



## TRACE TECHNOLOGY FOR ASSAYS OF NOVEL BIOMARKERS

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### **Abstract**

The availability of an easily measurable specific marker for diagnosis of a disease is an important but not always reachable objective. Emergency area is an area where decisions must be taken as quick as possible, and sometimes it is not clear if the patient is well enough to be treated as an outpatient or must be hospitalized.

During last few years many new biomarkers and techniques for their measurement have been developed, and surely some others will appear. The aim of this report was to present TRACE technology for specific assays such as: copeptin, proadrenomedullin and proatrial natriuretic peptide. Ongoing research is being done to decide in which diseases they might be useful and if they will be needed for diagnosis, prognosis or treatment monitoring. Results are not conclusive yet, but in the future some of these markers could be used in routine laboratory work if their utility is documented by new data.

## **INTRODUCTION**

A biomarker is any molecule used as an indicator of a biological state. It can be used for diagnosis, prognosis or /and follow-up of diseases. The "ideal" biomarker is specific, sensitive and easy to measure, but finding one is not easy.

One of the biggest problems when a biomarker is found is its measurement, because the concentration is usually very low, techniques are laborious and time-consuming. This makes difficult its posterior clinical use. During past few years, new biomarkers have been found and ongoing research is being done to prove their utility in different diseases.

## **TRACE<sup>®</sup> TECHNOLOGY**

Time-Resolved Amplified Cryptate Emission (TRACE) is a technology based on non-radiating energy transfer from donor molecule (europium cryptate) to acceptor molecule (XL665) as a result of the completed immune reaction. TRACE<sup>®</sup> technology is exclusively available on KRYPTOR<sup>®</sup> analysers. The specific fluorescence which is proportional to the antigen concentration is obtained through a double selection: spectral (separation depending on wavelength) and temporal (time resolved measurement). TRACE technology allows measurements in a homogeneous phase, providing precise results in a short reaction time. This makes possible biomarkers analysis in routine laboratory practice. Some of the specific novel biomarkers available with the use of TRACE technology are: copeptin, proadrenomedullin and proatrial natriuretic peptide.

## **COPEPTIN**

Vasopressin (AVP) is a polypeptide with a disulphide bond between the two cysteine amino acids. In humans AVP is encoded by the mRNA for preprorenophysin II. After cleavage of the signal peptide, the resulting prohormone

contains AVP (nine amino acids), neurophysin II (95 amino acids) and a glycopeptide (39 amino acids). At high doses it is capable of triggering capillary vasoconstriction. At low doses it inhibits urine output with no effect on the circulation, earning its name the 'antidiuretic hormone'.

AVP measurement is useful in certain endocrine disorders and as a guide therapy in pathologies in which osmotic and cardiovascular homeostasis are disturbed (shock, sepsis) (1,2). However, there is a problem with its measurement that is difficult because the molecule is very unstable, largely attached to the platelets, rapidly cleared (short half-life) and there are variations between the assays as well.

Copeptin is the carboxyterminal fragment of provasopressin, a 39 aminoacid glycoprotein of yet not well-known function. As stoichiometrically released, it would reflect AVP production. Finally, copeptin is a more stable molecule and its assay is automated. As correlation was found between AVP and copeptin concentration this allows to use copeptin as a specific novel biomarker in diagnosis of infection, severe sepsis, and septic shock in the Emergency Departments (1,2).

### **PROADRENOMEDULLIN**

Adrenomedullin (ADM) is an almost ubiquitous peptide, extracted from a pheochromocytoma in 1993. It has 52 aminoacids, with a disulfide bridge between the residues 16 and 21. Its main function is to decrease blood pressure, but it has many other functional actions such as: vasodilatation, increases diuresis and natriuresis, increases tolerance of cells to oxidative stress and hypoxia, stimulates angiogenesis, modulates cytokine production and exerts antibacterial action.

Many disease states have been associated with high levels of plasma adrenomedullin: cardiovascular (hypertension, acute myocardial infarction, heart failure), respiratory (asthma, COPD), endocrine (thyrotoxicosis, type I diabetes mellitus), renal (chronic renal failure, glomerulonephritis), cancer, hepatic cirrhosis etc. Measurement of ADM is very difficult as it has a very short half-life, is bound to a specific protein (binding protein H), adheres non specifically to the surfaces and because of other technical problems.

ADM gene is localized in chromosome 11 and is synthesized as a part of preproadrenomedullin, a 185 aminoacid precursor. During processing of preproADM other peptides are released and midregional-proadrenomedullin (MR-proADM) is one of them: the molecule consisting of 45-92 aminoacids. Stoichiometrically released, this molecule may directly reflect ADM concentration in plasma and is more stable (3).

### **PROATRIAL NATRIURETIC PEPTIDE (PROANP)**

Atrial natriuretic peptide (ANP) is a 28 aminoacid peptide with a 17 aminoacid ring closed by a disulfide bond between two cysteine residues (in position 7 and 23). ANP is closely related to BNP (brain natriuretic peptide) and CNP (C-type natriuretic peptide), which all share the same aminoacid ring. Predominant signal for the release is atrial stretch or atrial distension due to volume expansion. Besides, there are variety of other signals for its release like hypervolemia, exercise, caloric restriction, sympathetic stimulation of  $\beta$ -adrenoreceptors, hypernatremia (though it is not a direct stimulus), or a response to angiotensin-II and endothelin.

Main function of proANP is to ensure relative constancy of body electrolyte and water content and circulatory homeostasis. It does so by different biological actions like vasodilatation, increasing natriuresis and diuresis, suppression of renin-angiotensin-aldosterone system, suppression of sympathetic activity and antidiuretic hormone.

The peptide is synthesized as a part of preproANP, which after cleavage of signal peptide releases a 126 aminoacid precursor of ANP called proANP. ProANP directly reflects ANP levels, is a more stable molecule and easier to measure (4).

### **CLINICAL USE OF NOVEL BIOMARKERS**

Copeptin, proADM and proANP can be easily measured by TRACE technology. They have been studied for diagnosis, prognosis and follow-up of many diseases: heart failure (5,6), pneumonias (7-9), COPD (10), myocardial infarction (11,12), sepsis (3,13), etc.

Some reported very good results, others not so good, and some authors conclude these biomarkers do not add any valuable information. There are much more biomarkers, like C-terminal proendothelin-1, lipopolysaccharide binding protein, neopterin and some interleukins that are currently being investigated.

## CONCLUSION

Some of the above presented biomarkers, are nowadays easily and quickly measured with the use of TRACE technology which make them helpful in the process of taking decisions in the Emergency Department as quickly as possible. Their clinical utility has not been proven conclusively yet but in the future they might be used in the routine daily practice like other biomarkers that are nowadays in everyday use, for example C-reactive protein and procalcitonin.

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