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(54) Title: MEANS AND METHODS FOR DIAGNOSING A DIABETES MELLITUS ASSOCIATED KIDNEY DAMAGE IN INDIVIDUALS IN NEED OF A SUITABLE THERAPY

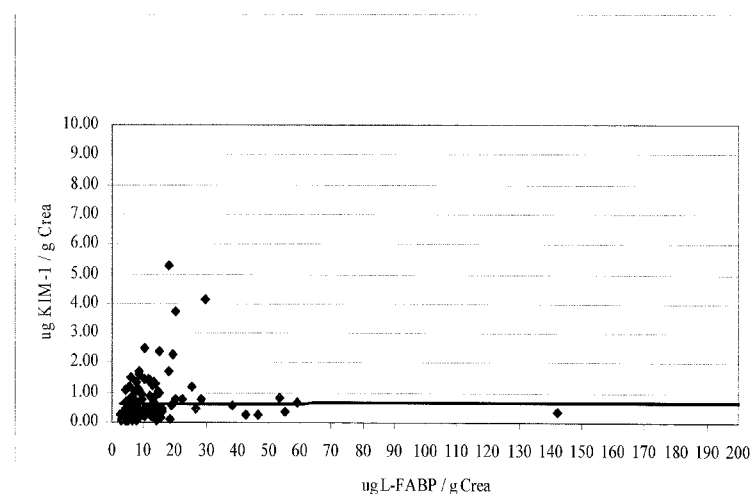


Figure 1

(57) Abstract: The present invention relates to a method for diagnosing kidney damage in a subject suffering from diabetes mellitus comprising the steps of : a) determining the amounts of liver-type fatty acid binding protein (L-FABP) and kidney injury molecule 1 (KIM-1) and optionally adiponectin in a urine-sample of a subject; b) comparing the amounts determined in step a) with reference amounts optionally forming the L-FABP/KIM-1 ratio and diagnosing the kidney damage; c) optionally forming the L-FABP/KIM-1 ratio. Moreover, the present invention relates to a device and a kit for carrying out said method.

5 **Means and methods for diagnosing a diabetes mellitus associated kidney damage in**
 individuals in need of a suitable therapy

The present invention relates to diagnostic methods and means. Specifically, it relates to a method for diagnosing kidney damage preferably chronic kidney damage, more preferably tubular damage and tubular repair and/or glomerular damage, in particular chronic tubular damage and tubular repair and/or chronic glomerular damage, in individuals suffering from diabetes mellitus who are in need of a suitable therapy. Moreover, the present invention relates to devices, kits for carrying out said method and a method of deciding on a suitable therapy in patients suffering from diabetes mellitus associated kidney damage.

There are two main categories of diabetes mellitus (DM) - type 1 and type 2, which can be distinguished by a combination of features known to the person skilled in the art.

20 In type 1 diabetes (previously called juvenile-onset or insulin-dependent diabetes), insulin
production continuously decreases because of autoimmune pancreatic beta-cell destruction,
possibly triggered by environmental exposure in genetically susceptible people.
Destruction progresses subclinically over months or years until beta-cell mass decreases to
the point that insulin concentrations are no longer adequate to control plasma glucose
25 levels. The type 1 diabetes generally develops in childhood or adolescence and until
recently was the most common form diagnosed before age 30; however, it can also develop
in adults.

In type 2 diabetes (previously called adult-onset or non-insulin-dependent diabetes), insulin secretion is inadequate. Often insulin levels are very high, especially early in the disease, but peripheral insulin resistance and increased hepatic production of glucose makes insulin levels inadequate to normalize plasma glucose levels. Insulin production then falls, further exacerbating hyperglycemia. The disease generally develops in adults and becomes more common with age. Plasma glucose levels reach higher levels after food

intake in older than in younger adults, especially after high carbohydrate loads, and take longer to return to normal, in part because of increased accumulation of visceral and abdominal fat and decreased muscle mass.

- 5 Years of poorly controlled diabetes lead to multiple, primarily vascular complications that may affect both small (microvascular) and large (macrovascular) vessels. Microvascular disease underlies the three most common and devastating manifestations of diabetes mellitus: retinopathy, nephropathy, and neuropathy.
- 10 Diabetes can lead to kidney damage or renal disorder. A first hint for kidney damage is the presence of protein in urine (micro- or macroalbuminuria) which can be assessed by simple dip stick. The most common test used to date is still creatinine while acknowledging its missing accuracy.
- 15 Early identification of kidney damage in subjects suffering from diabetes mellitus is highly desirable.

Renal function can be assessed by means of the glomerular filtration rate (GFR). For example, the GFR may be calculated by the Cockcroft-Gault or the MDRD formula

20 (Levey 1999, Annals of Internal Medicine, 461-470). GFR is the volume of fluid filtered from the renal glomerular capillaries into the Bowman's capsule per unit time. Clinically, this is often used to determine renal function. The GFR was originally estimated (the GFR can never be determined, all calculations derived from formulas such as the Cockcroft-Gault formula of the MDRD formula deliver only estimates and not the "real" GFR) by

25 injecting inulin into the plasma. Since inulin is not reabsorbed by the kidney after glomerular filtration, its rate of excretion is directly proportional to the rate of filtration of water and solutes across the glomerular filter. In clinical practice however, creatinine clearance is used to measure GFR. Creatinine is an endogenous molecule, synthesized in the body, which is freely filtered by the glomerulus (but also secreted by the renal tubules

30 in very small amounts). Creatinine clearance (CrCl) is therefore a close approximation of the GFR. The GFR is typically recorded in millilitres per minute (mL/min). The normal range of GFR for males is 97 to 137 mL/min, the normal range of GFR for females is 88 to 128 mL/min.

- 35 GFR is indicative of the kidneys' capacity of water and solutes filtration. A decreased GFR occurs in case of loss of renal tissue (e.g. by necrotic processes). GFR is not indicative for

certain renal disorders e.g. tubular damage. Tubular damage may be present even when GFR is normal.

One of the first hints for kidney damage is the presence of protein in urine (micro- or macroalbuminuria) which can be assessed by simple dip stick. The most common test used to date is still creatinine while acknowledging its missing accuracy.

Remuzzi et al (Kidney International, Vol. 68, Supplement 99 (2005), S57-S65) studied the role of renin-angiotensin-aldosterone system (RAAS) in the progression of chronic kidney disease. Angiotensin II contributes to accelerate renal damage. ACE inhibitors or angiotensin II receptor antagonists can be used in combination to maximize RAAS inhibition and more effectively reduce proteinuria and GFR decline in diabetic and non-diabetic renal disease. Add-on therapy with an aldosterone antagonist may further increase renoprotection.

According to the study of Kollerits et al there is evidence that adiponectin in blood serum may serve as a gender-specific independent predictor of chronic kidney disease progression associated with the metabolic syndrome (Kollerits et al. (2007), Kidney Int. 71(12):1279-86). The role of adiponectin in urine was not studied.

Kamijo et al. (Urinary liver-type fatty acid binding protein as a useful biomarker in chronic kidney disease. Mol. Cell Biochem. 2006; 284) reported that urinary excretion of L-FABP may reflect various kind of stresses that cause tubulointerstitial damage and may be a useful clinical marker of the progression of chronic renal disease.

Van Timmeren et al. (J. Pathol 2007; 212:209-217) reported that tubular kidney injury molecule (KIM-1) is upregulated in renal disease and is associated with renal fibrosis and inflammation. Moreover urinary KIM-1 reflects tissue KIM-1, indicating that it can be used as a non-invasive biomarker in renal disease. One advantage of KIM-1 as a urinary biomarker is the fact that its expression seems to be limited to the dysfunctional kidney (P. Devarajan, Expert Opin. Med, Diagn, (2008) 2(4):387-398).

However, reliable methods for diagnosing kidney damage in individuals suffering from diabetes mellitus who are in need of a suitable therapy have not been described yet.

The technical problem underlying the present invention can be seen as the provision of means and methods for complying with the aforementioned needs. The technical problem is solved by the embodiments characterized in the claims and herein below.

- 5 Accordingly, the present invention relates to a method of diagnosing or detecting kidney damage in a subject suffering from diabetes mellitus, based on the comparison of the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof and kidney injury molecule 1 (KIM-1) or a variant thereof, determined in a sample of said subject, preferably determined in a urine sample of the subject, to at least one reference amount.

10

The method of the present invention may comprise the following steps: a) determining the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof, preferably urinary liver-type fatty acid binding protein (L-FABP), and kidney injury molecule 1 (KIM-1) or a variant thereof, in a sample, preferably a urine-sample of a subject; b) comparing the amounts determined in step a) with reference amounts.

15

The diagnosis or detection of the kidney disease may be established based on the information obtained in step b) and preferably based on the information obtained in a) and b).

- 20 Accordingly, the present invention relates to a method for diagnosing or detecting kidney damage in a subject suffering from diabetes mellitus comprising the steps of:

a) determining the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof and kidney injury molecule 1 (KIM-1) or a variant thereof and optionally adiponectin, preferably in a urine-sample of a subject;

- 25 b) comparing the amounts determined in step a) with reference amounts and

c) diagnosing the kidney damage, based on the comparison in step b).

It is also provided a method for diagnosing or detecting kidney damage in a subject suffering from diabetes mellitus, comprising the steps of:

- 30 a) determining the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof and kidney injury molecule 1 (KIM-1) or a variant thereof in a urinary sample of a subject;

b) comparing the amounts determined in step a) with reference amounts;

whereby the kidney damage is diagnosed or wherein the comparison of the determined amounts with the reference amounts is indicative of the patient to suffer from kidney damage

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In a preferred embodiment of the present invention, in an optional step prior aa) to step b) of comparison, the L-FABP/KIM-1 ratio is formed. The kidney damage is then detected or diagnosed based on the ratio formed in the optional step aa).

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Moreover, the present invention relates to a method of deciding on a suitable therapy in a patient suffering from diabetes mellitus associated kidney damage, a device and a kit for carrying out said method.

10 The method of the present invention is, preferably, an in vitro method. Moreover, it may comprise steps in addition to those explicitly mentioned above where appropriate. For example, further steps may relate to sample pre-treatments or evaluation of the results obtained by the method.

15 Diagnosing as used herein refers to assessing the probability according to which a subject suffers from the diseases referred to in this specification. As will be understood by those skilled in the art, such an assessment is usually not intended to be correct for 100% of the subjects to be diagnosed. The term, however, requires that a statistically significant portion of subjects can be diagnosed to suffer from the said disease (e.g. a cohort in a cohort
20 study). Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student's t-test, Mann-Whitney test etc.. Details are found in Dowdy and Wearden, Statistics for Research, John Wiley & Sons, New York 1983. Preferred confidence intervals are at least 90%, at least
25 95%, at least 97%, at least 98% or at least 99 %. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001.

Diagnosing as used herein preferably refers to analyzing and where appropriate monitoring of the relevant disease. In particular, diagnosing means analyzing the pathology of specific
30 parts of an organ e.g. glomerulus and tubulus of the kidney, in particular of the tubules and glomerules and estimating the extent of damage and repair, in particular of the tubulus and glomerulus. Monitoring relates to keeping track of the already diagnosed disease, in particular to analyze the progression of the disease or the influence of a particular treatment on the progression of disease. Most preferably, diagnosing relates to analyzing
35 the pathology of tubules in the kidney and estimating the extent of damage and repair in the tubules.

The term “subject” as used herein relates to animals, preferably mammals, and, more preferably, humans. However, it is envisaged by the present invention that the subject shall be suffering from diabetes mellitus type 1 or type 2 as specified hereinafter. Except for the diabetes mellitus and kidney damage, the subject, preferably, shall be apparently healthy, in particular with respect to kidney function (based on the upper limit for serum creatinine).

The terms “kidney damage”, “kidney disease” or “renal disorders” are well known to the person skilled in the art.

10

In this context, the term "renal disorder" is considered to relate, preferably, to any dysfunction of the kidney or any dysfunction affecting the capacity of the kidney for waste removal and/or ultrafiltration, in particular any impairment of kidney function as determined by methods known to the person skilled in the art, preferably by GFR and/or creatinine clearance. Examples for renal disorders include congenital disorders and acquired disorders. Examples for congenital renal disorders include congenital hydronephrosis, congenital obstruction of urinary tract, duplicated ureter, horseshoe kidney, polycystic kidney disease, renal dysplasia, unilateral small kidney. Examples for acquired renal disorders include diabetic or analgesic nephropathy, glomerulonephritis, hydronephrosis (the enlargement of one or both of the kidneys caused by obstruction of the flow of urine), interstitial nephritis, kidney stones, kidney tumors (e.g. Wilms tumor and renal cell carcinoma), lupus nephritis, minimal change disease, nephrotic syndrome (the glomerulus has been damaged so that a large amount of protein in the blood enters the urine. Other frequent features of the nephrotic syndrome include swelling, low serum albumin, and high cholesterol), pyelonephritis, renal failure (e.g. acute renal failure and chronic renal failure).

In a preferred embodiment of the present invention, the terms “kidney damage” and “kidney disease” exclude any dysfunction of the kidney or any dysfunction affecting the capacity of the kidney for waste removal and/or ultrafiltration, in particular any impairment of kidney function as determined by methods known to the person skilled in the art, preferably by GFR and/or creatinine clearance. The terms in particular exclude congenital hydronephrosis, congenital obstruction of urinary tract, duplicated ureter, horseshoe kidney, polycystic kidney disease, renal dysplasia, unilateral small kidney, diabetic or analgesic nephropathy, glomerulonephritis, hydronephrosis, interstitial nephritis, kidney stones, kidney tumors (e.g. Wilms tumor and renal cell carcinoma), lupus nephritis, minimal change disease, nephrotic syndrome (swelling, low serum albumin, and

high cholesterol), pyelonephritis, renal failure, in particular acute kidney injury (acute renal failure) and chronic kidney disease (chronic renal failure) and cardiorenal syndrome. The terms “kidney damage” and “kidney disease” in particular refer to tubular damage optionally associated with tubular repair, and glomerular damage. Tubular damage, optionally associated with tubular repair, is also referred to as “progressive tubular disease” in the context of the present invention. Tubular damage, optionally associated with tubular repair, in connection with glomerular damage, is also referred to as progressive tubular and glomerular damage of the kidney. As in the context of the present invention subjects which suffer from diabetes mellitus are diagnosed, tubular damage and/or tubular repair are also referred to as “diabetes mellitus associated kidney damage”.

Renal disorders can be diagnosed by means known to the person skilled in the art. Particularly, renal function (which is used interchangeably with “kidney function” in the context of the present invention) can be assessed by means of the glomerular filtration rate (GFR). For example, the GFR may be calculated by the Cockcroft-Gault or the MDRD formula (Levey 1999, Annals of Internal Medicine, 461-470). GFR is the volume of fluid filtered from the renal glomerular capillaries into the Bowman's capsule per unit time. Clinically, this is often used to determine renal function. The GFR was originally estimated (the GFR can never be determined, all calculations derived from formulas such as the Cockcroft Gault formula of the MDRD formula deliver only estimates and not the “real” GFR) by injecting inulin into the plasma. Since inulin is not reabsorbed by the kidney after glomerular filtration, its rate of excretion is directly proportional to the rate of filtration of water and solutes across the glomerular filter. In clinical practice however, creatinine clearance is used to measure GFR. Creatinine is an endogenous molecule, synthesized in the body, which is freely filtered by the glomerulus (but also secreted by the renal tubules in very small amounts). Creatinine clearance (CrCl) is therefore a close approximation of the GFR. The GFR is typically recorded in millilitres per minute (mL/min). The normal range of GFR for males is 97 to 137 mL/min, the normal range of GFR for females is 88 to 128 mL/min.

GFR is indicative of the kidneys' capacity of water and solutes filtration. A decreased GFR occurs in case of loss of renal tissue (e.g. by necrotic processes). GFR is not indicative for certain renal disorders e.g. tubular damage. Tubular damage may be present even when GFR is normal.

One of the first hints for renal disorder is the presence of protein in urine (micro- or macroalbuminuria) which can be assessed by simple dip stick. The most common test used to date is still creatinine while acknowledging its missing accuracy.

- 5 Chronic kidney disease (CKD) and acute kidney injury (AKI) are known to the person skilled in the art and generally recognized as referring to renal failure as determined by GFR or creatinine clearance.

10 CKD is known as a loss of renal function which may worsen over a period of months or even years. The symptoms of worsening renal function are unspecific. In CKD glomerular filtration rate is significantly reduced, resulting in a decreased capability of the kidneys to excrete waste products by water and solute filtration. Creatinine levels may be normal in the early stages of CKD. CKD is not reversible. The severity of CKD is classified in five stages, with stage 1 being the mildest and usually causing few symptoms. Stage 5
15 constitutes a severe illness including poor life expectancy and is also referred to as end-stage renal disease (ESRD), chronic kidney failure (CKF) or chronic renal failure (CRF).

Acute kidney injury (AKI), previously also referred to as acute renal failure (ARF), is a rapid loss of kidney function which may originate from various reasons, including low
20 blood volume and exposure to toxins. Contrary to CKD, AKI can be reversible. AKI is diagnosed on the basis of creatinine levels, urinary indices like blood urea nitrogen (BUN), occurrence of urinary sediment, but also on clinical history. A progressive daily rise in serum creatinine is considered diagnostic of ARF.

25 The term “cardiorenal syndrome” (also “CRS”) as used in the context of the present invention is to be understood in the sense of the definition established by Ronco et al, in Intensive Care Med. 2008, 34, pages 957-962 and in J. Am. Coll. Cardiol. 2008, 52, p. 1527 - 1539. Accordingly, CRS refers, in the broadest sense, to a pathophysiologic disorder of the heart and kidneys whereby acute or chronic dysfunction of one of the cited
30 organs may induce acute or chronic dysfunction of the other. The most simple description of CRS is that a relatively normal kidney is dysfunctional because of a diseased heart, assuming that in the presence of a healthy heart, the same kidney would perform normally. 5 subtypes of CRS exist. Type 1 CRS reflects an abrupt worsening of cardiac function (e.g., acute cardiogenic shock or decompensated congestive heart failure) leading to acute
35 kidney injury. Type 2 CRS comprises chronic abnormalities in cardiac function (e.g., chronic congestive heart failure) causing progressive chronic kidney disease. Type 3 CRS consists of an abrupt worsening of renal function (e.g., acute kidney ischemia or

glomerulonephritis) causing acute cardiac dysfunction (e.g., heart failure, arrhythmia, ischemia). Type 4 CRS describes a state of chronic kidney disease (e.g., chronic glomerular disease) contributing to decreased cardiac function, cardiac hypertrophy, and/or increased risk of adverse cardiovascular events.

5

In the context of the present invention, the term „tubular damage“ refers to epithelial injury in tubule cells as a consequence of diabetes mellitus. The present invention preferably refers to chronic tubular damage. It is believed that in tubular damage tubule cells are ischemic following diabetes mellitus, but it is also believed that tubules have retained their functionality within the kidney entirely or at least to the greatest or a great part. This means that renal function is not impaired or only impaired to a lesser extent, such that CKD or AKI will not or cannot be diagnosed by the methods known in the art, i.e GFR and/or creatinine clearance. In tubular damage, tubule cells may become dysfunctional, in general by necrosis, and die. However, tubular epithelium regeneration is possible after ischemia and even after necrosis, referred to as „tubular repair“ in the context of the present invention. As the present invention preferably refers to chronic tubular injury, it likewise refers to chronic tubular repair or tubular repair from chronic tubular damage.

In the context of the present invention, the term „apparently healthy“ is known to the person skilled in the art and refers to a subject which does not show obvious signs of an underlying renal disorder. The disorder here is an impaired kidney function, in particular in respect to GFR, for example based on creatinine clearance, in particular its upper limit. The subject, thus, may suffer from an impaired kidney function as defined beforehand, but does not show obvious signs such that the impaired kidney function cannot be diagnosed or assessed without detailed diagnostic examination by a physician. In particular, the diagnosis by a specialist (here: a nephrologist) would be required to diagnose impaired kidney function in the apparently healthy subject not showing obvious symptoms of the disease.

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The term “apparently healthy” as used in the context of the present invention, accordingly, is restricted to individuals not showing obvious signs of an impaired kidney function (i.e. of a dysfunction of the kidney) or not having an impaired kidney function (i.e. of a dysfunction of the kidney). An apparently healthy individual, as understood in the context of the present invention, may however suffer from one or more pathophysiological states of the kidney in which kidney function is not impaired, or in which kidney function is not impaired at the onset of the respective disease but which may lead an impaired kidney

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function. The individual may suffer from microalbuminuria, albuminuria and/or proteinuria and/or any pathophysiological state associated therewith. The individual may also suffer from glomerular damage and/or any pathophysiological state associated therewith. These pathophysiological states are known to the person skilled in the art and include disease associated with glomerular syndromes, preferably: acute nephritic syndromes, in particular glomerulonephritis, nephropathy; nephrotic syndromes, in particular minimal change disease, glomerulosclerosis, glomerulonephritis, diabetic nephropathy; and glomerular vascular syndromes, in particular atherosclerotic nephropathy, hypertensive nephropathy.

The terms „diabetes mellitus type 1” and „diabetes mellitus type 2“ have been described in the introductory part of this application and are known to a person skilled in the art.

In type 1 diabetes (previously called juvenile-onset or insulin-dependent diabetes), insulin production continuously decreases because of autoimmune pancreatic beta-cell destruction, possibly triggered by environmental exposure in genetically susceptible people. Destruction progresses subclinically over months or years until beta-cell mass decreases to the point that insulin concentrations are no longer adequate to control plasma glucose levels. The type 1 diabetes generally develops in childhood or adolescence and until recently was the most common form diagnosed before age 30; however, it can also develop in adults.

In type 2 diabetes (previously called adult-onset or non-insulin-dependent diabetes), insulin secretion is inadequate. Often insulin levels are very high, especially early in the disease, but peripheral insulin resistance and increased hepatic production of glucose makes insulin levels inadequate to normalize plasma glucose levels. Insulin production then falls, further exacerbating hyperglycemia. The disease generally develops in adults and becomes more common with age. Plasma glucose levels reach higher levels after food intake in older than in younger adults, especially after high carbohydrate loads, and take longer to return to normal, in part because of increased accumulation of visceral and abdominal fat and decreased muscle mass.

The term “liver-type fatty acid binding protein” (L-FABP, frequently also referred to as FABP1 herein also referred to as liver fatty acid binding protein) relates to a polypeptide being a liver type fatty acid binding protein and to a variant thereof. Liver-type fatty acid binding protein is an intracellular carrier protein of free fatty acids that is expressed in the proximal tubules of the human kidney. For a sequence of human L-FABP, see e.g. Chan et

al.: Human liver fatty acid binding protein cDNA and amino acid sequence, Functional and evolutionary implications, J. Biol. Chem. 260 (5), 2629-2632 (1985) or GenBank Acc. Number M10617.1.

- 5 As L-FABP is preferably determined in a urine sample of the respective subject, it may also be referred to, in the context of the present invention, as “urinary liver-type fatty acid binding protein” or “urinary” L-FABP.

The term “L-FABP” encompasses also variants of L-FABP, preferably, of human L-FABP.

- 10 Such variants have at least the same essential biological and immunological properties as L-FABP, i.e. they bind free fatty acids and/or cholesterol and/or retinoids, and/or are involved in intracellular lipid transport. In particular, they share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA Assays using polyclonal or monoclonal
- 15 antibodies specifically recognizing the L-FABP. Moreover, it is to be understood that a variant as referred to in accordance with the present invention shall have an amino acid sequence which differs due to at least one amino acid substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least 50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% identical with the amino sequence of the
- 20 human L-FABP, preferably over the entire length of human L-FABP. How to determine the degree of identity is specified elsewhere herein. Variants may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of L-FABP or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to
- 25 above. Such fragments may be, e.g., degradation products of the L-FABP. Further included are variants which differ due to posttranslational modifications such as phosphorylation or myristylation. The term “L-FABP or a variant thereof”, preferably, does not include heart FABP, brain FABP and intestine FABP.

- 30 Adiponectin is a polypeptide (one of several known adipocytokines) secreted by the adipocyte. In the art, adiponectin is frequently also referred to as Acrp30 and apM1. Adiponectin has recently been shown to have various activities such as anti-inflammatory, antiatherogenic, preventive for metabolic syndrome, and insulin sensitizing activities. Adiponectin is encoded by a single gene, and has 244 amino acids, its molecular weight is
- 35 approximately 30 kilodaltons. The mature human adiponectin protein encompasses amino acids 19 to 244 of full-length adiponectin. A globular domain is thought to encompass

amino acids 107 – 244 of full-length adiponectin. The sequence of the adiponectin polypeptide is well known in the art, and, e.g., disclosed in WO/2008/084003.

5 Adiponectin is the most abundant adipokine secreted by adipocytes. Adipocytes are endocrine secretory cells which release free fatty acids and produce, in addition to adiponectin, several cytokines such as Tumor necrosis factor (TNF) alpha, leptin, and interleukins.

10 It is generally assumed that adiponectin sensitizes the body to insulin. Decreased adiponectin blood levels are observed in patients with diabetes and metabolic syndrome and are thought to play a key role in insulin resistance (see e.g. Han et al. Journal of the American College of Cardiology, Vol. 49(5)531-8).

15 Adiponectin associates itself into larger structures. Three adiponectin polypeptides bind together and form a homotrimer. These trimers bind together to form hexamers or dodecamers. Adiponectin is known to exist in a wide range of multimer complexes in plasma and combines via its collagen domain to create 3 major oligomeric forms: a low-molecular weight (LMW) trimer, a middle-molecular weight (MMW) hexamer, and high-molecular weight (HMW) 12- to 18-mer adiponectin (Kadowaki et al. (2006) Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest. 116(7): 1784–1792; Rexford S. Ahima, Obesity 2006;14:242S-249S). Adiponectin has been reported to have several physiological actions, such as protective activities against atherosclerosis, improvement of insulin sensitivity, and prevention of hepatic fibrosis.

25 Adiponectin as used herein, preferably, relates to total adiponectin, which encompasses low molecular weight adiponectin, mid molecular weight adiponectin and high molecular weight adiponectin. The terms high molecular weight adiponectin, low and mid molecular weight adiponectin and total adiponectin are understood by the skilled person. Preferably, said adiponectin is human adiponectin. Methods for the determination of adiponectin are, e.g., disclosed in US 2007/0042424 A1 as well as in WO/2008/084003. The amount of adiponectin is determined in a urine sample.

35 The adiponectin referred to in accordance with the present invention further encompasses allelic and other variants of said specific sequence for human adiponectin discussed above. Specifically, envisaged are variant polypeptides which are on the amino acid level preferably, at least 50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99%

identical, to human adiponectin, preferably over the entire length of human adiponectin. The degree of identity between two amino acid sequences can be determined by algorithms well known in the art. Preferably, the degree of identity is to be determined by comparing two optimally aligned sequences over a comparison window, where the fragment of amino acid sequence in the comparison window may comprise additions or deletions (e.g., gaps or overhangs) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Add. APL. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (USA)* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, PASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by visual inspection. Given that two sequences have been identified for comparison, GAP and BESTFIT are preferably employed to determine their optimal alignment and, thus, the degree of identity. Preferably, the default values of 5.00 for gap weight and 0.30 for gap weight length are used. Variants referred to above may be allelic variants or any other species specific homologs, paralogs, or orthologs. Substantially similar and also envisaged are proteolytic degradation products which are still recognized by the diagnostic means or by ligands directed against the respective full-length peptide. Also encompassed are variant polypeptides having amino acid deletions, substitutions, and/or additions compared to the amino acid sequence of human adiponectin as long as the said polypeptides have adiponectin properties.

The term "kidney injury molecule-1" (KIM-1) relates to a type 1 membrane protein containing a unique six-cysteine Ig domain and a mucin domain in its extracellular portion. KIM-1 which is the sequence of rat 3-2 cDNA contains an open reading frame of 307 amino acids.

The protein sequence of human cDNA clone 85 also contains one Ig, mucin, transmembrane, and cytoplasmic domain each as rat KIM-1. All six cysteines within the Ig domains of both proteins are conserved. Within the Ig domain, the rat Kim-1 and human cDNA clone 85 exhibit 68.3% similarity in the protein level. The mucin domain is longer,

and the cytoplasmic domain is shorter in clone 85 than rat KIM-1, with similarity of 49.3 and 34.8% respectively. Clone 85 is referred to as human KIM-1 (for the structure of KIM-1 proteins see e.g. Ichimura et al., *J Biol Chem*, 273 (7), 4135-4142 (1998), in particular Fig. 1). Recombinant human KIM-1 exhibits no cross-reactivity or interference to recombinant rat- or mouse-KIM-1.

KIM-1 mRNA and protein are expressed in high levels in regenerating proximal tubule epithelial cells which cells are known to repair and regenerate the damaged region in the postischemic kidney. KIM-1 is an epithelial cell adhesion molecule (CAM) up-regulated in the cells, which are dedifferentiated and undergoing replication after renal epithelial injury. A proteolytically processed domain of KIM-1 is easily detected in the urine soon after acute kidney injury (AKI) so that KIM-1 performs as an acute kidney injury urinary biomarker (*Expert Opin. Med. Diagn.* (2008) **2** (4): 387-398).

KIM-1 referred to in accordance with the present invention further encompasses allelic and other variants of said specific sequence for human KIM-1 discussed above. Specifically, envisaged are variant polypeptides which are on the amino acid level preferably, at least 50%, 60%, 70%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% identical, to human KIM-1, preferably over the entire length of human KIM-1. The degree of identity between two amino acid sequences can be determined by algorithms well known in the art. Preferably, the degree of identity is to be determined by comparing two optimally aligned sequences over a comparison window, where the fragment of amino acid sequence in the comparison window may comprise additions or deletions (e.g., gaps or overhangs) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Add. APL. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (USA)* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, PASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by visual inspection. Given that two sequences have been identified for comparison, GAP and BESTFIT are preferably employed to determine their optimal alignment and, thus, the

degree of identity. Preferably, the default values of 5.00 for gap weight and 0.30 for gap weight length are used. Variants referred to above may be allelic variants or any other species specific homologs, paralogs, or orthologs. Substantially similar and also envisaged are proteolytic degradation products which are still recognized by the diagnostic means or by ligands directed against the respective full-length peptide. Also encompassed are variant polypeptides having amino acid deletions, substitutions, and/or additions compared to the amino acid sequence of human KIM-1 as long as the said polypeptides have KIM-1 properties. "KIM-1 properties" as used in the context of the present invention refers to inducing dedifferentiation and replication after renal epithelial injury.

Determining the amount of adiponectin, L-FABP or a variant thereof, KIM-1 or a variant thereof or any other peptide or polypeptide referred to in this specification relates to measuring the amount or concentration, preferably semi-quantitatively or quantitatively. Measuring can be done directly or indirectly. Direct measuring relates to measuring the amount or concentration of the peptide or polypeptide based on a signal which is obtained from the peptide or polypeptide itself and the intensity of which directly correlates with the number of molecules of the peptide present in the sample. Such a signal – sometimes referred to herein as intensity signal – may be obtained, e.g., by measuring an intensity value of a specific physical or chemical property of the peptide or polypeptide. Indirect measuring includes measuring of a signal obtained from a secondary component (i.e. a component not being the peptide or polypeptide itself) or a biological read out system, e.g., measurable cellular responses, ligands, labels, or enzymatic reaction products.

In accordance with the present invention, determining the amount of a peptide or polypeptide can be achieved by all known means for determining the amount of a peptide in a sample. Said means comprise immunoassay devices and methods which may utilize labeled molecules in various sandwich, competition, or other assay formats. Said assays will develop a signal which is indicative for the presence or absence of the peptide or polypeptide. Moreover, the signal strength can, preferably, be correlated directly or indirectly (e.g. reverse- proportional) to the amount of polypeptide present in a sample. Further suitable methods comprise measuring a physical or chemical property specific for the peptide or polypeptide such as its precise molecular mass or NMR spectrum. Said methods comprise, preferably, biosensors, optical devices coupled to immunoassays, biochips, analytical devices such as mass- spectrometers, NMR- analyzers, or chromatography devices. Further, methods include micro-plate ELISA-based methods, fully-automated or robotic immunoassays (available for example on ElecsysTM analyzers), CBA (an enzymatic Cobalt Binding Assay, available for example on Roche-HitachiTM

analyzers), and latex agglutination assays (available for example on Roche-HitachiTM analyzers).

Preferably, determining the amount of a peptide or polypeptide comprises the steps of (α)
5 contacting a cell capable of eliciting a cellular response the intensity of which is indicative
of the amount of the peptide or polypeptide with the said peptide or polypeptide for an
adequate period of time, (β) measuring the cellular response. For measuring cellular
responses, the sample or processed sample is, preferably, added to a cell culture and an
internal or external cellular response is measured. The cellular response may include the
10 measurable expression of a reporter gene or the secretion of a substance, e.g. a peptide,
polypeptide, or a small molecule. The expression or substance shall generate an intensity
signal which correlates to the amount of the peptide or polypeptide.

Also preferably, determining the amount of a peptide or polypeptide comprises the step of
15 measuring a specific intensity signal obtainable from the peptide or polypeptide in the
sample. As described above, such a signal may be the signal intensity observed at an m/z
variable specific for the peptide or polypeptide observed in mass spectra or a NMR
spectrum specific for the peptide or polypeptide.

20 Determining the amount of a peptide or polypeptide may, preferably, comprise the steps of
(α) contacting the peptide with a specific ligand, (optionally) removing non-bound ligand,
(β) measuring the amount of bound ligand. The bound ligand will generate an intensity
signal. Binding according to the present invention includes both covalent and non-covalent
binding. A ligand according to the present invention can be any compound, e.g., a peptide,
25 polypeptide, nucleic acid, or small molecule, binding to the peptide or polypeptide
described herein. Preferred ligands include antibodies, nucleic acids, peptides or
polypeptides such as receptors or binding partners for the peptide or polypeptide and
fragments thereof comprising the binding domains for the peptides, and aptamers, e.g.
nucleic acid or peptide aptamers. Methods to prepare such ligands are well-known in the
30 art. For example, identification and production of suitable antibodies or aptamers is also
offered by commercial suppliers. The person skilled in the art is familiar with methods to
develop derivatives of such ligands with higher affinity or specificity. For example,
random mutations can be introduced into the nucleic acids, peptides or polypeptides. These
derivatives can then be tested for binding according to screening procedures known in the
35 art, e.g. phage display. Antibodies as referred to herein include both polyclonal and
monoclonal antibodies, as well as fragments thereof, such as Fv, Fab and F(ab)₂ fragments
that are capable of binding antigen or hapten. The present invention also includes single

chain antibodies and humanized hybrid antibodies wherein amino acid sequences of a non-human donor antibody exhibiting a desired antigen-specificity are combined with sequences of a human acceptor antibody. The donor sequences will usually include at least the antigen-binding amino acid residues of the donor but may comprise other structurally and/or functionally relevant amino acid residues of the donor antibody as well. Such hybrids can be prepared by several methods well known in the art. Preferably, the ligand or agent binds specifically to the peptide or polypeptide. Specific binding according to the present invention means that the ligand or agent should not bind substantially to ("cross-react" with) another peptide, polypeptide or substance present in the sample to be analyzed. Preferably, the specifically bound peptide or polypeptide should be bound with at least 3 times higher, more preferably at least 10 times higher and even more preferably at least 50 times higher affinity than any other relevant peptide or polypeptide. Non-specific binding may be tolerable, if it can still be distinguished and measured unequivocally, e.g. according to its size on a Western Blot, or by its relatively higher abundance in the sample. Binding of the ligand can be measured by any method known in the art. Preferably, said method is semi-quantitative or quantitative. Suitable methods are described in the following.

First, binding of a ligand may be measured directly, e.g. by NMR or surface plasmon resonance.

Second, if the ligand also serves as a substrate of an enzymatic activity of the peptide or polypeptide of interest, an enzymatic reaction product may be measured (e.g. the amount of a protease can be measured by measuring the amount of cleaved substrate, e.g. on a Western Blot). Alternatively, the ligand may exhibit enzymatic properties itself and the "ligand/peptide or polypeptide" complex or the ligand which was bound by the peptide or polypeptide, respectively, may be contacted with a suitable substrate allowing detection by the generation of an intensity signal. For measurement of enzymatic reaction products, preferably the amount of substrate is saturating. The substrate may also be labeled with a detectable label prior to the reaction. Preferably, the sample is contacted with the substrate for an adequate period of time. An adequate period of time refers to the time necessary for a detectable, preferably measurable, amount of product to be produced. Instead of measuring the amount of product, the time necessary for appearance of a given (e.g. detectable) amount of product can be measured.

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Third, the ligand may be coupled covalently or non-covalently to a label allowing detection and measurement of the ligand. Labeling may be done by direct or indirect methods. Direct

labeling involves coupling of the label directly (covalently or non-covalently) to the ligand. Indirect labeling involves binding (covalently or non-covalently) of a secondary ligand to the first ligand. The secondary ligand should specifically bind to the first ligand. Said secondary ligand may be coupled with a suitable label and/or be the target (receptor) of tertiary ligand binding to the secondary ligand. The use of secondary, tertiary or even higher order ligands is often used to increase the signal. Suitable secondary and higher order ligands may include antibodies, secondary antibodies, and the well-known streptavidin-biotin system (Vector Laboratories, Inc.). The ligand or substrate may also be "tagged" with one or more tags as known in the art. Such tags may then be targets for higher order ligands. Suitable tags include biotin, digoxigenin, His-Tag, Glutathion-S-Transferase, FLAG, GFP, myc-tag, influenza A virus haemagglutinin (HA), maltose binding protein, and the like. In the case of a peptide or polypeptide, the tag is preferably at the N-terminus and/or C-terminus. Suitable labels are any labels detectable by an appropriate detection method. Typical labels include gold particles, latex beads, acridan ester, luminol, ruthenium, enzymatically active labels, radioactive labels, magnetic labels ("e.g. magnetic beads", including paramagnetic and superparamagnetic labels), and fluorescent labels. Enzymatically active labels include e.g. horseradish peroxidase, alkaline phosphatase, beta-Galactosidase, Luciferase, and derivatives thereof. Suitable substrates for detection include di-amino-benzidine (DAB), 3,3'-5,5'-tetramethylbenzidine, NBT-BCIP (4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate, available as ready-made stock solution from Roche Diagnostics), CDP-Star™ (Amersham Biosciences), ECF™ (Amersham Biosciences). A suitable enzyme-substrate combination may result in a colored reaction product, fluorescence or chemoluminescence, which can be measured according to methods known in the art (e.g. using a light-sensitive film or a suitable camera system). As for measuring the enzymatic reaction, the criteria given above apply analogously. Typical fluorescent labels include fluorescent proteins (such as GFP and its derivatives), Cy3, Cy5, Texas Red, Fluorescein, and the Alexa dyes (e.g. Alexa 568). Further fluorescent labels are available e.g. from Molecular Probes (Oregon). Also the use of quantum dots as fluorescent labels is contemplated. Typical radioactive labels include ^{35}S , ^{125}I , ^{32}P , ^{33}P and the like. A radioactive label can be detected by any method known and appropriate, e.g. a light-sensitive film or a phosphor imager. Suitable measurement methods according to the present invention also include precipitation (particularly immunoprecipitation), electrochemiluminescence (electro-generated chemiluminescence), RIA (radioimmunoassay), ELISA (enzyme-linked immunosorbent assay), sandwich enzyme immune tests, electrochemiluminescence sandwich immunoassays (ECLIA), dissociation-enhanced lanthanide fluoro immuno assay (DELFA), scintillation proximity assay (SPA), turbidimetry, nephelometry, latex-

enhanced turbidimetry or nephelometry, or solid phase immune tests. Further methods known in the art (such as gel electrophoresis, 2D gel electrophoresis, SDS polyacrylamid gel electrophoresis (SDS-PAGE), Western Blotting, and mass spectrometry), can be used alone or in combination with labeling or other detection methods as described above.

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The amount of a peptide or polypeptide may be, also preferably, determined as follows: (α) contacting a solid support comprising a ligand for the peptide or polypeptide as specified above with a sample comprising the peptide or polypeptide and (β) measuring the amount peptide or polypeptide which is bound to the support. The ligand, preferably chosen from the group consisting of nucleic acids, peptides, polypeptides, antibodies and aptamers, is preferably present on a solid support in immobilized form. Materials for manufacturing solid supports are well known in the art and include, inter alia, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, membranes, sheets, duracytes, wells and walls of reaction trays, plastic tubes etc. The ligand or agent may be bound to many different carriers. Examples of well-known carriers include glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for the purposes of the invention. Suitable methods for fixing/immobilizing said ligand are well known and include, but are not limited to ionic, hydrophobic, covalent interactions and the like. It is also contemplated to use "suspension arrays" as arrays according to the present invention (Nolan 2002, Trends Biotechnol. 20(1):9-12). In such suspension arrays, the carrier, e.g. a microbead or microsphere, is present in suspension. The array consists of different microbeads or microspheres, possibly labeled, carrying different ligands. Methods of producing such arrays, for example based on solid-phase chemistry and photo-labile protective groups, are generally known (US 5,744,305).

The term "amount" as used herein encompasses the absolute amount of a polypeptide or peptide, the relative amount or concentration of the said polypeptide or peptide as well as any value or parameter which correlates thereto or can be derived there from. Such values or parameters comprise intensity signal values from all specific physical or chemical properties obtained from the said peptides by direct measurements, e.g., intensity values in mass spectra or NMR spectra. Moreover, encompassed are all values or parameters which are obtained by indirect measurements specified elsewhere in this description, e.g., response levels determined from biological read out systems in response to the peptides or intensity signals obtained from specifically bound ligands. It is to be understood that values

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correlating to the aforementioned amounts or parameters can also be obtained by all standard mathematical operations.

5 The term “sample” refers to a sample of a body fluid, to a sample of separated cells or to a sample from a tissue or an organ. Samples of body fluids can be obtained by well known techniques and include, preferably, samples of blood, plasma, serum, urine, samples of blood, plasma or serum. It is to be understood that the sample depends on the marker to be determined. Therefore, it is encompassed that the polypeptides as referred to herein are determined in different samples. L-FABP or a variant thereof or a variant thereof and
10 KIM-1 or a variant thereof and adiponectin or a variant thereof are preferably determined in a urine sample.

The term “forming a ratio” as used herein means calculating in each individual a ratio between the determined amounts of the specified peptides. All ratios were used to calculate
15 median and respective percentiles to obtain reference kidney damage information for the target disease.

The term “comparing” as used herein encompasses comparing the amount of the peptide or polypeptide comprised by the sample to be analyzed with an amount of a suitable reference
20 source specified elsewhere in this description. It is to be understood that comparing as used herein refers to a comparison of corresponding parameters or values, e.g., an absolute amount is compared to an absolute reference amount while a concentration is compared to a reference concentration or an intensity signal obtained from a test sample is compared to the same type of intensity signal of a reference sample or a ratio of amounts is compared to
25 a reference ratio of amounts. The comparison referred to in step (c) of the method of the present invention may be carried out manually or computer assisted. For a computer assisted comparison, the value of the determined amount may be compared to values corresponding to suitable references which are stored in a database by a computer program. The computer program may further evaluate the result of the comparison, i.e. automatically
30 provide the desired assessment in a suitable output format.

In general, for determining the respective amounts/amounts or amount ratios allowing to establish the desired diagnosis in accordance with the respective embodiment of the present invention, (“threshold”, “reference amount”), the amount(s)/amount(s) or amount
35 ratios of the respective peptide or peptides are determined in appropriate patient groups. According to the diagnosis to be established, the patient group may, for example, comprise only healthy individuals, or may comprise healthy individuals and individuals suffering

from the pathophysiological (state which is to be determined, or may comprise only individuals suffering from the pathophysiological state which is to be determined, or may comprise individuals suffering from the various pathophysiological states to be distinguished, by the respective marker(s) using validated analytical methods. The results
5 which are obtained are collected and analyzed by statistical methods known to the person skilled in the art. The obtained threshold values are then established in accordance with the desired probability of suffering from the disease and linked to the particular threshold value. For example, it may be useful to choose the median value, the 60th, 70th, 80th, 90th, 95th or even the 99th percentile of the healthy and/or non-healthy patient collective, in order
10 to establish the threshold value(s), reference value(s) or amount ratios.

A reference value of a diagnostic marker can be established, and the amount of the marker in a patient sample can simply be compared to the reference value. The sensitivity and specificity of a diagnostic and/or prognostic test depends on more than just the analytical
15 "quality" of the test-they also depend on the definition of what constitutes an abnormal result. In practice, Receiver Operating Characteristic curves, or "ROC" curves, are typically calculated by plotting the value of a variable versus its relative frequency in "normal" and "disease" populations. For any particular marker of the invention, a distribution of marker amounts for subjects with and without a disease will likely overlap.
20 Under such conditions, a test does not absolutely distinguish normal from disease with 100% accuracy, and the area of overlap indicates where the test cannot distinguish normal from disease. A threshold is selected, above which (or below which, depending on how a marker changes with the disease) the test is considered to be abnormal and below which the test is considered to be normal. The area under the ROC curve is a measure of the
25 probability that the perceived measurement will allow correct identification of a condition. ROC curves can be used even when test results don't necessarily give an accurate number. As long as one can rank results, one can create an ROC curve. For example, results of a test on "disease" samples might be ranked according to degree (say 1=low, 2=normal, and 3=high). This ranking can be correlated to results in the "normal" population, and a ROC
30 curve created. These methods are well known in the art. See, e.g., Hanley et al, Radiology 143: 29-36 (1982).

In certain embodiments, markers and/or marker panels are selected to exhibit at least about 70% sensitivity, more preferably at least about 80% sensitivity, even more preferably at
35 least about 85% sensitivity, still more preferably at least about 90% sensitivity, and most preferably at least about 95% sensitivity, combined with at least about 70% specificity, more preferably at least about 80% specificity, even more preferably at least about 85%

specificity, still more preferably at least about 90% specificity, and most preferably at least about 95% specificity. In particularly preferred embodiments, both the sensitivity and specificity are at least about 75%, more preferably at least about 80%, even more preferably at least about 85%, still more preferably at least about 90%, and most preferably at least about 95%. The term "about" in this context refers to +/- 5% of a given measurement.

In other embodiments, a positive likelihood ratio, negative likelihood ratio, odds ratio, or hazard ratio is used as a measure of a test's ability to predict risk or diagnose a disease. In the case of a positive likelihood ratio, a value of 1 indicates that a positive result is equally likely among subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a positive result is more likely in the diseased group; and a value less than 1 indicates that a positive result is more likely in the control group. In the case of a negative likelihood ratio, a value of 1 indicates that a negative result is equally likely among subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a negative result is more likely in the test group; and a value less than 1 indicates that a negative result is more likely in the control group. In certain preferred embodiments, markers and/or marker panels are preferably selected to exhibit a positive or negative likelihood ratio of at least about 1.5 or more or about 0.67 or less, more preferably at least about 2 or more or about 0.5 or less, still more preferably at least about 5 or more or about 0.2 or less, even more preferably at least about 10 or more or about 0.1 or less, and most preferably at least about 20 or more or about 0.05 or less. The term "about" in this context refers to +/- 5% of a given measurement.

In the case of an odds ratio, a value of 1 indicates that a positive result is equally likely among subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a positive result is more likely in the diseased group; and a value less than 1 indicates that a positive result is more likely in the control group. In certain preferred embodiments, markers and/or marker panels are preferably selected to exhibit an odds ratio of at least about 2 or more or about 0.5 or less, more preferably at least about 3 or more or about 0.33 or less, still more preferably at least about 4 or more or about 0.25 or less, even more preferably at least about 5 or more or about 0.2 or less, and most preferably at least about 10 or more or about 0.1 or less. The term "about" in this context refers to +/- 5% of a given measurement.

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In the case of a hazard ratio, a value of 1 indicates that the relative risk of an endpoint (e.g., death) is equal in both the "diseased" and "control" groups; a value greater than 1 indicates

that the risk is greater in the diseased group; and a value less than 1 indicates that the risk is greater in the control group. In certain preferred embodiments, markers and/or marker panels are preferably selected to exhibit a hazard ratio of at least about 1.1 or more or about 0.91 or less, more preferably at least about 1.25 or more or about 0.8 or less, still more preferably at least about 1.5 or more or about 0.67 or less, even more preferably at least about 2 or more or about 0.5 or less, and most preferably at least about 2.5 or more or about 0.4 or less. The term "about" in this context refers to +/- 5% of a given measurement.

While exemplary panels are described herein, one or more markers may be replaced, added, or subtracted from these exemplary panels while still providing clinically useful results. Panels may comprise both specific markers of a disease (e.g., markers that are increased or decreased in bacterial infection, but not in other disease states) and/or non-specific markers (e.g., markers that are increased or decreased due to inflammation, regardless of the cause; markers that are increased or decreased due to changes in hemostasis, regardless of the cause, etc.). While certain markers may not individually be definitive in the methods described herein, a particular "fingerprint" pattern of changes may, in effect, act as a specific indicator of disease state. As discussed above, that pattern of changes may be obtained from a single sample, or may optionally consider temporal changes in one or more members of the panel (or temporal changes in a panel response value).

The term "reference amounts" as used herein in this embodiment of the invention refers to amounts of the polypeptides which allow diagnosing kidney damage in a subject suffering from diabetes mellitus (in general, the subject is apparently healthy in respect to kidney damage).

Therefore, the reference amounts will in general be derived from subjects known to be physiologically healthy, or subjects known to suffer from kidney damage (which may be apparently healthy in respect to kidney function), or subjects suffering from diabetes mellitus or subjects suffering from diabetes mellitus and known to suffer from kidney damage.

Accordingly, the term "reference amount" as used herein either refers to an amount which allows diagnosing kidney damage in a subject with diabetes mellitus (in general, this subject is apparently healthy in respect to kidney function). The comparison with reference amounts permits to differentiate between these individuals and those known to suffer from diabetes mellitus, but not suffering from kidney damage. In the present invention,

“reference amount” also refers to the ratio L-FABP/KIM-1 and the ratio L-FABP/adiponectin.

Reference amounts for L-FABP or a variant thereof and KIM-1 or a variant thereof may be derived from subjects as defined above in the present invention which suffer from diabetes mellitus (and which, preferably, are apparently healthy in respect to kidney function), and where the subject was diagnosed to suffer from kidney damage, preferably tubular kidney damage and tubular kidney repair, in particular chronic tubular kidney damage and tubular kidney repair. The amounts of the respective peptide serving for establishing the reference amounts can be determined prior to the diagnosis established in accordance with the present invention.

In all embodiments of the present invention, the amount/amounts of the respective markers used therein (L-FABP or a variant thereof and KIM-1 or a variant thereof) are determined by methods known to the person skilled in the art.

In order to test if a chosen reference value yields a sufficiently safe diagnosis of patients suffering from the disease of interest, one may for example determine the efficiency (E) of the methods of the invention for a given reference value using the following formula:

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$$E = (TP / TO) \times 100;$$

wherein TP = true positives and TO = total number of tests = TP + FP + FN + TN, wherein FP = false positives; FN = false negatives and TN = true negatives. E has the following range of values: $0 < E < 100$). Preferably, a tested reference value yields a sufficiently safe diagnosis provided the value of E is at least about 50, more preferably at least about 60, more preferably at least about 70, more preferably at least about 80, more preferably at least about 90, more preferably at least about 95, more preferably at least about 98.

The diagnosis if individuals are healthy or suffer from a certain pathophysiological state is made by established methods known to the person skilled in the art. The methods differ in respect to the individual pathophysiological state.

The algorithms to establish the desired diagnosis are laid out in the present application, in the passages referring to the respective embodiment, to which reference is made.

Accordingly, the present invention also comprises a method of determining the threshold amount indicative for a physiological and/or a pathological state and/or a certain pathological state, comprising the steps of determining in appropriate patient groups the amounts of the appropriate marker(s), collecting the data and analyzing the data by statistical methods and establishing the threshold values.

The term "about" as used herein refers to +/- 20%, preferably +/-10%, preferably, +/- 5% of a given measurement or value.

The term "reference amount" as used herein refers to an amount which allows diagnosing kidney damage.

It is to be understood that if a reference from a subject is used which suffers from a disease or combination of diseases, an amount of a peptide or protein in a sample of a test subject being essentially identical to said reference amount shall be indicative for the respective disease or combination of diseases. The reference amount applicable for an individual subject may vary depending on various physiological parameters such as age, gender, or subpopulation. Moreover, the reference amounts, preferably define thresholds. Thus, a suitable reference amount may be determined by the method of the present invention from a reference sample to be analyzed together, i.e. simultaneously or subsequently, with the test sample. A suitable technique may be to determine the median of the population for the peptide or polypeptide amounts to be determined in the method of the present invention.

KIM-1 and L-FABP are urinary biomarkers which are increased expressed in the proximal tubule epithelial cells in the postischemic kidney. As L-FABP is considered a biomarker of tubular damage and KIM-1 is believed an indicator of tubular repair, the ratio of both markers reflects evidence of disease progression. As adiponectin appears to be an indicator of "glomerular health", combined determination of these markers disclose relevant information of pathogenic kidney processes.

According to a further embodiment of the invention, the L-FABP/ adiponectin ratio was calculated to obtain information on the predominant location of kidney damage (tubular vs glomerular).

Based on the comparison of the amounts of L-FABP or a variant thereof and KIM-1 or a variant thereof and optionally adiponectin determined in step a) and the corresponding reference amounts and the L-FABP/KIM-1 ratio formed in step b), the extent and

progression of the kidney damage of subjects suffering from diabetes mellitus type 1 or type 2 can be characterized.

Advantageously, it has been found that the combination of L-FABP or a variant thereof, KIM-1 or a variant thereof and adiponectin as biomarkers, in particular the amounts of L-FABP or a variant thereof, KIM-1 or a variant thereof and adiponectin present in a urine-sample of a subject in combination with the amounts of L-FABP and KIM-1 or, in a preferred embodiment, the ratio of the amounts of L-FABP/KIM-1 allow for the characterization of a diabetes mellitus associated kidney damage in a reliable and efficient manner. Moreover, it has been found that the concentrations of said biomarkers do not correlate (see Figures 1-2 and 3-4 for diabetes mellitus type 1 and type 2, respectively). Thus, each of said biomarkers is statistically independent from each other. Thanks to the present invention, subjects can be more readily and reliably diagnosed and subsequently treated according to the result of the inventive method.

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According to the method of the invention increased amounts of L-FABP or a variant thereof and decreased amounts of KIM-1 or a variant thereof in comparison to reference amounts measured in a urinary sample of a subject, accordingly leading to increased values of the L-FABP/KIM-1-ratio, are indicative for a progressive tubular damage of the kidney because the effect of damage is predominant over repair. Increased amounts of adiponectin in comparison to reference amounts are indicative for a progressive glomerular damage of the kidney. Increased values of the L-FABP/KIM-1-ratios in combination with increased amounts of adiponectin in comparison to reference amounts are indicative for a progressive tubular and glomerular damage of the kidney.

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Progressive kidney damage, in particular progressive glomerular and/or tubular damage, will result in end stage renal disease over variable time periods. The diagnosis of end stage renal disease is based on the kidney function (e.g. creatinine value).

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A reference amount of $> \text{about } 3 \mu\text{g/g}$, preferable $> 7 \text{ about } \mu\text{g/g}$, more preferable $> \text{about } 20 \mu\text{g/g}$, in particular $> \text{about } 40 \mu\text{g/g}$, creatinine for L-FABP or a variant thereof; and a L-FABP/KIM-1 ratio of $> \text{about } 18$, preferable $> \text{about } 20$, more preferable $> \text{about } 22$, in particular $> \text{about } 24$, alone or in combination with a reference amount of $> \text{about } 0,23 \mu\text{g/g}$, preferable $> \text{about } 0,30 \mu\text{g/g}$, more preferable $> \text{about } 0,40 \mu\text{g/g}$, in particular $> \text{about } 0,50 \mu\text{g/g}$, creatinine for adiponectin; are indicative for diabetes mellitus type 1 or type 2 associated kidney damage, in particular tubular damage.

35

As outlined elsewhere in the present application, L-FABP represents tubular kidney damage und KIM 1 tubular repair. As described L-FABP represents an indicator of tubular damage and increasing amounts of L-FABP indicate the extent (and severity) of tubular damage, which might or might not be associated with appropriate repair. A value of L-FABP above 7 ug/g creatinine indicates that the diabetes mellitus patient has tubular damage which is in edxcess of that found in the average diabetes mellitus patient. Similarly a ratio of L- FABP/KIM1 of 22 was found in the average diabetes patient, values above this indicate that damage exceeds repair indicating the likelihood of progressive kidney disease, vice versa a ratio below 22 suggests appropriate repair, making a progressive kidney disease unlikely. Similarly, average adiponectin levels in patients with diabetes mellitus are 0.4 ug/g creatinine, values above this value indicate excess glomerular damage when compared to the average diabetes mellitus patient, also suggesting progressive kidney disease, values below 0.4 ug/g creatinne however render a progressive kidney disease unlikely.

15

The higher the afore-mentioned reference amounts of adiponectin and of L-FABP or a variant thereof and the higher the afore-mentioned L-FABP/KIM-1 ratio of > about 18, preferable > about 20, more preferable > about 22, in particular > about 24, the more likely is a progressive and severe damage of the kidney, in particular tubular damage.

20

In particular a reference amount of > about 0,23 µg/g creatinine for adiponectin, a reference amount of > about 3 µg/g creatinine for L-FABP or a variant thereof and a L-FABP/KIM-1 ratio of < about 18 are indicative for a minor damage of the kidney, in particular minor tubular damage.

25

In particular a reference amount of > about 0,30 µg/g creatinine for adiponectin, a reference amount of > about 7 µg/g creatinine for L-FABP or a variant thereof and a L-FABP/KIM-1 ratio of > about 20 are indicative for a moderate damage of the kidney, in particular moderate tubular damage.

30

In particular a reference amount of > about 0,40 µg/g creatinine for adiponectin, a reference amount of > about 20 µg/g creatinine for L-FABP or a variant thereof and a L-FABP/KIM-1 ratio of > about 22 are indicative for a severe damage of the kidney, in particular severe tubular damage.

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In particular a reference amount of > about 0,50 µg/g creatinine for adiponectin, a reference amount of > about 22 µg/g creatinine for L-FABP or a variant thereof and a L-

FABP/KIM-1 ratio of $>$ about 24 are indicative for a very severe damage of the kidney, in particular very severe tubular damage.

5 In subjects suffering from diabetes mellitus type 1, a L-FABP/adiponectin ratio of less than about 19, about 19 to $<$ about 28, about 28 to about 57 and more than about 57 is indicative for a minor, moderate, severe and very severe kidney damage, in particular minor, moderate, severe and very severe tubular damage, respectively.

10 In subjects suffering from diabetes mellitus type 1, a L-FABP/adiponectin ratio of more than about 30, preferably more than about 35, more preferably more than about 40, most preferably more than about 45, in particular more than about 50, is indicative for a predominant tubular damage of the kidney whereas a L-FABP/adiponectin ratio of less than about 25, preferably less than about 20, more preferably less than about 15, in particular about 10 or less, is indicative for a predominant glomerular damage of the
15 kidney.

In subjects suffering from diabetes mellitus type 2, a L-FABP/adiponectin ratio of less than about 3, about 3 to $<$ about 11, about 11 to about 49 and more than about 49 is indicative for a minor, moderate, severe and very severe kidney damage, in particular minor,
20 moderate, severe and very severe tubular damage, respectively.

In subjects suffering from diabetes mellitus type 2, a L-FABP/adiponectin ratio of more than about 15, preferably more than about 20, more preferably more than about 25, most preferably more than about 30, in particular more than about 40 and very particular more
25 than about 50 is indicative for a predominant tubular damage of the kidney whereas a L-FABP/adiponectin ratio of less than about 10, preferably less than about 7, more preferably less than about 6, in particular about 3 or less, is indicative for a predominant glomerular damage of the kidney.

30 The present invention also provides a method of deciding, in a subject suffering from diabetes mellitus and, preferably, being apparently healthy in respect to kidney function, on a suitable therapy for diabetes mellitus associated kidney damage, based on the comparison of the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof and kidney injury molecule 1 (KIM-1) or a variant thereof, determined in a sample
35 of said subject, preferably determined in a urine sample of the subject, to at least one reference amount.

The method of the present invention may comprise the following steps: a) determining the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof, preferably urinary liver-type fatty acid binding protein (L-FABP), and kidney injury molecule 1 (KIM-1) or a variant thereof in a sample, preferably a urine-sample of a subject; b) comparing the amounts determined in step a) with reference amounts.

The decision on the suitable therapy may be established based on the information obtained in step b) and preferably based on the information in steps a) and b).

The present invention therefore also provides a method of deciding, in a subject suffering from diabetes mellitus associated kidney damage, on a suitable therapy, comprising at least one of the following steps:

- a) determining the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof and kidney injury molecule 1 (KIM-1) or a variant thereof and optionally adiponectin in a urine-sample of a subject;
- b) comparing the amounts determined in step a) with reference amounts, thereby diagnosing the kidney damage, and
- c) deciding on the suitable therapy.

In one embodiment of the present invention, the L-FABP/KIM-1 ratio is formed.

In a further embodiment of the present invention, the individual is apparently healthy in respect to kidney function

Suitable therapies are the administration of pharmaceuticals and/or life style recommendations which are effective in respect of:

1. inhibition of further progression of kidney disease,
2. diabetes mellitus as such (causing the kidney damage)
3. prevention of further kidney damage (in particular in case of a decreased repair process).

Typical pharmaceuticals of category 1 are among others Angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers and/or aldosterone antagonists.

Category 2 encompasses the administration of insulin sensitizers and insulin itself and life style recommendations such as diet and weight reduction.

Category 3 encompasses among others the administration of metformin (effective for the diabetes mellitus), ACE inhibitors applied in high doses, and avoiding use of radio contrast agents.

- 5 The afore-mentioned agents are known to a person skilled in the art. Suitable ACE inhibitors are in particular Enalapril, Captopril, Ramipril and/or Trandolapril. Suitable angiotensin II receptor blockers are Losartan, Valsartan, Irbesartan, Candesartan, Telmisartan and/or Eprosartan. Suitable aldosterone antagonists are in particular Spironolactone and/or Eplerenone .

10

The afore-mentioned therapies are in particular effective if combined with each other.

- As outlined above using L-FABP, KIM 1, and adiponectin in urine and forming the ratios of L-FABP/KIM 1 and LLFABP/adiponectin information can be obtained as to the severity
15 of tubular and glomerular damage, in case of tubular damage to what extent tubular damage will be repaired and by using the ratio of L-FABP/adiponectin by determining the predominant site of kidney damage. Taking the 50 % percentile of the diabetic population it can be assessed whether this damage and/or repair is average for the population or whether is exceeds average damage or is below this average values. Accordingly
20 interpretations have been provided as to whether kidney damage is minor, moderate, severe or very severe. Accordingly progressive or nonprogressive kidney disease is to be expected. Aggressiveness of treatment needs to be considered based on these assumptions, for example if minor kidney damage is diagnosed the patients can be followed without further treatment of low dose ACE inhibitors applied, conversely if severe kidney disease
25 is diagnosed, ACE inhibitors in combination with aldosterone antagonists and tight glucose control and narrow follow up is indicated and particularly any drug or intervention that might result in additional kidney damage should be avoided if possible.

- The terms “suitable therapy” and “susceptible” as used herein means that a therapy applied
30 to a subject will inhibit or ameliorate the progression of diabetes mellitus or its accompanying symptoms and/or of kidney damage or its accompanying symptoms. It is to be understood assessment for susceptibility for the therapy will not be correct for all (100%) of the investigated subjects. However, it is envisaged that at least a statistically significant portion can be determined for which the therapy can be successfully applied.
35 Whether a portion is statistically significant can be determined by techniques specified elsewhere herein.

The present invention also relates to a method of monitoring kidney damage in a subject suffering from diabetes mellitus, based on the comparison of the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof and kidney injury molecule 1 (KIM-1) or a variant thereof, determined in a sample of said subject, preferably determined
5 in a urine sample of the subject, to at least one reference amount, and repeating the comparison step.

In a preferred embodiment, the above method of monitoring comprises monitoring the therapy.

10

The method of the present invention may comprise the following steps: a) determining the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof, and kidney injury molecule 1 (KIM-1) or a variant thereof in a sample, preferably a urine-sample of a subject; b) comparing the amounts determined in step a) with reference amounts.

15

Diagnosis of the kidney disease may be established based on the information obtained in step b) and preferably based on the information obtained in a) and b), and monitoring is carried out by repeating step b, preferably by repeating steps a) and b) during therapy..

20

Accordingly, the present invention relates to a method for monitoring kidney damage in a subject suffering from diabetes mellitus comprising the steps of:

a) determining the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof and kidney injury molecule 1 (KIM-1) or a variant thereof and optionally adiponectin in a sample of a subject;

25

b) comparing the amounts determined in step a) with reference amounts and diagnosing the kidney damage,

c) deciding on a suitable therapy, and

d) repeating steps a) to c) during the therapy.

30

In one embodiment of the present invention, the L-FABP/KIM-1 ratio is formed. In a further embodiment of the present invention, the method includes deciding on the suitable therapy, after step b), in the case of therapy monitoring.

In an embodiment of the present invention, the amount of adiponectin or a variant thereof is determined in a sample of the subject, preferably a urine sample..

35

Monitoring relates to keeping track of the already diagnosed disease, in particular to analyze the progression of the disease or the influence of a particular treatment on the

progression of disease. Monitoring means control preferably after 2 weeks, more preferably after 1 month, most preferably after 3 , 6 or 12 months, respectively, depending on the state as clinically needed.

- 5 As described above in the present invention patients can be classified according to the severity of kidney damage and their putative progression, accordingly monitoring of such patients needs to take these findings into consideration, e.g. if the kidney is disease is classified as mild, follow up might be necessary and recommended only after 12 months, in case the kidney disease has been classified as very severe such follow up should be done
10 within 2 weeks or 1 month and depending on the result of the follow up, the interval being extended or kept.

Accordingly, the present invention relates to a method for diagnosing myocardial infarction in a subject comprising at least one of the following steps:

- 15 a) determining the amounts of a natriuretic peptide and/or troponin T in a sample of the subject;
b) comparing the amounts determined in step a) with reference amounts; and
c) diagnosing myocardial infarction.
- 20 Moreover, the present invention also envisages kits and devices adapted to carry out the method of the present invention.

Furthermore, the present invention relates to a device for diagnosing kidney damage in a subject suffering from diabetes mellitus, comprising:

- 25 a) means for determining the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof and kidney injury molecule 1 (KIM-1) or a variant thereof in a sample of a subject;
b) means for comparing the amounts determined in step e) with reference amounts;
whereby the device is adapted for diagnosing the kidney damage.

30

In a preferred embodiment of the present invention, the device furthermore comprises means for forming the L-FABP/KIM-1 ratio.

The sample, preferably, is a urinary sample.

35

In an embodiment of the present invention, the device furthermore comprises means for determining the amounts of adiponectin or a variant thereof in a serum sample of a subject;

and/or means for comparing the amounts determined with reference amounts; and optionally means for diagnosing the suspected disease;

The term "device" as used herein relates to a system of means comprising at least the
5 aforementioned means operatively linked to each other as to allow the characterization. Preferred means for determining the amount of a one of the aforementioned polypeptides as well as means for carrying out the comparison are disclosed above in connection with the method of the invention. How to link the means in an operating manner will depend on the type of means included into the device. For example, where means for automatically
10 determining the amount of the peptides are applied, the data obtained by said automatically operating means can be processed by, e.g., a computer program in order to obtain the desired results. Preferably, the means are comprised by a single device in such a case. Said device may accordingly include an analyzing unit for the measurement of the amount of the polypeptides in an applied sample and a computer unit for processing the resulting data
15 for the evaluation. The computer unit, preferably, comprises a database including the stored reference amounts or values thereof recited elsewhere in this specification as well as a computer-implemented algorithm for carrying out a comparison of the determined amounts for the polypeptides with the stored reference amounts of the database. Computer-implemented as used herein refers to a computer-readable program code tangibly included
20 into the computer unit. Alternatively, where means such as test stripes are used for determining the amount of the peptides or polypeptides, the means for comparison may comprise control stripes or tables allocating the determined amount to a reference amount. The test stripes are, preferably, coupled to a ligand which specifically binds to the peptides or polypeptides referred to herein. The strip or device, preferably, comprises means for
25 detection of the binding of said peptides or polypeptides to the said ligand. Preferred means for detection are disclosed in connection with embodiments relating to the method of the invention above. In such a case, the means are operatively linked in that the user of the system brings together the result of the determination of the amount and the diagnostic or prognostic value thereof due to the instructions and interpretations given in a manual.
30 The means may appear as separate devices in such an embodiment and are, preferably, packaged together as a kit. The person skilled in the art will realize how to link the means without further ado. Preferred devices are those which can be applied without the particular knowledge of a specialized clinician, e.g., test stripes or electronic devices which merely require loading with a sample. The results may be given as output of raw data which need
35 interpretation by the clinician. Preferably, the output of the device is, however, processed, i.e. evaluated, raw data the interpretation of which does not require a clinician. Further preferred devices comprise the analyzing units/devices (e.g., biosensors, arrays, solid

- 34 -

supports coupled to ligands specifically recognizing the polypeptides referred to herein, Plasmon surface resonance devices, NMR spectrometers, mass- spectrometers etc.) and/or evaluation units/devices referred to above in accordance with the method of the invention.

5 Moreover the present invention is concerned with a kit for diagnosing kidney damage in a subject suffering from diabetes mellitus, comprising:

- a) means for determining the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof and kidney injury molecule 1 (KIM-1) or a variant thereof in a sample of
10 a subject;
- b) means for comparing the amounts determined in step e) with reference amounts;
whereby the kit is adapted for diagnosing the kidney damage.

In a preferred embodiment of the present invention, the device furthermore comprises
15 means for forming the L-FABP/KIM-1 ratio.

The sample, preferably, is a urinary sample.

In an embodiment of the present invention, the device furthermore comprises means for
20 determining the amounts of adiponectin or a variant thereof in a serum sample of a subject;
and/or means for comparing the amounts determined with reference amounts; and
optionally means for diagnosing the suspected disease;

The term “kit” as used herein refers to a collection of the aforementioned compounds,
25 means or reagents of the present invention which may or may not be packaged together.
The components of the kit may be comprised by separate vials (i.e. as a kit of separate parts) or provided in a single vial. Moreover, it is to be understood that the kit of the present invention is to be used for practising the methods referred to herein above. It is, preferably, envisaged that all components are provided in a ready-to-use manner for
30 practising the methods referred to above. Further, the kit preferably contains instructions for carrying out the said methods. The instructions can be provided by a user’s manual in paper- or electronic form. For example, the manual may comprise instructions for interpreting the results obtained when carrying out the aforementioned methods using the kit of the present invention.

35

How to link the means in an operating manner will depend on the type of means included into the device. For example, where means for automatically determining the amount of

the peptides are applied, the data obtained by said automatically operating means can be processed by, e.g., a computer program in order to obtain the desired results. Preferably, the means are comprised by a single device in such a case. Said device may accordingly include an analyzing unit for the measurement of the amount of the peptides or polypeptides in an applied sample and a computer unit for processing the resulting data for the evaluation. Alternatively, where means such as test stripes are used for determining the amount of the peptides or polypeptides, the means for comparison may comprise control stripes or tables allocating the determined amount to a reference amount. The test stripes are, preferably, coupled to a ligand which specifically binds to the peptides or polypeptides referred to herein. The strip or device, preferably, comprises means for detection of the binding of said peptides or polypeptides to the said ligand. Preferred means for detection are disclosed in connection with embodiments relating to the method of the invention above. In such a case, the means are operatively linked in that the user of the system brings together the result of the determination of the amount and the diagnostic or prognostic value thereof due to the instructions and interpretations given in a manual. The means may appear as separate devices in such an embodiment and are, preferably, packaged together as a kit. The person skilled in the art will realize how to link the means without further ado. Preferred devices are those which can be applied without the particular knowledge of a specialized clinician, e.g., test stripes or electronic devices which merely require loading with a sample. The results may be given as output of raw data which need interpretation by the clinician. Preferably, the output of the device is, however, processed, i.e. evaluated, raw data the interpretation of which does not require a clinician. Further preferred devices comprise the analyzing units/devices (e.g., biosensors, arrays, solid supports coupled to ligands specifically recognizing the KIM-1 or a variant thereof, L-FABP or a variant thereof and a cardiac troponin. Plasmon surface resonance devices, NMR spectrometers, mass- spectrometers etc.) or evaluation units/devices referred to above in accordance with the method of the invention.

The present invention also relates to the use of a kit or device for determining the amount of KIM-1 or a variant thereof, L-FABP or a variant thereof and optionally adiponectin or a variant thereof in a sample of a subject, comprising means for determining the amount of KIM-1 or a variant thereof, L-FABP or a variant thereof and optionally a natriuretic peptide and/or means for comparing the amount of KIM-1 or a variant thereof, L-FABP or a variant thereof and optionally adiponectin or a variant thereof to at least one reference amount for: diagnosing kidney damage in a subject with diabetes mellitus and preferably being apparently healthy in respect to kidney function; and/or deciding whether a subject suffering from diabetes mellitus associated kidney damage and preferably being apparently

healthy in respect to kidney function is susceptible to a suitable therapy; and/or monitoring kidney damage in a subject suffering from diabetes mellitus associated kidney damage.

5 The present invention also relates to the use of: an antibody against KIM-1 or a variant thereof, an antibody against L-FABP or a variant thereof and optionally an antibody against adiponectin or a variant thereof, and/or of means for determining the amount of KIM-1 or a variant thereof, L-FABP or a variant thereof and optionally adiponectin or a variant thereof, and/or of means for comparing the amount of KIM-1, L-FABP or a variant thereof and optionally adiponectin or a variant thereof to at least one reference amount for
10 the manufacture of a diagnostic composition for: diagnosing kidney damage in a subject with diabetes mellitus and preferably being apparently healthy in respect to kidney function; and/or deciding whether a subject suffering from diabetes mellitus associated kidney damage and preferably being apparently healthy in respect to kidney function is susceptible to a suitable therapy; and/or monitoring kidney damage in a subject suffering
15 from diabetes mellitus associated kidney damage.

The present invention also relates to the use of: an antibody against KIM-1 or a variant thereof, an antibody against L-FABP or a variant thereof and optionally an antibody against adiponectin or a variant thereof, and/or of means for determining the amount of
20 KIM-1 or a variant thereof, L-FABP or a variant thereof and optionally adiponectin or a variant thereof, and/or of means for comparing the amount of KIM-1 or a variant thereof, L-FABP or a variant thereof and optionally adiponectin or a variant thereof to at least one reference amount for: diagnosing kidney damage in a subject with diabetes mellitus and preferably being apparently healthy in respect to kidney function; and/or deciding whether
25 a subject suffering from diabetes mellitus associated kidney damage and preferably being apparently healthy in respect to kidney function is susceptible to a suitable therapy; and/or monitoring kidney damage in a subject suffering from diabetes mellitus associated kidney damage.

30 All references cited in this specification are herewith incorporated by reference with respect to their entire disclosure content and the disclosure content specifically mentioned in this specification.

35 The following examples shall merely illustrate the invention. They shall not be construed, whatsoever, to limit the scope of the invention.

Examples

General

5 In all examples, urinary levels of said biomarkers were determined using the following commercially available immunoassay kits:

L-FABP was determined by using the L-FABP ELISA-Kit from CMIC Co., Ltd, Japan. The test is based on an ELISA 2-step assay. L-FABP standard or urine samples are firstly treated with pretreatment solution, and transferred into a L-FABP antibody coated microplate containing assay buffer and incubated. During this incubation, L-FABP in the reaction solution binds to the immobilized antibody. After washing, the 2nd antibody-peroxidase conjugate is added as the secondary antibody and incubated, thereby forming sandwich of the L-FABP antigen between the immobilized antibody and conjugate antibody. After incubation, the plate is washed and substrate for enzyme reaction is added, color develops according to the L-FABP antigen quantity. The L-FABP concentration is determined based on the optical density.

Adiponectin (multimeric) was determined by using the test EIA from ALPCO diagnostics® (USA), operating on the principle of a “sandwich” format ELISA. The specific antibodies used in the kit are anti-human adiponectin monoclonal antibodies (MoAbs) directed to two independent epitopes. The specimens are pre-treated as described below, and total adiponectin and individual multimers of adiponectin are determined selectively, directly or indirectly. Multimers of adiponectin are classified into four fractions with this kit:

- 1) Total adiponectin fraction: “Total-Ad”-assayed directly on the plate
- 25 2) High-molecular adiponectin fraction (equivalent of dodecamer -octodecamer): “HMW-Ad”-assayed directly on the plate
- 3) Middle-molecular adiponectin fraction (equivalent of hexamer): “MMW-Ad”-inferred value obtained by subtracting the concentration of HMW-Ad from the combined concentration of MMW-Ad + HMW-Ad
- 30 4) Low-molecular adiponectin fraction (equivalent of trimer including albumin-binding adiponectin): “LMWAd”-inferred value obtained by subtracting the combined concentration of MMW-Ad + HMW-Ad from the total concentration of Ad. The microtiter plate wells have been coated with an anti-human adiponectin monoclonal antibody. Adiponectin in the standards and pretreated specimens are captured by the antibody during the first incubation. Afterwards, a wash step removes all unbound material. Subsequently, an anti-human adiponectin antibody which has been biotin-labeled is added and binds to the immobilized adiponectin in the wells. Subsequently, an anti-human adiponectin antibody
- 35

which has been biotin-labeled is added and binds to the immobilized adiponectin in the wells. After the second incubation and subsequent wash step, HRP-labeled streptavidin is added. After the third incubation and subsequent wash step, substrate solution is added. Finally, stop reagent is added after allowing the color to develop. The intensity of the color development is read by a microplate reader. The absorbance value reported by the plate reader is proportional to the concentration of adiponectin in the sample.

Human KIM-1 was determined by the Human KIM-1 (catalogue number DY 1750) ELISA Development kit from R&D-Systems, containing a capture antibody (goat anti-human TIM-1) and a detection antibody (biotinylated goat anti-human TIM-1). A seven point standard curve using 2-fold serial dilutions in Reagent Diluent, and a high standard of 2000 pg/mL is recommended.

Example 1:

Patients suffering from diabetes mellitus type 1 (a total of 203 patients) and diabetes mellitus type 2 (a total of 134 patients) were investigated for urine levels of adiponectin, KIM-1 and L-FABP. The patients had a normal kidney function based on the upper limit for serum creatinine. The patients had evidence of acute infection and no evidence of acute metabolic changes.

As shown by figures 1-4 the concentrations of said biomarkers do not correlate.

Fig. 1: plot of L-FABP versus KIM-1 in patients with diabetes mellitus type 1

Fig. 2: plot of L-FABP versus adiponectin in patients with diabetes mellitus type 1

Fig. 3: plot of L-FABP versus KIM-1 in patients with diabetes mellitus type 2

Fig. 4: plot of L-FABP versus adiponectin in patients with diabetes mellitus type 2

Figure 1 is a plot of the amounts of the tubular damage marker L-FABP versus the tubular repair marker KIM 1 in diabetes type 1 patients. As can be seen both markers do not correlate, there are for example patients with high L-FABP but low KIM 1 values, these patients do have significant tubular damage but inappropriate repair indicative of

progressing kidney disease, vice versa there are cases with high levels of KIM 1 and thus repair but moderate tubular damage, indicating no progressive kidney disease.

Figure 2 is a plot of the amounts of L-FABP versus urinary adiponectin in type 1 diabetes mellitus patients. As can be seen there are cases with predominant tubular damage as indicated by high L-FABP levels but low adiponectin concentrations, in these patients tubular damage predominates over glomerular damage. There are also cases where the opposite is found or where tubular and glomerular damage appear to be equal. Thus testing for both marker demonstrates the predominant kidney injury in the diabetes patient and in addition its extent.

Figure 3 and Figure 4 provide the same information as Figures 1 and 2, the only difference lying in the patients being type 2 diabetes patients, where similar findings can be obtained when compared to type 1 diabetes patients.

15

The biomarker concentrations of said study, the L-FABP/Adiponectin ratio and the L-FABP/KIM-1 ratio are summarized in tables 1 and 2.

Tab. 1	Biomarker Concentrations in patients with diabetes mellitus type 1
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percentile	Adiponectin μg/g Creatinin	u-L-FABP μg/g Creatinin	KIM-1 μg/g Creatinin	L-FABP/ KIM-1 Ratio	L-FABP/ Adiponectin Ratio
50 th median	0,42	5,51	0,31	22,37	28,3
25 th	0,09	3,62	0,14	10,47	10
75 th	1,55	12,15	0,66	72,25	57

20

Tab. 2	Biomarker Concentrations in patients with diabetes mellitus type 2
--------	--

percentile	Adiponectin μg/g Creatinin	L-FABP μg/g Creatinin	KIM-1 μg/g Creatinin	L-FABP/ KIM-1 Ratio	L-FABP/ Adiponectin Ratio
50 th median	0,33	8,21	0,38	22,55	11,4
25 th	0,11	5,69	0,21	11,21	3

75 th	1,01	13,16	0,78	40,51	49
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It has been found that in a urine-sample of subjects suffering from diabetes mellitus type 1 or type 2 increased amounts of L-FABP and decreased amounts of KIM-1 in comparison to reference amounts, accordingly leading to high values of the L-FABP/KIM-1-ratio, are indicative for a severe tubular damage of the kidney because the effect of damage is predominant over repair. Increased amounts of adiponectin in comparison to reference amounts are indicative for a severe glomerular damage of the kidney.

10 **Example 2:**

A total of 52 patients with type 2 diabetes mellitus and microalbuminuria (30 to 300 ug/min, mean 93 ug/min) and unimpaired kidney function (apparently healthy) as assessed by creatinine levels within the normal range were included into the study. They were 41 males and 11 females, mean age 58 years. They received different doses of the AT blocker ibersatan for 2 months (each dose). Ibersatan doses were 300, 600 and 900 mg/day.

The study consisted of an initial 8-week washout period (discontinuation of all previous antihypertensive medication and initiation of bendroflumethiazide 5 mg daily in all patients). Following the washout period, patients were treated in random order with ibesartan 300, 600, and 900 mg once daily. Each treatment period consisted of an initial 7 weeks titration period with irbesartan 300 mg followed by 8 weeks with daily doses of irbesartan of 300, 600, and 900 mg respectively. End points were evaluated after the initial washout period (baseline and at the end of each of the three treatment periods).

The study was carried out as described in Rossing et al, Kidney International vol 68 (2005), pages 1190 – 1198.

Urine samples were taken at entry into the study (no treatment) and after each treatment cycle and at the end of treatment, Urine samples were stored at – 20 degrees Celsius before use. The following urine markers were tested: L-FABP, KIM 1 and Adiponectin as described previously.

Before the start of each treatment cycle urine samples were taken und analysed for L-FABP, KIM-1 and adiponectin and from the results obtained ratios were formed as

previously described. The same was done after completion of each treatment period (10, 28 and 38 weeks) results are presented.

Data summarised in Table 3 refer to each treatment period irrespective of the ibersartan dose (300, 600 or 900 mg daily, respectively).

Table 3:

10	treatment period	L-FABP ug /g Crea (mean)	KIM 1 ug/g Crea (mean)	Adiponectin ug/g Crea (mean)	L-FABP/ KIM 1 (mean)	L-FABP/ Adiponectin (mean)
	prior treatment	3,2	0,24	1,4	13,3	2,7
	10 weeks	3,2	0,28	0,7	14,4	8,0
15	28 weeks	4,3	0,29	0,3	15,2	23,8
	38 weeks	4,6	0,28	0,3	16,7	33,3

As can be seen from the Table the application of ibersatan was followed by a reduction of glomerular damage. Surprisingly there was no overall evidence of reduction in tubular damage, in contrast in average L-FABP as a marker of tubular damage increased as did the tubular repair marker KIM 1; on the average the ratio of L-FABP/KIM 1 increased from 13.3. to 16.7. With the reduction of adiponectin in urine the L-FABP/adiponectin ratio shifted from 2.7 to 33.3. This indicates a slight increase in tubular damage. This also indicates a reduction in glomerular damage, which is in accordance with a significant reduction in urinary albumin excretion in the average range of 49 - 59 %.

Data summarised in Table 4 to 6 refer to each treatment period, arranged depending on the ibersartan dose (300, 600 and 900 mg daily).

30

Table 4: 300 mg Ibersatan:

	L-FABP/Crea	KIM 1/Crea	Adiponectin/Crea
prio treatment	3,14	0,24	0,58
10 weeks	3,1	0,22	0,51
35 28 weeks	3,8	0,29	0,17
38 weeks	5,6	0,35	0,52

Table 5: 600 mg Ibersatan

5	prior treatment	3,14	0,24	0,58
	10 weeks	4,3	0,19	0,88
	28 weeks	6,3	0,25	0,37
	38 weeks	2,6	0,46	0,17

10

Table 6 900 mg Ibersatan

	prior treatment	3,14	0,24	0,58
	10 weeks	1,8	0,45	0,4
15	28 weeks	2,9	0,4	0,12
	38 weeks	5,2	0,2	0,26

As can be seen from Tables 4 to 6 effects of iberatan were not obviously different between different doses.

20

Comparison of amounts for L-FABP, KIM 1 und Adiponectin values measured at 38 weeks with baseline amounts shows that individual monitoring of patients is recommendable. Individuals respond differently to iberatan, which is shown in Table 7:

25 Table 7: comparison of visit 8 values with baseline levels

	L-FABP	KIM 1	Adiponectin
Increased	27/ 52	29/52	8/52
Decreased	25/52	23/52	44/52

30

Example 3:

35 A total of 52 patients with established type 1 diabetes and arterial hypertension were included into a study to receive the AT1 blocker losartan (50 mg daily for 2 months

followed by 100 mg/day for the rest of the study period) for a total of 36 months. All patients had nephropathy (albuminuria exceeding 300 mg/24 h) however a normal kidney function with creatinine levels within the normal range and a glomerular filtration rate exceeding 60 ml.min.1.73 m². Patients did not have a history of cardiac events (e.g. no myocardial infarction, no acute coronary syndrome).

Urine samples were taken before the start of treatment and then at 12 months intervals. Samples were stored at – 20 °C until tested.

L-FABP, KIM 1 and Adiponectin were tested as previously described.

Results are summarized in Table 6:

	L-FABP ug /g Crea	KIM 1 ug/g Crea	Adiponectin ug/g Crea	L-FABP/ KIM 1	L-FABP/ Adiponectin
Median					
Before	12,9	0,6	3,89	21,7	2,8
12 months	13,75	0,43	1,95	36,4	8,4
24 months	12,35	0,38	1,08	30,8	7,3
36 months	9,0	0,44	2,8	23,8	4,0

The results show that losartan did not significantly reduce tubular damage until the end of the study, this included the tubular repair marker KIM 1. In contrast to this, application of losartan was rather associated with an increase of tubular damage and a decrease in tubular repair. In contrast to this, adiponectin decreased throughout the study period suggesting a primary beneficial action of AT blockers on glomerular damage.

Similar individual variation in response to losartan when compared to ibersatan were seen This is exhibited in Table 7

Table 7: Increase/decrease in biomarker compared to baseline (before therapy at different timepoints (increase vs decrease in % of cases)

Timepoint	L-FABP	KIM 1	Adiponectin
12 months	52/48	13/87	28/72
24 months	36/64	25/75	23/77
36 months	32/68	7/93	32/68

A decrease of L-FABP with an increase in KIM1 and a decrease of adiponectin in urine is considered beneficial response to treatment.

5

Claims

1. A method for diagnosing kidney damage in a subject suffering from diabetes mellitus comprising the steps of:
 - a) determining the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof and kidney injury molecule 1 (KIM-1) or a variant thereof in a urinary sample of a subject;
 - b) comparing the amounts determined in step a) with reference amounts;
 - c) optionally forming the L-FABP/KIM-1 ratio; anddiagnosing the kidney damage.
2. The method according to claim 1, wherein the kidney damage is tubular damage and/or tubular repair..
3. The method according to claim 1 or 2, wherein with respect to the corresponding reference amounts an increased amount of L-FABP or a variant thereof and a decreased amount of KIM-1 or a variant thereof, resulting in a high value of the L-FABP/KIM-1 ratio, are indicative for progressive tubular damage of the kidney.
4. The method according to claim 3, wherein said reference amount for L-FABP or a variant thereof is $>$ about 7 $\mu\text{g/g}$ creatinine and said L-FABP/KIM-1 ratio is $>$ about 20.
5. The method according to any of claims 1 to 4, wherein the method comprises the additional step of determining the amount of adiponectin or a variant thereof in sample of a subject.
6. The method according to claim 5, wherein with respect to the corresponding reference amount an increased amount of adiponectin of is $>$ about 0,30 $\mu\text{g/g}$ creatinine is indicative for a progressive glomerular damage of the kidney.

7. The method according to claim 4 or 5, wherein additionally a L-FABP/adiponectin ratio is formed.
- 5 8. The method according to claim 7, wherein, in subjects suffering from diabetes mellitus type 1, a L-FABP/adiponectin ratio of more than about 30 is indicative for a predominant tubular damage of the kidney and a L-FABP/adiponectin ratio of less than about 25 is indicative for a predominant glomerular damage of the kidney.
- 10 9. The method according to claim 8, wherein, in subjects suffering from diabetes mellitus type 2, a L-FABP/adiponectin ratio of more than about 15 is indicative for a predominant tubular damage of the kidney and a L-FABP/adiponectin ratio of less than about 10 is indicative for a predominant glomerular damage of the kidney.
- 15 10. A method of deciding whether a subject suffering from diabetes mellitus associated kidney damage is susceptible to a suitable therapy comprising:
 - a) determining the amounts of liver-type fatty acid binding protein (L-FABP) or a variant thereof and kidney injury molecule 1 (KIM-1) or a variant thereof and optionally adiponectin in a urine-sample of a subject;
 - 20 b) optionally forming the L-FABP/KIM-1 ratio;
 - b) comparing the amounts determined in step a) and/or b) with reference amounts, thereby diagnosing the kidney damage, and
 - c) deciding on the suitable therapy
- 25 11. A method for diagnosing myocardial infarction in a subject comprising at least one of the following steps:
 - a) determining the amounts of a natriuretic peptide and/or troponin T in a sample of the subject;
 - b) comparing the amounts determined in step a) with reference amounts; and
 - 30 c) diagnosing myocardial infarction.
12. A method for monitoring kidney damage during therapy in a subject suffering from diabetes mellitus comprising the steps of:
 - a) determining the amounts of liver-type fatty acid binding protein (L-FABP) or a
35 variant thereof and kidney injury molecule 1 (KIM-1) or a variant thereof and optionally adiponectin in a sample of a subject;

- b) optionally forming the L-FABP/KIM-1 ratio;
- c) comparing the amounts determined in step a) and/or b) with reference amounts and diagnosing the kidney damage,
- d) repeating steps a) to c) during the therapy.

5

13. A device for diagnosing kidney damage in a subject suffering from diabetes mellitus, comprising
- a) means for determining the amounts of liver-type fatty acid binding protein (L-FABP) and kidney injury molecule 1 (KIM-1) and optionally adiponectin in a urine-
- 10 sample of a subject;
- b) means for comparing the amounts determined in step a) with reference amounts and whereby the device is adapted for diagnosing the kidney damage.

15

14. A kit for diagnosing kidney damage in a subject suffering from diabetes mellitus, comprising:
- a) means for determining the amounts of liver-type fatty acid binding protein (L-FABP) and kidney injury molecule 1 (KIM-1) and optionally adiponectin in a urine-sample of a subject;
 - b) means for comparing the amounts determined in step a) with reference amounts and
- 20 whereby the kit is adapted for diagnosing the kidney damage.

25

15. Use of a) a means for determining the amount of a liver-type fatty acid binding protein (L-FABP) or a variant thereof and of b) a means for determining the amounts of and
- 25 kidney injury molecule 1 (KIM-1) or a variant thereof, for diagnosing or detecting kidney damage in a subject suffering from diabetes mellitus, in a urinary sample from the subject.

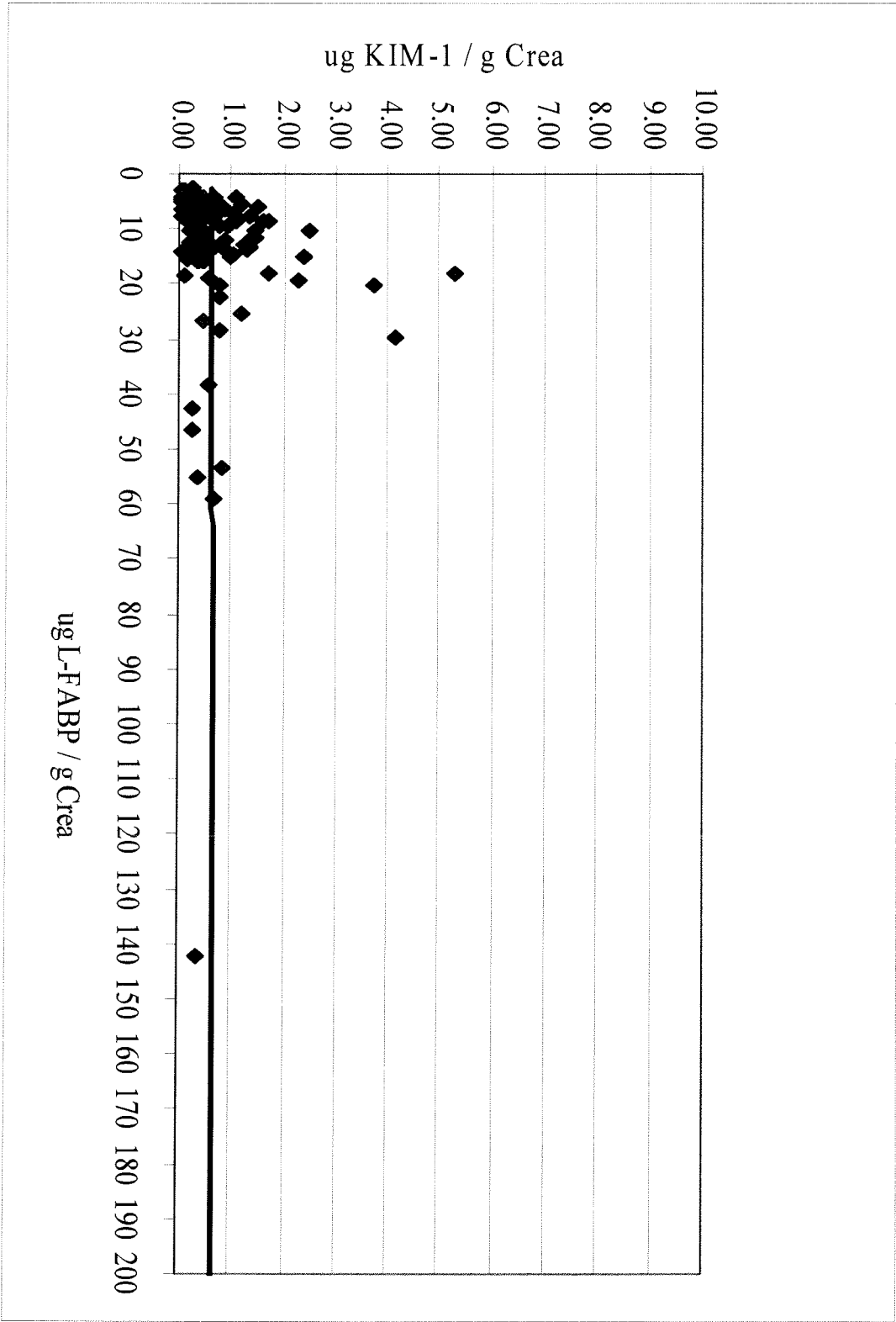


Figure 1

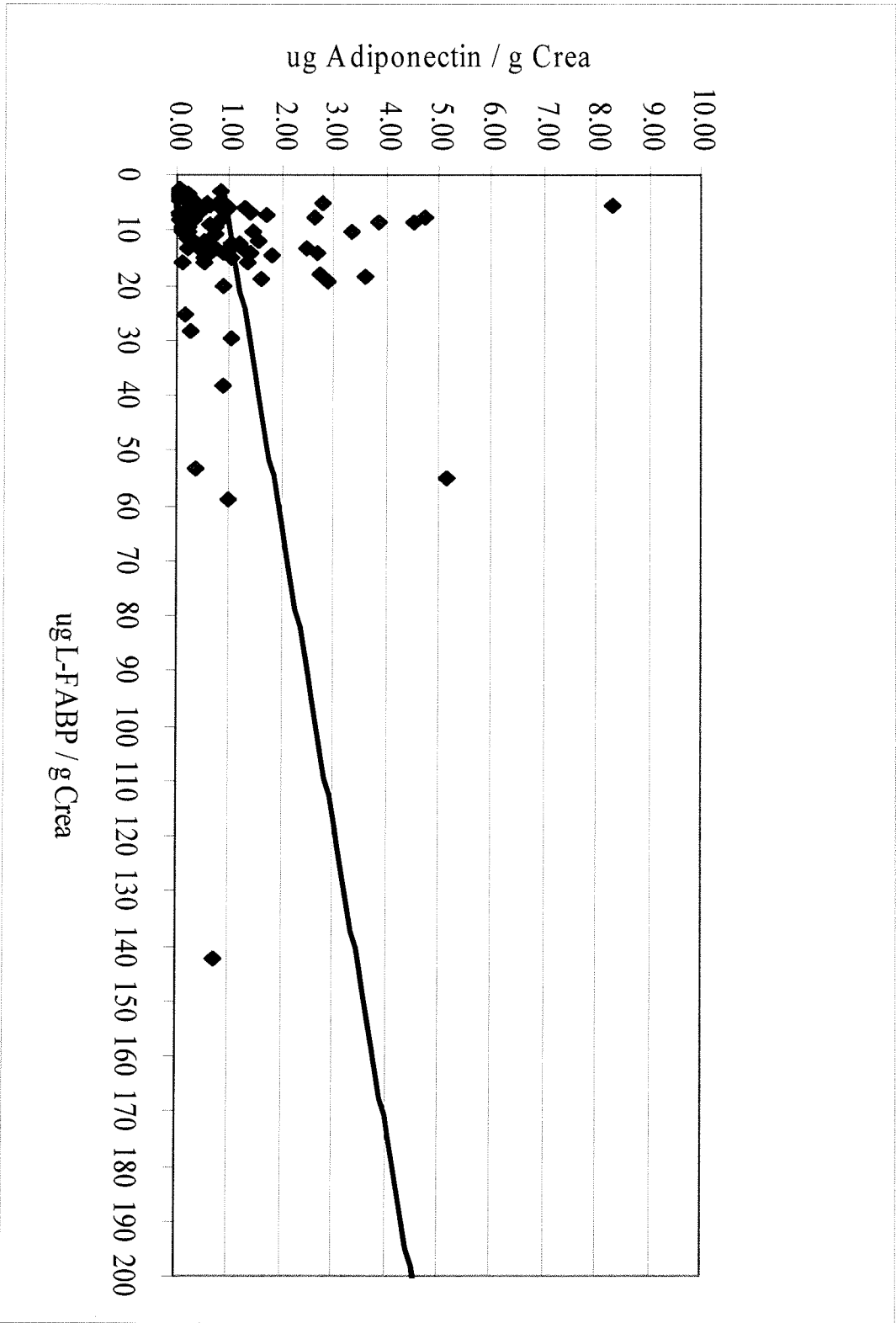


Figure 2

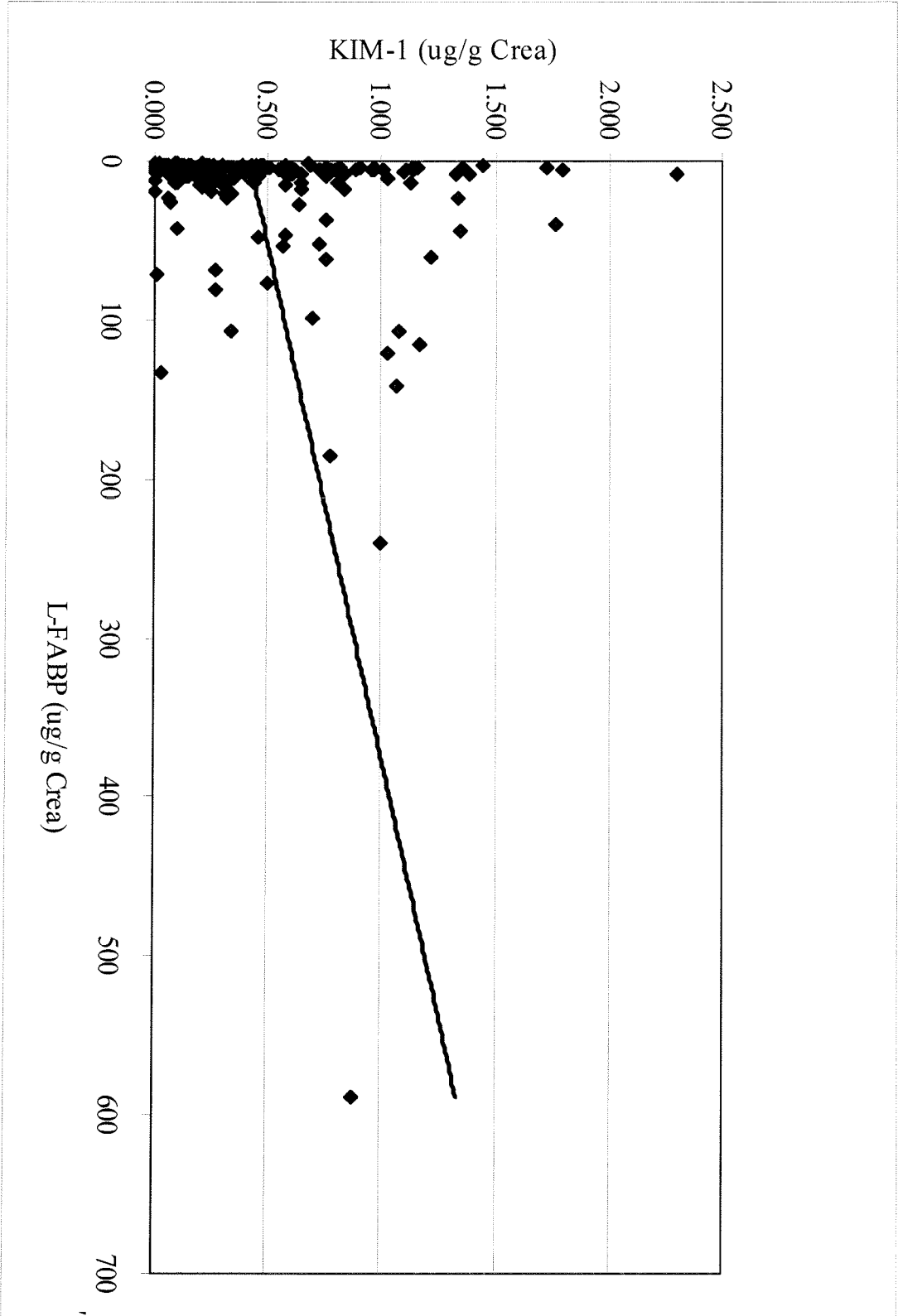
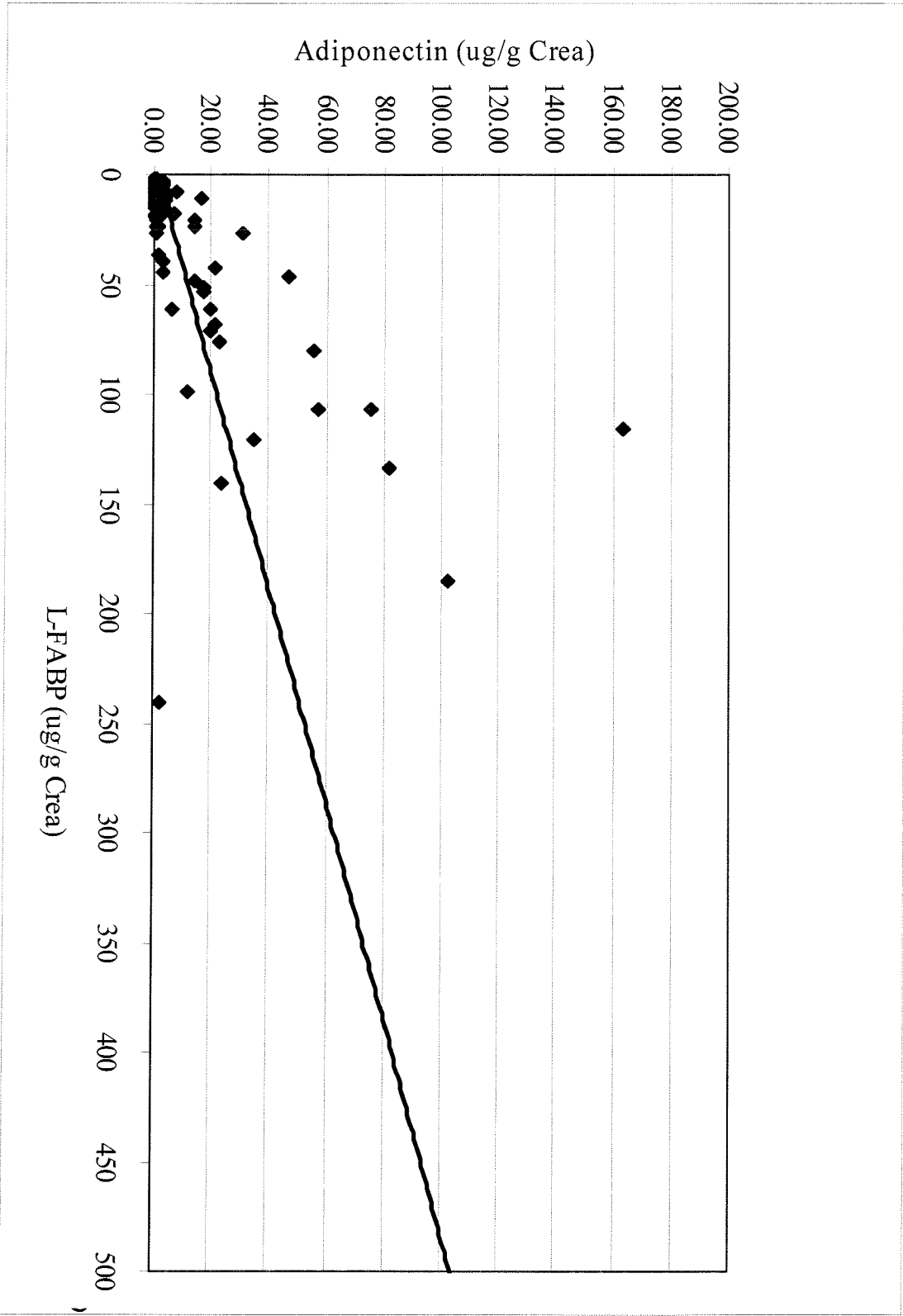


Figure 3

Figure 4



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/055855

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/154238 A (SIEMENS HEALTHCARE DIAGNOSTICS INC. [US]) 18 December 2008 (2008-12-18) paragraphs [0002], [0028] - [0032], [0041], [0071], [0077] - [0080] claims 1-24 paragraphs [0081] - [0083]	1-10, 13-15
X	FERGUSON M.A. ET AL.: "Biomarkers of nephrotoxic acute kidney injury" TOXICOLOGY, vol. 245, no. 3, 4 January 2008 (2008-01-04), pages 182-193, XP022508093 abstract page 184, right-hand column page 186 paragraphs [0006], [0009]	1,2,10, 12-15
-/--		

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

3 August 2010

Date of mailing of the international search report

27/09/2010

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/055855

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DEVARAJAN P: "Emerging urinary biomarkers in the diagnosis of acute kidney injury" EXPERT OPIN. MED. DIAGN., vol. 2, no. 4, 2008, pages 387-398, XP009121130 cited in the application abstract paragraphs [0005], [0006] -----	1,2,10, 12-15
A	US 2004/241774 A1 (KAZUO U. [JP]) 2 December 2004 (2004-12-02) abstract paragraphs [0041] - [0079] claims 1-10 -----	1-10, 12-15
A	WO 2009/040400 A (ROCHE DIAGNOSTICS GMBH [DE]) 2 April 2009 (2009-04-02) abstract page 17, lines 5-9 claims 1-17 -----	1-10, 12-15
X,P	VON EYNATTEN M. ET AL.: "Urinary L-FABP and anaemia: distinct roles of urinary markers in type 2 diabetes." EUR. J. CLIN. INVEST., vol. 40, no. 2, February 2010 (2010-02), pages 95-102, XP002595155 the whole document -----	1-10, 12-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2010/055855

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-10, 12-15

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-10, 12-15

Method for diagnosing kidney damage in a subject suffering
from diabetes mellitus

2. claim: 11

Method for diagnosing myocardial infarction.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2010/055855

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008154238 A	18-12-2008	EP 2160478 A1 US 2010184110 A1	10-03-2010 22-07-2010
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WO 2009040400 A	02-04-2009	EP 2042873 A1 EP 2203748 A1	01-04-2009 07-07-2010