Chronic kidney disease

Proteins are responsible for structure and signaling in living organisms and in every organ. Based on this consideration, it appears only logical investigate proteomic changes in the context of chronic kidney disease (CKD), aiming at identifying molecular changes associated with CKD onset and progression that can be linked to molecular pathophysiology and that could serve as more appropriate biomarkers or even as therapeutic targets. **Figure 1** illustrates this concept of how early diagnosis and/or prognosis of diseases, based on proteomic changes involved in pathology, improves chances for better outcomes for patients.

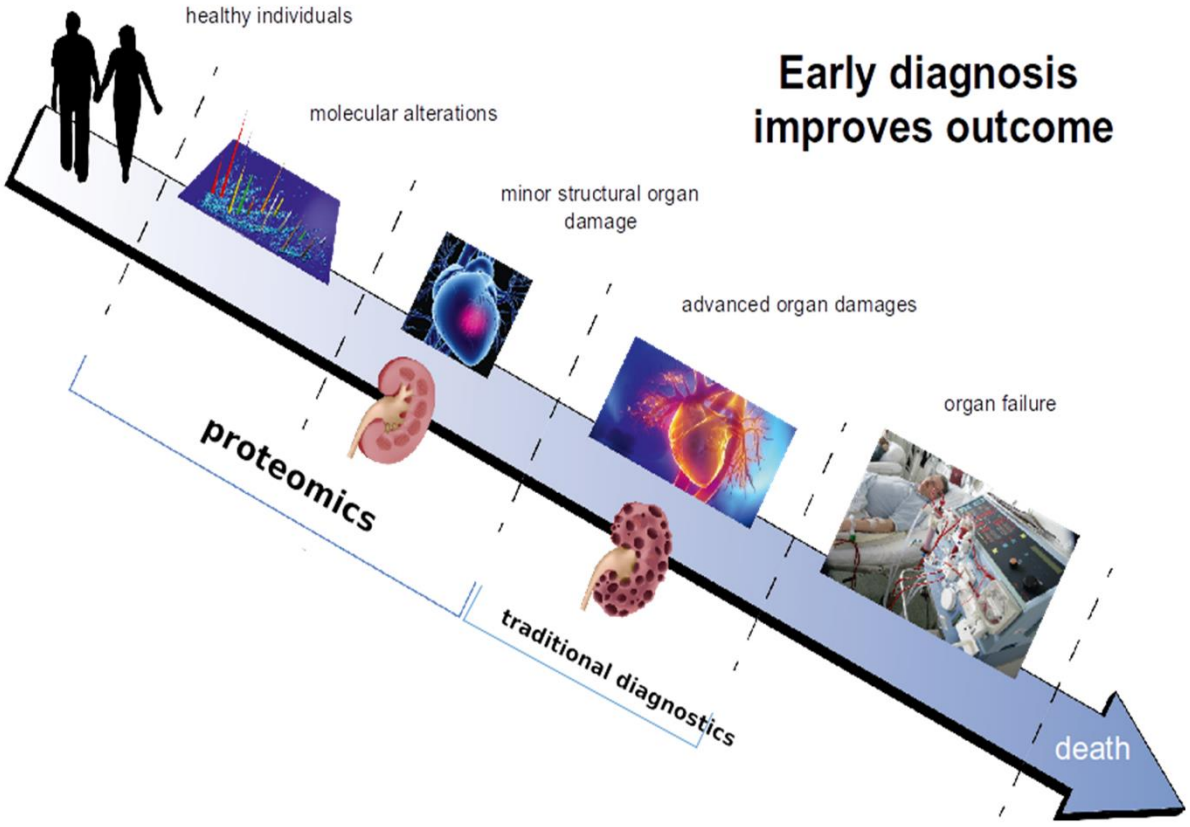

Figure 1. Early diagnosis and/or prognosis of diseases improves chances for a better outcome for the patient. The initiation of molecular processes that result in (chronic) diseases can be detected based on molecular changes, using proteomic technologies, prior to advanced organ damage. This could allow earlier intervention where drugs are most effective (Stepczynska et al.).

Diagnosis and Prognosis of CKD

The urinary peptide-based DiaPat® RenOM test is a general test for the diagnosis of CKD. It was developed in 2010 by Good et al.[1]. The rational was to identify biomarkers associated with

CKD in general and enable the early detection of molecular changes that predict the development or progression of CKD. In the above study, 273 urinary peptides were identified that significantly differed between CKD and healthy controls. The first validation of the test using 144 samples showed a sensitivity of 85% and specificity of 100% with an area under the curve (AUC) of 0.96 for the diagnosis of CKD. To establish an added value in patient management, DiaPat® RenOM test was assessed in several studies[2-14]. A graphic depiction of the studies published to date using DiaPat® RenOM test is presented in **figure 2**.

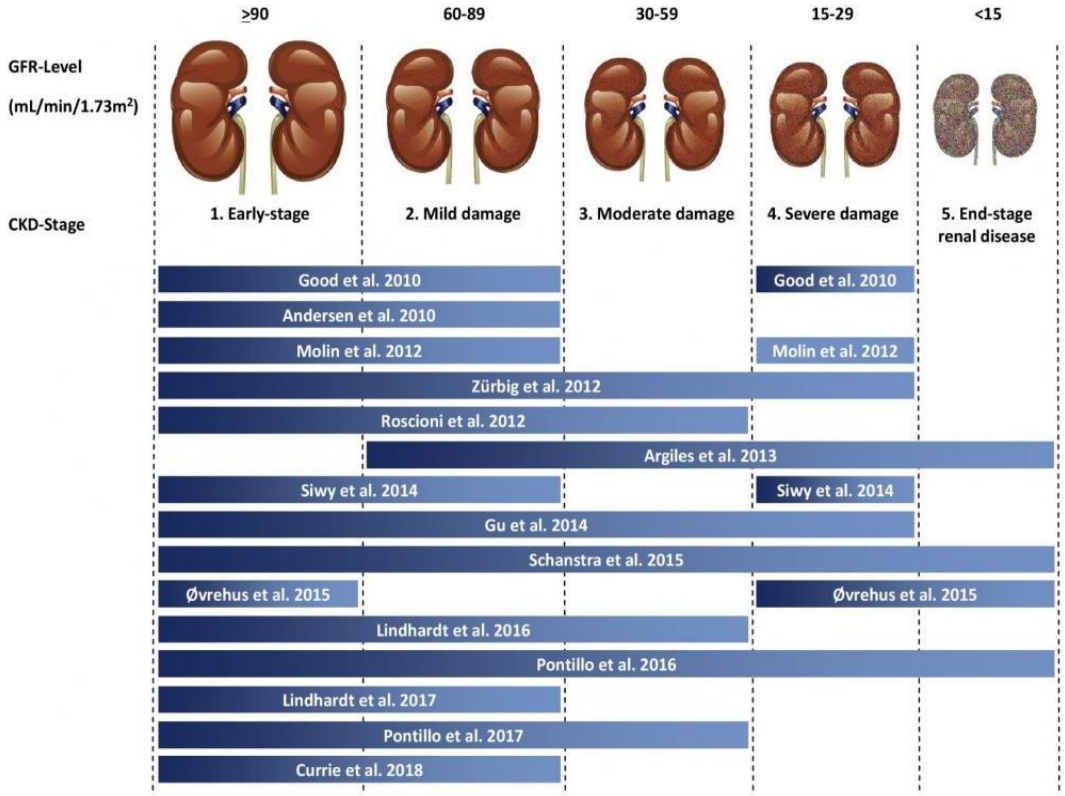

Figure 2. Graphical distribution of the studies evaluating the performance of the DiaPat® RenOM test in the diagnosis and prognosis of CKD according to disease stage (Critselis at al.).

Characteristics of DiaPat® RenOM test:

- is validated in independent multicenter cohorts[1;7;13] (**Figure 3A**)
- is validated in longitudinal cohorts[12;14]
- shows better results than currently used biomarkers[10;12;14] (**Figure 3B**)
- can predict endpoints of CKD[3;10;11]
- is validated concerning its stability, intra- and intermediate precision, reproducibility, and interference[1;15]

- shows treatment effects[2;5;6] (**Figure 3C**)
- was used in an interventional trial (PRIORITY)[16,17], (**Figure 3D**)
- is associated with mortality[18]
- achieves high evidence levels[19]
- shows cost-effectiveness compared to urinary albumin excretion[20] (**Figure 3E**)
- is used for pilot studies with pharmaceutical companies
- got "letter of support" from American Food and Drug Administration (FDA)

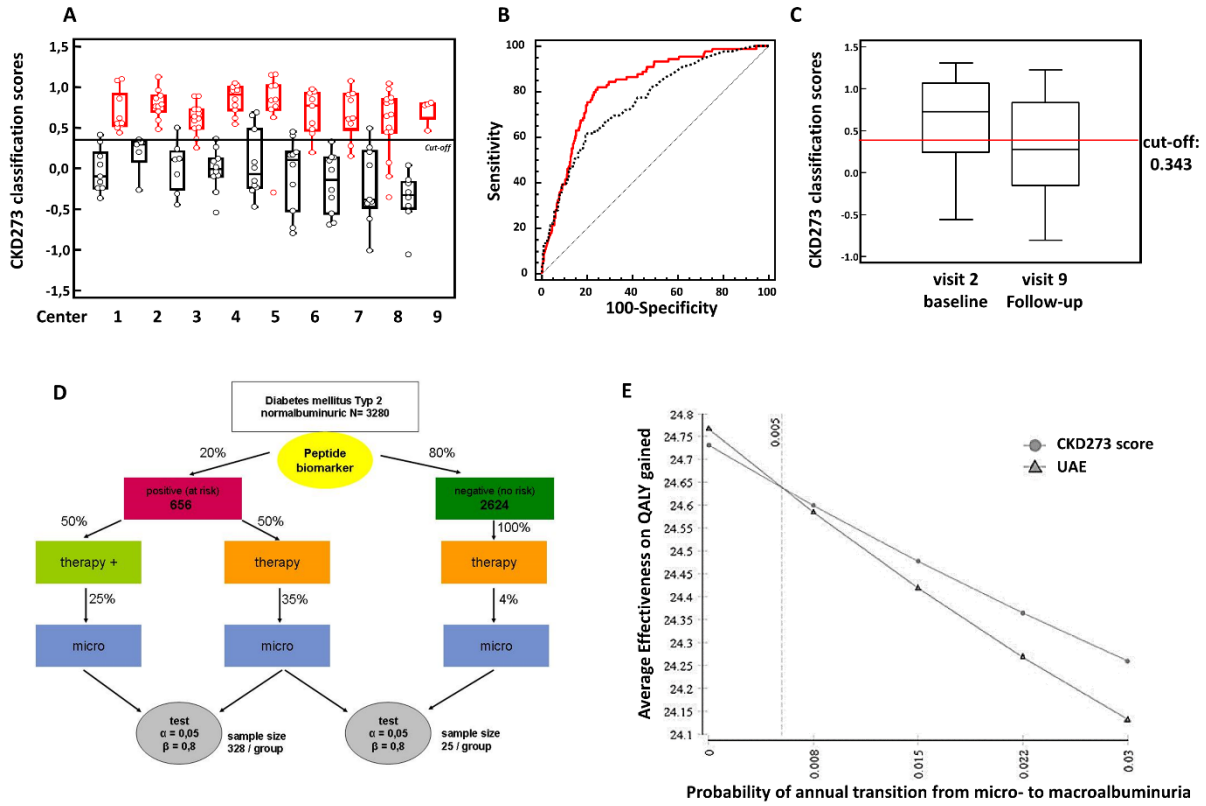

Figure 3. The DiaPat® RenOM test (CKD273) highlights. **A.** validation of the test in nine independent cohorts shoved consistent high diagnostics accuracy with area under curve above (AUC) 0.95 (Siwy et al.). **B.** Comparison of the test with clinical parameters with significant higher AUC for the DiaPat® RenOM test (AUC=0.82, black line) than for albuminuria (AUC=0.76, red line) using patients with fast-progressing CKD (eGFR slope decline of >−5% per year). **C.** The classification results of microalbuminuric patients before (visit 2) and after two years (visit 9) treatment with 200 mg Irbesartan with significant (p=0.024) decline of classification scores after Irbesartan treatment indicating an improvement of kidney physiology (Andersen et al.). **D.** Interventional study in which the DiaPat® RenOM test was used for the patient stratification and participants with a high-risk, the test scores are included in the randomized intervention study with active drug or placebo in addition to standard care (Tofte et al.). **E.** One-way sensitivity analyses for the cost-effectiveness from an European prospective of screening T2D patients using CKD273 as compared to screening annually with UAE over 40-year time frame that indicates that with increasing probability of annual CKD progression, the CKD273 screening was associated with a greater gain in QALYs in diabetic patients (Critselis at al.).

Non-invasive discrimination of various types of CKD

Currently, our technology is capable of detecting over 80% of all CKDs using DiaPat® RenOM test:

- Focal segmental glomerulosclerosis (FSGS)
- Diabetic nephropathy
- Nephrosclerosis
- IgA-nephropathy (IgAN)
- Minimal Change Disease (MCD)
- Membranous glomerulonephritis (MGN)
- Lupus nephritis
- Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV)

In addition, the DiaPat® RenOM test clearly differentiated various types of CKD. The DiaPat® RenOM test includes specific urinary peptide classifiers[19] which are composites multiple peptides specific for a single CKD type. These classifiers enable a differential diagnosis of certain types of CKD (**Figure 5**). They may also serve as an excellent basis for the assessment of the different types of CKD, to understand molecular pathophysiology and to identify the best-suited therapeutic targets. In contrast to kidney biopsy, DiaPat® RenOM test offers the possibility of being applied early in the course of the disease when the benefit of intervention is optimal and of being repeatable without any risk for the patient and, thus, can be used to monitor progression of disease and/or treatment response.

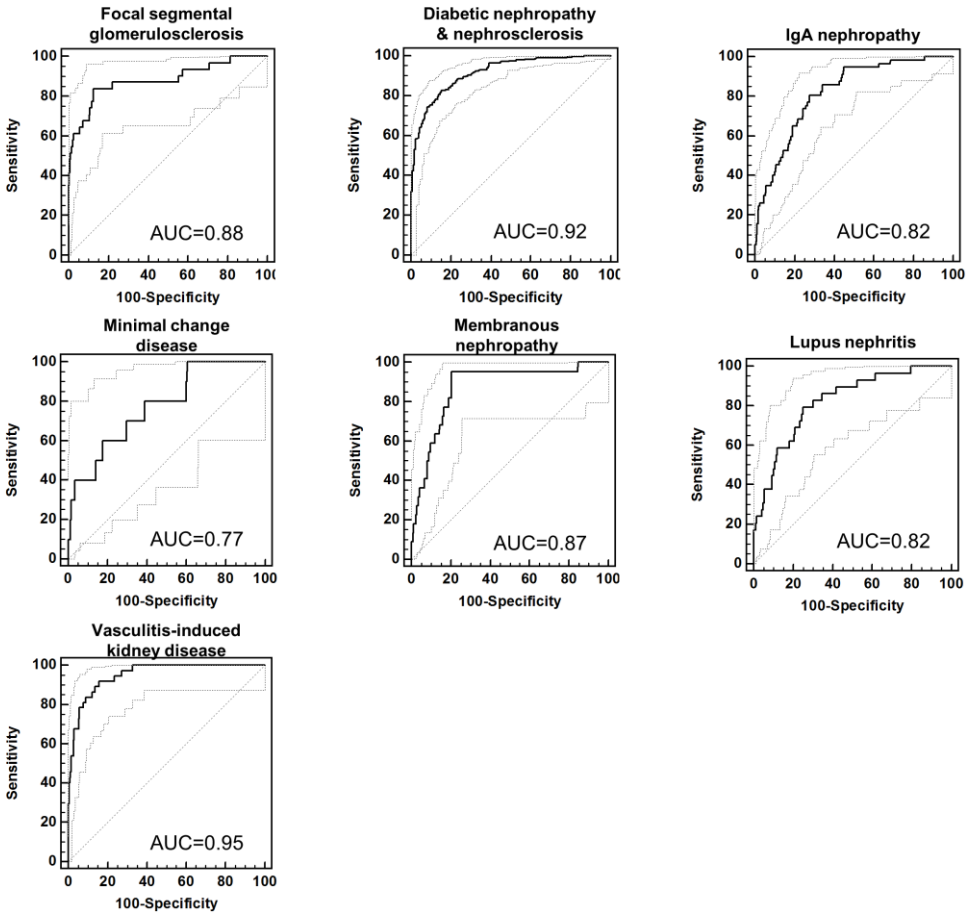

Figure 5. Discrimination of the individual CKD types from all others CKD types based on 474 samples resulted in high accuracy with AUC-values ≥ 0.8 (Siwy et al.).

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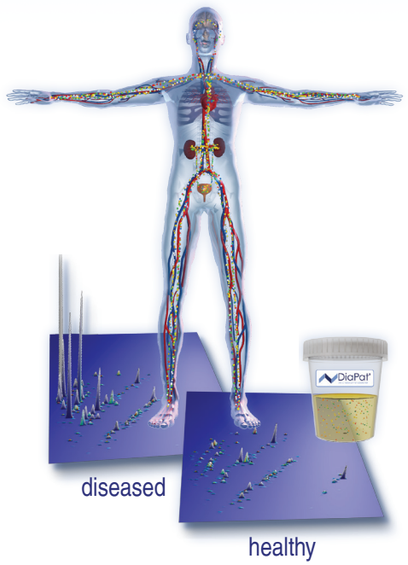
Uniqueness of the DiaPat® Test

Diseases originate mainly on the molecular level, which can be depicted by proteome analysis, utilising the unique DiaPat® method with a single urine sample.

Blood and urine, the filtrate of blood, carry proteins from every part of the body. 1700 liters of blood are filtered through the kidneys every 24 hours. This filtration process produces about 180 liters of primary urine from which 1.5 liters are excreted from the body.

The DiaPat® Test detects early pathological alterations and therefore provides timely disease recognition, enabling an early and succesful therapeutic intervention to follow.

As the DiaPat® Test utillises proteins, which are targeted by most of the drugs, an individual succesfull therapy can be achieved.



DiaPat® Technology

The DiaPat® Test enables identification of disease specific protein signatures using body fluids such as urine.

From a single urine sample up to 6 gigabytes of data can be obtained and over 100,000 proteins and peptides can be analyzed. Using this approach, hundreds of proteins and protein fragments can be detected and disease specific patterns can be identified with exceptional precision.

Utilising the DiaPat® technology, several diagnostic patterns have been developed including: (i) 75 biomarkers for the prognosis of myocardial infarction risk, (ii) 238 biomarkers for the diagnosis of coronary artery disease, (iii) 96 biomarkers for the prognosis of heart failure, and (iv) 237 biomarkers for chronic kidney diseases, including diabetic nephropathy. Due to high number of biomarkes within each pattern, the DiaPat® Tests provide high accuracy, which cannot be achieved with commercially available single biomarkers.

Scientific evidence for the DiaPat® Tests:

- over 70 clinical studies
- over 200 scientific publications in leading journals
- involvement of about 500 world-renowned scientists from over 65 university hospitals

Projects supported by the European Commission through the Seventh Framework Programme (FP7)

Overview

DiaPat® KardiOM + RenOM Test

- detects risk of myocardial infarction up to 5 years in advance due to unstable plaques
- indicates significant narrowing of blood vessels
- detects heart failure up to 5 years in advance
- detects chronic kidney disease, including diabetic nephropathy
- allows differential diagnosis of kidney diseases
- enables personalised medicine

Heart and kidney check up

Cardiovascular and renal diseases are interrelated and often demonstrate themselves in the form of cardiorenal syndrome.

In additon to the DiaPat® KardiOM + RenOM Test, we offer a test for determination of age-specific proteome on request.

To receive further information about products and prices, please call our hotline 0511 – 554744 44 0 or visit our website.

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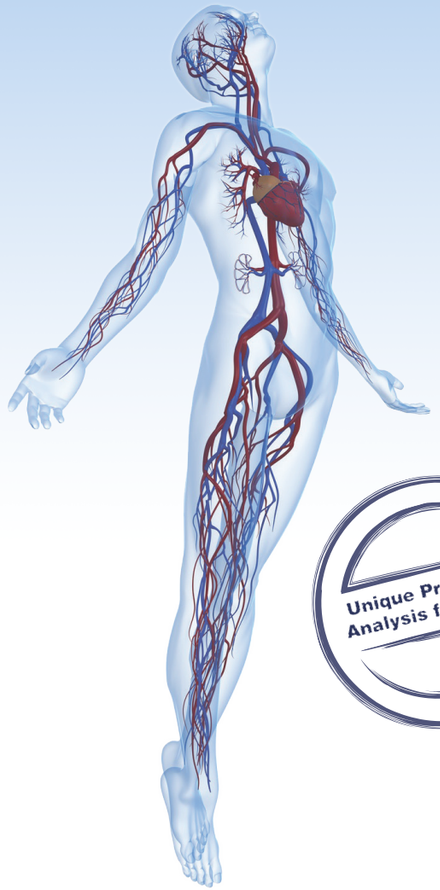
V1.1 -April 2016

KardiOM + RenOM Test

Early Heart and Kidney Diseases detection



Unique Proteomic Analysis from Urine

DiaPat®
the key to your health

Significant advantage of DiaPat®

The cardiorenal syndrome, which displays the interdependence of cardiovascular and renal diseases, can be diagnosed with the DiaPat® KardiOM + RenOM Test. Cardiovascular and renal diseases influence each other and increase risk of mortality.

The DiaPat® Test allows collection of information from a urine sample without utilisation of chemicals, such as contrast agents used for imaging, which can be hazardous to your health. Thus, the repetitive application of the DiaPat® Test, either to determine disease risk at different time points or to monitor the effect of therapy, is safe.

The DiaPat® proteome analysis depicts diseases at the molecular level, the level of proteins, which is targeted by drugs.

Only effective implementation of diagnosis and treatment leads to succesful therapy.

DiaPat® for asymptomatic heart diseases

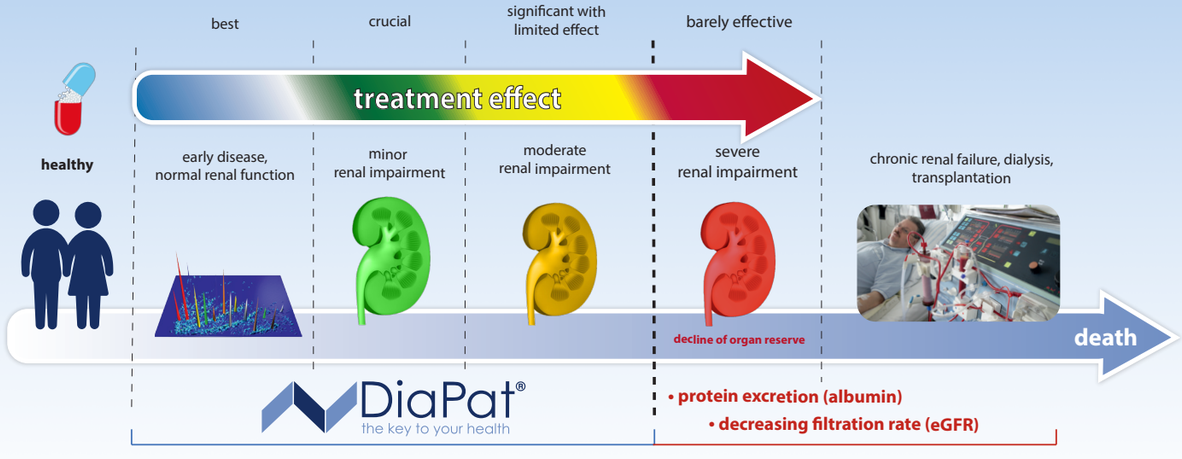
DiaPat® detects heart disease independly of the appearance of symptoms, such as fatigue from minimal physical activity, shortness of breath, palpitations, chest pain or edema (body fluid retention). Cardiovascular diseases can arise silently, ie. asymptomatically.

For doctors:
The DiaPat® detects preclinical left ventricular dysfunctions (systolic and diastolic) already at the NYHA stage I and allows prognosis of progression to overt heart failure.

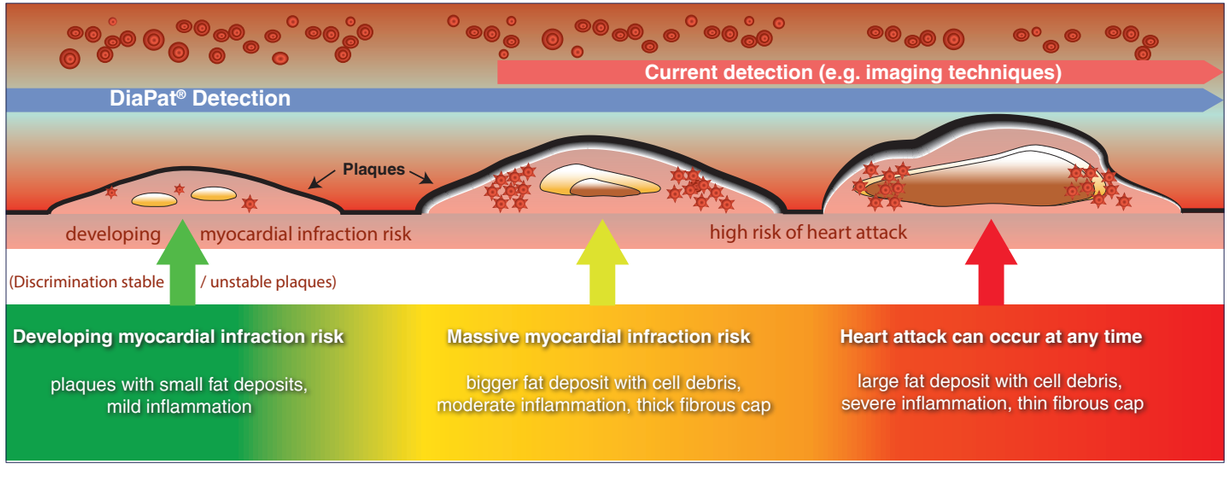
Molecular diagnosis of kidney diseases

Dangers of diabetes

DiaPat® guided diagnostics and therapy



Early detection prevents myocardial infraction



Blood pressure is controlled in part by the kidney (adrenal gland).

Chronic kidney disease (CKD) patients have 5-10 times increased risk of cardiovascular disease, especially coronary artery disease and heart failure.

The interconnected chronic heart and kidney diseases often arise unnoticed.Thus, so far these chronic diseases have been detected too late and are associated with considerably shorter life expectancy. The DiaPat® Test early and accurately detects both diseases based on a single urine sample.

Between 30% and 40% of diabetics develop diabetic nephropathy (DN) in their life times. The kidney dysfunction in DN is usually assessed by the presence of albumin (a protein) in urine and glomerular filtration rate (measure of kidney ability to filtrate blood). Unfortunately these methods detect abnormalities, when already 50% of kidney function is irreversibly lost. We propose our technology and biomarker panels to detect abnormalities at a very early, molecular stage, before structural alternations occur and the kidney is still fully functional. Only intervention at a very early stage has potential to prevent or slow down disease development.