (NNT) values associated with statin therapy among those with elevated CRP and low LDL-C were comparable, if not superior, to published NNT values for several widely accepted interventions for primary cardiovascular prevention, including the use of statins among those with overt hyperlipidemia[49]. Additionally, not only was cost-effectiveness of the intervention tested in the JUPITER trial similar to the one already accepted for the treatment of hyperlipidemia, but for many patient subsets it was even cost saving[50]. Finally, the Food and Drug Administration has formally expanded the labeling of statin therapy to include individuals with elevated CRP and at least one additional risk factor, even if LDL-C is low[51].

Another important ongoing study investigating CRP-guided therapy is the CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study) trial[52]. The trial was designed to investigate whether IL-1β inhibition by canakinumab as compared with placebo reduces the rates of recurrent MI, stroke and cardiovascular death among stable CAD patients who remain at high vascular risk due to persistent elevation of CRP (>2 mg/L) despite contemporary secondary prevention strategies. A total of 17 200 of patients will be randomly assigned either to groups receiving placebo or canakinumab, administered subcutaneously, at doses of 50, 150 or 300 mg every three months.

Evidence arising from genetic studies

Genetic studies conducted so far do not support the causal role of CRP in the pathogenesis of CAD. The application of Mendelian randomization, a sophisticated research tool used in order to determine cause-effect relationships, showed in an individual participant-level meta-analysis, including 194 418 persons, that CRP genetic variants were associated with CRP concentration, but not with the prevalence of CAD, despite a clear link existing between CRP concentration and the prevalence of CAD[53]. Consistent results were obtained in a combined analysis of four large independent Danish cross-sectional studies and prospective cohorts as well as in a British research project including a genome-wide association study ($n = 17\,967$) followed by a replication study ($n = 13\,615$)[54]. The authors conclude that CRP is unlikely to be even a modest causal factor in CAD.

Analytical concerns and recommendations on clinical use

For cardiovascular risk assessment, CRP should be measured using the third generation of highly sensitive assays (hsCRP) with low imprecision (5–10%) in the range of 3 and 10 mg/L and detection limits ≤0.15 mg/L that are capable of measurement of CRP concentrations in the healthy population[44]. Numerous such tests are currently commercially available. Due to their increased sensitivity, good reproducibility and assay standardization, CRP in terms of its analytical validation is most appropriate for routine clinical use among other inflammatory markers discussed in this review. Importantly, no specific patient preparation before blood sampling is necessary and the CRP *in vitro* stability is excellent[44].

Despite the lack of its causality in the pathogenesis of CAD, assessment of CRP concentration may be useful in everyday cardiovascular risk stratification and decision-making, particularly in individuals without established CAD and in those with ACS. This opinion is reflected in recommendations and statements of scientific societies regarding the role of CRP in the cardiovascular risk stratification summarized in Table 2[16–18,44]. Increased CRP concentration accounts for the presence of multiple measured and, in many clinical situations, unmeasured risk factors and therefore may reflect a substantial part of the overall cardiovascular risk. Many of these risk factors are not included in risk scores recommended in various clinical settings (e.g. primary prevention: the Framingham Risk Score [FRS] and the Systematic COronary Risk Evaluation [SCORE] risk assessment system developed by the European Society of Cardiology [ESC]; ACS patients: the Global Registry of Acute Coronary Events [GRACE] Risk Score). However, the question, whether CRP provides an incremental prognostic value when assessed on top of high-sensitivity cardiac troponin (cTn), remains open both in primary and secondary prevention due to the lack of scientific data.

Fibrinogen

Background

Fibrinogen was the first clotting factor, discovered and described in the first half of the nineteenth century[55]. Studied intensively for decades, it has played a major role not only as a precursor of fibrin, but also as a factor associated with many diseases.

Fibrinogen is a soluble plasma glycoprotein synthesized by hepatocytes[56]. The homodimeric fibrinogen molecule that is found in the blood contains three different polypeptide chains called α, β and γ. Although the molecular structure of fibrinogen has already been known since the seventies, the use of X-ray crystallography for better identification and explanation of its pleiotropic biological properties has become possible[56]. In fact, several binding sites on the fibrinogen molecule have been recognized that are responsible for its interaction with different receptors or adhesion molecules, expressed on the various cells of the hematopoietic, immune and nervous systems[56]. Since fibrinogen, similarly as CRP, is an acute phase protein, it increases several-fold in pathological conditions, whereas under physiological conditions plasma concentrations of fibrinogen range from 2–4 g/L. Higher concentrations >3.5 g/L are, among others, associated with human diseases with an inflammatory component, including CAD[55,56].

Table 2. Recommendations and statements of scientific societies regarding the role of CRP in cardiovascular risk stratification.

Scientific organization and year of publication	Recommendation/Statement	Class of recommendation/ Level of evidence
ESC 2013[44]	*Statements concerning biochemical and analytical issues*	
	CRP concentrations are reported in mg/L.	Not reported
	CRP test results are method-dependent, but classification of patients into risk categories is usually comparable.	Not reported
	Third-generation hs-CRP assays are recommended.	Not reported
	No specific patient preparation before blood sampling is necessary.	Not reported
	The in vitro stability of CRP is high.	Not reported
	Statements concerning clinical use of CRP in ACS	
	CRP is an established marker for diagnosing and monitoring infection, inflammation, and tissue injury.	Not reported
	CRP measurement in primary prevention predicts future cardiovascular events with significance similar to that of total and HDL cholesterol.	Not reported
	CRP measurement in secondary prevention predicts risk of recurrent MI, stroke, and cardiovascular death.	Not reported
	CRP measurements have no value for diagnosing AMI.	Not reported
	CRP release is related to infarct size and risk in STEMI patients.	Not reported
	CRP is not helpful for the choice of an invasive or conservative strategy in ACS.	Not reported
	CRP measurement after ACS and after PCI can be used to identify patients in whom an intensive risk factor modification is useful.	Not reported
	For cardiovascular prevention, a CRP value of >3 mg/L is considered high risk but a limit ≥10 mg/L seems more appropriate in ACS patients.	Not reported
ESC 2012[18]	Hs-CRP may be measured as a part of risk assessment in patients at unusual or moderate CVD risk.	IIb/B Benefit ≥ risk; additional studies with broad objectives needed; procedure may be reasonable, usefulness is less well established; greater conflicting evidence from single randomized trial or nonrandomized studies/limited populations evaluated; data derived from a single randomized trial or nonrandomized studies
	In asymptomatic low-risk individuals and high-risk patients, hs-CRP should not be measured to assess 10-year cardiovascular risk.	III/B No benefit or harm; recommendation that procedure is not useful and may be harmful; evidence from single randomized trial or nonrandomized studies/limited populations evaluated; data derived from a single randomized trial or nonrandomized studies
ACCF/AHA 2010[16]	In men ≥50 years of age or women ≥60 years of age with LDL-C<130 mg/dL; considered as low-risk, measurement of CRP can be useful in the selection of patients for statin therapy.	IIa/B Benefit≫risk; additional studies with focused objectives needed; it is reasonable to perform procedure, recommendation in favor of procedure being useful; some conflicting evidence from single randomized trial or nonrandomized studies/limited populations evaluated; data derived from a single randomized trial or nonrandomized studies
	In asymptomatic men ≤50 years of age or women ≤60 years of age; considered as intermediate-risk, measurement of CRP may be reasonable for cardiovascular risk assessment.	IIb/B Benefit ≥ risk; additional studies with broad objectives needed; procedure may be reasonable, usefulness is less well established; greater conflicting evidence from single randomized trial or nonrandomized studies/limited populations evaluated; data derived from a single randomized trial or nonrandomized studies
	In asymptomatic adults at high-risk, measurement of CRP is not recommended for cardiovascular risk assessment.	III/B No benefit or harm; recommendation that procedure is not useful and may be harmful; evidence from single randomized trial or nonrandomized studies/limited populations evaluated; data derived from a single randomized trial or nonrandomized studies
	In low-risk men ≤50 years of age or women ≤60 years of age, measurement of CRP is not recommended for cardiovascular risk assessment.	III/B No benefit or harm; recommendation that procedure is not useful and may be harmful; evidence from single randomized trial or nonrandomized studies/limited populations evaluated; data derived from a single randomized trial or nonrandomized studies

(*continued*)

Scientific organization and year of publication	Recommendation/Statement	Class of recommendation/ Level of evidence
NACB 2009[17]	*Recommendations concerning clinical science*	
	1a. After standard global risk assessment, if the 10-year-risk is <5%, hsCRP should not be measured.	I/A Conditions for which there is evidence and/or general agreement that a given procedure is useful and effective/data derived from multiple randomized clinical trials that involved large numbers of patients
	1b. If the 10-year-risk is 5 to 10% it is expected that adult at 10% of risk might be reclassified to higher risk group with the CRP measurement.	II/B Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of a procedure or treatment/data derived from a limited number of randomized trials that involved small numbers of patients or from careful analyses of nonrandomized studies or observational registries
	1c.If risk is intermediate (10 to 20%) and uncertainty remains as to the use of preventive therapies, hsCRP measurement might be useful for further stratification.	I/A Conditions for which there is evidence and/or general agreement that a given procedure is useful and effective/data derived from multiple randomized clinical trials that involved large numbers of patients
	2. Treatment based on hsCRP concentration should be introduced on the basis of clinical judgment.	IIb/B Usefulness/efficacy is less well established by evidence/data derived from a limited number of randomized trials that involved small numbers of patients or from careful analyses of nonrandomized studies or observational registries
	3. There are insufficient data on the therapeutic monitoring using hsCRP to evaluate the effect of treatment in primary prevention.	III/C Conditions for which there is evidence and/or general agreement that the procedure/treatment is not useful/effective and in some cases may be harmful/expert consensus as the primary basis for the recommendation
	4. The utility of hsCRP measurement to improve patient's lifestyle has not been demonstrated.	IIb/C Usefulness/efficacy is less well established by evidence/opinion/expert consensus as the primary basis for the recommendation
	5. Measurement of other inflammatory markers in addition to hsCRP for coronary risk assessment is not supported due to lack of sufficient evidence.	IIb/C Usefulness/efficacy is less well established by evidence/opinion/expert consensus as the primary basis for the recommendation
	Recommendations concerning clinical science and laboratory testing	
	Measurement of hsCRP should be done in fasting in metabolically stable patients, free of infections or acute illness. If the hsCRP concentration is >3 mg/L the test should be repeated at least 2 weeks later. hsCRP ≥10 mg/L might be related to cardiovascular risk.	IIa/A Weight of evidence/opinion is in favor of usefulness/efficacy/data derived from multiple randomized clinical trials that involved large numbers of patients
	Of the examined inflammatory markers for cardiovascular risk assessment, hsCRP has the analyte and assay characteristics most appropriate for use in clinical practice.	I/A Conditions for which there is evidence and/or general agreement that a given procedure is useful and effective/data derived from multiple randomized clinical trials that involved large numbers of patients
	hsCRP should be expressed in mg/L, regardless of the method used.	I/C Conditions for which there is evidence and/or general agreement that a given procedure is useful and effective/expert consensus as the primary basis for the recommendation
	Standardized CRP assays classified patients into the following risk categories: a. Low risk <1.0 mg/L b. Average risk 1.0 to 3.0 mg/L c. High risk >3.0 mg/L d. Very high risk ≥10.0 mg/L	IIa/A Weight of evidence/opinion is in favor of usefulness/efficacy/data derived from multiple randomized clinical trials that involved large numbers of patients
	Manufacturers of assays for hsCRP should follow approved laboratory protocols.	I/C Conditions for which there is evidence and/or general agreement that a given procedure is useful and effective/expert consensus as the primary basis for the recommendation
	Categorization for risk prediction in certain population such as non-Caucasian and the elderly should be performed with caution.	IIa/C Weight of evidence/opinion is in favor of usefulness/efficacy/expert consensus as the primary basis for the recommendation

ACCF/AHA: American College of Cardiology Foundation/American Heart Association; ACS: acute coronary syndrome; AMI: acute myocardial infarction; CRP: C-reactive protein; CVD: cardiovascular disease; ESC: European Society of Cardiology; HDL: high-density lipoprotein; hs-CRP: high-sensitivity C-reactive protein; LDL-C: low-density lipoprotein cholesterol; NACB: National Academy of Clinical Biochemistry; PCI: percutaneous coronary intervention; STEMI: ST-segment elevation acute myocardial infarction.

Nowadays, due to a more critical approach to the role of inflammatory markers in cardiovascular diseases and not fully documented causal relationship between its plasma concentration and the incidence of CAD, fibrinogen plays a less important role in cardiovascular risk stratification. Because of these inconsistencies pertaining to the role of fibrinogen, we undertook efforts to assess the rationale for or against its use in clinical practice.

Evidence for the role of fibrinogen in cardiovascular risk assessment

The most important scientific evidence for establishing the role of fibrinogen for cardiovascular risk assessment was provided by the large international meta-analyses: the Fibrinogen Studies Collaboration (FSC) and the ERFC[34,57–59]. The FSC assessed the relationship of fibrinogen concentrations with the risk of both major vascular and non-vascular outcomes based on 154 211 individual participants' data without known cardiovascular disease from 31 prospective studies. In this meta-analysis, moderately strong associations were found between fibrinogen concentration and the risks for CAD, stroke and other vascular and non-vascular mortality. After adjustment for age and sex, HRs per 1 g/L increase in fibrinogen were 2.42 (95% CI 2.24–2.60), 2.06 (95% CI 1.83–2.33), 2.76 (95% CI 2.28–3.35) and 2.03 (95% CI 1.90–2.18) for CAD, stroke, and other vascular mortality and non-vascular mortality, respectively[57]. However, the HRs were significantly reduced (to about 1.8) after the adjustment for several established cardiovascular risk factors (such as smoking, body mass index, total cholesterol, hypertension and diabetes). A subsequent study based on the FSC data indicated that approximately one third of the variation in fibrinogen concentrations is determined by age, sex and cohort characteristics. Another 7% of the variation is explained by established cardiovascular risk factors and a further 10% by inflammatory markers[58]. In the ERFC study involving 246 669 participants without a history of cardiovascular disease, Kaptoge et al. analyzed data from 53 prospective studies and found that the assessment of CRP or fibrinogen concentrations was associated with a significant improvement in the prediction of the first-onset of a cardiovascular event, even when added to established cardiovascular risk factors[34]. They concluded that the measurement of CRP or fibrinogen concentrations in healthy persons at intermediate risk (risk of 10% to <20% over a period of 10 years) could help prevent one additional event over a period of 10 years for every 400 to 500 persons screened[34]. Despite the fact that these large meta-analyses confirmed the data from smaller clinical trials and epidemiological studies conducted in the last decades, with increased fibrinogen concentration being an independent risk factor for cardiovascular disease, the causality of this relationship continues to be less accurately explained. Furthermore, the lack of fibrinogen specificity for determining the risk, in which the role of an inflammatory component seems to play a key role, simultaneously diminishes its importance as an independent risk marker of CAD.

The association between fibrinogen and cardiovascular events in populations with pre-existing cardiovascular disease, particularly in various subgroups of patients with CAD, seems to be less extensively investigated. However, a recently published study on the relationship showed results similar to those presented for primary prevention[60]. In fact, in all subsets of patients with CAD, fibrinogen was an independent predictor of all-cause and cardiac mortality, but it did not provide any additional prognostic information than that provided by traditional cardiovascular risk factors[60].

Evidence arising from genetic studies

It has already been shown that genetic variants influence plasma fibrinogen concentration, affecting its variability, making estimates of its heritability range from 34 to 50%. However, genetic studies conducted so far delivered only a very poor explanation of its variation (<2%). As evidenced by a recent multiethnic meta-analysis of 28 genome-wide association studies that included over 90 000 individuals, the causal relationship between fibrinogen and cardiovascular disease could not be established. In this study, 23 fibrinogen loci were identified, of which 15 were new, but the clinical outcome analysis of these loci did not support a causal relationship between plasma fibrinogen concentration and cardiovascular disease, particularly in clinically apparent CAD[61].

Analytical concerns and recommendations

For fibrinogen, the most important analytical considerations relate to assay standardization, or rather the absence of it. In 2009, the National Academy of Clinical Biochemistry (NACB) stated that evidence supporting the clinical usefulness of fibrinogen in cardiovascular risk assessment is inconclusive[17]. Because of the lack of selective agents lowering fibrinogen and analytical aspects resulting from difficulties in assay standardization, the measurement of this biomarker is not recommended by the NACB[60].

However, the European guidelines on cardiovascular disease prevention in clinical practice as of 2012 allow fibrinogen measurement as a part of risk assessment in patients with an unusual or moderate cardiovascular risk (class of recommendation IIb [benefit $\geq$ risk, usefulness/efficacy is less well-established], level of evidence B [data derived from a single randomized trial or non-randomized studies]), but not in asymptomatic low-risk individuals and high-risk patients (class of recommendation III [recommendation that a procedure or treatment is not useful/effective and may be harmful], level of evidence B [data derived from a single randomized trial or non-randomized studies])[18].

Lp-PLA2

Background

Lp-PLA2, also known as platelet-activating factor acetylhydrolase (PAFA), is a lipid-related inflammatory biomarker, a member of the human A2 phospholipase superfamily, derived from a variety of inflammatory cells including macrophages, T-lymphocytes, monocytes and mast cells[62]. Lp-PLA2 is transported in the circulation bound with the low-density lipoprotein (LDL) molecules, more specifically with electronegative domains on apolipoprotein B (apoB)[63]. Less than 30% of Lp-PLA2 is associated with high-density lipoprotein

(HDL) and very low-density lipoprotein (VLDL)[62,64]. Lp-PLA2 as an enzyme is able to modify the surface of LDL particles in the process of phospholipid hydrolysis, which in turn increases their susceptibility to oxidation. After LDL oxidation Lp-PLA2 causes the release of lysophosphatidylocholine and oxidized fatty acids, which have pro-inflammatory and pro-atherogenic properties[62]. Beyond LDL modification, Lp-PLA2 is involved in different biological processes including lipid and cellular protein metabolism, monocyte chemotaxis and regulation of inflammatory response. Moreover, enhanced Lp-PLA2 activity found in coronary vulnerable plaques seems to be essential for its potential inflammatory contribution to both atherosclerosis progression and ACS occurrence.

Evidence supporting the role of Lp-PLA2 in cardiovascular risk assessment

Interestingly, Lp-PLA2 was initially recognized as an anti-inflammatory enzyme in different animal models[65]. However, despite the preliminary reports on its anti-atherogenic features, there was an increasing amount of evidence that showed its pro-atherogenic and pro-inflammatory properties in relation to plaque rupture and the prevalence of cardiovascular events[64]. More than a decade ago, in a five-year case-control trial – the West of Scotland Coronary Prevention Study (WOSCPS) – Lp-PLA2 was first demonstrated to be an independent risk factor for CAD[66]. In the Atherosclerosis Risk in Communities (ARIC) study with a total number of 12 762 healthy middle-aged subjects, concentrations of both Lp-PLA2 and CRP were shown to have a complementary value beyond traditional risk factors in identifying those at an increased risk for ischemic stroke[67]. Subsequent studies, including the Rotterdam Study, the Rancho Bernardo Study, the Bruneck Study and the Cardiovascular Health Study[68], consistently confirmed the predictive value of elevated Lp-PLA2 concentrations for cardiovascular events in apparently healthy adults[69]. Epidemiological studies on the evaluation of the associations of increased concentrations of Lp-PLA2 and clinical outcomes provided substantial evidence for the role of Lp-PLA2 in secondary prevention. For instance, in the Prevention of Events with Angiotensin-Converting Enzyme Inhibition (PEACE) study, Sabatine and colleagues evaluated the prognostic utility of Lp-PLA2 for cardiovascular outcomes in patients with stable CAD. Lp-PLA2 concentrations were measured in 3766 patients during a follow-up of five years. Patients with Lp-PLA2 concentrations in the highest quartile had a HR of 1.41 (95% CI 1.17–1.70) for an adverse cardiovascular outcome compared to those from the lowest quartile[70]. Moreover, a number of systematic reviews summarized the research evidence on the association of Lp-PLA2 and cardiovascular disease. Despite the promising results from epidemiological studies that have generally established the independent association between increased concentrations of Lp-PLA2 and the risk of future cardiovascular events, there are inconsistencies regarding the population selection (for both primary and secondary prevention), a precisely specified endpoint and the type of assay tested (Lp-PLA2 activity or mass)[71]. Garza et al. in a meta-analysis of 14 observational studies with 20 549 patients reported an odds ratio (OR) of 1.60 (95% CI 1.36–1.89) for the development of future cardiovascular events[72]. The ERFC performed a patient-level meta-analysis of the independent associations of novel lipid factors with cardiovascular risk[73]. In 11 studies including 32 075 participants, Lp-PLA2 was an independent risk factor for cardiovascular events with a HR of 1.12 (95% CI 1.09–1.21) per 1 standard deviation increase in Lp-PLA2 activity. However, there was no significant improvement in risk reclassification after the addition of Lp-PLA2, with a net reclassification improvement (NRI) of 0.21 (95% CI −0.45–0.86)[73]. In a recent large meta-analysis of 79 036 participants from 32 prospective studies, both Lp-PLA2 mass and activity were found to be associated with vascular disease, including CAD, ischemic stroke and mortality[74]. RRs per 1 standard deviation higher value, adjusted for conventional risk factors, were: 1.10 (95% CI 1.05–1.16) with Lp-PLA2 activity and 1.11 (95% CI 1.07–1.16) with Lp-PLA2 mass for CAD; 1.08 (95% CI 0.97–1.20) with Lp-PLA2 activity and 1.14 (95% CI 1.02–1.27) with Lp-PLA2 mass for ischaemic stroke; 1.16 (95% CI 1.09–1.24) with Lp-PLA2 activity and 1.13 (95% CI 1.05–1.22) with Lp-PLA2 mass for vascular mortality; and 1.10 (95% CI 1.04–1.17) with Lp-PLA2 activity and 1.10 (95% CI 1.03–1.18) with Lp-PLA2 mass for non-vascular mortality, respectively. However, the magnitude of these associations was similar to that observed for non-HDL-C and systolic blood pressure, and was comparable for Lp-PLA2 mass and Lp-PLA2 activity[74]. In contrast, in the JUPITER trial Lp-PLA2 activity, but not Lp-PLA2 mass, was predictive of incident vascular events[75].

Although most studies have shown a positive correlation between Lp-PLA2 and the risk of cardiovascular disease, its strength was decreased after the adjustment of other classical risk factors, and especially of atherogenic lipoproteins. This may result from biological properties of Lp-PLA2, which are strongly associated with atherogenic lipoproteins containing the apoB molecule. Therefore, the effect induced by Lp-PLA2 may be dependent not only on the inflammatory component, but also on the lipid component of the atherosclerotic process[76].

Evidence arising from genetic studies

Evidence from genetic studies did not confirm the link between Lp-PLA2 gene polymorphism and cardiovascular risk. In a meta-analysis including a total of 12 studies with 26 118 participants, Casas et al. examined the association of Lp-PLA2 activity with an increased risk of CAD, and the causal relationship between common PLA2G7 genetic variants (encoding Lp-PLA2)[77]. In contrast to Lp-PLA2 activity, PLA2G7 variants were not associated with other cardiovascular risk markers, angiographic CAD or CAD events. Other findings from five community-based studies comprising 13 664 subjects showed that both Lp-PLA2 mass and its activity were associated with PLA2G7, but Lp-PLA2 activity, and not Lp-PLA2 mass, was strongly associated with genetic variants related to LDL-C[78]. Although recent meta-analyses failed to provide evidence to support the hypothesis that Lp-PLA2 is a risk factor for CAD, it cannot be ruled out that it actually is. This may, however, be related to the strong affinity with atherogenic lipoproteins containing apoB.

Therefore, genetic studies provided insufficient data to detect any genetic effect consistent with the causal role of Lp-PLA2 in CAD.

Current evidence from clinical trials

To assess the efficacy and clinical benefits from targeting treatment to Lp-PLA2, randomized, placebo-controlled trials are required. At least three large clinical trials, the Heart Protection Study (HPS), the JUPITER trial and the Long-term Intervention with Provastatin in Ischemic Disease Study (LIPID) assessed Lp-PLA2 mass and/or activity in relation to cardiovascular risk and have further examined whether the utility of Lp-PLA2 for risk stratification is modified by statin treatment[75,79,80]. In the HPS trial, only Lp-PLA2 activity was significantly associated with cardiovascular events in an unadjusted model, however after the adjustment for non-lipid risk factors, the strength of this relationship decreased and after considering lipid risk factors it was not statistically significant[79]. In contrast, in the JUPITER trial the association of Lp-PLA2 activity with cardiovascular outcomes remained significant even after the adjustment for LDL-C and other risk factors[75].

In both the HPS and in the JUPITER trial, effective use of statin therapy significantly influenced the relationship between Lp-PLA2 and cardiovascular risk. Although the treatment with statins resulted in a reduction in both Lp-PLA2 mass and activity, no added value was observed as to the effect caused by the statin treatment[75,79]. In contrast, the results of the LIPID sub-study, involving over 6500 patients, showed that decreased Lp-PLA2 activity strongly predicts reduction in subsequent CAD events after adjustment for treatment, 23 baseline risk factors, and other biomarkers, including LDL-C and LDL-C changes ($p < 0.001$). Novel findings from the LIPID study suggest that changes in Lp-PLA2 activity account for more than a half (59%) of provastatin treatment effects, independently of changes in LDL-C. This is the first study to demonstrate that the benefits of statin treatment may at least partly result from changes in Lp-PLA2 activity, thus making the existence of additional mechanisms of statin effects on cardiovascular risk reduction more likely[80]. The mechanism for inhibition of Lp-PLA2 activity by statins is not well-defined. However, statin (simvastatin) has been shown to decrease Lp-PLA2 expression and activity in lipopolysaccharide-stimulated human monocyte-derived macrophages through inhibition of the mevalonate-geranylgeranyl pyrophosphate-RhoA-p38 mitogen-activated protein kinase pathway[81].

Clinical studies concerning the use of Lp-PLA2 activity inhibitors were unsuccessful or yielded very little promising results[82,83]. Currently, high expectations of clinicians have been concentrated on two clinical trials: STABILITY (STabilisation of Atherosclerotic plaque By Initiation of darapLadIb TherapY) and its successor SOLID-TIMI-52 (The Stabilization Of pLaques usIng Darapladib-Thrombolysis In Myocardial Infarction 52 Trial)[84,85]. More recently, results have been announced from the phase III STABILITY trial, evaluating the direct inhibition of Lp-PLA2 with darapladib in 15 828 patients with chronic CAD[84,86] Unexpectedly, the study failed to meet the primary endpoint measure, which was the time to the first occurrence of any composite of a major adverse cardiovascular event (MACE). It seems plausible that the clinical benefits of darapladib in patients with chronic CAD may be less significant than initially expected. A further analysis of the second phase III study, SOLID-TIMI-52, which evaluates the efficacy of darapladib in approximately 13 000 patients with ACS is ongoing and expected to be completed in 2014. Results concerning the pre-defined secondary endpoints are more promising than those for the primary endpoints reported in findings of the STABILITY trial[86]. However, results from the phase III STABILITY trial did not establish the causal role of Lp-PLA2 in cardiovascular events, which makes the question of its clinical usefulness still disputable.

Analytical concerns and recommendations

Although commercially available analytical methods allow the determination of both Lp-PLA2 mass and activity, mean levels of Lp-PLA2 mass have varied widely in prior epidemiological studies, which might make measurements of its activity more attractive[76]. For instance, as seen in the JUPITER trial, the mean values of the Lp-PLA2 mass in the placebo group decreased by 11.7% when measured again a year later, whereas Lp-PLA2 activity and LDL-C values changed only minimally[75].

Due to the analytical problems with the automated and manual Lp-PLA2 mass assays a need arises for an automated, validated assay for Lp-PLA2 activity measurement. Recently presented results on performance characteristics of an automated assay on the Cobas 6000/c501 (Roche Diagnostics, Indianapolis, IN) confirmed its readiness for implementation into clinical practice[87].

The initial guidelines of the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) Task Force on Practice Guidelines recommended Lp-PLA2 measurements for the risk assessment in asymptomatic patients (class of recommendation IIb [benefit $\geq$ risk, additional studies with broad objectives needed, procedure may be reasonable, usefulness is less well-established], level of evidence B [data derived from a single randomized trial or non-randomized studies])[16]. In 2012, both the American and European guidelines recommended the incorporation of Lp-PLA2 measurements into patients' cardiovascular risk assessment[18,88]. The American Association of Clinical Endocrinologists (AACE) supported Lp-PLA2 testing for assessing the inflammatory component of the atherosclerotic process and further established Lp-PLA2 as a vascular-specific inflammatory risk factor (grade B [intermediate strength of recommendation], evidence level 1 [recommendation based on strong evidence])[88]. The Fifth Joint Task Force of the ESC on Cardiovascular Disease Prevention in Clinical Practice accepted the possibility of Lp-PLA2 measurement in patients at higher risk of a recurrent acute atherothrombotic event (class of recommendation IIb [usefulness/efficacy is less well-established by evidence/opinion], level of evidence B [data derived from a single randomized clinical trial or large non-randomized studies])[18].

MPO

Background

Myeloperoxidase (MPO) is a pro-inflammatory enzyme stored in azurophilic granules of polymorphonuclear neutrophils and macrophages that generates reactive oxygen species. Its biological function is to catalyze the conversion of chloride and hydrogen peroxide to hypochloride in the heme-dependent reaction in inflammatory conditions[89]. MPO has a negative impact on various key molecules due to its pro-oxidative action. For instance, it intensifies the process of LDL particle oxidation in the arterial wall, modifies apolipoprotein AI molecules causing the loss of anti-inflammatory properties of HDL, consumes nitric oxide, which finally affects endothelial dysfunction, and impairs vasodilatation, thus increasing local inflammation and accelerating the progression of atherosclerosis[89–91]. Further, MPO activates metalloproteinases and inactivates their tissue inhibitors[92]. Since MPO, similarly as CRP and Lp-PLA2, is present within atherosclerotic plaques, it may actively contribute to plaque instability and rupture[32,90,93,94]. MPO has also been shown to be involved in both proapoptotic and prothrombotic pathways that are likely to contribute to plaque fissuring, superficial erosions and thrombus generation during ACS[95]. However, research findings suggest that enhanced expression of MPO is caused by an ongoing inflammatory process rather than tissue response to ischemia[95].

Epidemiological and clinical evidence for MPO measurement in patients with and without established CAD

Although most studies on the prognostic role of MPO focused on the settings following MI and HF, as well as on the assessment of chest pain or in stable CAD, several studies have examined its application in healthy, asymptomatic individuals without diagnosed CAD[94,96].

The first scientific evidence on the relationship between increased MPO concentrations and CAD emerged in 2001. Results reported by Zhang et al. indicated that CAD patients had significantly higher MPO concentrations compared to controls. As demonstrated by multivariate regression analysis, blood MPO was the strongest predictor for CAD (OR 20.4; 95% CI 8.9–47.7) even after the adjustment for traditional risk factors[97]. In another prospective study, Meuwese et al. examined the association of MPO with the risk of CAD development in an initially healthy population of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study[98]. This study included 1138 case-subjects who developed CAD during the eight-year follow-up, and 2237 matched control-subjects who remained free of CAD. As previously reported, MPO concentrations were significantly higher in case subjects. The strongest linear positive association was observed between MPO and CRP and leukocyte count, whereas a negative association was observed with HDL-C concentration. Individuals in the top quartile of MPO concentrations had higher risk of future CAD compared to those from the lowest quartile (OR 1.49; 95% CI 1.20–1.84), which remained significant after adjustment for traditional risk factors, but was weakened after adjustment for CRP. Moreover, increased concentrations of MPO (>728 pmol/L) were predictive of CAD even in subjects with low risk (LDL-C <130 mg/dL, HDL-C >50 mg/dL and CRP <2 mg/L)[98].

Another study prospectively examined the usefulness of MPO in patients with stable CAD undergoing coronary angiography. In a cohort of 885 patients who were followed up over 13 years, MPO independently predicted risk for cardiovascular mortality (HR for patients from the highest versus the lowest tertile 2.4; 95% CI 1.5–3.0), even after the adjustment for traditional risk factors and CRP. The strongest ability to risk stratification was observed when MPO and CRP were evaluated together[99].

A majority of studies, yet not all of them, presented a significant association of MPO with an increased risk of future CAD. In a recent study published by Wong and colleagues, MPO was measured in 1302 asymptomatic patients without known CAD followed over 3.8 years[100]. In this study, coronary artery calcium (CAC) was evaluated as a surrogate of subclinical atherosclerosis. Patients with higher MPO concentrations were more likely to have an unfavorable profile of other traditional risk factors, however no direct significant association between CAC levels and MPO concentrations was found. The study results also demonstrated that MPO alone did not have any additional value-to-risk prediction. In contrast, combining CAC and MPO measurements resulted in the improvement in cardiovascular risk prediction. Patients with highest CAC and MPO were at an increased risk of developing CAD[100]. One of the limitations of this study was an insufficient number of primary endpoints to be able to examine each of them separately. Additionally, the results did not establish the causal role of MPO in the CAD progression because the effect seems to be partially attributed to CAC.

Role of MPO in patients with acute chest pain

Several large studies have focused on the role of MPO in patients with chest pain suspected of ACS, and risk stratification of these patients with emphasis on multimarker strategies. Unfortunately, these studies were heterogeneous in terms of sample type, pre-defined endpoints and population characterization. In the CAPTURE trial, Baldus et al. investigated the prognostic information of circulating MPO concentrations in 1090 patients with ACS. The primary endpoint of the study was defined as mortality and nonfatal MI during the 30 days and 6 months of the follow-up period. Patients with higher concentrations of MPO (>350 μg/L) were at an increased risk of adverse cardiac outcomes (HR 2.25; 95% CI 1.32–3.82). Interestingly, patients with low and high cTnT concentrations did not differ in terms of MPO concentration. Moreover, MPO was capable of identifying subjects at an increased risk for cardiovascular events if they had low baseline cTnT (adjusted HR 7.48; 95% CI 1.98–28.29) and CRP concentrations. This may be explained by the fact that, from a pathophysiological point of view, the release of MPO from neutrophils substantially precedes the damage and necrosis of cardiomyocytes, and even the release of other systemic inflammatory mediators. Interestingly, increased risk of death and MI depending on MPO tertiles was observed

only after 72 hours from the last episode of chest pain and was most strongly expressed after six months of follow-up[101]. In contrast, Nicholls et al. showed that MPO concentrations are predictive of MACE up to 16 hours after a presentation with chest pain[96]. It seems that despite the initial process of leukocyte activation and MPO release, which precedes cardiomyocyte damage and inflammation induced by necrosis, the risk stratification using MPO is possible only in the early phase from the onset of chest pain. Furthermore, along with the progress of ischemia, systemic inflammatory response is enhanced as a result of necrosis[97]. The risk assessment in patients after MI, involving solely MPO, may be at least partly explained by systemic inflammatory activation and complicity, therefore its role in identifying patients with unstable coronary plaques who are at risk for MI is limited.

The potential diagnostic value of MPO in patients with suspected ACS was investigated in a large 18-center prospective Motivational Interventions for Drugs & Alcohol misuse in Schizophrenia (MIDAS) study. Despite the promising results of earlier studies conducted in smaller cohorts, this study showed a limited clinical utility of the MPO measurement in patients presenting to the emergency department (ED) with suspected MI. Moreover, MPO was not clinically useful and had a limited clinical value in initially troponin-negative patients[90].

Another important issue is a multimarker strategy for the diagnosis of MI in patients presenting with chest pain and suspected of ACS[102,103]. Apple et al. evaluated the clinical utility of seven biomarkers, including MPO, for the early diagnosis of MI in 457 patients having chest pain[102]. In this study, only cTnI measured with the use of the second generation assay had the highest clinical and diagnostic value for the early diagnosis of MI. In addition, none of the biomarkers, whether it was a marker of inflammation, plaque destabilization or plaque rupture, had incremental diagnostic value beyond troponin. Later, in a subsequent study by the same author regarding the clinical utility of MPO for the risk stratification in patients with ischemia, suggestive of ACS and with normal cTnI concentrations at admission, MPO was a powerful predictor of MACE. The combined use of cTnI and MPO identified a significantly greater proportion of patients at risk of MACE during the 30-day follow-up, however when these markers were considered separately, the adjusted HR for MACE was significantly higher for troponin[104]. A reliable assessment of this study is hampered by the lack of information of the heparin administration before or at the time of admission, which is known to significantly affect the concentration of MPO[105,106]. In another more recently published study, MPO had an independent overall prognostic value for the prognosis of MACE and, most importantly, in patients with a negative contemporary sensitive cTnI concentration at admission[107].

The MERLIN-TIMI 36 (Metabolic Efficiency with Ranolazine for Less Ischaemia in Non-ST Elevation Acute Coronary-Thrombolysis In Myocardial Infarction 36) trial simultaneously evaluated the incremental prognostic value of cTnI, N-terminal pro B-type natriuretic peptide (NT-proBNP), CRP and MPO among 4352 well-characterized patients with NSTE-ACS. After including all biomarkers in the clinical model, only NT-proBNP and cTnI were independently associated with cardiovascular death. Moreover, the model adjusted for the TIMI Risk Score was significantly improved in terms of discrimination and reclassification after the addition of both natriuretic peptide and troponin, but not after the addition of either MPO or CRP[108].

Altogether, the measurement of MPO in patients presenting with acute chest pain seems to be clinically proven, however with the introduction of hs-cTn assays along with increased sensitivity and specificity, the role of MPO in ACS patients is likely to be very limited. Given the different nature of both markers new strategies using these markers should be investigated. The potential combined prognostic value of MPO and hs-cTn has not yet been well studied and requires further research. Preliminary findings, however, showed the use of hs-cTn to be superior for the early diagnosis of MI among patients presenting with chest pain in the ED[109].

Since 2001 there has been accumulation of large body of literature related to the clinical utility of MPO in predicting future cardiovascular events and risk stratification in patients with chest pain with suspected ACS. However, their evaluation, direct comparison and the impact on the explanation of the true causal relationship is difficult due to inconsistencies in assay characteristics, sample types and population used.

Analytical concerns and recommendations

It has already been recognized that preanalytical handling is very important in the MPO measurement. Both the use of an improper sample type and its incorrect storage may dramatically affect the concentration of MPO, thus causing false-positive results[105,106]. Recent data indicated that only ethylenediaminetetraacetic acid (EDTA) inhibits leukocyte MPO leakage and therefore should be used as the preferred anticoagulant[106]. In contrast to the current laboratory recommendations, previous studies have utilized serum (EPIC-Norfolk, CAPTURE) instead of EDTA plasma and have included patients treated with heparin, which is known to significantly increase the release of MPO from leukocytes and contributes to its higher concentration in circulation[105,106].

Because current findings did not provide evidence as to the direct causality of MPO in the risk of adverse clinical outcomes, routine measurement of this biomarker is not recommended in any clinical settings.

GDF-15

Background

Cytokines are the propagators of inflammation and many of them might be valuable biomarkers, adding to what is known about the pathophysiological processes underlying CAD. GDF-15, also known as macrophage inhibitory cytokine-1 (MIC-1), is a pleiotropic cytokine which may play opposing roles in various pathological processes including inflammation, atherosclerosis, stress responses, cardiovascular diseases, tissue injury and repair, obesity and its complications, chronic kidney disease, pulmonary embolism and cancer[110–112]. GDF-15 is a divergent member of the transforming growth factor-β superfamily expressed in most mammalian tissues. Enhanced expression of GDF-15 was found in the myocardium and vascular cell types in response to oxidative stress and

inflammation[113]. GDF-15 was shown to be a lesion-induced factor implicated in several different pathological pathways[111].

Evidence supporting the role of GDF-15 in cardiovascular risk assessment

The expression of GDF-15 was upregulated after the induction of apoptosis, stimulation by oxidatively-modified LDL and proinflammatory cytokines in cultured human macrophages, and by triglyceride-rich lipoproteins in smooth muscle cells of human coronary arteries[111,114]. Bonaterra et al. suggested a proatherogenic function of GDF-15 showing that in mice it plays an important role in the progression of atherosclerotic lesions, thus regulating cell apoptosis and IL-6-dependent inflammatory response to vascular injury[111]. Others recently reported that GDF-15 exerted an anti-inflammatory response after MI in a mouse model or that it was associated with a decreased size of atherosclerotic lesions suggesting a protective effect on the disease process[115,116]. In elderly subjects plasma concentrations of GDF-15 correlated weakly with a number of biomarkers of endothelial activation and vascular inflammation, and reflected different biological pathways involved in the development and progression of atherosclerosis[117]. Moreover, increasing GDF-15 concentrations in the plasma were independently associated with subclinical coronary atherosclerosis in a low-risk young multiethnic population[24]. Thus, it seems that GDF-15, depending on the circumstances, may have a protective or harmful effect on atherosclerosis development but its role still requires further explanation.

Circulating GDF-15 concentration predicts all-cause mortality in the general population, suggesting a fundamental role in the biological processes associated with aging. The association of GDF-15 with cardiovascular diseases such as ACS, stable CAD and HF makes it a novel promising biomarker for risk assessment, independent of other established risk factors and biomarkers[118].

GDF-15 was suggested to predict a disease course and mortality in cardiovascular disease (CVD), end-stage renal disease and pulmonary diseases. Wiklund et al. followed two large cohorts of male and female subjects, aged 35–80 years, for several years to determine all-cause mortality[119]. This study demonstrated that GDF-15 concentration is an important independent predictor of raised all-cause mortality risk. Moreover, it also emphasized the more prominent role of environmental rather than genetic factors on GDF-15 levels in relation to mortality risk, which was later confirmed by Ho et al.[118,119]. The prognostic power of a single GDF-15 value and its change over time for the future cardiovascular, non-cardiovascular and all-cause mortality was recently shown in elderly Caucasian subjects, aged 70 years, participating in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study[23].

Risk prediction in CAD remains a difficult task, however the multimarker strategy with the use of biomarkers representing distinct pathophysiological processes seems promising. Schnabel et al. investigated twelve different biomarkers in relation to cardiovascular events over time in patients with stable angina[120]. They found GDF-15 to be the second, -strongest predictor (after NT-pro-BNP) of cardiovascular outcome in stable angina and reported its power for the reclassification of patients.

GDF-15 was also associated with the prediction of acute and six-month risk in patients with NSTE-ACS or in patients with acute chest pain, but at low risk of future cardiac events[121,122]. In the first study, GDF-15 was shown to be related to cardiovascular risk factors and biochemical markers of hemodynamic stress, renal dysfunction, and inflammation, and was independently associated with a five-year risk of death and/or recurrent MI[121]. Serial measurements of this biomarker allowed better discrimination of patients according to their level of risk. In spite of several limitations listed by the authors, this study demonstrates the prognostic ability of GDF-15 in all stages of CAD.

In the second study, 14 novel biomarkers of cardiac damage, inflammation, plaque rupture, and neurohormonal activation were assayed in patients with ACS, at the presentation of which only three, including GDF-15, were independently associated with future death or MI[122]. In this group of patients GDF-15 was the best predictor of death/MI in the long-term follow-up characterized by good diagnostic utility (area under the receiver operating characteristic curve [AUC] 0.78) if compared to hs-cTnT and midregional pro-adrenomedullin (AUC 0.73 and 0.71, respectively)[122]. The results of such studies should promote further research on the utility of GDF-15 in the clinical management of CAD.

Very recently-published data demonstrate the incremental prognostic information on GDF-15 in NSTE-ACS patients when used in combination with the GRACE risk score and hs-cTnT[123]. In this report GDF-15 showed the best performance out of different biomarkers under study such as: copeptin, CRP, cystatin C, fibroblast growth factor 23, galectin-3, NT-proBNP and sST2 (soluble suppression of tumorigenicity 2). GDF-15 was most strongly associated with death or nonfatal MI at six months. Moreover, it showed better discrimination of outcome events than GRACE score alone (AUC 0.771 versus 0.749; $p < 0.001$) and when added to GRACE score (AUC 0.788; $p < 0.001$) or GRACE plus hs-cTnT (AUC 0.791; $p < 0.001$), it significantly improved the prognostic information. Out of eight biomarkers studied only GDF-15 and NT-proBNP added incremental value beyond the GRACE risk score and hs-cTnT, however the improvement was greater for GDF-15. This study clearly shows the potential of GDF-15 for reclassification of NSTE-ACS patients, which may lead to changes in the decision-making process.

The contribution of GDF-15 to the development of atherosclerosis requires further explanation, whereas the association between increased GDF-15 and mortality risk across the spectrum of cardiovascular diseases seems to be undisputable. Nevertheless, before the implementation of GDF-15 as a routine clinically useful biomarker takes place, several important issues have to be addressed. Whether the improvement in the discrimination and reclassification of patients using GDF-15 alone or in combination with other biomarkers will affect treatment strategies or patient monitoring still remains to be established[24]. It is also important to assess whether its clinical utility is age-dependent and whether its concentration will help to identify individuals at-risk in the general population years before the onset of

clinically overt disease. Moreover, it has to be clarified whether GDF-15 is a risk marker or a causative factor. Finally, it is of great importance to make sure that GDF-15, as an analyte of low biological variation, would be a useful monitoring parameter to guide treatment decisions in high-risk patients[124]. A better understanding of the pathophysiology and potential utility of GDF-15 requires continued evaluation.

Analytical concerns and recommendations

Wollert et al. previously reported two cut-offs for GDF-15. The value of 1200 ng/L was considered an optimal cut-off for presumably healthy individuals, whereas the value of 1800 ng/L was considered optimal in patients with NSTE-ACS and for the purposes of risk stratification in ACS patients[125]. Although the majority of clinical studies on GDF-15 were conducted in a selected population of patients, GDF-15 has shown a tendency to vary significantly by gender, ethnicity, obesity, renal function and hormonal status[126,127]. Moreover, the impact of diurnal variation has not been examined yet, but high inter-individual variation has been demonstrated[126]. In addition, the *in vitro* stability of GDF-15 is high[127].

The current guidelines do not recommend measurements of this biomarker, nevertheless promising results of clinical trials may suggest that GDF-15 is a potential tool for risk-stratification and therapeutic decision-making.

Conclusions

Although according to the traditional approach all discussed inflammatory markers were shown to be associated with the risk of future cardiovascular events in individuals with and without CAD, their clear clinical utility remains not fully elucidated. Current recommendations of numerous scientific societies predominantly advocate routine assessment of CRP in healthy people with intermediate cardiovascular risk. However, these recommendations substantially vary in their strength among particular societies. These discrepancies have a multifactorial background, including: (i) the strong prognostic value of CRP supported by solid scientific evidence and proven to be comparable in magnitude with that of total and HDL cholesterol, or hypertension, (ii) favourable analytical characteristics of commercially available CRP assays, (iii) lack of CRP specificity and causal relationship between CRP concentration and cardiovascular risk, and (iv) CRP dependence on other classical risk factors. Of major importance is that CRP measurement in healthy men $\geq$50 years of age or healthy women $\geq$60 years of age with low-density lipoprotein cholesterol $<$130 mg/dL may be helpful in the selection of patients for statin therapy. Additionally, evaluation of CRP and fibrinogen or Lp-PLA2 may be considered to facilitate risk stratification in ACS patients and in healthy individuals with intermediate cardiovascular risk, respectively. Nevertheless, the clinical utility of CRP requires further investigation in a broad spectrum of CAD patients, while other promising inflammatory markers, particularly GDF-15 and Lp-PLA2, should be tested in individuals both with and without established CAD. Further studies should also focus on novel performance metrics such as measures of discrimination, calibration and reclassification, in order to better address the clinical utility of investigated biomarkers and to avoid misleadingly optimistic results. It also has to be emphasized that due to the multifactorial pathogenesis of CAD, detailed risk stratification remains a complex process also involving, beyond assessment of inflammatory biomarkers, the patient's clinical characteristics, results of imaging examinations, electrocardiographic findings and other laboratory parameters (e.g. lipid profile, renal function assessed by estimation of glomerular filtration rate or cystatin concentration, markers of left ventricular overload and/or fibrosis, including brain natriuretic peptide [BNP] or galectin-3, and biomarkers of myocardial necrosis, preferably cTnI or TnT).

Declaration of interest

The authors declare that they have no conflicts of interest.

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