

**AN UPDATE ON THE CLINICAL USEFULNESS OF CRP, TNF- $\alpha$ , IL-6, NT-pro BNP, HOMOCYSTEINE AND LIPOPROTEIN (a) IN THE DIAGNOSTIC AND PROGNOSTIC EVALUATION OF CARDIO VASCULAR DISEASES****Dr. Swaminathan S.<sup>\*1</sup>, Rajeswari S.<sup>2</sup> and Dr. Wasim Mohideen<sup>3</sup>**<sup>1</sup>Director of Laboratory Services & Consultant biochemist, Techmed Health Centre & Diagnostic Pvt Ltd, No: 01, Siva Building, Krishna Street, off North Usman Road, T. Nagar, Chennai 600 017.<sup>2</sup>Senior Specialist Biomedical Scientist, Blood Sciences Department, Frimley Park Hospital NHS Foundation Trust, Camberley, Surrey, United Kingdom GU 16 7UJ.<sup>3</sup>Director of Preventive & Wellness Medicine, Techmed Health Centre & Diagnostic Pvt Ltd, No: 01, Siva Building, Krishna Street, off North Usman Road, T. Nagar, Chennai 600 017.**\*Corresponding Author: Dr. Swaminathan S.**

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**ABSTRACT**

In the evaluation and treatment of cardiovascular diseases, the routine measurement of lipid profile is being used for many years and even today is the first line of laboratory test used in CVD screening. However, extensive research in this field has shown that 50% of patients who died due to cardiac arrest have normal lipid profile levels at the time of death. Hence, there may be many hidden, genetically linked inflammatory markers which may have caused such deaths and there is need to diagnose all cardiac dysfunction related patients by measuring markers like CRP, TNF- $\alpha$ , IL-6, BNP and Homocysteine and Lipoprotein (a) as studies have shown increased levels of these in many cardiac dysfunctional patients. This review article is an attempt to bring out the research findings during the last two decades on the clinical usefulness of the above markers and to recommend the measurements of these along with the traditional lipid profile so as to modify the treatment modalities and to evaluate the prognosis. Among the 6 markers reviewed in this article, while standardized methods with reference values are available for the first 5 parameters, methodological variations still exist for Lipoprotein (a) measurement and hence its clinical usefulness could be realised only after an acceptable method is established. The aim of this review article is to discuss the merits and better clinical usefulness of these additional biomarkers which may facilitate fast and accurate diagnosis of CVD, heart failure and disorders associated with them.

**KEYWORDS:** CVD, CRP, IL-6, BNP, HCY, TNF- $\alpha$ .**INTRODUCTION**

Present review aims to discuss new emerging biomarkers that could facilitate more authentic and fast diagnosis of CVDs, Heart Failure (HF), and various lipid abnormalities and disorders.

Along with traditional biomarkers such as lipid profile, glucose, and hormone levels and physiological biomarkers based on measurement of levels of important biomolecules such as serum ferritin, triglyceride to High Density Lipoproteins (HDLp) ratio, lipophorin-cholesterol ratio, lipid-lipophorin ratio, Low Density Lipoprotein Cholesterol (LDL-c) cholesterol level, HDLp and apolipoprotein levels, lipophorin and LTPs ratio, sphingolipids, Omega-3 Index, and ST2 level, immunohistochemical, oxidative stress (OT), inflammatory, anatomical, imaging, genetic, and therapeutic biomarkers have been explained in detail

with their investigational specifications. Many of these biomarkers, alone or in combination, can play an important role in prediction of risks, its types, and status of morbidity.<sup>[1]</sup>

Traditional risk factors—including age, gender, hypertension, dyslipidaemia, smoking, and diabetes—form the foundation for all cardiovascular risk prediction models. Yet, there is substantial interest in identifying novel cardiovascular risk factors, to improve our understanding of disease biology and to account for the cases of heart disease that can not be explained by known risk factors. Novel 'risk factors' cited in the cardiovascular literature more often than not involve biochemical markers measurable in the plasma or serum, including CRP, lipoprotein (a), homocysteine, and others. In recent years, a spirited debate has arisen regarding the validity and usefulness of these new measures, as evidenced by the growing number of

epidemiological studies and reviews addressing this question. CRP and homocysteine—although some have argued that they are causal risk factors, the biological evidence is not conclusive, and no clinical trial data yet exist to suggest that they are bonafide targets for therapy. Risk factors that constitute viable targets for therapy, such as LDL-c, warrant routine clinical measurement, as long as measurement of the risk factor is practical and cost-effective. CRP substantially improve the correspondence of predicted and actual risks.<sup>[2]</sup>

Risk markers related to atherosclerosis, thrombosis, inflammation, cardiac injury, and fibrosis are introduced in the context of their pathophysiology. Rapidly developing new areas, such as assessment of micro-RNA, are also explored.<sup>[3]</sup> Models have been developed that allow physicians to stratify the asymptomatic population in Sub groups at low, moderate, high, and very high total CVD risk. Detection of subclinical vascular damage may improve total CVD risk estimation in asymptomatic subjects who are close to a threshold that could affect management decisions and in whom the chances of re-classification in a different risk category are great.<sup>[4]</sup> Measurements of the presence of preclinical atherosclerotic CVD are essential to enhance early detection. The traditional approach to reduction of risk for CVD events has been to screen the healthy population for “risk factors”. It also meant for aggressive intervention in those individuals, who have suffered from a cardiovascular event with therapy aimed at secondary prevention.<sup>[5]</sup>

Previous studies have shown an association between serum CRP and CVD risk. The roles of interleukin-6 (IL-6) and Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) are less well established. Both CRP and TNF- $\alpha$  predicted all CVD events and total mortality among men. Among women the findings were non significant. CRP and TNF- $\alpha$  were significant, independent predictors of Coronary Heart Disease (CHD) and CVD events and total mortality among men. These findings provide further support to the important role of inflammation in the pathogenesis of CVD. Coronary calcification was identified in 22.5% participants and 14% had significant ( $\geq 50\%$ ) coronary stenosis. Multiple logistic regression analyses suggested that IL-6 levels were independently associated with the presence of coronary calcification and significant coronary stenosis, while TNF- $\alpha$ , sICAM-1 and hs-CRP levels were not. IL-6 in atherosclerosis may be a marker for significant coronary stenosis in cardiovascularly asymptomatic individuals.<sup>[6]</sup>

### C-Reactive Protein and CVD

CRP is a liver-derived pattern recognition molecule that is increased in inflammatory states. It rapidly increases within hours after tissue injury, and it is suggested that it is part of the innate immune system and contributes to host defense. Since CVD is at least in part an inflammatory process, CRP has been investigated in the context of arteriosclerosis and subsequent vascular

disorders. Based on multiple epidemiological and intervention studies, minor CRP elevation followed by High-Sensitivity CRP (hs-CRP) has been shown to be associated with future major cardiovascular risk. It is recommended by the American Heart Association that patients at intermediate or high risk of CHD may benefit from measurement of hs-CRP with regard to their individual risk prediction. Elevation of hs-CRP is associated with increased risk of Type 2 Diabetes Mellitus (T2DM) development in patients with all levels of metabolic syndrome (MetS). In T1 and T2DM, hemoglobinA1c significantly correlates with hs-CRP levels and future cardiovascular risk. Also, hs-CRP levels increase with the stage of beta-cell dysfunction and insulin resistance (IR).<sup>[7]</sup> Of the wide array of inflammatory biomarkers that have been studied, hs-CRP has received the most attention for its use in screening and risk reclassification and as a predictor of clinical response to statin therapy. Although CRP is involved in the immunologic process that triggers vascular remodeling and plaque deposition and is associated with increased CVD, definitive randomized evidence for its role as a causative factor in atherothrombosis is lacking.<sup>[8]</sup>

Despite substantial differences in ethnicities, habits, cultures, the prevalence of traditional cardiovascular risk factors and affordable therapies, atherosclerosis remains the major cause of death in developing and developed countries. However, irrespective of these differences, inflammation is currently recognized as the common pathway for the major complications of atherosclerosis, stroke, and Ischemic Heart Disease (IHD). The complications associated with vulnerable atherosclerotic plaques are triggered by the major mechanisms of dyslipidemia and inflammation; whereas both mechanisms are influenced by classic risk factors and hs-CRP contributes additional information regarding cardiovascular events and mortality.<sup>[9]</sup> The role of low grade systemic inflammation as evidenced by elevated hs-CRP levels in the pathogenesis of atherosclerotic vascular disease has been intensely investigated through observational studies and clinical trials in the past two decades. Most Indian studies had small sample sizes and short term follow ups. There were no large population based prospective studies where patients were followed up for long periods of time for major cardiovascular end points.<sup>[10]</sup>

CRP may help predict short- and long-term cardiovascular outcomes and may have a role in CVD screening analogous to that of lipid. In the future, CRP may modify treatment and preventive therapies.<sup>[11]</sup> hs-CRP predicts CHD events in subjects with T2DM. In a large cohort of T2DM patients, hs-CRP was found to be an independent risk factor for CHD deaths.<sup>[12]</sup> CVD is the major cause of premature death worldwide. CRP is one possible marker of vascular inflammation and plays a direct role in promoting vascular inflammation, vessel damage and clinical CVD events. The CRP level over

10mg/L is correlated with 4% risk of developing, a fatal CVD in 10 years. The acute phase reactant, CRP, a simple down stream marker of inflammation, has now emerged as a major cardiovascular risk factor.<sup>[13]</sup>

At all levels of LDL-c, MetS and at all levels of Framingham risk, CRP provides additional information on vascular risk. CRP is one of the most consistent risk stratifiers that we have. But it is important to think beyond CRP as a simple marker for high risk of disease, as it predicts something about the underlying biology.<sup>[14]</sup> The level of systemic inflammation as measured by circulating levels of CRP has been linked to prognosis in patients with atherosclerotic disease, congestive heart failure, atrial fibrillation, myocarditis, aortic valve disease and heart transplantationin disease initiation, progression, and clinical manifestations.<sup>[15]</sup>

Modestly elevated baseline concentrations of CRP, are associated with the long-term risk of CHD in general populations, whilst the major acute phase response of CRP following myocardial infarction (MCI) is associated with death and cardiac complications. The pathogenic and clinical significance of these associations is controversial. CRP concentrations in individuals with substantial tissue damage and modest but persistent increases in baseline values in generally healthy subjects.<sup>[16]</sup> CRP may be used as a tool for further risk stratification of intermediate-risk patients in order to select candidates who may benefit the most from additional interventions and therapies. The use of hs-CRP for cardiovascular risk stratification remains highly controversial. CRP is, at best, a weak independent risk factor for clinical cardiovascular events. Without the inclusion of patients with CRP < 2, it cannot be concluded that the CRP level of >2 conferred any increased risk, nor does it identify patients who would have received additional benefit with statin therapy. This has led many to question whether patients with CRP < 2 would have received similar benefits from statin therapy if they had been included in the trial.<sup>[17]</sup>

CRP and N-terminal pro-brain natriuretic peptide (NT-proBNP) provide prognostic information in patients with stable CHD. NT-proBNP-CRP was the strongest independent correlate of mortality hazard ratio (HR) for high NT-proBNP-high CRP vs low NT-proBNP-low CRP). Combined use of NT-proBNP and CRP improves long-term risk prediction of mortality in patients with stable CHD.<sup>[18]</sup> Elevated hs-CRP increases the risk of CVD in the general population, but its role as a predictive marker in HIV-positive patients remains unclear. Higher IL-6 and P-selectin levels were also independently associated with increased CVD risk, although the association was weaker than for hs-CRP. Higher total cholesterol and lower HDL-c cholesterol increased CVD risk, independent of hs-CRP and hs-CRP may be a useful additional biomarker to predict CVD risk in HIV-infected patients receiving Anti Retroviral Therapy (cART).<sup>[19]</sup>

Serum *hs*-CRP was related to the combined end point “death and/or any cardiovascular event” during a median 24-month follow-up period. Serum *hs*-CRP was related to the severity of Peripheral Arterial Diseases (PAD), showing a relation to future hemodynamic function and cardiovascular events in PAD patients. In addition to coronary plaques, aneurysmal aortas, and failed venous coronary bypasses, femoral plaques also produce CRP, thus illustrating that the production of CRP may represent a universal response to vascular injury and may contribute to plaque development.<sup>[20]</sup> *hs*-CRP and hypoalbuminemia are associated with increased risk of mortality in patients with kidney failure (CHD). There are limited data evaluating the relationships between CRP, albumin, and outcomes in chronic kidney disease (CKD) stages 3 and 4. Both high CRP and low albumin, measured in CKD stages 3 and 4, are independent risk factors for all-cause mortality. High CRP, but not serum albumin, is a risk factor for cardiovascular mortality. These results suggest that high CRP and hypoalbuminemia provide prognostic information independent of each other in CKD.<sup>[21]</sup>

CRP is an acute phase reactant largely produced by the liver in response to inflammatory cytokines such as interleukin-6 that has been viewed as an inactive downstream marker of low-grade vascular inflammation. Several other more sophisticated measures of cytokine activation, cellular adhesion, and immune and enzyme function have also been found to predict risk of future cardiovascular events, including IL-6 and IL-18, TNF- $\alpha$ , soluble intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), lipoprotein-associated phospholipase A<sub>2</sub>, and soluble CD40 ligand. Nonetheless, the overall weight of evidence favors CRP as the best currently available risk predictor, a finding supported by head-to-head comparisons of various inflammatory markers.<sup>[22]</sup> *hs*-CRP was strongly positively associated with all definitions of radiographic OA, but this association was not independent of Body Mass Index (BMI). The pathogenic significance of *hs*-CRP elevations in this subgroup is unclear. Serum *hs*-CRP for predicting risk of CVD is confounded by obesity, ethnicity, gender and comorbidities.<sup>[23]</sup>

Although CRP is associated with adverse cardiovascular events, unlike BNP, multiple stimuli increase production of CRP. Therefore, elevation in CRP is not specific to CVD. Partition values for CRP and cardiovascular risk based on epidemiological studies predict risk for populations but may not always be useful when used alone to predict individual risk or to direct therapy. Given the non-specific stimuli which affect circulating concentrations of CRP, using CRP to monitor treatment to reduce cardiovascular risk may provide little benefit without understanding or targeting the underlying causes for its elevation.<sup>[24]</sup>

### IL-6 and CVD

IL-6 has been emphasized by reports of elevated circulating as well as intracardiac IL-6 levels in patients with congestive heart failure (CHF). Myocardial IL-6 concentrations are also significantly higher in Left ventricular assist device (LVAD) candidates compared with advanced heart failure patients. Although the IL-6 family plays a central role in the pathophysiology of CVD, it remains to be determined whether the IL-6 family is beneficial or detrimental. Future study will be needed to resolve this question.<sup>[25]</sup> The presence of CVD, however, strongly affected the risk of mortality associated with high IL-6. Among women with prevalent CVD, those with high IL-6 levels had >4-fold risk of death compared with women in the lowest tertile, whereas the relative risk associated with high IL-6 among those without CVD was much lower and not significant. Adjustment for all chronic diseases and disease severity measures, including ankle-brachial index, forced expiratory volume, and exercise tolerance, did not change the results. IL-6 level is helpful in identifying a subgroup of older CVD patients with high risk of death over a period of 3 years. Systemic inflammation, as measured by IL-6, may be related to the clinical evolution of older patients with CVD.<sup>[26]</sup>

Biomarkers such as IL-6, soluble interleukin-6 receptor (sIL-6R), and hs-CRP have been reported to be elevated in AMI. After 24h post-AMI, hs-CRP levels were increased compared to stable CAD and were preceded by increased IL-6 at presentation. IL-6 and sIL-6R are associated with AMI and cardiac injury. These data support the hypothesis that trans-IL-6 receptor binding may alter intracellular signaling, and blocking of IL-6 receptor binding may be pathogenic in AMI. These data may be predictive of mechanism(s) by which plaques become unstable and rupture.<sup>[27]</sup> Plasma IL-6 concentrations are related to decreasing functional status of the patient and provide important prognostic information, strongly suggest that IL-6 and IL-6 related cytokines are intricately involved in the pathophysiology of the failing heart.<sup>[28]</sup> The basic principles of IL-6 signalling and its roles in diabetes and associated cardiovascular complications, with emphasis on the different outcomes mediated by the two modes of IL-6 signalling and the value of developing therapeutic strategies to specifically target the deleterious trans-signalling of IL-6 are to be established by further studies.<sup>[29]</sup>

An increased serum IL-6 level is associated with a risk of CVD. Elevated IL-6 levels are also involved in the pathogenesis of IR and can predict the development of T2 DM. Kaplan–Meier curves revealed that patients with a high IL-6 concentration had a higher incidence of cardiovascular events than those with non-high IL-6 concentration. These observations results suggest that measurement of serum IL-6 concentration is a useful tool to identify high-risk patients for cardiovascular events in T2DM.<sup>[30]</sup> Although a large number of pro- and anti-

inflammatory cytokines are of importance, available data suggest that the anti-inflammatory cytokine interleukin IL-10 and the mainly proinflammatory cytokines IL-6 and TNF- $\alpha$  may play important roles in the development of helpercell imbalance, CVD and wasting in the uremic milieu. Given the strong association between proinflammatory cytokines and complications common in End Stage Renal Disease (ESRD), such as vascular calcification and wasting, the potential role of both general and targeted anticytokine treatment strategies in ESRD patients needs further evaluation.<sup>[31]</sup>

An increased IL-6 level are 25.0 times more likely to have IHD than those without increased IL-6 levels. Furthermore, it was found that for every 1 pg/mL increase in IL-6, the chances of developing the disease increases by 1.24 times. From these findings, it can be inferred that IL-6 can be used as a biomarker of IHD.<sup>[32]</sup>

### NT-ProBNP and CVD

Heart ventricles produce BNP in response to increased mechanical load and wall stretch. In Acute coronary syndrome (ACS) concentrations are strong predictors of recurring MCI, HF, and death. In acute dyspnea, high BNP and NT-proBNP point to a cardiac rather than a pulmonary origin of the symptoms. BNP and NT-proBNP help in the assessment of the severity of ventricular dysfunction and HF and as a prognostic predictor, regardless of the primary cause of the condition. They can be used to guide the therapy of HF and left ventricular dysfunction. BNP and NT-proBNP work better when they are used for specific clinical purposes, rather than for screening in the general population. Their main strength is the excellent negative predictive value with regard to left ventricular dysfunction and heart failure. BNP and NT-proBNP are nonspecific biomarkers of cardiac dysfunction. Specific diagnostic tools, such as echocardiography, are required to define the actual abnormality.<sup>[33]</sup>

Many pregnant women with heart disease have increased BNP levels during pregnancy. Incorporating serial BNP levels into clinical practice can be helpful, specifically in adjudicating suspected adverse cardiac events during pregnancy.<sup>[34]</sup> BNP levels in children with CHD undergoing cardiac catheterization. BNP levels ranged from < 5 to > 1300 pg/mL, with a median BNP concentration of 19.0 pg/mL. BNP concentration may prove to be a useful clinical tool in managing children and adults with CHD.<sup>[35]</sup> Perioperative BNP correlates to severity of illness and lengths of therapy in the CHD population, overall. Substantial variation in BNP across time as well as within and between CHD lesions limits its practical utility as an isolated point-of-care measure. BNP commonly peaks 6-12 Hours post operatively, but the timing and magnitude of BNP elevation demonstrates notable age-dependency, peaking earlier and rising an order of magnitude higher in neonates. In spite of higher clinical acuity, non-neonatal univentricular CHD

paradoxically demonstrates lower BNP levels compared with biventricular physiologies.<sup>[36]</sup>

The relationship between BNP and both left ventricular ejection fraction and left-sided filling pressures is weak, and data on the prognostic impact of high BNP levels in patients with sepsis are conflicting. Highlights the potential benefits of BNP in the recognition and management of HF, and defines the gray zones of BNP levels; it also identifies conditions influencing BNP levels in relation to a certain HF and describes conditions of no cardiac origin with increased BNP.<sup>[37]</sup> BNP and NT-pro-BNP are frequently used in the diagnosis of CHF and distinguishing between patients with dyspnoea of cardiac or pulmonary origin. Values of NT-pro-BNP are affected by age or the presence of one or several comorbidities such as CRF, T2DM, and ACS. 'Normal' values of these peptides also vary depending on the type of test used. The performance characteristics of these tests vary depending on the patients on whom they are used and the manufacturer. For this reason, the determination of reference values for this peptide represents such a challenge.<sup>[38]</sup>

Interest in BNP and NT-pro BNP in the management of children with CHD has increased. There are, however, no current guidelines for their routine use. Prospective, randomised clinical trials designed to evaluate the clinical utility and cost-effectiveness of routine BNP/ NT pro BNP use in CHD are lacking. The results of well-designed, prospective clinical trials should assist in formulating guidelines and expert consensus recommendations for its use in patients with CHD. Finally, the use of new point-of-care testing methods that use less invasive sampling techniques - capillary blood specimens - may contribute to a more widespread use of the BNP assay, especially in neonates and infants, as well as contribute to the development of screening programmes for CHD using this biomarker.<sup>[39]</sup> The plasma concentration of BNP is raised in various pathological states, especially HF. Many studies suggest that measurement of plasma BNP has clinical utility for excluding a diagnosis of HF in patients with dyspnea or fluid retention and for providing prognostic information in those with HF or other cardiac disease. It may also be of value in identifying patients after MCI in whom further assessment of cardiac function is likely to be worthwhile. Preliminary evidence suggests that measuring the plasma concentration of BNP may be useful in fine tuning therapy for HF. Artificially raising the circulating levels of BNP shows considerable promise as a treatment for HF. With simpler assay methods now available, it is likely that many physicians will measure plasma BNP to aid them in the diagnosis, risk stratification, and monitoring of their patients with HF or other cardiac dysfunction.<sup>[40]</sup>

In patients with suspected HF., BNP testing decreased the need for hospitalization, shortened length of hospitalization, and reduced costs. Outside of the

emergency department, it is less clear that BNP measurement improves clinical outcomes in patients with HF. Although an elevated BNP is associated with adverse outcomes including mortality and hospital readmission, what the physician should do in response to an elevated BNP, and whether such an intervention would improve outcomes, is uncertain.<sup>[41]</sup>

### **TNF- $\alpha$ and CVD**

Increased levels of TNF- $\alpha$  or of its soluble receptors have been implicated in the pathophysiology of ischaemia-reperfusion injury, myocarditis, cardiac allograft and, more recently, also in the progression of CHF. The hypothesis that TNF- $\alpha$  may be involved in the progression of CHF may be of clinical relevance as anti-TNF strategies are considered for therapeutical strategies.<sup>[42]</sup> The functional role of TNF- $\alpha$  in the heart has been extensively studied over the last 15 years. Collectively, these studies have demonstrated that TNF- $\alpha$  has both diverse and potentially conflicting roles in cardiac function and pathology. TNF- $\alpha$  antagonist therapy recently has been demonstrated to be clinically applicable in inflammatory conditions, and clinical trials are currently in progress in the use of these agents in CVD. The scope for clinical applications of anti-TNF- $\alpha$  therapy in CVD is potentially extensive.<sup>[43]</sup>

TNF- $\alpha$  functions within a complex and tightly regulated cytokine network, activating multiple signal transduction pathways and inducing or suppressing a wide variety of genes, including those encoding for other cytokines, adhesion molecules and the inducible nitric oxide synthase. TNF- $\alpha$  has recently been implicated as a transducer of CVD, CAD and CHF.<sup>[44]</sup> Investigators are now evaluating the clinical efficacy of novel anticytokine and anti-TNF strategies in patients with HF; one such strategy is the use of a recombinantly produced chimeric TNF- $\alpha$  soluble receptor.<sup>[45]</sup> The increase in TNF- $\alpha$  expression induces the production of reactive oxygen Species (ROS), resulting in endothelial dysfunction in many pathophysiological conditions. Lipid metabolism, dietary supplements and physical activity affect TNF- $\alpha$  expression. The interaction between TNF- $\alpha$  and stem cells is also important in terms of vascular repair or regeneration. Careful scrutiny of these factors may help elucidate the mechanisms that induce vascular dysfunction. Available evidence shows the role of TNF- $\alpha$  in vascular dysfunction in CVD. These findings may prompt new directions for targeting inflammation in future therapies.<sup>[46]</sup> The increased risk of premature CVD in rheumatoid arthritis (RA) patients may depend on traditional risk factors but may also be attributable to RA-specific risk factors such as disease-related dyslipidemia, or cytokines such as TNF- $\alpha$ . The effects of TNF- $\alpha$  blockers on incident cases of CHF in RA remains controversial. Due to the lack of evidence of a beneficial effect of anti-TNF- $\alpha$  agents in treatment of CHF, they should not be used to treat patients with New York Heart Association (NYHA) class III or IV HF.<sup>[47]</sup>

### Homocysteine and CVD

High homocysteine (HCY) levels are associated with cardiovascular morbidity and mortality. In CKD, HCY levels rise, and cardiovascular risk increases with declining kidney function. While some studies in this population have found an association between elevated HCY and cardiovascular risk, others have noted that this association is largely attenuated by adjustment for kidney function, and several studies of patients with kidney failure have found that lower HCY levels predict mortality. HCY levels can be lowered with folate, vitamin B<sub>6</sub> and vitamin B<sub>12</sub>. Three large, randomized, controlled trials of patients with pre-existing CVD and two smaller, randomized, controlled trials of patients with kidney failure failed to detect any cardiovascular benefit from HCY-lowering vitamins. Several more interventional trials are ongoing, but the available data thus far do not support screening for or treatment of hyperhomocysteinemia.<sup>[48]</sup> Inborn errors of HCY metabolism result in markedly elevated plasma HCY and thromboembolic (mainly venous) disease: there is evidence of endothelial dysfunction with both markedly and mildly elevated concentrations, atherosclerotic patients is also associated with most standard vascular risk factors, and importantly, with early decline in renal function, which is common in atherosclerosis. Decline in renal function alone causes elevated plasma HCY (and cysteine). These observations suggest that mild hyperhomocysteinemia could often be an effect rather than a cause of atherosclerotic disease. There is recent evidence suggesting an acute antioxidant effect of folic acid independent of its effect on HCY concentrations. This antioxidant mechanism may oppose an oxidant effect of HCY and be relevant to treatment of patients with vascular disease, especially those with chronic renal insufficiency. Such patients have moderately elevated plasma HCY and greatly increased cardiovascular risk that is largely unexplained.<sup>[49]</sup>

Plasma HCY is determined by both genetic and nutritional factors. The B-vitamins folate, B-12 and B-6 all play a key role in HCY metabolism and in fact it has been proposed that about two-thirds of all cases of hyperhomocysteinemia are due to an inadequate status of one or all of these vitamins. Of the three, folate appears to be the most important determinant and has been shown to significantly lower HCY concentration, in light of the evidence that folate may play a role in primary prevention of CVD via HCY-lowering the protective effect of fruit and vegetables may be partly explained by folate.<sup>[50]</sup> That HCY was associated with subsequent MCI. However, the association was limited to those above a threshold level of HCY. In the general population serum total HCY is an independent risk factor for CHD with no threshold level.<sup>[51]</sup>

The mean HCY concentration adjusted for significant correlates (serum creatinine, uric acid, and LDL-c) was 12.0  $\mu\text{mol/L}$  in proband cases compared with 10.1

$\mu\text{mol/L}$  in controls. Many (17.6%) of the proband cases had - concentrations exceeding the 95th percentile for the controls. HCY among cases was bimodally distributed even after adjustment for concentrations of plasma vitamins. concordant high HCY was seen in at least 12% of 85 families with two or more affected siblings. Substantial proportion of early familial CAD is probably related to production of high concentrations of HCY by one or more major genes.<sup>[52]</sup> Patients with renal impairment have markedly elevated HCY levels and are at particularly high risk for IHD and stroke, but the relevance of elevated HCY levels in this population is uncertain. Among prospective studies, a 25% lower blood HCY level was associated with an 11% lower risk of IHD and about a 20% lower risk of stroke, after adjustment for known cardiovascular risk factors. Individuals who had the TT genotype for MTHFR compared with those with CC had 25% higher HCY levels and a 16% higher risk of IHD. Among individuals aged over 65 years, a 1% higher serum creatinine level was associated with about a 1% higher HCY concentration. The concordance of the IHD risks obtained in the studies of genetically determined differences in HCY and the population-based studies of HCY suggest that these associations are likely to be causal. Renal function is an important determinant of circulating HCY concentrations. results on randomized trials of folic acid-based vitamins in patients with renal disease are required to clarify the relevance of lowering HCY for vascular disease.<sup>[53]</sup>

### Lipoprotein (a) and CVD

Elevated lipoprotein(a) (Lp[a]) is a causal genetic risk factor for CVD. Although Lp(a) is a major causal risk factor for CHD, no currently available controlled studies have suggested that lowering it through either pharmacotherapy or LDL apheresis specifically and significantly reduces coronary risk. Further research is needed to optimize management in order to reduce CHD risk associated with elevated Lp(a) and to determine what other intermediate- or high-risk groups might benefit from Lp(a) screening.<sup>[54]</sup>

Recent genetic findings, indicates that elevated Lp(a), like elevated LDL-cholesterol, is causally related to premature CVD/CHD. The association is continuous without a threshold or dependence on LDL- or non-HDL-cholesterol levels. Elevated Lp(a) levels may either induce a prothrombotic/anti-fibrinolytic effect as apolipoprotein(a) resembles both plasminogen and plasmin but has no fibrinolytic activity, or may accelerate atherosclerosis because, like LDL, the Lp(a) particle is cholesterol-rich, or both. It is recommended to screen for elevated Lp(a) in those at intermediate or high CVD/CHD risk, a desirable level <50 mg/dL as a function of global cardiovascular risk, and use of niacin for Lp(a) and CVD/CHD risk reduction.<sup>[55]</sup>

Many observations have pointed out that Lp(a) levels may be a risk factor for CVD. Lp(a) inhibits the

activation of transforming growth factor (TGF) and contributes to the growth of arterial atherosclerotic lesions by promoting the proliferation of vascular smooth muscle cells and the migration of smooth muscle cells to endothelial cells. Moreover Lp(a) inhibits plasminogen binding to the surfaces of endothelial cells and decreases the activity of fibrin-dependent tissue-type plasminogen activator. Lp(a) may act as a proinflammatory mediator that augments the lesion formation in atherosclerotic plaques. Elevated serum Lp(a) is an independent predictor of CAD and MCI. Furthermore, Lp(a) levels should be a marker of rest enosis after percutaneous transluminal coronary angioplasty, saphenous vein bypass graft atherosclerosis, and accelerated coronary atherosclerosis of cardiac transplantation. The possibility that Lp(a) may be a risk factor for ischemic stroke has been assessed in several studies. Recent findings suggest that Lp(a)-lowering therapy might be beneficial in patients with high Lp(a) levels. A future therapeutic approach could include apheresis in high-risk patients in order to reduce major coronary events.<sup>[56]</sup>

Circulating concentration of lipoprotein(a) (Lp[a] ), a large glycoprotein attached to a low-density lipoprotein-like particle, may be associated with risk of CHD and stroke. Under a wide range of circumstances, there are continuous, independent, and modest associations of Lp(a) concentration with risk of CHD and stroke that appear exclusive to vascular outcomes.<sup>[57]</sup>

As promising compounds to lower Lipoprotein(a) (Lp(a)) are emerging, the need for a precise characterization and comparability of the Lp(a)-associated cardiovascular risk is increasing. Regional differences exist within the European population. Elevated Lp(a) was robustly associated with an increased risk for MCE and CVD in particular among individuals with diabetes. These results may lead to better identification of target populations who might benefit from future Lp(a)-lowering therapies.<sup>[58]</sup>

## CONCLUSION

This review article has highlighted the merits of measuring CRP, TNF- $\alpha$ , IL-6, BNP and homocysteine and Lipoprotein(a) in the diagnostic and prognostic evaluation of all types of CVD. The levels of all the above markers have been found to be elevated in all types of cardiac related dysfunctions compared to normal populations. All these 6 markers have shown equally promising treatment options and screening with these markers during emergency is also found to be very useful in deciding/modifying the treatment options. Further, these markers have been found to be highly specific and sensitive to MCI. Further studies are required in this field to identify sex specific markers in the given population. All these markers, except Lipoprotein (a) for which an accepted reference method is still not available, may be grouped as emerging CVD markers, which if done at regular intervals could be very useful to bring down cardiac related deaths.

**Conflicts of interest:** None

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