Proteomics in Management of Acute Kidney Injury

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Acute Kidney Injury (AKI) is currently recognized as a life-threatening disease, leading to an exponential increase in morbidity and mortality worldwide. At present, AKI is characterized by a significant increase in serum creatinine (SCr) levels, typically followed by a sudden drop in glomerulus filtration rate (GFR). Changes in urine output are usually associated with the renal inability to excrete urea and other nitrogenous waste products, causing extracellular volume and electrolyte imbalances. Several molecular mechanisms were proposed to be affiliated with AKI development and progression, ultimately involving renal epithelium tubular cell-cycle arrest, inflammation, mitochondrial dysfunction, the inability to recover and regenerate proximal tubules, and impaired endothelial function. Diagnosis and prognosis using state-of-the-art clinical markers are often late and provide poor outcomes at disease onset. Inappropriate clinical assessment is a strong disease contributor, actively driving progression towards end stage renal disease (ESRD). Proteins, as the main functional and structural unit of the cell, provide the opportunity to monitor the disease on a molecular level. Changes in the proteomic profiles are pivotal for the expression of molecular pathways and disease pathogenesis. Introduction of highly-sensitive and innovative technology enabled the discovery of novel biomarkers for improved risk stratification, better and more cost-effective medical care for the ill patients and advanced personalized medicine.

acute kidney injury proteomics biomarkers

1. Introduction

In the recent years, there has been a steady and substantial increase of patients suffering from acute kidney injury (AKI), affecting 13.3 million people worldwide with a mortality rate of up to 1.7 million deaths [1][2]. This complex disorder is defined by many pathophysiological distinct conditions, and it is still considered as under-recognized outcome, usually associated with secondary aetiologies like cardiovascular complications or sepsis [3]. By definition, AKI is characterized by a significant reduction of the renal function and a subsequent increase in serum creatinine levels (SCr ≥ 26.4 μmol/L), associated with short- and/or long-term complications. Usually, the early signs originate in the proximal tubular cells of the renal cortex, where symptoms are asymptomatic until disease progression is advanced [4]. The spectrum of kidney injuries is manifested within hours or a few days without reduced urine output. The outcome is extremely serious, causing the accumulation of unfiltrated waste blood products, impaired electrolyte homeostasis, and inflammation, which in turn, induce an imbalance of normal kidney function [5].

AKI is classified into three stages: prerenal, intrinsic renal, and/or postrenal. Prerenal renal injury is characterized by diminished renal blood flow, often due to hypovolemia, which leads to a decrease in glomerular filtration rate (60 to 70 percent of cases). In intrinsic renal injury, there is damage to the renal parenchyma, often from prolonged or severe renal hypoperfusion (25 to 40 percent of cases). The medical intervention, drug induced acute interstitial nephritis, accelerated hypertension, surgery correlated embolism, intrarenal deposition are considered as an intrinsic acute renal injuries. Postrenal injury occurs because of urinary tract obstruction due to tumor, benign prostatic hyperthropy or neurogenic bladder with decreased function of the urinary collection system (5 to 10 percent of cases) [5][6].

Nowadays, AKI management is of high importance due to the fact that clinical data are constantly showing an association with progressive loss of the kidney function and an increased risk of initiation of renal replacement therapy (RRT). The awareness of such a situation is evident because early recognition of AKI to improve kidney function and reduce long-term burdens is really at a moderate level. Lack of consistency and standardization in diagnostic classification for AKI has been an issue for real estimation of disease severity [7]. Current diagnosis based on patient history, physical examination, laboratory analysis, ultrasound, and kidney biopsy is limited due to non-early AKI detection and inability to predict disease course [7][8]. Often, this is associated with over-or-under treatment of the patients with dramatic increase in medical costs as well as a multifactorial unpleasant experience of physiological issues [9]. In addition, there is no approved medical therapy to prevent, treat, or enhance AKI recovery, which is a significant problem for the critically ill patients.

Within the last two decades, the study of proteomics has progressed enormously and most importantly, has revolutionized our understanding of molecular biology. Proteins and their smaller molecular units, called peptides, display the physiological and pathophysiological processes inside the cell or organism. This empowers us to utilize the complete set of proteins (proteome) to examine their structure, function, and expression in the cell, ultimately improving human health [10]. Proteome in general is highly dynamic and occasionally responds to different environmental stimuli. As we know, disease mechanisms and drug effects have a tremendous impact on the protein profiles, which is why it is important to reveal crucial information for an in-depth understanding of the disease and therapy on a molecular level [10][11].

Latest developments in high-resolution technologies enable high-speed levels and exceptional analytical performance designed for the assessment of complex biological samples. This in turn, has opened new avenues for the identification and characterization of novel biomarkers, especially in the field of proteomics and body fluids [12][13][14]. Proteins can be indicative of molecular changes during the disease state at first, and at the same time, might be a signal for disease progression. In fact, application of those molecular targets, features, and signatures in biomarker-guided therapies has been a major interest for the scientific community not only in the past few years but also it is the future prospective [15][16]. Especially, assessment of novel biomarkers for improved diagnostics but also prognostic accuracy, patient risk stratification, prediction of disease outcome, and monitoring of response to treatment are of special interest [17][18][19]. Therefore, a better and more comprehensive understanding of the protein's dynamically driven biological functions, including metabolic cross-talk interactions, is an unmet need for a more precise understanding of disease onset and progression.

2. Proteomics in Management of Acute Kidney Injury

2.1. Acute Kidney Injury (AKI)—Related Protein Biomarkers

In light of the three stages of AKI, the examined biomarkers are sorted into categories as prerenal, intrinsic renal injuries -intrinsic renal after medical intervention- and postrenal injuries. The biomarkers are also defined under three types as diagnostic, prognostic and monitoring biomarkers according to their characteristics, stated in recent studies. The proteins that are utilized to detect and confirm AKI are named as diagnostic AKI biomarkers. The ones that provide information on AKI stage and affected cells or areas of the kidney are named as prognostic, and the ones that support the research if the treatment effect is different for biomarker positive patients are classified as monitoring biomarkers. Together with the biomarkers, the affected kidney areas and cells are summarized in **Table 1** based on the findings of the investigators.

Table 1. The list of biomarkers evaluated in AKI clinical studies.

Biomarkers	Biomarker Type	Study Type	Affected Area of Kidney	Affected Kidney Cell Types	AKI Category
NGAL	Diagnostic	Urine analysis	Renal pelvis	Collecting duct epithelial cells	Prerenal
B2M	Diagnostic	Urine analysis	Proximal tubule	Tubular epithelial cells	Intrinsic renal
SERPINA1 (AAT)	Diagnostic	Urine analysis	Proximal tubule	Tubular epithelial cells	Intrinsic renal
RBP4	Diagnostic	Plasma analysis	Proximal tubule	Tubular epithelial cells	Postrenal
FBG	Diagnostic	Urine analysis	Glomerulus	Tubular epithelial cells	Intrinsic renal (after MI) **
GDF15	Diagnostic	Urine analysis	Nephron	Renal endothelial cells	Intrinsic renal (after MI) **
LRG1	Diagnostic	Urine analysis	Nephron	Renal endothelial cells	Intrinsic renal (after MI) **
SPP1	Diagnostic	Urine analysis	Nephron	Renal endothelial cells	Intrinsic renal (after MI) **
ANXA5	Diagnostic	Urine analysis	Nephron	Renal endothelial cells	Prerenal

Biomarkers	Biomarker Type	Study Type	Affected Area of Kidney	Affected Kidney Cell Types	AKI Category
6-PGLS	Diagnostic	Urine analysis	Nephron	Renal endothelial cells	Prerenal
TIMP-2 IGFBP7 *	Diagnostic	Urine/serum	Proximal tubule	Proximal tubular epithelial cells	Intrinsic renal (after MI) **
C3	Diagnostic or prognostic	Urine analysis	Glomerulus	Tubular epithelial cells	Intrinsic renal (after MI) **
C4	Diagnostic or prognostic	Urine analysis	Glomerulus	Tubular epithelial cells	Intrinsic renal (after MI) **
GAL-3BP	Prognostic	Urine analysis	Glomerulus	Tubular epithelial cells	Intrinsic renal (after MI) **
Cys C	Prognostic	Plasma analysis	Proximal tubule	Tubular epithelial cells	Prerenal
S100P	Prognostic	Urine analysis	Glomerulus	Urothelium cells	Prerenal
α2M	Prognostic	Urine analysis	Glomerulus	Tubular epithelial cells	Intrinsic renal (after MI) **
CD26 *	Prognostic	Urine analysis	Glomerulus/Proximal tubule	Renal brush border epithelium	Intrinsic renal (after MI) **
sTNFR1, sTNFR2	Monitoring	Plasma analysis	Glomerulus	Tubular epithelial & mesangial cells	Intrinsic renal
ANXA-2	Monitoring	Urine analysis	Glomerulus	Renal glomerular endothelial cells	Intrinsic renal
CRP	Monitoring	Blood analysis	Renal cortex	Renal Cortical Epithelial Cells	Intrinsic renal (after MI) **
OPN	Monitoring	Blood analysis	Nephron-loop of Henle	Renal epithelial cells	Intrinsic renal (after MI) **

Biomarkers	Biomarker Type	Study Type	Affected Area of Kidney	Affected Kidney Cell Types	AKI Category
CD5 & Factor VII *	Monitoring	Blood analysis	Nephron	Filtrating cells	Intrinsic renal (after MI) **
IgHM	Monitoring	Urine analysis	Glomerulus	Tubular epithelial cells	Intrinsic renal (after MI) **
Serotransferrin	Monitoring	Urine analysis	Glomerulus	Tubular epithelial cells	Intrinsic renal (after MI) **
HRG	Monitoring	Urine analysis	Glomerulus	Proximal tubule epithelial cells	Intrinsic renal (after MI) **
CFB	Monitoring	Urine analysis	Glomerulus	Proximal tubule epithelial cells	Intrinsic renal (after MI) **
CD59	Monitoring	Urine analysis	Glomerulus/Proximal tubule	Renal brush border epithelium	Intrinsic renal (after MI) **
AGT	Monitoring	Urine analysis	Glomerulus	Proximal tubule epithelial cells	Intrinsic renal (after MI) **
KRK1 *	Monitoring	Urine analysis	Glomerulus	Proximal tubule epithelial cells	Intrinsic renal (after MI) **

* indicates downregulation of protein biomarkers associated with disease outcome ** denotes for intrinsic renal injury after medical intervention.

2.2. AKI—Related Protein Biomarker Types and Their Association

A deep profiling of proteins involved in AKI development provides crucial evidence for the identification of new biomarkers and their classification as diagnostic, prognostic, or monitoring biomarkers. A special interest, in context to AKI, was protein-based biomarkers detectable in various bodyfluids, which likely can play a significant role in providing more specific, accurate, and medical knowledge for early diagnosis and future optimization of disease treatments. Efforts towards reaching these goals and all aspects of better patient management have been discussed under biomarker type subsections. The details of clinical studies, including their most important discoveries, are presented in **Table 2**.

Table 2. Characteristics of the AKI clinical studies and their most important discoveries.

Authors	Biofluid	Method	Patient Cohort	Investigated Biomarkers	Most Significant Biomarkers	Conclusion
Ibrahim et al. [20] [21]	Blood	Luminex xMAP immunoassay	44 AKI 745 non-AKI	109	CRP; OPN; CD5; FACTOR VII	The biomarker panel using machine learning was developed and showed a performance with an AUC of 0.79 for predicting procedural AKI. The optimal score cutoff had 77% sensitivity, 75% specificity, and a negative predictive value of 98% for procedural AKI. An elevated score was predictive of procedural AKI in all subjects (odds ratio = 9.87; $p < 0.001$).
Zhu et al. [21][22]	Urine	LC-MS/MS	4 CI-AKI 20 CI-non AKI	99	NGAL; S100- P; ANXA2; B2M; SERPINA1; RBP4	In relatively small patient cohort, urine proteome of CI-AKI vs. non-CI-AKI were compared. Upregulation was observed in CI-AKI with ratio of 7.40 (B2M), 6.63(S100-P), 4.25 (NGAL) and 4.27 (SERPINA1).
Awdishu et al. [23]	Urine/blood	LC-MS/MS	10 V-AKI 12 HC	251	C3; C4; GAL-3BP, FBG, α2M; IgHM; SEROTRANSFERRIN	Urinary exosome proteins in response to V-AKI might provide vulnerable molecular information that

Authors	Biofluid	Method	Patient Cohort	Investigated Biomarkers	Most Significant Biomarkers	Conclusion
						helps elucidate mechanisms of injury and identify novel biomarkers among patients with confirmed drug-induced kidney injury.
Jung et al. [24]	Urine	LC-MS/MS	14 AKI 14 non-AKI	174	NGAL; ANXA5;GAL3; 6-PGLS; S100-P	Proteomic urinary-based biomarkers that can predict early AKI occurrences in infants were identified. Three biomarkers performed well, showing AUC values of 0.75, 0.88 and 0.74 for NGAL, ANXA5 and S100-P, respectively. There was higher beneficial effect of the classifier performance when NGAL + AXA5 (AUC of 0.92) and NGAL + AXA5 + S100-P (AUC of 0.93) were applied.
Du et al. [25]	Urine	Flow cytometry	133 AKI 68 non-AKI	1	CD26	Urinary exosomal CD26 was negatively correlated with AKI compared with non-AKI patients (β = −15.95, p < 0.001). Similar results were obtained for the AKI cohort with major adverse

Authors	Biofluid	Method	Patient Cohort	Investigated Biomarkers	Most Significant Biomarkers	Conclusion
						events. On the other hand, AKI survivors exhibited high-CD26 levels compared AKI patients with low-CD26 levels for early reversal, recovery and reversal, respectively, after adjustment for clinical factors (ORs (95% CI) were 4.73 (1.77–11.48), 5.23 (1.72–13.95) and 6.73 (2.00–19.67), respectively). Prediction performance was moderate for AKI survivors (AUC 0.65; 95% CI, 0.53–0.77; p = 0.021) but improved for non-septic AKI survivors (AUC, 0.83; 95% CI, 0.70–0.97; p = 0.003)
Wilson et al. [22]	Plasma	Randox's multiplexed Biochip Arrays	500 AKI	11	sTNFR1; sTNFR2; CYSTATIN C; NGAL	A multivariable panel containing sTNFR1, sTNFR2, cystatin C, and eGFR discriminated between those with and without kidney disease progression (AUC 0.79 [95% CI, 0.70–0.83]).

Authors	Biofluid	Method	Patient Cohort	Investigated Biomarkers	Most Significant Biomarkers	Conclusion
						Optimization of the panel showed 95% sensitivity and a negative predictive value of 92% used to stratify patients at low risk for disease severity.
Merchant et al. [26]	Urine	ELISA	15 AKI 32 non-AKI	29	HRG; CFB; CD59; C3; AGT	Two proteins, HRG and CFB were upregulated in AKI patients, showing moderate predictive performance (AUC 0.79; 95% CI, 0.65–0.94; $p = 0.001$ and AUC 0.75; 95% CI, 0.57–0.93; $p = 0.007$). Significant improvement in the risk prediction for primary outcome was observed, specifically for NRI, IDI in addition to CFB and HRG. Only HRG was a significant predictor in the 21 patients with AKI defined by KDIGO criteria.
Coca et al. [27]	Serum	Randox's multiplexed Biochip Arrays	769 AKI 769 non-AKI	2	sTNFR1; sTNFR2	Plasma sTNFR1 and sTNFR2 measured 3 months after discharge were associated with renal

Authors	Biofluid	Method	Patient Cohort	Investigated Biomarkers	Most Significant Biomarkers	Conclusion
						deterioration independent of AKI (HR 4.7, 95% CI, 2.6–8.6) and significant association with renal failure. In this regards, clinical classifier performance was with AUC of 0.83. There was also association of the both biomarkers with Heart failure ((sTNFR1-1.9 (95% CI, 1.4–2.5) and sTNFR2-1.5 (95% CI, 1.2–2.0)) and death ((sTNFR1- 3.3 (95% CI, 2.5–4.2) and sTNFR2-1.5 (95% CI, 1.9–3.1)).
Jiang et al. [28]	Urine	LC-MS/MS	90 CP-AKI	12	GDF15; LRG1; SPP1	Urinary proteomic profiles of GDF15 (1.77-fold) and LRG1 (4.25-fold) were significantly elevated by CP treatment compared to the baseline.
Di Leo et al. [29]	Urine/serum	NephroCheck® (NC) Immunoassay	719 patients at ICU	2	TIMP-2; IGFBP7	TIMP-2 and IGFBP7 levels yielded good performance in prediction AKI development at first 4 days at ICU and in all critically ill

Authors	Biofluid	Method	Patient Cohort	Investigated Biomarkers	Most Significant Biomarkers	Conclusion
						patients (AUC of 0.65). The Kaplan-Meier analysis predicted lower risk for AKI development only for those patients who NC test was negative.
Navarrete et al. [30]	Urine/serum	ELISA assay	21 AKI 21 non-AKI	1	PLA2G15/LPLA2	Urinary PLA2G15/LPLA2 activity was associated with subsequent AKI development during/ongoing CPB. There was similar association with PLA2G15/LPLA2 activity from serum. No association was observed between PLA2G15/LPLA2 activity from both biofluids, suggesting that this biomarker might be an early sign of renal response to CPB events.
Navarrete et al. [31]	Urine	Nano RPLC-MS/MS	8 AKI 8 non-AKI	28	KRK1	Investigation on KLK1, confirmed the activity of this enzyme in AKI and non-AKI patients. In fact, increased action of KLK1 was confirmed only in AKI patients who arrived at ICU

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Authors	Biofluid	Method	Patient Cohort	Investigated Biomarkers	Most Significant Biomarkers	Conclusion
						and had highest activity in comparison to other enzymes, hence providing novel finding related to intraoperative events in human ischemia reperfusion injury during CPB.

LC-MS/MS—liquid chromatography coupled with tandem mass spectrometry; ELISA—enzyme-linked immunosorbent assay; CI-AKI—contrast-induced acute kidney injury; AUC—area under the curve; 95% CI—confidence interval at 95%; CPB—cardiopulmonary bypass; VI-AKI Vancomycin-associated AKI; HC—healthy volunteer; OR—odd ratios; KDIGO—kidney disease: improving global outcomes; CP-AKI—cisplatin-induced acute kidney injury; ICU—intensive care unit; HR—hazard ratio.

2.3. Diagnostic AKI Biomarkers

β2 microglobulin (B2M) is a blood protein that is present on the surface of nucleated cells as a part of the normal immune system. This protein has a molecular weight of 12 kDa and is released by the cells into the blood, generally being a highly concentrated circulating protein compared to its lower levels or traces found in urine, the spinal cord, and other biofluids [32]. Until now, it is known that B2M associates and forms complexes together with the major histocompatibility complex I (MHC-I) and human leukocyte antigen I (HLA-I) on the cell surface [33]. Another important linkage is with the Fc receptor, which supports the regulation of immunoglobulins G, albumin, and hepcidin [34]. Previous experimental data suggested the potential implication of B2M in several diseases, not only in glomerulonephritis and/or AKI but also in hemochromatosis [35]. In the kidneys, B2M is usually filtered in the glomeruli, and then 99% of the content is reabsorbed in the renal proximal tubule structures. Higher concentration in urine could be detected, and this is due to renal impairment and the inability of proper protein reabsorption which leads to reduced renal function [33][36].

Alpha-1-Antitrypsin (AAT) is one of the most abundant and active proteins, with a molecular size of 54 kDa. AAT belongs to the serine protease inhibitor family (also known under the SERPIN acronym) which is mainly produced in the liver. It was initially discovered in human plasma as a glycoprotein that was characterized to have inhibitory effect on several proteases, including elastase and/or proteinase-3 [37][38]. Due to its inhibitory effect, AAT can cause anti-inflammatory effects and improvement of injured tissue during evaluated molecular pathways. Despite the molecular function, AAT has a high affinity for building complexes with hemin salt in order to prevent forming of porphyrias and hemin-induced reactive species in neutrophils [39][40]. Regarding AKI, it has been previously reported that AAT was identified in urine and considered as a biomarker for ischemic injury [16][41].

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Retinol-binding protein (RBP) is a low-molecular-weight protein with a molecular mass of 21 kDa [42]. It is known as a circulating plasma protein synthesized in the liver which is responsible for the transportation of the fat-soluble vitamin A, called retinol. RBP forms complexes with transthyretin and preserves glomerular filtration in the kidneys [42][43]. The majority, or approximately 95%, of the protein is reabsorbed in the proximal tubule. Only a small portion (4–5%) of serum RBP freely passes the glomerular barrier and is excreted in urine [44][45]. Urinary RBP is relatively stable in urine and therefore serves as a diagnostic marker for tubular dysfunction and tubularpathies [46][47].

Fibrinogen (FBG) is a macromolecular glycoprotein belonging to the group of glycans (glycoproteins) found in plasma. It has a molecular weight of 340 kDa and is synthesized in the liver. Structurally, fibrinogen is composed of three different polypeptide chains, namely alpha, beta, and gamma chains, which are linked through disulfide bridges to develop the stable chemical structure of the fibrinogen molecules [48][49]. In return, fibrinogen creates structural complexes with other molecules and represents one of the major proteins responsible for blood clotting.

2.4. Prognostic AKI Biomarkers

Cystatin C (CysC) is a small and low-molecular-weight protein with a molecular mass of 13 kDa. It consists of 122 amino acids, and because of its small size, protein levels can be freely reabsorbed by the glomerulus and metabolized after reabsorption [50]. Cystatin C is a protein that is produced mainly by nucleated cells at a constant rate. It's known as a member of the cysteine protease inhibitors released in the blood system [51]. In general, CysC concentration can be detected in urine and plasma. Urinary CysC is a biomarker for the deterioration of proximal tubular cells, and its potential has been studied in terms of the prediction of kidney injury and its prognosis. Experimental investigation of the urinary CysC's role has confirmed its capability to provide early signs of renal impairment compared to well-known clinical markers like creatinine [52]. In contrast, serum CysC has been monitored in several clinical studies, showing a strong association with GFR estimation [53][54].

S100 calcium-binding protein P (S100P) is a member of the S100 family of proteins and contains helix-loop-helix (EF-hand) Ca^{2+}-binding motifs. It has a molecular mass of 10 kDa, and it is expressed in various organs like the human placenta, stomach, urinary bladder, and bone marrow [55][56]. As a result of its molecular structure, S100P actively regulates calcium homeostasis and, at the same time, acts as a calcium signaling molecule.

Galectin-3-binding protein (Gal-3BP), also known as 90k or Mac-2, is a glycoprotein secreted in the body fluids. As a member of the glycoprotein family, Gal-3BP is a typical protein prone to modifications with other amino acids and was initially identified to be involved in cellular transformation in different cancers [57][58][59][60][61][62][63][64]. It contains a carbohydrate recognition domain (CRD), which allows molecules and enzymes to oligomerize and form pentamers macromolecules. The molecular mass of Gal-3BP is calculated 65.3 kDa, although some reports indicate that the secreted form of the protein into body fluids could reach up to 100 kDa [65].

Alpha-2-microglobulin (α_2M) is a tetrameric protein that has a molecular size of 720 kDa and is one of the most abundant proteins found in blood plasma. α_2M has a special ability to inhibit different kinds of proteases, affecting distinct biological processes not only in plasma but also in cerebral spinal fluid, spinal fluid, synovial fluid, ocular

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fluid, and urine fluid [66][67][68][69]. The mechanism of action relies on the formation of tetrameric 'traps', which disturb the function of the active proteases while the actual substrate [70]. In this way, trapped proteases are exempted from further digestion of collagens and other large polypeptides, preventing conformational changes of small polypeptides entering into the 'trap'. This so called 'selectivity' of α_2M protein is based on the presence of a polypeptide 'target' region in the chemical structure of α_2M which became attractive for most of the proteolytic peptidases [70][71][72][73].

CD26 protein, also known as dipeptidyl peptidase-IV (DPP4), belongs to the group of glycoproteins that are expressed in epithelial cells in the liver, kidney, and intestine. Based on some previous laboratory measurements, CD26 is a large protein with a molecular size of 110 kDa [74]. As an enzyme, it has the ability to hydrolyze amino acids like proline and/or alanine from N-terminal residue. At the same time, CD26 can regulate and enhance the expression of T-cells during signal transduction pathway activity [75].

Complement C3 (C3) is a large plasma protein with a molecular weight of 180 kDa [76]. As one of the most abundant members of the complement system, C3 has a central role in the immune response system, in which detection and elimination of foreign molecules by activation of macrophages occur. This process is carried out by complement cascade mobilization when C3 associates with and forms complexes with other amino acids in the host [77][78].

2.5. Monitoring AKI Biomarkers

Tumor necrosis factor (TNF) is a cytokine with a molecular mass of 25.6 kDa. This molecule is secreted by the macrophages, and it is well-known for its central role during inflammation and stress response cascades. TNF-α has a high affinity for binding with other receptors, and therefore it can be found in a soluble and membrane-bounded form. Usually, it is in close interaction with the soluble receptors (sTNFR1 and sTNFR2), and its main function is related to host defense, cell proliferation, and differentiation. In terms of AKI, experimental studies showed associations with sepsis, sepsis shock, and the inflammatory response during severe conditions as well as in autoimmune diseases [79]. Functional analysis indicated TNF-α involvement in hypertension and regulation of blood pressure and NaCl retention [80].

Annexins represent another group of large proteins with a molecular mass of between 33–39 kDa. They are characterized as a protein superfamily that shares a very similar homological structure among all members. As a result of their core structure, they have an affinity to bind phospholipid- and calcium-based proteins [81][82]. There are more than 1000 proteins identified in different species, but in humans only 12. Moreover, annexins have been recognized as intracellular and extracellular proteins that can be attached to various cellular membrane ligands and receptors, involved in coagulation, inflammation, and molecular transport through membranes [83]. Evaluation of the annexin levels have been also assessed in the prediction of AKI progression, particularly when ischemic renal dysfunction significantly contributes towards apoptosis. Importantly, the latest proteomic investigation performed in patients with CI-AKI showed significant levels of annexins A1, A2, and A3 [21]. Annexin A1, also known as lipocortin, has been mainly controlled by glucocorticoids hormons and cytosolic phospholipase A2 (PLA2) inhibition,

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responsible for the mechanism of arachidonic acid (ARA) release and the prevention of signaling molecules (eicosanoids) synthesis [84][85][86]. The protein itself has binding affinity to the phospholipid layers of the cell membranes, hence making it a molecular target for monitoring treatment response through cross-linkage with formyl peptide receptors [87]. With similar properties, cytoplasmatic annexin A2 has been largerly studied for its roles in intracellular regulation and cell signaling cascades [88].

Osteopontin (OPN) is another type of glycoprotein with a molecular mass of 35 kDa [89]. OPN is prone to post-translational modifications (PTMs), especially phosphorylation on serine and threonine, O-glycosylation, and transglutamination, which may enlarge the molecular size up to 75 kDa [90]. It is also known as a pleotropic glycoprotein expressed in a variety of cells throughout the human body, such as activated T cells, macrophages, natural killer (NK) cells, neutrophils, and dendritic cells, among many others [91]. OPN has a crucial role in the formation of the bone and during bone metabolism. Normally, it is upregulated during inflammation and plays the role of a regulator of the immune response. During normal conditions, OPN is present in the loop of Henle and in the nephrons of the kidneys. After renal damage, significant expression of OPN may be observed in the urine as a result of tubular and glomerular deterioration [92][93][94].

C-reactive protein (CRP) is a blood-circulating protein with a molecular mass of 115 kDa that is a member of the pentraxin protein family [95]. CRP has been mainly known as a protein that could be sensitive to a variety of changes in the host due to inflammation, infection, or trauma, including additional pathophysiological changes during tissue damage [96]. In the diagnostic area, it is recognized and used as a clinical marker for inflammation during cardiovascular and kidney diseases [97][98]. Based on the previous laboratory data, CRP is primarily synthetized in the liver and also in smooth muscle cells, macrophages, endothelial cells, and some other types of cells [99].

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