REVIEW ARTICLE

Biomarkers for the Prediction of Mortality and Morbidity in Patients with Renal Replacement Therapy

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SUMMARY

The mortality of end-stage renal disease (ESRD) patients on dialysis remains high despite great improvement of dialysis technologies in the past decades. These patients die due to infectious diseases (mainly sepsis), cardiovascular diseases such as myocardial infarction, heart failure, stroke, and, in particular, sudden cardiac death. End stage renal disease is a complex condition, where the failure of kidney function is accompanied by numerous metabolic changes affecting almost all organ systems of the human body. Many of the biomarker characteristics of the individually affected organ systems have been associated with adverse outcomes. These biomarkers are different in patients with ESRD compared to the general population in the prediction of morbidity and mortality. Biomarker research in this field should aim to identify patients at risk for the different disease entities.

Traditional biomarkers such as CRP, BNP, and troponins as well as new biomarkers such as fetuin, CD154, and relaxin were analyzed in patients on dialysis. We will include observational as well as prospective clinical trials in this review. Furthermore, we will also discuss proteomics biomarker studies. The article assess the potential diagnostic value of different biomarkers in daily clinical practice as well as their usefulness for clinical drug development in end stage renal disease patients.

INTRODUCTION

Patients on dialysis have a substantially higher mortality rate as compared to the general population [1]. In spite of a clear improvement in dialysis technologies during the last years, the death rate among uremic patients treated by hemodialysis (HD) or peritoneal dialysis (PD) remains as high as in certain patients with solid organ cancer.

A well accepted, albeit complex, set of guidelines has been established in the United States and Europe to ensure the quality of dialysis care. However, outcomes remain poor [2,3]. Cardiovascular disease (CVD) is the most common cause of death in this population of patients [4]. Dialysis-related strategies have not been successful in reducing mortality rates. The underlying renal disease, preexisting coronary artery disease, malnutrition, systemic inflammation, and anemia have all been identified as risk factors [5].
There has been increasing interest in the role of biomarkers that can identify development of life-threatening complications of the disease early or that correlate well with progression and mortality. The underlying premise is that early detection of the complications and understanding of disease can then lead to interventions that stop or retard complications and increase survival. Applying new technologies of genomic analysis (e.g., RNA subtraction or DNA microarrays) and proteomic approaches to identify novel proteins that encode for renal injury are on-going.

1. Classical markers of cardiovascular dysfunction

The leading causes of mortality in patients with end-stage renal disease (ESRD) are cardiovascular disorders, such as left ventricular (LV) abnormalities, including LV hypertrophy, systolic dysfunction and congestive heart failure, and sudden cardiac death due to malignant arrhythmias as well as sepsis/infectious diseases. Because of the high cardiovascular death rate in the dialysis population (almost 40 times greater than in the general population), the burden of cardiovascular disease when entering dialysis and its impact on the survival of dialysis patients has recently received increased attention (Collins 2003). Initial studies thus focused on the analysis of morbidity and mortality biomarkers in patients with end-stage renal disease that were initially characterized in the general population.

The factors leading to an increased risk of cardiovascular disease among patients with ESRD are multifaceted and in many cases not yet fully understood. Along with conventional cardiovascular risk factors in patients with uremia, multiple mediators related to the metabolic changes due to the loss of kidney function contribute to this increased risk by causing multiple functional and structural changes in the heart and blood vessels. These factors include increased inflammation, greater sympathetic-nerve activity, oxidative stress, disturbed mineral balance, and profound endothelial dysfunction (Figure 1) (Himmelfarb and Ikizler).

Based on the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines, echocardiography is recommended at the initiation of dialysis in all ESRD patients and every 3 years thereafter (2005). However, echocardiography is not a procedure routinely performed in most dialysis centres. Therefore, circulating biomarkers that identify patients with subclinical heart disease or diseased myocardium may have a potentially important clinical value in allowing early detection, intervention, and possibly ongoing surveillance of high-risk patients (Wang 2009).

1.1 BNP, pro-BNP

BNP is a hormone that is secreted predominantly by the ventricles and reaches very high plasma concentrations in subjects with congestive heart failure (HF) or acutely decompensated heart failure (AHF). BNP is synthesized in the heart as a reaction to cardiac wall distension and stretching and neurohormonal activation. The cardiomyocytes synthesize a pre-propeptide (preproBNP 134 amino acids) which is split into a signal peptide and a propeptide (proBNP 108 amino acids). During secretion from the cardiomyocytes, proBNP is split at a ratio of 1:1 into the physiologically active BNP (32 amino acids) which corresponds to the C-terminal fragment and the biologically inactive N-terminal fragment (NTproBNP, 76 amino acids) [6]. BNP levels are well known to correlate with LV filling pressures and are elevated in patients with LV dysfunction [7]. According to the New York Heart Association classification of heart failure, both BNP and NT-pro-BNP levels increase in proportion to the severity of the disease [8]. Instage 5 chronic kidney disease (CKD) patients receiving hemodialysis or peritoneal dialysis, BNP and NT-pro-BNP are almost invariably increased compared to healthy individuals [9]. These elevations were partly attributed to decreased renal clearance, chronic volume overload as well as to the high prevalence of cardiac abnormalities [10]. NT-pro-BNP correlated with residual renal function in both hemodialysis and peritoneal dialysis patients [11]. BNP and NT-pro-BNP were markedly elevated in patients with renal dysfunction and normal LV systolic function, as reported in a study on a non-ESRD population [12]. NT-pro-BNP was also associated with coronary artery disease in non-dialysis CKD patients [13]. Thus, in dialysis patients, residual renal function must be taken into account by interpretation of BNP and NT-pro-BNP levels. In both hemodialysis and peritoneal dialysis patients, BNP and NT-pro-BNP have been shown to be strongly associated with LV hypertrophy and dilation and systolic dysfunction [14].

1.1.1 BNP and NT-pro-BNP levels as prognostic markers of cardiovascular dysfunction and mortality in dialysis patients

Recently, BNP and NT-pro-BNP were recognized as powerful predictors of mortality and cardiovascular death in the dialysis population.

In the first prospective clinical studies in hemodialysis patients, NT-pro-BNP was associated with 2 year all-cause mortality and with 3 year cardiac morbidity and mortality [9]. In the CREED study plasma BNP taken on a non-dialysis day was predictive of overall and cardiovascular death in hemodialysis patients [15]. In a more recent study [16] both pre- and posthemodialysis NT-pro-BNP was shown as predictive of 2-year mortality. These data clearly suggested BNP and NT-pro-BNP as powerful prognostic biomarkers irrerespectively of time point of measurement: before, shortly after or in-between dialysis. There is a study showing correlation of both pre- and post-dialysis BNP and NT-pro-BNP [17]. Because the levels of BNP and NT-pro-BNP are altered by the hemodialysis-procedure, the timing of plasma samplings for BNP or NT-pro-BNP measurement in relation to the dialysis session in hemodialysis patients is very important. Similar to hemodialysis, in peritoneal dialysis patients, NT-pro-BNP was predictive of 3 year all-cause and car-
Biomarkers for the Prediction of Mortality and Morbidity in Patients with Renal Replacement Therapy

Figure 1. Pathobiology of Increased Cardiovascular Risk in End-Stage Renal Disease (ESRD), modified according to [105].

Cardiovascular death, as well as cardiovascular events irrespectively of echocardiographic parameters as it was shown in the post hoc analysis of the Adequacy of Dialysis in Mexico (ADEMEX) [18]. In one of the recent studies it was shown that increased NT-pro-BNP levels strongly correlated with LV systolic dysfunction mortality [19]. Moreover NT-pro-BNP was a marker of mortality also after adjusting for LV mass index and midwall fractional shortening. These data suggest that NT-pro-BNP may be a powerful prognostic marker of cardiovascular dysfunction and mortality.

Serial measurements of NT-pro-BNP as markers of cardiovascular dysfunction and mortality in dialysis patients in a recent prospective cohort study performed in 2,990 hemodialysis patients reported increased NT-pro-BNP levels independently associated with increased risk of mortality at 3 months and 1 year follow-up [20]. Furthermore, in a smaller trial on 585 incident HD patients, repeated measurement of NT-pro-BNP was performed at 3 months. The greatest increase in NT-pro-BNP after 3 months of dialysis correlated with higher risk of mortality of dialysis patients unlike the greatest decrease of NT-pro-BNP. This suggests serial changes in NT-pro-BNP levels as an important prognostic evaluation, independent of baseline NT-pro-BNP level [20]. Interestingly, post hoc analysis of the German Diabetes Dialysis Study in 1,255 type 2 diabetic patients with end stage renal disease on hemodialysis has revealed that increased NT-pro-BNP over a 6-month follow up is a significant predictor of an increased risk of cardiovascular events and mortality. A doubled increase in NT-pro-BNP levels was associated with a 46 % increase in the risk of death independently of baseline NT-pro-BNP and baseline co-morbidity [21].

1.1.2 Perspectives of BNP/NT-pro-BNP as a routine measurement in dialysis patients

Based on data published so far, serial monitoring of NT-pro-BNP levels may be suggested as a useful clinical tool in assessment of treatment efficacy in the dialysis population and deserves further investigation. Lowering plasma NT-pro-BNP levels has been shown as a better predictor of reduction of cardiovascular events and delay time to first cardiovascular event compared with other clinical parameters in patients with chronic heart failure [21]. A BNP-guided treatment has also been reported to reduce the risk of cardiovascular death or duration of hospital stay for heart failure compared to standard clinical care in the general population [22]. Recently, the correlation of changes in the LV mass index with changes in NT-pro-BNP levels over a 6-month and 12-month period was shown in a prospective study of 21 stable hemodialysis patients with preserved systolic function [23]. All these data suggest further investiga-
tion of serial BNP and NT-pro-BNP monitoring as biomarkers of changes in LV mass index or systolic dysfunction in hemodialysis patients. Further prospective evaluation of BNP or NT-pro-BNP guided therapy in monitoring of treatment efficacy and improving clinical outcomes of hemodialysis patients is needed.

1.2 Troponin T
Cardiac troponin T (cTnT) is a low molecular weight (39 kDa) protein component of the thin actin filament of the cardiac muscle, including troponins C and I (cTnI) [24]. Following damage to myocytes, cTnT and cTnI are released into the circulation and their detection has been used as a sensitive marker of myocardial necrosis. The superiority of these markers over more traditional cardiac enzymes (creatine kinase and its muscle brain [MB] isoform) has been confirmed. Serial measurements of cTnT or cTnI have become an important tool for risk stratification in patients presenting with acute coronary syndromes [25].

Cardiac troponin T (cTnT) is a sensitive marker of myocardial injury and is the current gold standard test for diagnosis of acute myocardial necrosis in the general population. However, levels of cTnT are frequently elevated in the absence of acute coronary syndrome among patients with varying degrees of kidney disease [25].

1.2.1 cTnT as prognostic markers of cardiovascular dysfunction and mortality in dialysis patients
A number of studies report on increased troponin concentration as a predictor of mortality in hemodialysis patients. In a 1 year follow-up study [26], a significant difference in survival was shown between patients with >0.1 µg/L or <0.1 µg/L cTnT concentrations. cTnT was higher in patients with diagnosed ischemic heart disease [26]. A number of studies [15,16,27,28] reported higher initial cTnT concentrations during a long-term follow-up study in patients who died compared with survivors and as a predictor of adverse cardiovascular outcome. A continuous relationship was found between increasing cTnT concentration and mortality from all causes and between cTnT concentration and the extent of coronary artery disease [29]. Further reports confirmed a positive association between mortality and cTnT concentrations [30]. In hemodialysis patients cTnT values ≥0.1 µg/L were associated with accelerated asymptomatic atherosclerosis (higher intima-media thickness and more plaques in carotid arteries) [28]. A level of 0.1 µg/L may be usefully applied as a threshold to cardiovascular mortality risk stratification in hemodialysis patients [28].

Studies of serum troponin in peritoneal dialysis patients demonstrated elevated cTnT concentrations in peritoneal dialysis patients are not necessarily associated with acute myocardial infarction [30]. At the same time, initial cTnT concentration was a strong predictor of mortality from all causes [30]. In a recent meta-analysis based on 28 studies including 3,931 patients, cTnT was shown to be a useful risk stratification tool in the ESRD population. The pooled analysis confirmed that an increased cTnT (>0.1 µg/L) can be used in identifying a subgroup of asymptomatic ESRD patients with poor prognosis and a higher risk of cardiac death [39]. Recently, the Food and Drug Administration has approved the use of cTnT as a biomarker for mortality risk stratification in ESRD patients. The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI™) recommended cTnT as a prognosis biomarker in patients with ESRD [31]. More importantly, it was shown that the prognostic value of cTnT was independent of inflammation, residual renal function, LV hypertrophy, and dysfunction, clearly supporting the additional value of measuring cTnT for early identification of high-risk ESRD patients [32]. Recently, cTnT was reported as a useful biomarker in predicting the development of circulatory disorders in patients on chronic peritoneal dialysis [33]. This suggests that cTnT may be used as an important adjunct to echocardiography in identifying peritoneal dialysis patients at risk of circulatory disorders.

1.2.2 Perspectives of cTnT as a routine measurement in dialysis patients
Because cTnT is increased in patients with residual kidney function, there is a necessity to define a baseline level applicable for dialysis patients in order to distinguish subsequent cTnT elevation due to cardiac disorders. Given the increase of cTnT levels post-dialysis, measurement should be obtained before dialysis [34]. Even minimally increased cTnT is suggested to be associated with an increased risk of mortality and cardiovascular death in peritoneal dialysis patients [35], as it is in the general population where a minimally increased cTnT concentration correlates with increased cardiovascular risk [36]. According to the recent National Academy of Clinical Biochemistry (NACB) laboratory medicine practice guidelines [37], a dynamic change in cardiac troponins of ≥20 % after presentation should be used to define acute coronary syndrome in ESRD. In addition, testing for cardiac troponins may be used as a prognostic biomarker for assessment of the risk of mortality in ESRD patients [32].

1.3 Dyslipidemia
Patients with CKD already have an altered lipemic profile in the early stages of the disease accelerating after the begin of dialysis. Up to 50 % higher triglyceride (TG), LDL, and Lp(a) values are reported in PD patients compared with the general population [38]. Similarly, elevated Lp(a) plasma was found in HD patients contributing to the high risk of atherosclerosis in this population. Several studies demonstrated a more atherogenic lipid profile in PD patients compared with HD patients [38]. A comparison of 564 HD and 168 PD patients [39] revealed that the patients on peritoneal dialysis had significantly higher total cholesterol (TC), LDL,
TG, and Lp(a) serum levels. Only HDL levels were similar in both populations.

1.3.1 Dislipidemia as prognostic markers of cardiovascular dysfunction and mortality in dialysis patients
Reports on the link between dyslipidemia and increased mortality in dialysis patients are controversial. A couple of prospective studies in HD patients failed to show association between dyslipidemia and mortality in dialysis patients [40], while a cross-sectional study of 95 diabetic PD patients followed over 9 years found increased apo-AI levels to be independent predictors of overall and CV mortality [41]. Habib et al published a retrospective analysis of 1,053 PD patients from the US Renal Data System (USRDS) [42] where they found correlations between TG levels higher than 200 mg/dL and increased risk of mortality. However, in this study neither LDL nor HDL serum levels were investigated. The possible explanation of this confounding result is the potential role of malnutrition and chronic inflammation, which independently cause lower TC levels and, at the same time, higher mortality rates. Both, low or elevated serum cholesterol levels are causes of increased risk of death. Low cholesterol is known as a surrogate marker for malnutrition, with a U-shaped correlation between cholesterol levels and mortality. Inflammation was considered as a confounding factor in a prospective study on 823 patients at the initiation of dialysis [43].
In this study, patients were classified by the absence of inflammation (C-reactive protein level lower than 10 mg/L or an IL-6 level less than 3.09 pg/mL) and the absence of malnutrition (serum albumin level lower than 36 g/L). There was a correlation between cholesterolemia and CV mortality in this cohort of patients [43]. It suggests dyslipidemia as an independent marker of higher mortality risk.

2. Vascular calcification
Accelerated vascular calcification (VC) is but one of the important mechanisms of cardiovascular disease in dialysis patients. In ESRD, VC is more severe and develops in both the intima and the media of the blood vessels. VC is an active and regulated process mediated by vascular smooth muscle cells (Figure 2), which undergo a phenotypic change to osteoblasts or chondrocytes, which in turn release promoters of VC and apoptosis. VC is markedly upregulated in dialysis patients. This may be explained by the upregulation of such promoters of VC as hyperphosphatemia, hypercalcemia, cholesterol, hyperleptinemia, and downregulation of the inhibitors of VC such as the matrix Gla protein fetuin-A.

2.1 Fetuin-A
Fetuin-A is a 62 kilodalton glycoprotein which belongs to the cystatin superfamily of proteins [44]. In humans, the 349-amino acid protein, secreted from the liver, consists of two chains: a heavy and a light chain joined by a connecting segment and linked by disulfide bonds [45]. The N-terminal of the heavy chain consists of two cystatin domains, D1 and D2. The acidic amino acids in the D1 domain appear to account for fetuin’s ability to inhibit precipitation of calcium and phosphorus [45]. Indeed, fetuin-A accounts for up to one-half of the in vitro capacity of the serum to prevent the precipitation of calcium and phosphorus. It is now recognized that fetuin-A can actively regulate the cell-mediated process of osteogenesis in the vessel wall, inhibit mineralization in a concentration-dependent manner, and enhance the phagocytosis of apoptotic bodies by vascular smooth muscle cells, limiting their ability to nucleate calcium phosphate [46]. Finally, fetuin-A is an antagonist of bone morphogenetic protein-2, the promoter of VC in vascular cells [45].
A number of studies have demonstrated an association between serum fetuin-A levels and all-cause mortality of dialysis patients [47,48]. This association of low fetuin-A levels and mortality was confirmed by clinical trial on 664 HD and 323 PD patients during a median follow-up of 2.8 years. In this study, an increase in serum fetuin-A by 0.1 g per liter corresponded to a 9% lower death risk [49]. The death predictive value of fetuin-A in this study was independent of serum CPR levels. At the same time, multivariate analysis of biomarkers of prediction of mortality in dialysis patients where serum C-reactive was entered, fetuin-A lost its predictable value [48,50]. The last fact suggests further investigation of the role of fetuin-A in dialysis patients to fully elucidate pathomechanisms lowering serum fetuin-A levels in ESRD.

2.2 Fibroblast growth factor 23 (FGF-23)
FGF-23 is a hormone secreted by osteoblasts. It plays a role in the regulation of phosphorus and the metabolism of vitamin D. Depletion of FGF-23 causes hyperphosphatemia, upregulation of 1,25-dihydroxyvitamin D, ectopic calcification, and early death [51,52]. FGF-23 is involved in the physiologic maintaining of normal serum phosphate levels in the settings of variable dietary phosphorus intake [53,54]. In the settings of impaired, reduced nephron mass, normal serum phosphate levels are maintained in part by a reactive increase of FGF-23, which promotes excretion of phosphate via the remaining nephrons and decreases the absorption of dietary phosphorus by inhibiting the synthesis of 1,25-dihydroxyvitamin D [55]. Depletion of FGF-23 with the CKD progressions leads to hyperphosphatemia, ectopic calcification and premature death [56,57]. It was previously reported that increased serum phosphate levels and decreased 1,25-dihydroxyvitamin D levels are associated with increased mortality [58]. In the recent study of Gutiérrez et al., multivariable adjusted analyses showed that an increase in serum phosphate levels higher than 5.5 mg/dl and an increase of FGF-23 was associated with a 20% increase in the mortality risk, suggesting hyperphosphatemia and an in-
Figure 2. Major mechanisms of vascular calcification. Six different mechanisms that have been proposed to regulate the initiation or progression of vascular calcification are illustrated, along with key molecular mediators where known. The extent to which each of these mechanisms plays a role in vascular calcification in various disease states, including hyperphosphatemia and ESRD, is currently unknown. cMGP, gamma carboxylated matrix gla protein; pOPN, phosphorylated osteopontin [106].

crease of FGF-23 as a sensitive biomarker for the assessment of the risk of death [59].

3. Inflammation
Inflammation in CKD patients is a local process which is also reflected systemically. The inflammatory response is mediated by multiple factors such as cytokines, complement, adhesion molecules, acute phase proteins, and white blood cells (WBCs) [60] (see also Figure 3). Recently, the prognostic value of several markers of inflammation, such as C-reactive protein (CRP), interleukin-6 (IL-6), adhesion molecules, tumor necrosis factor-α (TNF-α), fetuin etc. have been evaluated in normal populations as well as in patients with CKD. An independent association between cardiovascular outcomes and different markers of inflammation including CRP, IL-6, and fibrinogen was reported in patients with CKD as well as in the general population [60,61].

3.1 C-reactive protein (CRP)
CRP is considered a classical marker of all-cause and cardiovascular mortality not only in the general population but also in CKD patients [60,61]. Recently, numerous studies have shown an association between minor elevations in CRP and an increased risk of cardiac events or stroke in apparently healthy individuals [62]. Some reports show that even modest increases in baseline CRP values are associated with an increased risk of future atherothrombotic events in patients with unstable stenocardia [63]. Because of great consistency of CRP data in the general population, CRP was suggested as a clinical biomarker for cardiovascular risk prediction [61,62].

The association of CRP with cardiovascular morbidity and mortality and its prognostic significance as an inflammatory marker becomes more pronounced with CKD progression and reaches maximal significance in patients with ESRD [63]. An increase of CRP is associated with a higher death risk independent of predialysis levels [64]. CRP is elevated up to 10-fold in HD patients compared to healthy individuals [65]. There are reports showing a 25 % increase in CRP levels in patients during a single hemodialysis session [64]. At the same time, HD appears to be associated with increased CRP levels on the long term. CRP is a powerful indicator of all-cause and cardiovascular mortality as is evident from a follow-up study over a period of 4 years in hemodialysis patients [66]. In patients on PD, CRP is associated with vascular calcification [67] and higher cardiovascular morbidity [68].
3.2 IL-6
IL-6 is a major mediator of the acute phase response strongly associated with CRP. IL-6 was shown as a key mediator of the inflammatory response and atherosclerosis through various metabolic, endothelial, and coagulant pathways [69]. In 40 % to 50 % of patients with ESRD IL-6 is markedly up-regulated [70]. IL-6 was suggested as a reliable and strong prognosis marker in both renal patients [69,70] and the general population [71]. In HD patients, plasma IL-6 levels are strongly associated with cardiovascular morbidity and hypoalbuminemia and are associated with all-cause mortality [71]. Moreover, it was reported that IL-6 was a stronger predictor of cardiovascular mortality than CRP in hemodialysis patients and may be used as an additional prognosis marker in these patients [72]. In addition, some studies have shown better correlation of IL-6 with atherosclerosis than CRP [73]. Moreover, levels of IL-6 appear to be more constant than those of CRP [72] making IL-6 a better predictive marker than CRP for atherosclerosis and its cardiovascular morbidity in HD patients.

3.3 Adhesion Molecules
Cell surface glycoproteins ICAM-1 and VCAM-1, so called adhesion molecules, are induced by endothelial cells at the sites of inflammation and are responsible in part for the adherence of hematopoietic cells to endothelium. Therefore ICAM-1 and VCAM-1 are thought to participate in the pathogenesis of atherosclerosis. Normal endothelial cells in normal arterial segments express low level of ICAM-1, while VCAM-1 is found only in inflammatory regions predisposed to atherosclerotic lesions. Although the expression of both ICAM-1 and VCAM-1 is upregulated in atherosclerotic lesions, VCAM-1 appears to play a more important role in the development of atherosclerosis [74].

P-selectin is another adhesion molecule and receptor of platelet and endothelial cells that mediates adhesion between vascular cells. It promotes the migration of inflammatory cells into sites of atherosclerotic lesions [75]. Experimental data report that monoclonal antibodies directed against adhesion molecules may ameliorate an inflammatory response in atherosclerotic plaques [76]. Adhesion molecules are upregulated in CKD patients due to impaired clearance and enhanced synthesis. The upregulation of adhesion molecules is considered a con-
sequence of endothelial dysfunction, but its mechanisms still have to be fully elucidated [77,78]. Although ICAM-1 was a strong predictor of survival in predialysis CKD patients [78], serum ICAM-1 and VCAM-1 appeared to be unrelated to all-cause mortality in dialysis patients [77]. In conclusion, the utility and applicability of adhesion molecules as prognosis biomarkers in clinical practice must still be determined.

3.4 Homocysteine
Homocysteine is a sulfur-containing amino acid and a non-traditional prognosis marker for CVD that has recently received much interest in the general population and in patients with CKD [79]. Homocysteine has been described as playing a role in the development of endothelial dysfunction, oxidative stress, and vascular abnormalities via many mechanisms. Mildly elevated plasma total homocysteine (tHcy) in the general population is a significant risk factor of CVD [79]. 80 - 85 % of patients on dialysis have a 4-fold increase in plasma tHcy levels [80]. Plasma tHcy levels were associated with higher CVD risk in patients with CKD. However, lowering of plasma tHcy levels has failed to show a beneficial effect in clinical trials [79]. Moreover, it was demonstrated that low levels of homocysteine are associated with poor outcome in ESRD patients. The last may be explained by the confounding effect of malnutrition/protein-energy wasting in dialysis patients [80]. Associations between homocysteine levels and patient outcomes still must be fully elucidated.

3.5 Comparison of different inflammation-markers
Multivariate modelling was used in a prospective study on a large cohort of chronic dialysis patients to evaluate a prognostic value for all-cause and cardiovascular mortality of CRP, proinflammatory cytokines (IL-1α, IL-6, IL-18, and TNF-α) and adhesion molecules (ICAM-1 and VCAM-1). It was found that independent association of IL-6 and CRP with all-cause and cardiovascular death in ESRD patients and the prognostic value of these molecules was apparently higher than cytokines and adhesion molecules. IL-6 is strongly linked to outcomes and was shown to be the best biomarker of risk stratification in ESRD patients. However, given the expense of IL-6 evaluation, CRP may be considered an alternative to IL-6 in a routine clinical practice. The predictive value of CRP is close to that of IL-6 [73]. Comparative multivariate analysis of a cohort of incident dialysis patients revealed the better quality of IL-6 as a predictor of CVD and mortality compared to other biomarkers (serum albumin, CRP, and fetuin A), suggesting the value of IL-6 measurement in clinical practice [81].

4. Malnutrition
In CKD patients, malnutrition and wasting are common factors [82] that are associated with higher morbidity and mortality [83]. Malnutrition develops in CKD patients before initiation of renal replacement therapy [84], and after starting dialysis it is aggravated due to dialyzer membrane bio-incompatibility and nutrient losses. Abnormal protein and energy metabolism, hormonal disturbances, spontaneous reductions in dietary energy and protein intake contribute to the decline in nutritional status in patients with ESRD [85] despite optimal dialysis regimen and sufficient protein intake [85]. Such comorbidities as chronic inflammation, CVD, and diabetes mellitus may cause anorexia and also contribute to malnutrition in dialysis patients. On the other hand, it was reported that malnutrition per se is associated with inflammation in ESRD [86]. Moreover, malnutrition is associated with a greater risk for cardiovascular death in ESRD patients in both predialysis and dialysis cohorts [87]. Conversely, body mass index (BMI) was not associated with acute coronary syndromes in a large cohort of dialysis patients [88]. Malnutrition and low BMI cause increased oxidative stress [89] which leads to reduced bioavailability of nitric oxide and impaired endothelium-dependent vasodilation [89]. These mechanisms may be involved in the development of CVD in malnourished CKD patients. Strong association of nutritional status with disease outcome suggests better indicators for defining nutritional status for use in the clinical settings must be found.

4.1 Serum albumin
Over the past few years, the evaluation of nutritional status in CKD patients was challenged. The reason was that classical parameters previously used for the assessment of nutritional status such as visceral stores, serum albumin, and prealbumin were found to be independently altered by systemic inflammation. A number of studies have reported an association between a low serum albumin concentration and cardiovascular morbidity and death in ESRD patients on either PD or HD [90]. Moreover, in a clinical trial on 1,411 HD patients, a low albumin level was significantly associated with a higher incidence of cardiovascular disease [90]. However, in studies in which the predictable power of the malnutrition marker serum albumin was compared with inflammation marker levels, multiple-regression analysis revealed that the CRP level has a higher predictive power than serum albumin [90] suggesting that inflammation may be a more powerful prognosis factor for poor outcome. In support of this suggestion, it was recently shown [91] that the risk of mortality in HD patients is strongly associated with the neutrophil count. Both inflammation and dietary protein uptake affect the serum albumin levels, making the assessment of nutritional status in the setting of inflammation even more complex [92]. Changes in serum protein levels and body composition under inflammatory condition with adequate calorie and protein intake are the same as those developed in the settings of malnutrition. It may be explained by the fact that inflammatory cytokines, such as TNF-α and IL-6, are involved in the processes of catabolism and downregulation of albumin synthesis. [70] Serum albumin did not significantly correlate with other nutritional
parameters [93] suggesting a higher importance of non-nutritional factors for determination of serum albumin levels than dietary protein intake in CKD patients. Indeed, a number of factors unrelated to nutritional status such as inflammation, age, co-morbidities CVD and diabetes, have been shown to be strongly associated with serum albumin levels in CKD patients [94]. Furthermore, albuminuria and the dialysis procedure per se cause additional external albumin loss, significantly contributing to hypoalbuminemia in CKD patients. And last but not least, over-hydration, commonly observed in dialysis patients, may also play a role in the lowering of serum albumin levels.

4.2 Prealbumin
Prealbumin, also known as transthyretin, is a thyroxin-binding protein, which is not structurally related to albumin. Among a number of other available indicators, prealbumin is one of the most valuable and sensitive biochemical nutritional markers. In anabolic settings, hepatic protein synthesis switches to the production of acute-phase proteins, which cause changes in serum albumin and transthyretin levels, but transthyretin levels change more rapidly. Therefore, transthyretin is suggested as a better marker of anabolic protein synthesis compared to serum albumin. However, the usefulness of transthyretin as a biomarker in CKD patients is questionable because it is reabsorbed and/or metabolized by the proximal tubule [95], and serum levels of transthyretin are directly correlated with the decline of kidney function [91]. On the other hand, the prognostic value of transthyretin was shown in HD patients independent of the albumin level [96].

4.3 Serum creatinine
Serum creatinine levels reflect muscle mass, protein stores of the body, and dietary protein intake, which makes it another useful marker of nutritional status. Association of serum creatinine concentration and the prediction of outcome has also been shown in CKD patients [97]. However, creatinine levels are highly variable in CKD patients because they are affected by disease-related factors such as residual kidney function, inflammation and dialysis dose as well as disease-unrelated factors such as age, sex, race, [98] which makes its value as a biomarker questionable.

5. Novel Markers
Recently, new tools such as proteomics and epigenetics are used more and more for the identification of novel biomarkers. Relaxin was shown to predict morbidity and mortality in male patients on hemodialysis, whereas this was not the case in female patients on dialysis [99]. These findings were evidenced by observations in relaxin knockout mice, where only male mice develop renal and cardiac fibrosis [100,101]. In this context, it is of note that risk factors predicting mortality and morbidity in patients with end-stage kidney disease are gender-dependent [102]. Soluble CD40 ligand, a factor involved in the destabilization of atherosclerotic plaques, is another novel predictor of mortality due to myocardial infarct and stroke in end-stage kidney disease patients [103].

The proteomics approach allows comprehensive analysis of proteins in different fluids (such as serum, urine, dialysate) enabling identification of unknown molecular changes associated with uremia and novel biomarkers related to newly opened pathways [104]. As an example, capillary electrophoresis (CE)-mass spectrometry (MS) has been used in several studies to identify specific uremic retention molecules [104]. Of roughly 1400 polypeptides, more than 30 showed significant changes in dialysis patients as compared to controls with normal renal function. The great value of proteomics as a tool for the identification of new biomarkers is currently challenged due to difficulties in the validation of proteomic profiling and standards for quality control [104]. Another challenge in identification of novel biomarkers of morbidity and mortality is the time needed for follow-up patients. An effective way in which proteomics can be integrated in everyday clinical practice along with classical laboratory markers still needs to be established.

CONCLUSIONS

CKD patients are at high risk for developing cardiovascular complications. Classical cardiovascular risk factors applicable to the general population are not completely applicable to patients with CKD, suggesting that non-conventional risk factors caused by the uremic condition may be particularly important in this population. Markers of cardiovascular dysfunction such as BNP, NT-pro-BNP, and Troponin-T are classical prognostic markers of cardiovascular dysfunction and mortality in dialysis patients. Dislipidemia contributes to the development of cardiovascular complications. The increased circulating levels of markers of dislipidemia such as apo-AI and triglycerides were associated with increased risk of cardiovascular mortality in CKD and in the general population. Vascular calcification is another important reason for the high incidence of cardiovascular disease among chronic dialysis patients. Low circulating levels of fetuin-A, inhibitor of vascular calcification, are associated with a greater prevalence and/or severity of VC and increased risk for all-cause and cardiovascular mortality. Increased levels of FGF-23, another promoter of vascular calcification, are independently associated with mortality among patients who are beginning hemodialysis treatment.

The contribution of chronic inflammation to the development of atherosclerosis and the high morbidity and mortality rates in this patient population is receiving more and more recognition. Although numerous pro- and anti-inflammatory cytokines have been described as playing a role in the inflammatory response, most of the
studies suggest IL-6 along with CRP as major mediators of the acute phase response. The significant contribution of IL-6 and CRP to the development of atherosclerosis through various metabolic, endothelial, and procoagulant mechanisms was recently also well characterized. Malnutrition is associated with adverse outcomes such as CVD, inflammatory state, and increased mortality in ESRD patients. Identification of malnutrition in CKD patients is challenging, because classical malnutrition markers such as albumin and prealbumin are dependent on residual kidney function and dialysis. Nevertheless, assessment of malnutrition in CKD patients is of great value, because correction of the anabolic state improves the outcome in ESRD patients. The issue of the ideal prognosis biomarker remains open. Using new technologies such as proteomics and epigenomics in prospective comparative studies may be helpful in identifying a better predictor of outcome in this population.

Declaration of Interest:
There is no conflict of interest for any of the authors.

References:


Biomarkers for the prediction of mortality and morbidity in patients with renal replacement therapy


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