Libertas Academica
FREEDOM TO RESEARCH

Open Access: Full open access to this and thousands of other papers at http://www.la-press.com.

Biomarker Insights

Folate Receptor Alpha, Mesothelin and Megakaryocyte Potentiating Factor as Potential Serum Markers of Chronic Kidney Disease

Elizabeth B. Somers and Daniel J. O'Shannessy

Department of Translation Medicine and Diagnostics, Morphotek, Inc., Exton, PA, USA.

ABSTRACT: Renal disease is the eighth leading cause of death in the United States. Early diagnosis is usually based on the detection of proteinuria or elevated serum creatinine, a relatively poor biomarker that does not accurately predict renal disease progression. As a result, more predictive biomarkers of renal function are sought. We present preliminary data on three protein biomarkers, folate receptor alpha (FRA), mesothelin (MSLN), and megakaryocyte potentiating factor (MPF), currently being pursued for applications in oncology diagnostics, and evaluate serum and urine levels in subjects with renal disease. Compared to healthy subjects, a significant ($P < 0.0001$) increase in all three biomarkers in both serum and urine of subjects with renal disease was demonstrated. Further, serum levels of these three protein biomarkers increased with increasing stage of disease suggesting their potential value in predicting progression in subjects with renal disease and raising caution in interpretation of data in oncology applications.

KEYWORDS: renal disease, folate receptor alpha, mesothelin, megakaryocyte potentiating factor, predictive biomarkers

CITATION: Somers and O' Shannessy. Folate Receptor Alpha, Mesothelin and Megakaryocyte Potentiating Factor as Potential Serum Markers of Chronic Kidney Disease. *Biomarker Insights* 2014:9 29–37 doi: 10.4137/BMI.S15245.

RECEIVED: March 5, 2014. **RESUBMITTED:** April 2, 2014. **ACCEPTED FOR PUBLICATION:** April 6, 2014.

ACADEMIC EDITOR: Karen Pulford, Associate Editor

TYPE: Original Research

FUNDING: Authors disclose no funding sources.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

CORRESPONDENCE: doshannessy@morphotek.com

This paper was subject to independent, expert peer review by a minimum of two blind peer reviewers. All editorial decisions were made by the independent academic editor. All authors have provided signed confirmation of their compliance with ethical and legal obligations including (but not limited to) use of any copyrighted material, compliance with ICMJE authorship and competing interests disclosure guidelines and, where applicable, compliance with legal and ethical guidelines on human and animal research participants.

Introduction

According to the Centers for Disease Control and Prevention, more than 10% of adults in the United States have some form of kidney disease. Chronic kidney disease (CKD), characterized by a progressive loss in renal function over a period of months or years, is the eighth leading cause of death in the United States.[1] Progression of CKD to end-stage renal disease (ESRD) occurs when the kidneys are no longer able to remove enough wastes and excess fluids from the body. Kidney transplantation and dialysis are the only available therapies for ESRD management. Early detection of renal disease using biomarkers may help to prevent and/or predict progression to CKD and ESRD resulting in better patient management and would be more cost-effective than highly invasive renal transplantation or dialysis procedures.

The initial diagnosis of renal disease is usually based on detection of proteinuria or an elevation of serum creatinine, freely filtered by the glomerulus,[2] and most commonly used to determine an estimated glomerular filtration rate (eGFR), an indicator of renal function. However, use of creatinine as a diagnostic marker has limitations. Serum creatinine varies by age,[3] race,[3] sex,[3] muscle mass,[2,4] metabolism,[4] nutritional status,[4] co-morbid conditions, hydration status, and medication use[5–9] and, consequently, significant renal disease can exist with minimal or no change in creatinine.[8] Therefore, markers of early injury, especially those that correlate with early fibrosis and progression, are needed and would prove beneficial in both diagnosis and patient management settings. An ideal renal disease biomarker should be accurate, reliable, and easy to measure with a standard non-invasive, reproducible, and sensitive assay. Three biomarkers, folate receptor alpha (FRA), mesothelin (MSLN), and megakaryocyte potentiating factor (MPF), described in detail below, were selected for evaluation as potential markers of renal disease. Each of these biomarkers is currently being evaluated as potential diagnostics in oncology and each is known to be filtered by the kidneys. As such,

we reasoned that they may serve as viable candidate markers of renal function. Robust and reproducible assays are available for all three biomarkers.

FRA is a 38–40 Da glycosylphosphatidylinositol (GPI)-anchored protein that binds plasma folate (5-methyltetrahydrofolate) with high affinity (K_D ~1nM) and transports it into the cell via endocytosis. In normal human tissues, FRA expression is restricted to polarized epithelial cells in a number of tissues,[10–13] including high expression in the proximal tubules of the kidney where it has been shown to play a role in the tubular reabsorption of folate into the circulation.[14] Therefore, impairment of kidney function may prevent reabsorption of folate into the circulation and could cause folate deficiency,[15] which might then result in upregulation of FRA synthesis.[16–20] FRA has also been shown to be expressed on a number of epithelial tumors including ovarian, endometrial, lung adenocarcinoma, renal clear cell cancer, and triple negative breast cancer.[10–13,21–26]

MSLN is a 70 kDa protein, which upon proteolytic cleavage results in a 32 kDa secreted product termed MPF[27,28] and MSLN, a 40 kDa GPI-anchored glycoprotein that is also shed into the circulation.[29,30] Serum MSLN is currently used for mesothelioma diagnosis, prognosis, and monitoring.[31–33] MSLN is hypothesized to be involved in cell adhesion through interactions with MUC16 and intracellular signaling[34] and is highly expressed in ovarian cancer,[34–37] mesothelioma,[34,37–39] pancreatic cancer,[37] and a subset of lung adenocarcinoma.[37] MSLN also demonstrates restricted expression in normal tissues and is primarily expressed on mesothelial cells of the peritoneum, pericardium, and pleura.[34,37,40] Little is known about the biological function of MPF. However, given that it is a cleavage product from the MSLN precursor protein, its expression should mimic that of MSLN. Owing to its value as a diagnostic for mesothelioma, some studies on MSLN relative to kidney function have been reported. Studies have shown that serum MSLN levels were increased in individuals with renal impairment and that these levels were dependent on the stage of CKD.[17,31,41,42] Further, Hollevoet et al (2010) showed that an increase in MSLN correlated with an increase in creatinine levels.[31]

While some literature on the variation of serum MSLN levels relative to kidney function exists, no such literature exists to our knowledge on serum MPF or FRA, and no comparison relative to the urinary levels of these markers is available. A urine-based assay would of course be ideal as a diagnostic or monitoring tool as it requires totally non-invasive procedures for sample acquisition. As such, we undertook a preliminary investigation of the potential clinical utility of measurements of FRA, MSLN, and MPF, in serum *and* urine, as an aid in the diagnosis or assessment of renal disease. The results indicate that these markers may indeed have potential value in predicting progression in subjects with renal disease and are worthy of further study. Further, the demonstration of significant changes in the serum concentrations of these biomarkers relative to renal function may have implications to their use in oncology diagnostics, in particular, monitoring of disease, as some chemotherapeutics are known nephrotoxics.

Methods

Subject samples. This preliminary study included matched serum and urine samples from 200 subjects with varying stages of renal disease and 100 age-matched healthy subjects (Table 1). Samples were obtained from various commercial vendors with Institutional Review Board approvals and patient consent and were collected between 2009 and 2011 by standard techniques and processed/frozen within 30 minutes of collection. Urine samples represented spot collections collected at the time of blood draw and were centrifuged prior to freezing. All samples were stored at −80 °C, and thawed and aliquoted prior to analysis. Patient demographics, including sex, race, age, and stage (Table 1) were obtained from the suppliers.

Electrochemiluminescence (ECL) assays. The ECL assays for FRA, MSLN, and MPF used in the present analyses have been described previously.[43,44] Samples (serum, urine) from healthy or diseased subjects and standards were added to wells of 96-well plates previously coated with marker specific capture monoclonal antibody (MAb) and incubated at room

Table 1. Demographic and clinical characteristics of healthy subjects and renal disease subjects.

VARIABLE	HEALTHY SUBJECTS N (%)	RENAL DISEASE SUBJECTS N (%)
Total sample size	100	200
Age (Mean, SD)	60.2, 6.9	67.9, 13.7
Gender		
Male	40 (40)	82 (41)
Female	60 (60)	118 (59)
Race		
White/Caucasian	51 (51)	123 (61.5)
Black/African American	38 (38)	14 (7)
Native American/ Alaskan	0	1 (0.5)
Asian	4 (4)	2 (1)
Hispanic	0 (0)	7 (3.5)
Other	0 (0)	53 (26.5)
Unspecified	7 (7)	0 (0)
renal disease stage[1,2]		
Stage III		124 (62)
Stage IV		22 (11)
Stage V		4 (2)
Unspecified		50 (25)

Notes: [1]Numbers and percentages exclude healthy subjects. [2]GFR was used in the determination of renal disease stage.

temperature for two hours. The ruthenium labeled detection MAbs were diluted in assay buffer, added to washed plates, and incubated for an additional two hours at room temperature. Plates were washed, read buffer added, and signals measured using an MSD DISCOVERY WORKBENCH® (Mesoscale Discovery, Gaithersburg, MD). Optimal sample dilutions were: FRA (80-fold dilution of urine and a 20-fold dilution of serum), MSLN (60-fold dilution of urine and an 80-fold dilution of serum), and MPF (4-fold dilution of urine and a 20-fold dilution of serum).

Creatinine analysis. Creatinine concentrations in serum were determined using the QuantiChrom™ Creatinine Assay Kit (BioAssay Systems, Hayward, CA).

Statistical analyses. Pearson's correlation coefficient was performed to determine the correlation between the various biomarkers. Pairwise comparisons of biomarker levels between healthy subjects and stages of renal disease were performed using ANOVA followed by Tukey's post-hoc analysis. Receiver operating characteristic (ROC) analysis was employed to determine the performance of each marker by presence and stage of disease. ROC area under the curve (AUC) calculations were based on 95% confidence intervals. All comparisons were two-sided and a *P*-value ≤0.05 was considered statistically significant except where otherwise stated. Statistical analyses were performed in GraphPad Prism version 6.00 (GraphPad Software, Inc., La Jolla, CA).

Results

The ECL assays for FRA, MSLN, and MPF had intraday variability between 2–16% and excellent sensitivity with lower limits of detection (LLOD) of 1.22, 0.29, and 3.35 pg/mL, for FRA, MSLN, and MPF, respectively.[43]

Matched serum/urine sample pairs from healthy subjects and subjects with renal disease were measured for FRA, MSLN, and MPF using the described ECL assays and for creatinine using a commercial assay. The patient cohort investigated in the present study was limited and skewed toward stage III disease making some comparisons difficult.

Importantly, all three protein markers were readily detected in both serum and urine matrices. A Pearson's correlation matrix for the serum and urinary levels of these protein biomarkers and serum creatinine in both healthy subjects and subjects with renal disease is presented in Table 2. No significant correlation was observed between serum and urine levels for any of the three protein biomarkers, with correlation coefficients of r = −0.02–0.22 (Table 2) in healthy subjects. There was no correlation between serum and urine FRA levels in subjects with renal disease and a moderate to strong correlation between serum and urine MSLN and MPF levels in subjects with renal disease.

As can be seen, in healthy subjects and in subjects with renal disease, serum FRA was moderately correlated with MSLN and MPF and, as expected, MSLN was strongly correlated with MPF since those two proteins derive from the same gene product through proteolytic processing. Creatinine only weakly correlated with FRA, MSLN, or MPF. Further, no significant correlation was noted for any of the three protein biomarkers relative to age or gender (data not shown).

Serum levels of FRA, MSLN, MPF, and creatinine are shown in Table 3 and demonstrate a highly significant

Table 2. Pearson's correlation coefficients[1] between biomarkers in urine (u) and serum (s) from healthy subjects or renal disease subjects.

	HEALTHY SUBJECTS						
BIOMARKER	**sFRA**	**sMSLN**	**sMPF**	**sCREAT**	**uFRA**	**uMSLN**	**uMPF**
sFRA	1	0.68***	0.74****	0.56***	0.02	0.02	0.32**
sMSLN	0.68***	1	0.75****	0.47***	0.04	0.22*	0.38**
sMPF	0.74****	0.75****	1	0.31**	−0.08	0.03	0.12
sCreat	0.56***	0.47***	0.31**	1	0.01	0.22*	0.76****
uFRA	0.02	0.04	−0.08	−0.01	1	0.45***	−0.02
uMSLN	0.02	0.22*	0.03	0.22*	0.45***	1	0.22*
uMPF	0.32**	0.38**	0.12	0.76****	−0.02	0.22*	1
	RENAL DISEASE SUBJECTS						
BIOMARKER	**sFRA**	**sMSLN**	**sMPF**	**sCREAT**	**uFRA**	**uMSLN**	**uMPF**
sFRA	1	0.54***	0.67***	0.68***	0.02	0.30**	0.44***
sMSLN	0. 54***	1	0.82****	0.44***	−0.02	0.36**	0.31**
sMPF	0.67***	0. 82****	1	0.63**	−0.04	0.41***	0.63***
sCreat	0.68***	0.44***	0.63***	1	−0.08	0.19	0.62***
uFRA	0.02	−0.02	−0.04	−0.08	1	0.23*	−0.06
uMSLN	0.30**	0.36**	0.41***	0.19	0.23*	1	0.30**
uMPF	0.44***	0,31**	0.63***	0.62***	−0.06	0.30**	1

Notes: [1]Correlations were classified as follows: ****very strong. ***strong. **moderate. *weak.

Table 3. Serum biomarker levels for healthy subjects and renal disease subjects.

BIOMARKER	STATISTICS	HEALTHY SUBJECTS (N = 100)	RENAL DISEASE SUBJECTS (N = 200)	*P*-VALUE
FRA (pg/mL)	Mean, SD	366.35, 251.39	860.49, 581.81	<0.0001
	Median	313.86	668.07	
	Min, Max	140.47, 2173.20	243.15, 4296.04	
MSLN (pg/mL)	Mean, SD	18999.45, 14238.76	31752.16, 19897.75	<0.0001
	Median	14946.09	26138.54	
	Min, Max	6065.98, 83304.74	7198.91, 132260.13	
MPF (pg/mL)	Mean, SD	2957.77, 3240.11	4716.55, 2746.52	<0.0001
	Median	2343.22	4103.03	
	Min, Max	671.62, 30462.65	1130.17, 18608.72	
Creatinine (mg/dL)	Mean, SD	0.86, 0.73	2.11, 2.26	<0.0001
	Median	0.75	1.36	
	Min, Max	0, 7.09	0, 16.82	

($P < 0.0001$) discrimination between healthy subjects and renal disease subjects, evaluated as a single cohort. These data are shown graphically in Figure 1: FRA (Fig. 1A), MSLN (Fig. 1B), MPF (Fig. 1C), and creatinine (Fig. 1D). Further, urinary levels of FRA (Fig. 2A), MSLN (Fig. 2B), and MPF (Fig. 2C) were also shown to discriminate between subjects with renal disease and healthy subjects with $P = 0.0004$ for FRA and MPF and $P < 0.0001$ for MSLN (Table 4). These data demonstrate that while urinary measurements of these biomarkers are discriminatory, they appear somewhat less sensitive than serum determinations that may reflect inherent difficulties in analysis of urine samples and/or the fact that these were spot urine collections. This is reflected in the ROC analysis of urine data (Fig. 2D), which shows AUC values of: FRA = 0.68 ($P < 0.0001$); MSLN = 0.72 ($P < 0.0001$); and MPF = 0.60 ($P = 0.003$). However, these findings suggest further work is warranted to better understand the urinary excretion of these biomarkers, especially with respect to standardization of urine collections. Although little is known of the biological function of this growth factor, it is interesting to speculate that urinary MPF is not only a reflection of glomerular filtration *per se*, but also of an altered inflammatory environment.

ROC analysis (Fig. 1E) of serum levels of these markers resulted in AUCs of 0.89 ($P < 0.0001$) for FRA, 0.76 ($P < 0.0001$) for MSLN, 0.79 ($P < 0.0001$) for MPF, and 0.88 ($P < 0.0001$) for creatinine. These data suggest that serum determinations of FRA perform similarly to the standard serum creatinine measurements and may therefore be useful in the diagnosis of renal disease. In addition, serum determinations of FRA (Fig. 3A), as well as MSLN (Fig. 3C), MPF (Fig. 3E), and creatinine (Fig. 3G) were shown to increase with increasing stage of disease suggesting that one, or a combination, of markers may be useful in quantifying disease progression. The ROC analyses presented for these four markers (Fig. 3B (FRA), 3D (MSLN), 3F (MPF), and 3H (creatinine)) also support the potential of a combination of markers for evaluation of disease progression as each marker varies in its ability to distinguish between stages of disease. Clearly more work is warranted to further establish the clinical value of a multi-marker panel. Furthermore, FRA (Fig. 3B) and creatinine (Fig. 3H) were able to accurately distinguish stages 3, 4, and 5 of renal disease. MSLN (Fig. 3D) and MPF (Fig. 3F) poorly distinguished stage 3 of renal disease, but were able to distinguish stage 4 and stage 5 of renal disease.

These data suggest that additional studies aimed at combining a number of markers, including those described herein, may yield higher diagnostic accuracy and monitoring potential for renal disease.

Discussion

Creatinine remains the gold standard in the diagnosis and monitoring of impaired kidney function due to acute kidney injury (AKI), CKD, or subsequent to renal transplantation. Nevertheless, creatinine as a diagnostic marker has limitations, with levels being affected by many factors.[3–9] Furthermore, studies have shown that significant renal disease can exist with minimal or no change in creatinine.[9] Owing to these limitations, substantial effort is directed toward the discovery and development of new markers of kidney function, or dysfunction. In the present work, we report preliminary data on three such protein biomarkers, FRA, MSLN, and MPF, measured in both serum and urine samples, to assess their potential value in the diagnosis or monitoring of progression of renal disease in comparison to the standard serum creatinine analysis.

Levels of FRA, MSLN, and MPF were significantly increased in both the serum and urine of subjects with renal disease compared to healthy subjects. Only a weak correlation was observed between serum and urine biomarker values.

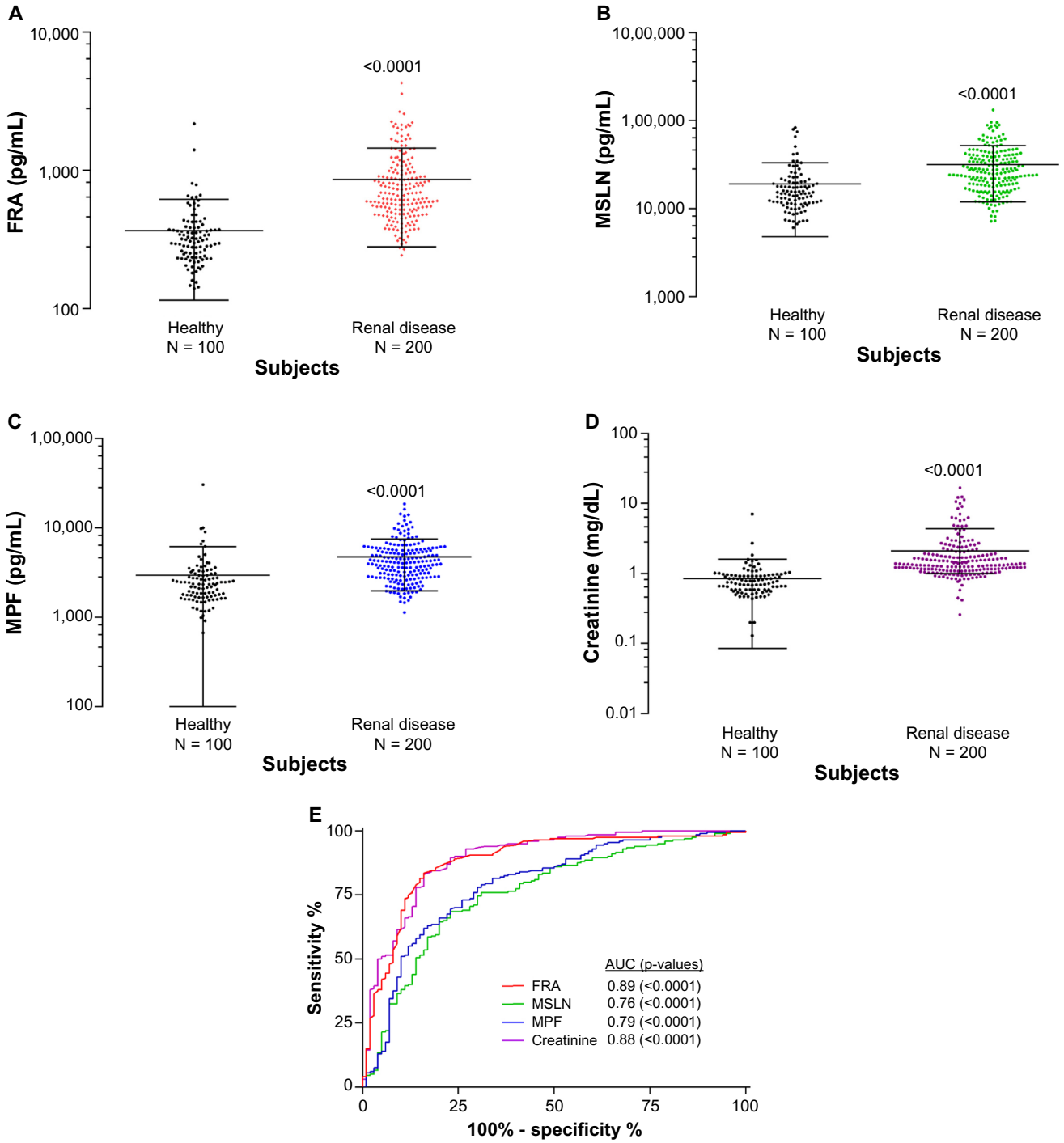

Figure 1. Scatter plots of the serum levels of: (**A**) FRA, (**B**) MSLN, (**C**) MPF, and (**D**) creatinine in healthy subjects and renal disease subjects. Data is plotted on a log scale. The line and error bars depict mean and standard deviation, respectively. *P*-values reflect differences between healthy subjects and renal disease subjects. (**E**) ROC analysis for individual markers: red line, FRA; green line, MSLN; blue line, MPF; purple line, creatinine. AUC values for each ROC are depicted; *P*-values reflect differences between the AUC of .healthy subjects and renal disease subjects for each marker.

The three biomarkers showed moderate to strong correlations with each other in serum, with Pearson's correlation coefficients ranging from 0.68 to 0.75. MSLN correlated well (Pearson's coefficient = 0.75) with MPF, but this is not surprising since these two proteins derive from the same gene product. There is no known biological connection between FRA and MSLN except for the fact that they are both GPI-anchored proteins. As such, it can be speculated that the relatively high correlation between the serum levels of these two proteins reflects a more generalized biologic process related to clearance of these two molecules. While MSLN has been shown to be a binding partner for Muc16 with unknown consequences on its circulatory half-life, FRA has been shown to bind to megalin in both the kidney and liver,

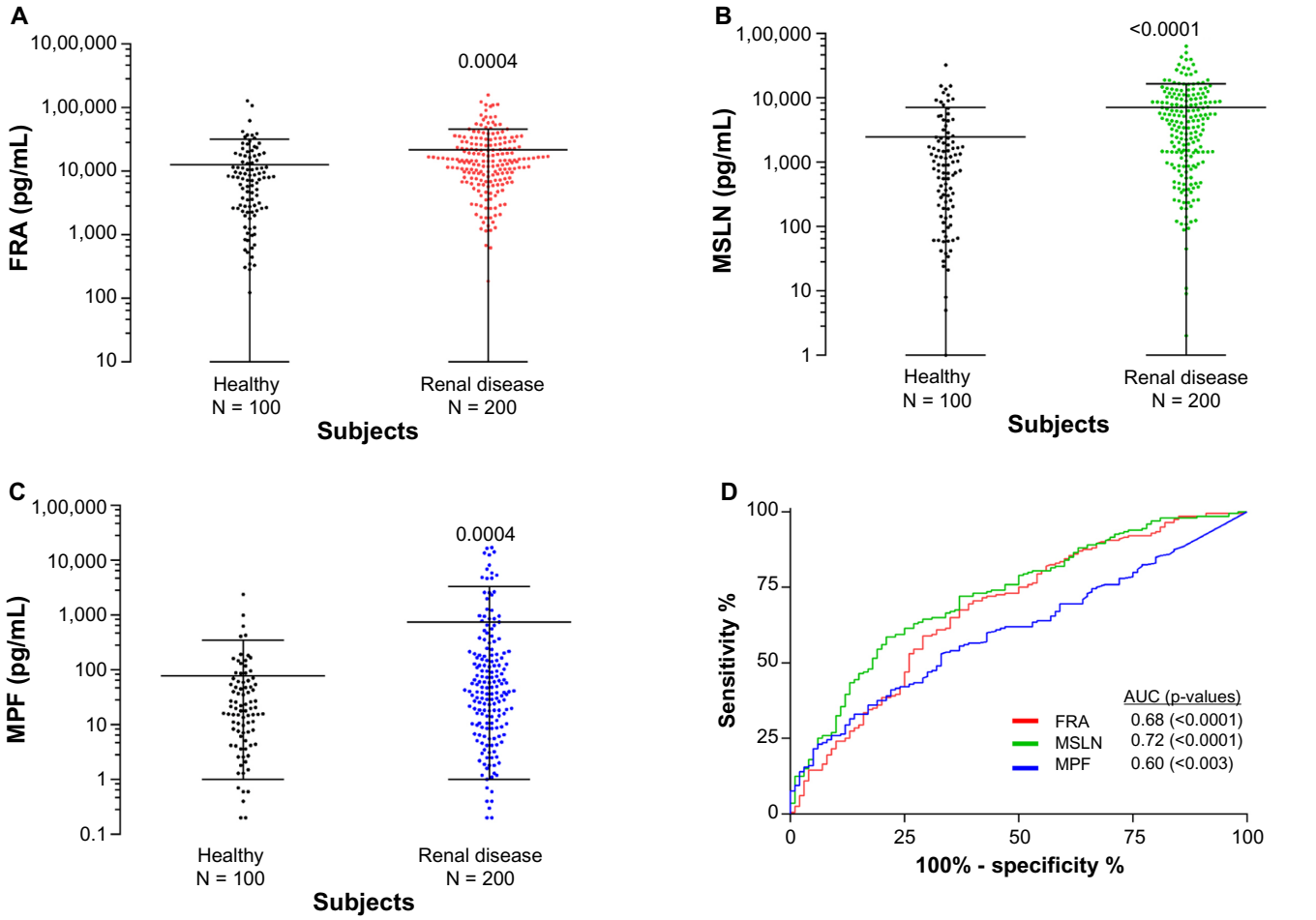

Figure 2. Scatter plots of the urine (spot collection) levels of: (**A**) FRA, (**B**) MSLN, and (**C**) MPF in healthy subjects and renal disease subjects. Data is plotted on a log scale. The line and error bars depict mean and standard deviation, respectively. *P*-values reflect differences between healthy subjects and renal disease subjects. (**D**) ROC analysis for individual markers: red line, FRA; green line, MSLN; and blue line, MPF. AUC values for each ROC are depicted; *P*-values reflect differences between the AUC of healthy subjects and renal disease subjects for each marker.

and may be removed from circulation, impacting its serum half-life.[45] Creatinine was demonstrated to weakly correlate with all three protein biomarkers in serum (Pearson's coefficients = 0.31–0.56), which may lend itself to the development of multi-marker panels. Of note, only weak correlations were observed between the three protein biomarkers in urine. This may be a reflection of not only their glomerular clearance but of the sample and sampling *per se*. Urine samples used in the present study were spot urines collected at the time of venipuncture but otherwise not controlled. Further, no studies were performed relative to potential interfering substances in urine. Be that as it may, it is clear that these three proteins are

Table 4. Urinary biomarker levels for healthy subjects and renal disease subjects.

BIOMARKER	STATISTICS	HEALTHY SUBJECTS (N = 100)	RENAL DISEASE SUBJECTS (N = 200)	*P*-VALUE
FRA (pg/mL)	Mean, SD	12652.86, 18824.77	21658.97, 23872.92	0.0004
	Median	7737.11	14558.71	
	Min, Max	122.71, 127187.14	186.33, 158325.80	
MSLN (pg/mL)	Mean, SD	2479.55, 4555.67	7136.96, 9418.64	<0.0001
	Median	844.44	3993.69	
	Min, Max	0, 32318.28	2.26, 63141.51	
MPF (pg/mL)	Mean, SD	78.22, 267.97	735.89, 2542.52	0.0004
	Median	15.28	32.37	
	Min, Max	0, 2389.48	0, 17164.88	

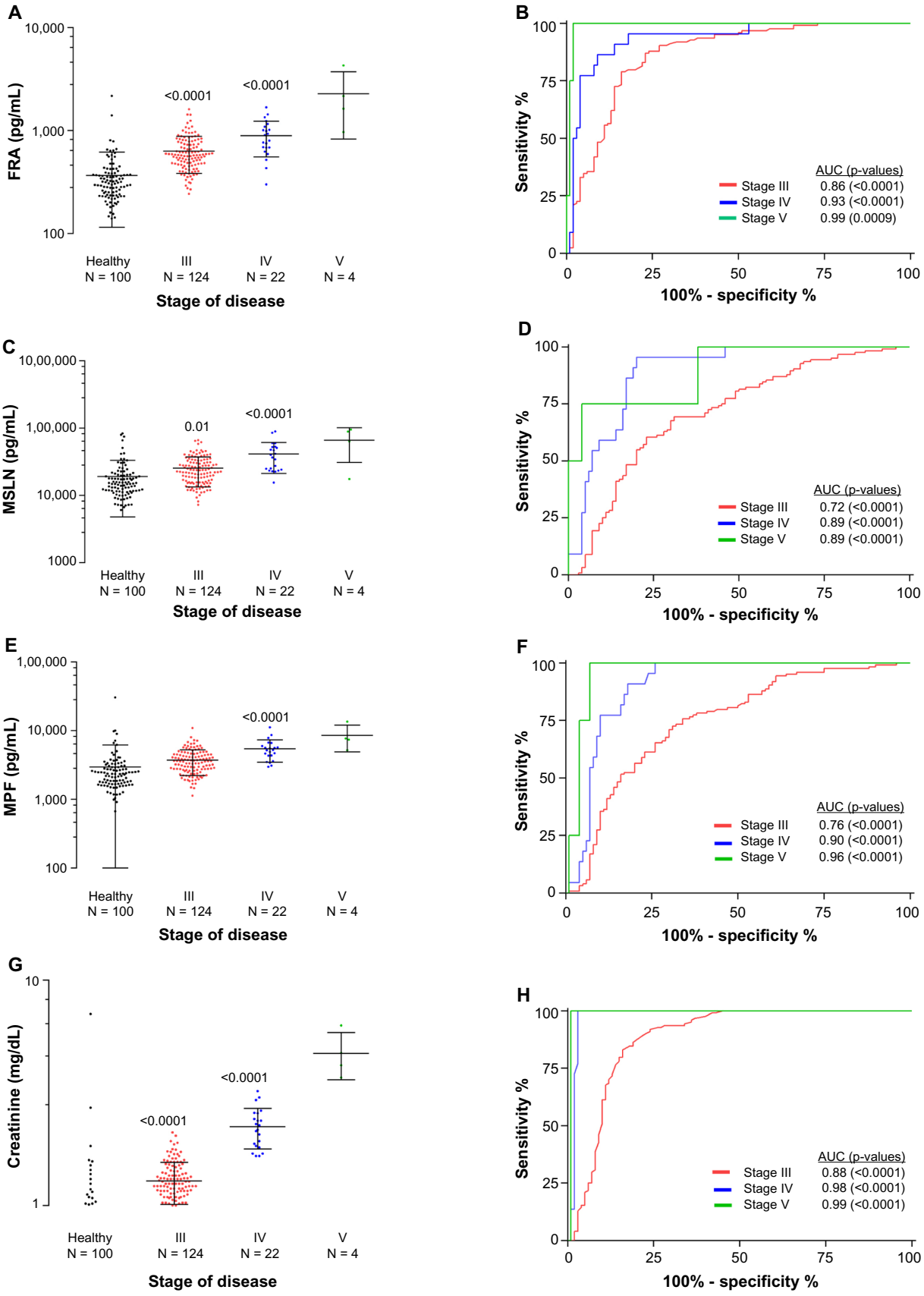

Figure 3. Scatter plots of the serum levels of: (**A**) FRA, (**C**) MSLN, (**E**) MPF, and (**G**) creatinine in healthy subjects and renal disease subjects by stage of disease. Data is plotted on a log scale. The line and error bars depict mean and standard deviation, respectively. *P*-values reflect differences between each stage of renal disease and healthy subjects. ROC curves showing the performance of: (**B**) FRA, (**D**) MSLN, (**F**) MPF, and (**H**) creatinine in discriminating healthy subjects from renal disease subjects by stage (III, red line; IV, blue line; V, green line). AUC values for each ROC are depicted; *P*-values reflect differences between the AUC of healthy subjects and renal disease subjects with various stages of disease.

in fact filtered by the kidneys and may, therefore, lend themselves to non-invasive sampling techniques for the diagnosis and/or monitoring of renal dysfunction. Of particular note is urinary MPF, which is largely undetectable in healthy subjects but can reach quite high levels in subjects with renal disease.

When measured in serum, each of the biomarkers investigated showed significant associations with increasing stage of disease, although the level of significance varied. However, further work is required to validate these proteins as potential biomarkers of value for renal disease progression. Unfortunately, the present study was limited by the distribution of stages within the patient cohort and as such should be considered preliminary in nature. Since FRA is expressed at high levels in the proximal tubules, it is interesting to speculate that the increased levels of FRA observed in the serum of subjects with renal disease may be a direct reflection of tubule damage rather than a consequence of or in addition to decreased glomerular filtration *per se* and further studies are warranted to elucidate the mechanism of increased serum FRA relative to renal disease.

The three protein biomarkers described herein, namely FRA, MSLN, and MPF, have been evaluated as biomarkers in ovarian cancer,[34–37,43] mesothelioma,[34,37–39] pancreatic cancer,[37] and a subset of lung adenocarcinoma.[37] In the present work, serum levels of these three markers are described to be impacted by impaired renal function, thus suggesting their use as diagnostic biomarkers in cancer need to be evaluated cautiously. Further studies are required to elucidate the relationship between serum and urine concentrations of these markers relative to eGFR and disease state, including the possibility that renal disease of different pathologies, eg glomerular versus interstitial, might result in differential levels of one or more of these markers, and importantly, the impact of renal function on the clinical value of these markers in cancer.

Finally, renal disease is a complex family of diseases involving multiple pathophysiological processes. As a result, a number of biomarkers spanning the known causes of/processes related to kidney disease have been investigated to various degrees. These include markers related to impairments in renal function (Cys-C),[46–50] oxidative stress [γ-Glutamyl transpeptidase (GGT)],[51] inflammation or fibrosis [Interleuken (IL)-18],[52] metabolic factors [Apolipoprotein A-IV,[53] fibroblast growth factor-23[54,55]], and damage to the kidney structure [Liver-type fatty acid-binding protein [L-FABP)[56]; kidney injury molecule (KIM)-1[57,58]; neutrophil gelatinase-associated lipocalin (NGAL[59,60])]. Similar to cancer therefore, kidney disease is not only a family of diseases but a disease of multiple etiologies. As such, it is likely that no single biomarker will have the required properties of sensitivity and specificity to be universally applicable to the diagnosis or monitoring of kidney function. Panels of biomarkers may be the only solution to this problem and the markers described herein should be considered potential candidates for inclusion in such a diagnostic panel.

Acknowledgments

The authors wish to acknowledge Lisa Marcucci for performing the assays described herein and Jennifer Venzie for assistance in the preparation of this manuscript.

Author Contributions

ES, DO and JV contributed to the writing of the MS. ES and DO conceived and designed the experiments. DO analyzed the data. DO wrote the first draft of the manuscript. ES and DO contributed to the writing of the manuscript. ES and DO agree with manuscript results and conclusions. ES and DO jointly developed the structure and arguments for the paper. ES and DO made critical revisions and approved final version. All authors reviewed and approved of the final manuscript.

REFERENCES

1. Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis*. 2003;41:1–12.
2. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem*. 1992;38:1933–53.
3. James GD, Sealey JE, Alderman M, et al. A longitudinal study of urinary creatinine and creatinine clearance in normal subjects. Race, sex, and age differences. *Am J Hypertens*. 1988;1:124–31.
4. Baxmann AC, Ahmed MS, Marques NC, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin J Am Soc Nephrol*. 2008;3:348–54.
5. Duncker D, Oswald H, Gardiwal A, et al. Stable cystatin C serum levels confirm normal renal function in patients with dronedarone-associated increase in serum creatinine. *J Cardiovasc Pharmacol Ther*. 2013;18:109–12.
6. Hilbrands LB, Artz MA, Wetzels JF, Koene RA. Cimetidine improves the reliability of creatinine as a marker of glomerular filtration. *Kidney Int*. 1991;40:1171–6.
7. Rocci ML Jr, Vlasses PH, Ferguson RK. Creatinine serum concentrations and H2-receptor antagonists. *Clin Nephrol*. 1984;22:214–5.
8. Kemperman FA, Silberbusch J, Slaats EH, Prins AM, Krediet RT, Arisz L. Follow-up of GFR estimated from plasma creatinine after cimetidine administration in patients with diabetes mellitus type 2. *Clin Nephrol*. 2000;54:255–60.
9. Tomlanovich S, Golbetz H, Perlroth M, Stinson E, Myers BD. Limitations of creatinine in quantifying the severity of cyclosporine-induced chronic nephropathy. *Am J Kidney Dis*. 1986;8:332–7.
10. Weitman SD, Lark RH, Coney LR, et al. Distribution of the folate receptor GP38 in normal and malignant cell lines and tissues. *Cancer Res*. 1992;52:3396–401.
11. Weitman SD, Weinberg AG, Coney LR, Zurawski VR, Jennings DS, Kamen BA. Cellular localization of the folate receptor: potential role in drug toxicity and folate homeostasis. *Cancer Res*. 1992;52:6708–11.
12. O'Shannessy DJ, Somers EB, Albone E, et al. Characterization of the human folate receptor alpha via novel antibody based probes. *Oncotarget*. 2011;2:1227–43.
13. O'Shannessy DJ, Yu G, Smale R, et al. Folate receptor alpha expression in lung cancer: diagnostic and prognostic significance. *Oncotarget*. 2012;3:414–25.
14. Birn H. The kidney in vitamin B12 and folate homeostasis: characterization of receptors for tubular uptake of vitamins and carrier proteins. *Am J Physiol Renal Physiol*. 2006;291:F22-F36.
15. Teschner M, Kosch M, Schaefer RM. Folate metabolism in renal failure. *Nephrol Dial Transplant*. 2002;17S:24–7.
16. Zhu WY, Alliegro MA, Melera PW. The rate of folate receptor alpha (FR alpha) synthesis in folate depleted CHL cells is regulated by a translational mechanism sensitive to media folate levels, while stable overexpression of its mRNA is mediated by gene amplification and an increase in transcript half-life. *J Cell Biochem*. 2001;81:205–19.
17. Antony A, Tang YS, Khan RA, et al. Translational upregulation of folate receptors is mediated by homocysteine via RNA-heterogeneous nuclear ribonucleoprotein E1 interactions. *J Clin Invest*. 2004;113:285–301.
18. Kane MA, Elwood PC, Portillo RM, et al. Influence on immunoreactive folate-binding proteins of extracellular folate concentration in cultured human cells. *J Clin Invest*. 1988;81:1398–406.

19. Henderson GB, Tsuji JM, Kumar HP. Mediated uptake of folate by a high-affinity binding protein in sublines of L1210 cells adapted to nanomolar concentrations of folate. *J Membr Biol*. 1988;101:247–58.
20. Kim HW, Choi YJ, Kim KN, Tamura T, Chang N. Effect of paternal folate deficiency on placental folate content and folate receptor α expression in rats. *Nutr Res Pract*. 2011;5:112–6.
21. Franklin WA, Waintrub M, Edwards D, et al. New anti-lung-cancer antibody cluster 12 reacts with human folate receptors present on adenocarcinoma. *Int J Cancer Suppl*. 1994;8:89–95.
22. Ross JF, Chaudhuri PK, Ratnam M. Differential regulation of folate receptor isoforms in normal and malignant tissues in vivo and in established cell lines. Physiologic and clinical implications. *Cancer*. 1994;73:2432–43.
23. Wu M, Gunning W, Ratnam M. Expression of folate receptor type alpha in relation to cell type, malignancy, and differentiation in ovary, uterus, and cervix. *Cancer Epidemiol Biomarkers Prev*. 1994;8:775–82.
24. Bueno R, Appasani K, Mercer H, Lester S, Sugarbaker D. The alpha folate receptor is highly activated in malignant pleural mesothelioma. *J Thorac Cardiovasc Surg*. 2001;121:225–33.
25. Parker N, Turk MJ, Westrick E, Lewis JD, Low PS, Leamon CP. Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay. *Anal Biochem*. 2005;338:284–93.
26. Shia J, Klimstra DS, Nitzkorski JR, et al. Immunohistochemical expression of folate receptor alpha in colorectal carcinoma: patterns and biological significance. *Hum Pathol*. 2008;39:498–505.
27. Yamaguchi N, Hattori K, Oh-Eda M, Kojima T, Imai N, Ochi N. A novel cytokine exhibiting megakaryocyte potentiating activity from a human pancreatic tumor cell line HPC-Y5. *J Biol Chem*. 1994;269:805–8.
28. Kojima T, Oh-eda M, Hattori K, et al. Molecular cloning and expression of megakaryocyte potentiating factor cDNA. *J Biol Chem*. 1995;270:21984–90.
29. Shiomi K, Miyamoto H, Segawa T, et al. Novel ELISA system for detection of N-ERC/mesothelin in the sera of mesothelioma patients. *Cancer Sci*. 2006;97:928–32.
30. Scholler N, Fu N, Ye Z, Goodman GE, Hellstrom KE, Hellstrom I. Soluble members of the mesothelin/megakaryocyte potentiating factor family are detectable in sera from patients with ovarian cancer. *Proc Natl Acad Sci U S A*. 1999;96:11531–6.
31. Hollevoet K, Nackaerts K, Thimpont J, et al. Diagnostic performance of soluble mesothelin and megakaryocyte potentiating factor in mesothelioma. *Am J Respir Crit Care Med*. 2010;181:620–5.
32. Creaney J, Francis RJ, Dick IM, et al. Serum soluble mesothelin concentrations in malignant pleural mesothelioma: relationship to tumor volume, clinical stage and changes in tumor burden. *Clin Cancer Res*. 2011;17:1181–9.
33. Wheatley-Price P, Yang B, Patsios D, et al. Soluble mesothelin-related peptide and osteopontin as markers of response in malignant mesothelioma. *J Clin Oncol*. 2010;28:3316–22.
34. Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. *Proc Natl Acad Sci U S A*. 1996;93:136–40.
35. Rump A, Morikawa Y, Tanaka M, et al. Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. *J Biol Chem*. 2004;279:9190–8.
36. Gubbels JA, Belisle J, Onda M, et al. Mesothelin-MUC16 binding is a high affinity, N-glycan dependent interaction that facilitates peritoneal metastasis of ovarian tumors. *Mol Cancer*. 2006;5:50.
37. Hassan R, Ho M. Mesothelin targeted cancer immunotherapy. *Eur J Cancer*. 2008;44:46–53.
38. Ordonez NG. Value of mesothelin immunostaining in the diagnosis of mesothelioma. *Mod Pathol*. 2003;16:192–7.
39. Robinson BW, Creaney J, Lake R, et al. Mesothelin-family proteins and diagnosis of mesothelioma. *Lancet*. 2003;362:1612–6.
40. Chang K, Pastan I, Willingham MC. Isolation and characterization of a monoclonal antibody, K1, reactive with ovarian cancers and normal mesothelium. *Int J Cancer*. 1992;50:373–81.
41. Boudville N, Paul R, Robinson BW, Creaney J. Mesothelin and kidney function—analysis of relationship and implications for mesothelioma screening. *Lung Cancer*. 2011;73:320–4.
42. Creaney J, Sneddon S, Dick IM, et al. Comparison of the diagnostic accuracy of the MSLN gene products, mesothelin and megakaryocyte potentiating factor, as biomarkers for mesothelioma in pleural effusions and serum. *Dis Markers*. 2013;35:119–27.
43. O'Shannessy DJ, Somers EB, Palmer LM, et al. Serum folate receptor alpha, mesothelin and megakaryocyte potentiating factor in ovarian cancer: association to disease stage and grade and comparison to CA125 and HE4. *J Ovarian Res*. 2013;6:29.
44. Debad J, Glezer EN, Leland JK, Sigal GB, Wohlstadter J. Clinical and biological applications of ECL. In: Bard AJ ed. *Electrogenerated Chemiluminescence*. New York: Marcel Dekker; 2004:359–96.
45. Birn H, Zhai X, Holm J, et al. Megalin binds and mediates cellular internalization of folate binding protein. *FEBS J*. 2005;272:4423–30.
46. Randers E, Erlandsen EJ. Serum cystatin C as an endogenous marker of the renal function—a review. *Clin Chem Lab Med*. 1999;37:389–95.
47. Madero M, Sarnak MJ, Stevens LA. Serum cystatin C as a marker of glomerular filtration rate. *Curr Opin Nephrol Hypertens*. 2006;15:610–6.
48. Newman DJ, Thakkar H, Edwards RG, et al. Serum cystatin C measured by automated immunoassay: a more sensitive marker of changes in GFR than serum creatinine. *Kidney Int*. 1995;47:312–8.
49. Spanaus KS, Kollerits B, Ritz E, et al. Serum creatinine, cystatin C, and beta-trace protein in diagnostic staging and predicting progression of primary nondiabetic chronic kidney disease. *Clin Chem*. 2010;56:740–9.
50. Stevens LA, Coresh J, Schmid CH, et al. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. *Am J Kidney Dis*. 2008;51:395–406.
51. Teppala S, Shankar A, Li J, Wong TY, Ducatman A. Association between serum gamma-glutamyltransferase and chronic kidney disease among US adults. *Kidney Blood Press Res*. 2010;33:1–6.
52. Parikh CR, Jani A, Melnikov VY, Faubel S, Edelstein CL. Urinary interleukin-18 is a marker of human acute tubular necrosis. *Am J Kidney Dis*. 2004;43:405–14.
53. Boes E, Fliser D, Ritz E, et al. Apolipoprotein A-IV predicts progression of chronic kidney disease: the mild to moderate kidney disease study. *J Am Soc Nephrol*. 2006;17:528–36.
54. Manghat P, Fraser WD, Wierzbicki AS, Fogelman I, Goldsmith DJ, Hampson G. Fibroblast growth factor-23 is associated with C-reactive protein, serum phosphate and bone mineral density in chronic kidney disease. *Osteoporos Int*. 2010;21:1853–61.
55. Isakova T. Fibroblast growth factor 23 and adverse clinical outcomes in chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2012;21:334–40.
56. Nielsen SE, Sugaya T, Hovind P, Baba T, Parving HH, Rossing P. Urinary liver-type fatty acid-binding protein (u-LFABP) predicts progression to nephropathy in type 1 diabetic patients. *Diabetes Care*. 2010;33:1320–4.
57. van Timmeren MM, van den Heuvel MC, Bailly V, Bakker SJ, van Goor H, Stegeman CA. Tubular kidney injury molecule-1 (KIM-1) in human renal disease. *J Pathol*. 2007;212:209–17.
58. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV. Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney Int*. 2002;62:237–44.
59. Malyszko J, Malyszko JS, Bachorzewska-Gajewska H, Poniatowski B, Dobrzycki S, Mysliwiec M. Neutrophil gelatinase-associated lipocalin is a new and sensitive marker of kidney function in chronic kidney disease patients and renal allograft recipients. *Transplant Proc*. 2009;41:158–61.
60. Bolignano D, Lacquaniti A, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. *Clin J Am Soc Nephrol*. 2009;4:337–44.