Proteomic Biomarkers in the Cardiorenal Syndrome: Toward Deciphering Molecular Pathophysiology

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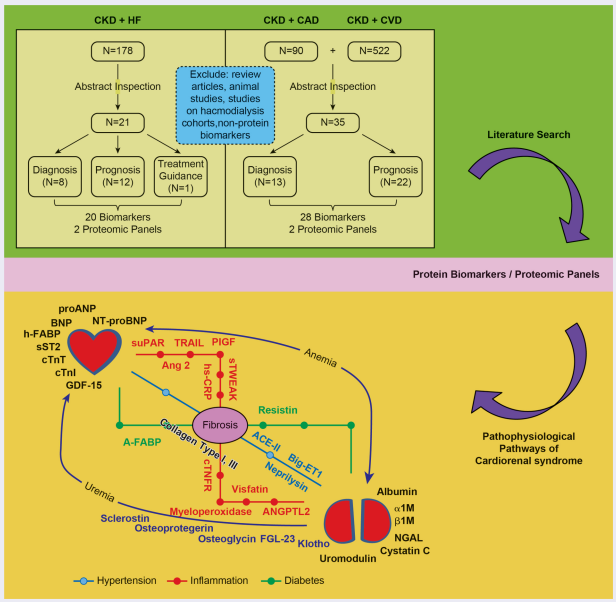
ABSTRACT

Cardiorenal syndrome (CRS) is defined by coexisting heart and renal dysfunctions. Malfunction of 1 organ may cause dysfunction of the other with variable causative disease that defines the type of CRS (1–5). Numerous studies showed that the prevalence of cardiovascular disease is increased in patients with chronic kidney disease (CKD). Similarly, CKD affects a large proportion of patients with heart failure. This overlap between primary heart or primary kidney disease blurs cause–effect inferences of the initiator/target organ. The classical subdivision of CRS in 5 categories does not provide pathophysiological suggestions for targeted intervention. It seems timely to revisit the value of CRS biomarkers in a pathophysiology-centered approach. We systematically reviewed the literature in CRS, which revealed 53 clinical studies describing the use of 44 biomarkers and 4 proteomic panels. All biomarkers are involved in at least one of the CRS comorbidities. Among the pathways affected, inflammation, aberrant glucose metabolism, neurohormonal activation, and oxidative stress are well described. There is growing evidence that fibrosis may be the "cornerstone" that unifies most of the pathways leading to CRS. Formation of excess fibrous connective tissue antedates CRS in many cases. This review highlights that biomarkers reflecting fibrosis may be of substantial clinical value in the early detection, prognostication, and guiding treatment of CRS. Biomarkers detecting changes in collagen turnover in the extracellular matrix of heart and kidney appear able to depict subclinical changes in the fibrotic remodeling of tissues and constitute a promising approach toward personalized intervention in CRS.

GRAPHICAL ABSTRACT



Keywords: biomarker; blood pressure; cardiorenal syndrome; hypertension; precision medicine; proteome

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Cardiovascular and chronic kidney disease (CKD) create a uge socioeconomic burden on the health care system. The 2 complications seldom exist in solitude. In a European-wide study CKD, defined as an estimated glomerular filtration rate below 60 ml/min/1.72 m^2, affects 17.3% of the coronary artery disease (CAD) population[1]; while the estimated global CKD prevalence is 8.7%.[2] In a study based on the British heart failure cohorts, the prevalence of CKD was 63%.[3] Furthermore, different forms of cardiovascular disease are present in 64.5% of elderly (aged ≥66) with CKD, while the percentage is 32.4% in elderly without CKD, according to the national annual report of US Renal Data System. The overlap of "cardiac" and "renal" diseases is illustrated in Figure 1.

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The prevalence of cardiovascular events in CKD patients and renal insufficiency in patients with heart dysfunction initiated the concept of "cardiorenal syndrome" (CRS). CRS is defined as the coexistence of malfunctions of kidney and heart, possibly malfunction in 1 organ causing deterioration of the other.[4] Pathophysiological changes, as well as therapeutic response in CRS, may not be identical to that in CKD, acute kidney injury, or cardiovascular disease including heart failure (HF) and CAD alone.[5] CRS is currently classified into 5 categories,[6] mostly based on disease-initiating events and its acuity (type 1 and 3 comprising acute events and type 2 and 4 chronic diseases), but not on molecular pathophysiology. Moreover, the specific underlying cardiac or renal disease (e.g., congestive HF vs. CAD or diabetic nephropathy vs. lupus nephritis is not considered). Consequently, biomarkers that are linked to molecular pathophysiology and that may be beneficial in effectively guiding (personalized) intervention poorly reflect these 5 categories.

A premise of biomarker research is that a biomarker is considered "useful" if it has foreseeable benefits on patient welfare.[7] Research in CRS biomarker consequently should be based on pathophysiology-centered approaches, to address the comorbidity targets in CRS, enabling prevention and intervention in patients without symptomatic disease. We investigated CRS biomarkers in the literature and interlinked them with CRS pathophysiological pathways, aiming at providing insight toward the identification of valuable CRS biomarkers.

BIOMARKERS IN CRS

Literature search strategy

A systematic search strategy was employed to retrieve the relevant literature. Based on the pathophysiological mechanism of CRS, the search was divided into 2 topics, focusing on the 2 most common cardiovascular comorbidities: (i) CKD and HF (CKD–HF) and (ii) CKD and CAD (CKD–CAD). In the core database of Web of Science, we searched for articles that contained the search items "chronic kidney disease" AND "heart failure" AND "biomarker." Only human studies published after 2009 were included; studies containing solely hemodialysis patients were omitted. Only protein biomarkers were considered and studies of poor quality or containing a low number of participants (n < 20) were discarded. The same strategy was used for CKD–CAD, with "heart failure" substituted by "cardiovascular" OR "coronary artery disease." It returned 178 and 612 original research articles for the 2 foci, respectively. In-depth independent inspection of the articles by 3 of the authors and the requirement for agreement by at least 2 yielded 20 biomarkers and 2 proteomic panels from 21 research articles on CKD–HF; while CKD–CAD results in 28 biomarkers and 2 proteomic panels from 35 studies. (There are 3 overlapping studies between CKD–HF and CKD–CAD.)

Acute kidney injury without glomerular diseases and its related heart complications are not addressed in this review. Hemodynamic acute kidney injury is predominantly the consequence of abrupt hemodynamic changes[8] which should be distinguished from the more or less progressive and gradual loss of renal function in CKD and its chaperoning cardiac dysfunction/HF.[9] The definition of the 2 foci is displayed as a 2 × 2 matrix in Figure 2a, and the literature search strategy is illustrated in Figure 2b.

CRS biomarkers in focus of CKD–HF

Detailed information and references of the 21 studies about CKD–HF are summarized in Supplementary Table S1A (diagnostics), S1B (prognostics), and S1C (treatment guidance) online. Eight studies investigated biomarkers for diagnosis, twelve for risk stratification, and one for treatment guidance, where N-terminal pro-B-type natriuretic peptide (NT-proBNP) level was used to stratify non-diabetic CKD patients, to examine if subgroups had an improved treatment benefit of a low-sodium diet, diuretics,

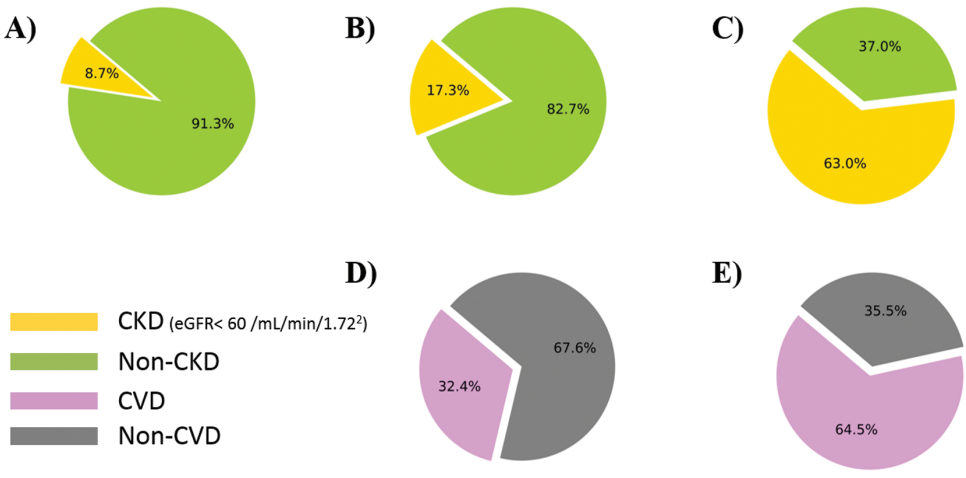

Figure 1. Overlap between CKD and cardiovascular diseases in 3 large-scale studies or reports. (**a**) Estimated global prevalence of CKD by the World Bank. (**b**) Prevalence of CKD in CAD in a trans-European study (n = 7,998). (**c**) Prevalence of CKD in HF in a British cohort (n = 50,114). (**d**) Prevalence of CVD in the elderly population without CKD (n = 108,6232) and (**e**) prevalence of CVD in the elderly population with CKD (n = 175,840) according to the US RDS 2018 Annual Data report. Abbreviations: CAD, coronary artery disease; CKD, chronic kidney disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HF, heart failure.

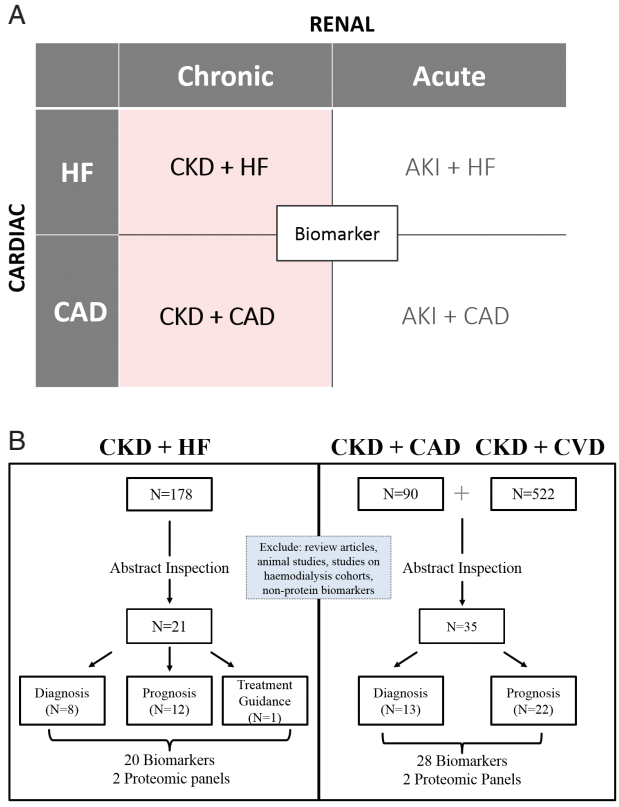

Figure 2. Schematic illustration of CRS biomarkers shortlisting from literature search focused on CKD–HF and CKD–CAD on Web of Science. (**a**) The definition of the 2 foci is displayed as a 2 × 2 matrix in the context of CRS. Biomarkers related to AKI are not considered in this review. (**b**) Flow chart of the literature search strategy for each focus: the left and right half represent the search strategy from focus CKD–HF and focus CKD–CAD, respectively. Abbreviations: AKI, acute kidney injury; CAD, coronary artery disease; CKD, chronic kidney disease; CRS, cardiorenal syndrome; HF, heart failure.

or renin–angiotensin–aldosterone system (RAAS) inhibitor.[10] Two studies involved the discovery and validation of proteomic panels with mass spectrometry (MS): Glorieux *et al.* investigated biomarkers related to cardiovascular disease in plasma samples of 29 CKD patients and controls,[11] whereas Farmakis *et al.* built a classifier for diagnosing HF with reduced ejection function in CKD patients based on the intensity of 107 urinary peptides.[12] These peptides were mostly collagen fragments, indicating that the turnover of collagen I and III may be altered in cardiovascular disease.

Of the 20 protein biomarkers listed, 7 are heart derived: cardiac troponin T (cTnT), troponin I, NT-proBNP, BNP, proANP (pro atrial natriuretic peptide), soluble suppressor of tumorigenicity (sST2), and growth differentiation factor 15 (GDF-15). They are highly expressed in cardiomyocytes and were shown to be associated with HF in clinical studies.[13–16] Neprilysin is a peptidase responsible for degrading natriuretic peptides and angiotensin 2[17] and a successfully used target for a recently invented therapeutical principle.[18] Big-endothelial-1 (Big-ET1) is a powerful vasoconstrictor produced by endothelial cells.[19] Three kidney-specific biomarkers were identified, including urinary α1-microglobulin and β2-microglobulin, which are biomarkers for kidney damage, and klotho, which mainly expressed on the cell surface of renal tubules, is linked to the progression of CKD.[20] Serum albumin is a classical indicator of CKD. Neutrophil gelatinase-associated lipocalin is produced in either tubular epithelial cells in kidney or neutrophils and is advocated as a biomarker in detecting acute kidney injury and CKD.[21] Several studies made use of collagen-derived biomarkers or collagen turnover-related biomarkers to investigate the severity of heart dysfunction,[22] predict the progression of kidney disease and HF mortality.[23,24] There is an established link between collagen-related biomarkers to organ fibrosis.[25,26]

CRS biomarkers in focus of CKD–CAD

Twenty-eight protein biomarkers and 2 proteomic panels were identified in CKD–CAD, reported in 35 studies. Detailed information and references of the studies are summarized in Supplementary Table S2A (diagnostics) and S2B (prognostics) online. Two studies investigated proteomic peptide classifiers serving different purposes: Schiffer *et al.* examined the severity of cardiovascular disease in CKD patients,[27] while Currie *et al.* scored diabetic patients with a classifier called CKD273, consisting of 273 urine peptides, to predict their risk of microalbuminuria and mortality.[28] Both classifiers contain a large number of collagen fragments. Additionally, collagen peptides including PIIINP and CITP were also used by Dellegrottaglie *et al.* to detect arterial stiffness in CKD patients.[29] In this context, matrix metalloproteinase 10 (MMP-10), responsible for collagen breakdown, was examined for its value in detecting CKD severity and the presence of CVD.[30]

Low serum uromodulin levels were suggested to indicate the onset of kidney dysfunction, and are linked to CRS-related mortalities and comorbidities.[31] Fibroblast growth factor 23 (FGF-23), sclerostin, osteoglycin, and osteoprotegerin are important components of calcium–phosphate metabolism, often elevated as a result of kidney dysfunction. Cystatin C reflects kidney function and enables the estimation of glomerular filtration rate.

Soluble TNF-like week inducer of apoptosis (sTWEAK), angiopoietin-like 2 (ANPTL2), angiopoietin, and placental growth factor (PlGF) are inflammatory messengers participating in the formation and development of atherosclerotic plaque.[29] Circulating tumor necrosis factor receptor (cTNFR) and soluble urokinase-type plasminogen activator receptor (suPAR) are inflammatory mediators. Plasma visfatin and resistin are believed to be associated with endothelial damage in chronic diseases.[32] Angiotensin-converting enzyme II (ACE-II) is an effector of RAAS activation. C-reactive protein is an acute-phase reactant and a cardiovascular biomarker due to its role in plaque formation.[33] Myeloperoxidase is a leukocyte-derived enzyme. It catalyzes the formation of proatherogenic reactive oxygen species, which involve in tissue damage during inflammation.[34] Lastly, A-FABP (adipocyte fatty acid-binding protein) and h-FABP (heart-type fatty acid-binding protein), that belong to the family of fatty acid transport proteins, were reported to be elevated in patients with worse cardiovascular outcome.[35]

Clinical availability of the CRS biomarkers

The clinical availability of the CRS biomarkers is summarized in Supplementary Table S3 online. Among all the biomarkers presented in this review, 9 were approved for diagnostic use by FDA (U.S. Food and Drug Administration), while 11 of them were approved in other countries (in the European Union). A large portion of biomarkers remained in the experimental or clinical trial phase.

BIOMARKERS TO CRS PATHOPHYSIOLOGY

The classical "five-subtype" definition (Table 1) categorizes CRS according to the initiator and target organ, plus the onset of disease (acute/chronic). Although this raised awareness and invigorated discussion of the disease, its descriptive nature impeded the exploration of CRS pathophysiology, generating little impact on the clinical management of CRS. It may be time to revise the definition of CRS and the associated biomarkers from a pathophysiology-centered perspective, which addresses the comorbidity factors and may lead to better therapeutic strategies.

Comorbidity factors of CRS

There are some comorbidity and underlying disease-specific factors that can initiate or exacerbate the simultaneous deterioration of cardiac and/or kidney function. They trigger different organ manifestations depending on the site of action. In the heart, remodeling of the left ventricular wall leads to loss of systolic and diastolic function and therefore is a forerunner of HF with reduced or preserved ejection fraction. Atherosclerosis in heart vessels leads to myocardial infarction or CAD. Arteriosclerosis in the kidney can induce chronic kidney damage. Vasculitis in lupus nephritis and/or granulomatous polyangiitis may affect both heart and kidney vasculature. Most importantly, as each comorbidity participates in at least 1 biochemical pathway, it should be traceable by biomarker(s), allowing monitoring the disease even at the subclinical level.

Inflammation

Based on the biomarkers currently known, local and systemic inflammation appears to be a major driving force in CRS.[9] Inflammation can be associated with lifestyle-related risk factors, such as smoking and obesity, with common cardiorenal comorbidities, such as hypertension, hyperglycemia, dyslipidemia, and hyperhomocysteinemia,[36] end-stage organ failure as exemplified by hemodialysis-related symptoms,[37] uremia or chronic HF.[3] Chronic inflammation alters and activates cell-mediated immunity, recruiting and potentiating immune cells. The immune activation results in the generation of proinflammatory cytokines, angiogenic growth factors, cell adhesion molecules, and chemokines, which spill over in circulation, damage the endothelium and initiate tissue remodeling in the target organs. In myocardium, alterations in vascular tone may lead to atherosclerosis-related coronary heart disease, which in turn causes vascular and myocardial fibrosis, and left ventricular hypertrophy, ultimately leading to HF.[29] Similar processes in the kidney induce tubulointerstitial fibrosis, glomerulosclerosis, and tubular hypertrophy, the hallmarks of CKD.

Hypertension

Hypertension is a major risk factor in both CKD and cardiovascular disease. Hypertension is associated with activation of RAAS and the sympathetic nervous system. Among the series of neurohormonal upregulation, angiotensin 2 induces the most direct and widespread effect. Apart from vasoconstriction, angiotensin 2 upregulates inflammatory and profibrotic cytokines that stimulate fibroblasts and cause fibrotic damage in renal parenchyma and the myocardium.[38] It also exacerbates vascular inflammation and growth, promoting endothelial dysfunction and structural remodeling. Moreover, it stimulates the release of aldosterone from the adrenal cortex. Aldosterone further enhances the hypertrophic response of angiotensin 2 and binding of the AT1 receptor.[39,40] Angiotensin 2 also increases the oxidative burden by activating NADPH and NADH oxidase. The 2 oxidases act on vascular smooth muscle cells, cardiomyocytes, and renal tubular epithelial cells, introducing further tissue damage and fibrosis via the production of reactive oxygen species.[41,42] Stimulated by angiotensin 2, endothelin-1 produced by endothelium directly contributes to the hypertrophic response of cardiomyocytes and thereby to cardiac hypertrophy.[43]

Diabetes

Diabetes results in several complications due to the uncontrolled blood sugar level, leading among others to increase in chemical modifications (e.g., advanced glycation end-products).[44] Advanced glycation end-products are described as key drivers of CRS.[45] Among the complications, diabetic nephropathy, diabetic cardiomyopathy, and atherosclerotic cardiovascular disease are most closely associated with CRS.[46] Prolonged exposure of renal cells to high glucose level induces hemodynamic and metabolic changes that

Table 1. The "five-subtype" classification of cardiorenal syndrome

Type	1	2	3	4	5
Denomination	Acute cardiorenal	Chronic cardiorenal	Acute renocardiac	Chronic renocardiac	Secondary
Induced by	Acute heart failure	Chronic heart failure	Acute worsening of renal function	Chronic kidney disease	Systemic diseases
Resulted in	Acute kidney injury	Chronic kidney disease	Acute worsening of cardiac function	Decreased cardiac function	Heart and kidney dysfunction

eventually lead to oxidative damage with the upregulation of NADPH oxidase in glomerular mesangial cells and cardiomyocytes.[47–49] Oxidative damage leads to structural alterations by increasing extracellular matrix accumulation, increasing permeability of glomerular basement membrane, and the initiation of interstitial fibrosis with the influx of fibroblasts.[50,51] The structural changes result in glomerular hyperperfusion and hyperfiltration, and compensatory activation of RAAS,[52] which in turn leads to hypertension, contributing to the pathogenesis of CRS.

The aberrant glucose metabolism in the diabetic heart over time compromises heart contractility and efficiency, leading to left ventricular systolic or diastolic dysfunction, or both.[53,54] The increased release of adipokines like leptin and resistin due to metabolic disruption in diabetes has hypertrophic effects on cardiomyocytes, contributing to left ventricular mass increase and left ventricular dysfunction.[55,56] Diabetes causes atherosclerotic-related heart disease, including coronary heart disease and myocardial infarction via multiple synergistic pathways. Hyperglycemia, dyslipidemia, inflammation, oxidative stress, endothelial dysfunction, and hypercoagulability altogether favor a prothrombotic environment while prohibiting fibrinolysis.[46,57]

Anemia

The pathophysiological relationship between anemia, CKD, and HF has been termed "cardio-renal-anemia syndrome."[58] Erythropoietin, a hormone produced by the kidney, stimulates the bone marrow to produce erythrocytes, the oxygen carrier. In advanced CKD patients, circulating erythropoietin level is reduced, resulting in a decrease in bone marrow erythrocyte production and hemoglobin levels, and ultimately anemia.[59] In this context, hemoglobin is negatively correlated with left ventricular mass increase, affecting both systolic and diastolic function and eventually progressing to HF in CKD patients.[60] On the other hand, HF patients have normal or elevated circulating erythropoietin levels.[61] The increased kidney production of erythropoietin is a reaction of the kidney hypoperfusion and tissue hypoxia caused by HF.[62] It is suggested that anemia in HF is the consequence of erythropoietin resistance,[63] and it consequently expose them to increased oxidative stress.[62]

Uremia

Uremia, a consequence of CKD especially in end stage renal disease, can augment the existing CRS and heart disease, expressed as type 4 CRS. Plasma levels of uremic toxins, such as indoxyl sulphate increase significantly due to decreased excretion capacity.[64] They increase oxidative burden and trigger ROS–endothelial dysfunction–fibrosis in kidney and heart.[65] Uremia also leads to hyperphosphatemia, increased serum parathyroid hormone and FGF-23, and vitamin D deficiency that hinders the absorption of calcium. The impaired phosphate homeostasis can increase cardiovascular toxicity and left ventricular mass, further promoting vascular stiffness and calcification in heart and kidney.[66]

All roads lead to fibrosis?

Fibrosis is a key driver of pathophysiology in CRS,[5] permeating almost every relevant biological process or pathology, including inflammation, neurohormonal activation, and uremia that lead to CRS. A schematic illustration of the biomarkers identified and their connection to these pathways is shown in Figure 3. It is comparable to the transport plan of a city, with fibrosis being the central hub connected by different railway systems. Most biomarkers are well connected in protein–protein interaction networks. There is a close interplay between biomarkers in the CKD–HF network (Figure 4a) as well as the CKD–CAD network (Figure 4b), indicating connections at the molecular level. Enrichment analysis using REACTOME identified several pathways that are common between CKD–HF and CKD–CAD (Supplementary Table S4 online). This includes among other pathways related to the collagen chain trimerization, collagen degradation, extracellular matrix organization, assembly of collagen fibrils, and immune system. Considering the multiple collagen-centered pathways and those related to immune system regulating fibrosis,[67] a fibrosis-centered pathophysiology of CRS is very well supported. The idea echoes with findings from a large-scale urinary peptide profiling in CKD patients conducted by Good *et al.* in 2010, a case–control peptidomic analysis from a cohort of hypertensive patients with left ventricular diastolic dysfunction conducted by Kuznetsova *et al.* in 2012,[68–71] and a proteome-mechanistic study by Wendt *et al.* identifying fibrosis as a central issue in obesity-related nephropathy.[72] These studies identified a large proportion of collagen fragments, a hallmark of extracellular matrix turnover, that differed significantly in abundance in the disease samples. In subsequent studies, the significant association of collagen fragments with progression in CKD and cardiovascular disease could be further verified, both in blood and in urine.[70,73,74] In addition, the reports on the potential of peptides derived from collagen type 1, 3, 6, and collagen degradation-related molecules (MMP-1, MMP-10, TIMP-1) as serum biomarkers in CRS highlighted the relevance of fibrosis in CRS pathophysiology.[22–24,28]

In conclusion, multiple protein biomarkers in CRS appear indicative of fibrosis: Among others, neutrophil gelatinase-associated lipocalin was shown to participate in cardiac remodeling in a mouse model after myocardial infarction and is suggested to be a mediator of vascular profibrotic effects of mineralocorticoids.[75,76] Circulating sST2 is a heart-specific biomarker for myocardial fibrosis and cardiac remodeling.[15] Natriuretic peptides, secreted by the heart under myocyte stretch, were extensively used in the diagnosis and investigated for the prognosis of cardiac hypertrophy or HF initiated by myocardial fibrosis[14] (but do not identify patients with silent left ventricular dysfunction[77]). Cardiac troponin levels can reflect the extent of myocardial fibrosis in patients with non-ischemic HF.[78] However, the value of these biomarkers in detecting subclinical fibrotic changes in CRS and potentially guiding intervention awaits additional studies. Interestingly, biomarkers for liver fibrosis (tumor growth factor (TGF) α and TGF-β1, platelet-derived growth factor BB, microfibrillar-associated protein 4, Cytokeratin-18 fragments)[79] are absent from the current

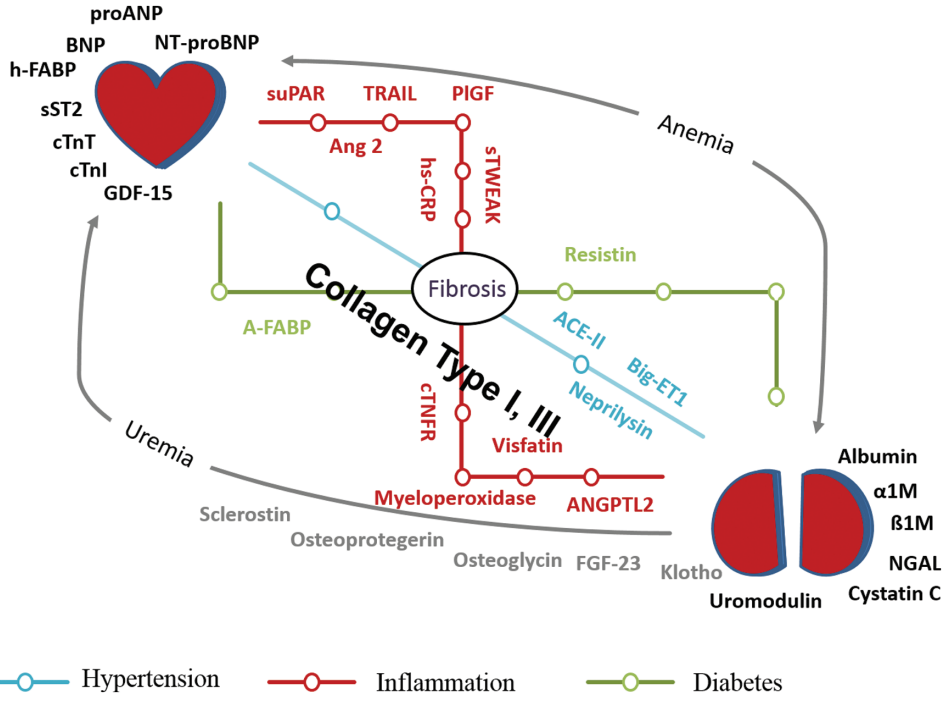

Figure 3. Schematic illustration of biomarkers and major pathophysiological pathways in CRS. Abbreviations: ACE-II, angiotensin-converting enzyme II; Ang 2, angiopoietin 2; α1M, α1-microglobulin; BNP, brain-type natriuretic peptide; β1M, β1-microglobulin; CRS, cardiorenal syndrome; cTNFR, circulating tumor necrosis factor receptor; cTnI, cardiac troponin I; cTnT, cardiac troponin T; FGF-23, fibroblast growth factor 23; GDF-15, growth differentiation factor 15; hs-CRP, high-sensitivity C-reactive protein; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sST2, soluble suppression of tumorgenicity 2; sTWEAK, soluble tumor necrosis factor-like weak inducer of apoptosis; suPAR, soluble urokinase-type plasminogen activator receptor.

collection of CRS biomarkers. It appears likely that these biomarkers may have substantial value in CRS, but were never tested in this context, making them prime candidates for future studies.

PERSPECTIVE

Among the studies that served as the basis for this review, only one investigated a biomarker guiding treatment.[80] In this study, patients that exhibited elevated levels of NT-proBNP experienced a larger reduction of blood pressure and proteinuria after sodium targeting (93 ± 52 mmol Na^+/24 hours) than those having lower plasma NT-proBNP. However, there was no significant difference in response to angiotensin 2 receptor blockers in the 2 groups, indicating that an excellent diagnostic/prognostic biomarker does not necessarily qualify for treatment guidance. In the recent PRIMA-II randomized controlled trial in acute decompensated HF patients, the NT-proBNP-guided therapy arm did not show any improvement in mortality and HF re-admission in comparison with the traditional therapy arm.[80] Although these traditional biomarkers were established in standard medical care for diagnosis and explored extensively in prognosis, the success in treatment guidance was so far limited.

The proteome as a key molecular indicator

Advancements in high-resolution MS enable assessing the subtle changes in epithelium and fibrotic status for pre-CRS.[74,81] During the initiation of fibrosis, the release of numerous fragments from the extracellular matrix is expected to be significantly altered, such as peptides derived from collagen and elastin. These peptides can be identified and quantified using MS. Based on the hypothesis that fibrosis is the major driver in CRS pathophysiology, it antedates CRS clinical manifestation. Further, fibrosis is associated with inflammation, RAAS activation, reduced glomerular filtration rate/cardiac output/vascular stiffness. Monitoring fibrosis, especially in the context of antifibrotic or general therapy and based on the assessment of multiple peptides may enable detecting and treating patients with clinically silent fibrosis, at high risk of progressing toward CRS.

We identified 4 studies using MS for biomarker assessment in the context of CRS. Schiffer *et al.* employed a panel of 13 biomarkers identified from capillary electrophoresis coupled MS (CE-MS)-based plasma proteomics.[27] The biomarkers, predominantly collagen fragments, were combined into a classifier that could assess disease severity in independent cohorts with 89% accuracy. The results were promising, albeit preliminary due to the small sample size. Farmakis *et al.* generated a classifier based on 107 urinary peptides discovered from 59 CKD patients and 67 matched controls. This high-dimensional classifier distinguished HFrEF from CKD background with 84% sensitivity and 91% specificity in an independent cohort of 58 subjects. Subsequent studies focusing on the urinary proteome further confirmed the abundance of collagen peptide in CKD.[50,58,59,82] The performance

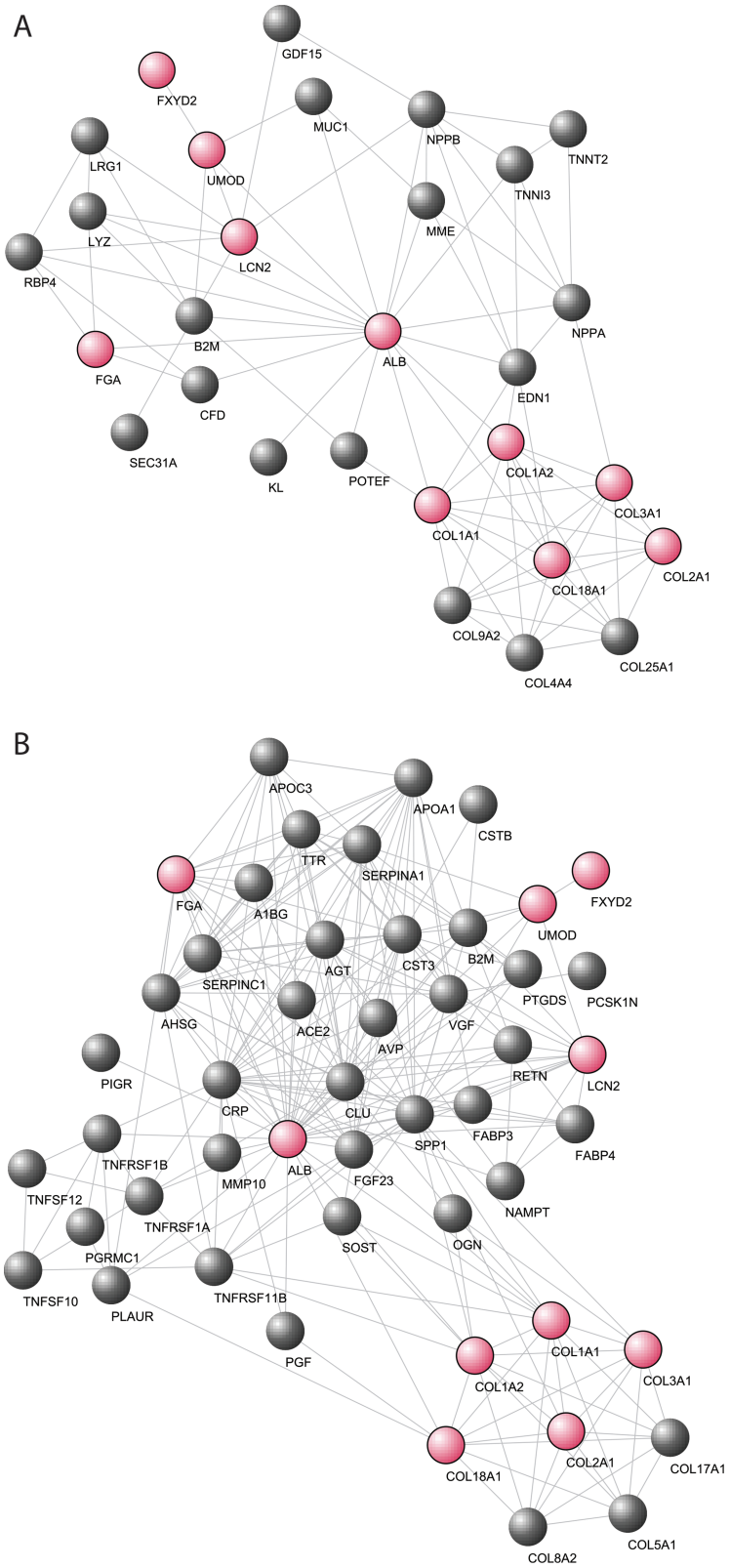

Figure 4. Graphical depiction of protein–protein interaction networks between biomarkers. Biomarkers found in the context of CKD–HF (**a**) and CKD–CAD (**b**) are depicted, with common biomarkers marked in red. Protein–protein interactions were retrieved using STRING database version 11 (https://string-db.org/) based on default settings, while networks were drawn using Cytoscape. Only connected nodes are displayed. Biomarkers are annotated using HGNC Gene Symbols. Abbreviations: CAD, coronary artery disease; CKD, chronic kidney disease; HF, heart failure.

of the high-dimensional classifier CKD273 in predicting kidney dysfunction and mortality was investigated in diabetic patients.[28] CKD273 consists of 273 CKD-specific urinary peptides,[68] and was previously validated for the detection of early-stage diabetic nephropathy.[83] Most of the peptides contained in CKD273 are derived from collagen, inflammatory proteins, apolipoproteins, and fibrinogen. CKD273 was an independent determinant of mortality, retaining significance in a multivariate Cox regression model. Of particular relevance is the exploitation of CKD273 in guiding intervention in PRIORITY (Proteomic prediction and RAAS Inhibition prevention Of early diabetic nephropathy in Type 2 diabetic patients with normoalbuminuria) to stratify high nephropathy risk diabetic patients with normoalbuminuria, for the randomization to spironolactone or placebo.[84] The results are relevant in CRS, as diabetes is among the top CRS morbidity factors. The study results support further implementations of proteomic classifier in CRS, e.g., to guide intervention with sodium-glucose co-transporter-2 (SGLT-2) inhibition.

The high-throughput nature of MS allows assessing multiple peptides, which can be combined in a classifier that takes into account the abundance of each peptide and gives an unbiased judgment accordingly. In a typical proteomic-based classifier, the number of targeted peptides is usually close or over one hundred. This large number of biomarkers confers stability, reduces variability, and allows more detailed biomarker phenotyping and risk assessment.[85] Such assessment may be the basis for personalized intervention, typically anti-inflammatory, RAAS blockade or mineralocorticoid receptor antagonist (MRA), and/or antifibrotic, and a new generation of drugs such as nonsteroidal antimineralocorticoid and SGLT-2 inhibitors. In several recent manuscripts, fibrosis was described as being attenuated as a result of SGLT-2 inhibition.[86,87] Although data are currently mostly from animal experiments, they coincide well with the evident benefit of SGLT-2 inhibition in both kidney and cardiovascular disease[88,89] (we are not aware of a specific study on SGLT-2 inhibition in multifactor CRS but not congestive HF or CKD). MRA are well established in congestive HF, but their renal-antifibrotic effect is not finally defined and comes with clinical problems like hyperkalemia. As a result of the relatively high costs associated with SGLT-2 inhibition and the risk assessment of MRA and based on their antifibrotic effect, the combination of assessing fibrosis based on collagen-derived biomarkers with SGLT-2 inhibition or MRA may currently be the most beneficial and cost-effective approach in the management of CRS. Currently, the prognostic biomarkers do not meet a specific treatment, which is to be expected as they have been described only recently. However, improved accuracy of prognosis and especially earlier detection can guide earlier intervention, which has been demonstrated to be of increased benefit in CKD.[90] Based on these considerations and facts, it is expected that the biomarkers available today that indicate changes in collagen turnover will lead to early intervention, e.g., with SGLT-2 inhibitors. The benefit of such an intervention has to be demonstrated in an appropriate clinical trial. In addition, first efforts are undertaken toward personalization of treatment at an early point in time, guided by proteomic biomarkers.

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DISCLOSURE

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