suPAR – a Unique Biomarker in Infectious Diseases and Cancer

The plasma level of **s**oluble **u**rokinase **p**lasminogen **a**ctivating **r**eceptor (suPAR) reflects immune activation and is increased in several infectious diseases, such as HIV-1-infection, malaria, tuberculosis, *Streptococcus pneumonia* bacteraemia, sepsis, pneumococcal pneumonia and bacterial and viral CNS infection. Furthermore, high suPAR levels are associated with increased inflammation, disease progression and fatal outcome. Measuring suPAR levels can thus serve as a marker to monitor disease progression and treatment [1,2,3,4,5,6].

suPAR levels can be measured easily with the novel **suPARnostic® ELISA kit**. Compared to other applicable products, this CE/IVD approved kit provides fast and reproducible results. Moreover, this product is backed by patent rights to use the marker in various disease conditions.

What is suPAR?

suPAR is the soluble form of the urokinase-type plasminogen activator receptor (uPAR), a three domain receptor[7] mainly expressed on immune cells, including neutrophils, activated T-cells, and macrophages.

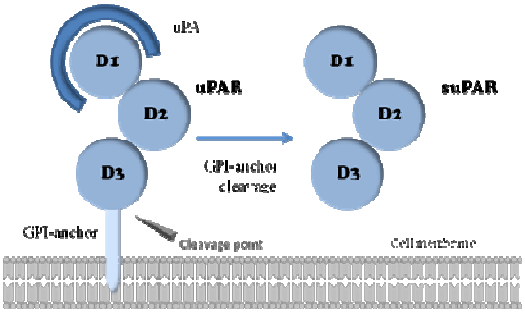

Figure 1 | Schematic representation of urokinase receptor The GPI-anchor links uPAR to the cell membrane making it available for uPA to bind to the receptor. When the receptor is cleaved between the GPI-anchor and D3, it becomes soluble (suPAR). suPAR is a stable protein that can be measured in various body fluids. uPA: urokinase-type plasminogen activator, uPAR: uPA receptor, suPAR: soluble uPAR, 1: Domain 1, D2: Domain 2, D3: Domain 3

Localization
uPAR is linked to the cell membrane by a glycosyl phosphatidylinositol (GPI)-anchor and binding of urokinase plasminogen activator (uPA) to uPAR, facilitates cleavage of the anchor and hence shedding of the receptor (Fig 1.)[7]. The soluble receptor, suPAR, is measurable in human body fluids including plasma, serum, urine, sputum, saliva and cerebrospinal fluid.

Biological Function
uPAR and its ligand are involved in numerous physiological and pathological pathways which include the plasminogen activating pathway, regulation of pericellular proteolysis, modulation of cell adhesion, migration and proliferation through interactions with proteins present in the extracellular matrix[8]. The involvement of the soluble form of the receptor in the inflammation process is well documented although the actual biological function of the molecule is still not clear. Studies suggest that suPAR is a regulator of uPAR/uPA actions through competitive inhibition of uPAR and several studies conclude that the cleaved receptor is a chemotatic agent promoting the immune response[9].

Monitoring and Disease Progression
suPAR levels reflect the inflammatory state of the individual and are therefore an obvious choice when monitoring disease development and treatment efficacy. As a disease progresses the suPAR level will correspondingly increase and this can be reversed by effective intervention. Successful treatment results in decreasing suPAR level.

Prognosis and Prediction
Several published studies on suPAR suggest the biomarker as an important prognostic tool. suPAR's diagnostic ability in specific infectious diseases is very limited [10], but its prognostic value is at the top of the spectrum of currently available tools, in both specificity and sensitivity. ROC curves comparing suPAR measurements with those of other markers is shown in the figures below.

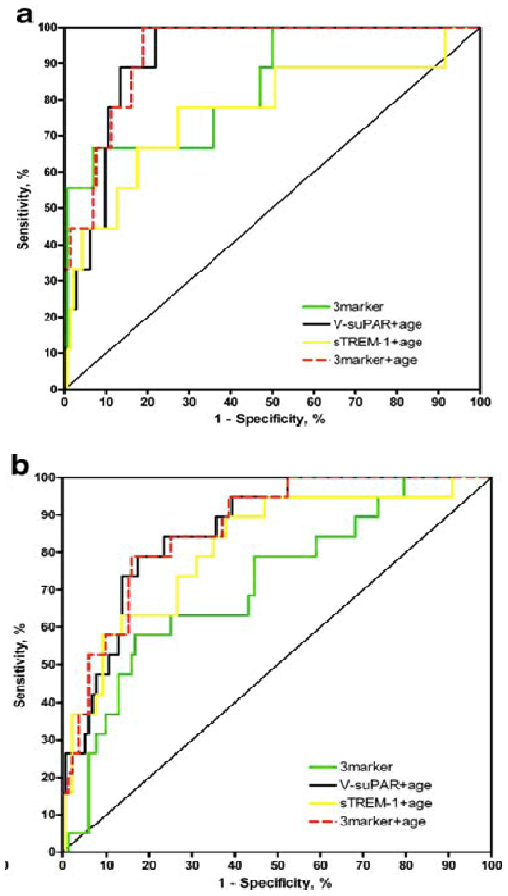

Figure 2 | ROC curve comparing composite marker's ability to predict mortality.
ROC curves comparing suPAR measured with suPARnostic® (V-suPAR) combined with age (AUC=0.92), soluble triggering receptor expressed on myoloid cells-1 (sTREM-1) combined with age, V-suPAR, sTREM-1 and macrophage migration inhibitory factor ROC curves combined as the 3-marker, and the 3-marker combined with age. Prediction of 30-day mortality(a) and 180-day mortality (b)[4]

The results were obtained from patients diagnosed with SIRS/sepsis. In particular, the combination of suPARnostic® measurement combined with age resulted in an AUC of 0.92, which is comparable to the AUC of the 3-markers combined with age and supersedes the predictive power of SAPSII and SOFA scores[4].

Both measuring suPAR alone and combining it with other markers provide great value. Sneider and co-workers found suPAR to have independent prognostic value to CD4 T cell count when predicting mortality in HIV-1 infected patients[11].

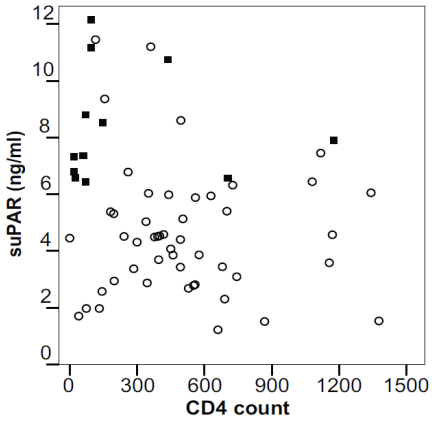

Figure 3 | Scatter plot of CD4 T cell counts and suPAR. No significant correlation was observed between suPAR level and CD4 T cell count. ○ survivors within 2 years of blood sampling, ■: patients that died within 2 years of blood sampling[11].

A scatter plot of CD4 T cell counts and suPAR is shown in figure 3. No significant correlation between suPAR level and CD4 T cell counts was observed and it is shown that patients with low CD4 T cell count are not necessarily at very high risk of dying unless they have a high value of suPAR. This emphasizes how valuable immune activation measurement using suPARnostic® is in prognosis and prediction of mortality.

suPAR Stability
suPAR's high stability and uniform kinetics make it a reliable clinical biomarker. Kofoed and co-workers examined suPAR in a stability test and found that suPAR showed very high stability compared to other sepsis markers[12] confirming results obtained from earlier studies[13]. The results are shown in figure 4 and 5.

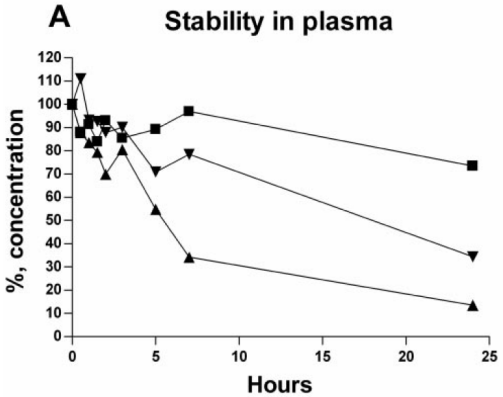

Figure 4 | Stability test. Stabilities of suPAR, sTREM-1, and MIF at room temperature. ■: suPAR, ▲: sTREM-1, ▼: MIF [12].

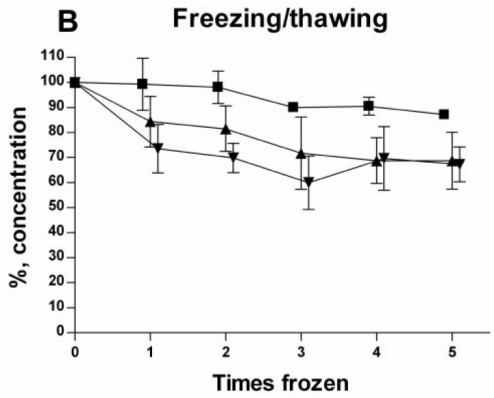

Figure 5 | Freezing/thawing test. Effect on suPAR, sTREM-1, and MIF after several freezing/thawing cycles(b). ■: suPAR, ▲: sTREM-1, ▼: MIF [12].

Figure 4.a shows that suPAR is the biomarker with the least degradation at room temperature and, as seen in figure 4.b, suPAR remains stable through several freezing/thawing cycles. This makes suPAR an obvious and reliable biomarker[12]. Furthermore, Kofoed and co-workers concludes that the suPARnostic® assay is very robust to differences in sample handling while this is not the case when obtaining results for sTREM-1 and MIF.

Why measure suPARnostic®?

- Valuable information about progression of several infectious diseases (e.g. sepsis/SIRS, meningitis, TB, HIV, malaria) and cancer[1,2,3,4,5,6]
- Monitoring tool for treatment response
- Tool for stratification of study populations for homogenous background
- Reliable risk status marker predicting mortality risk[4,11]
- Status marker of the immune system and immune activation[1,2,5,6]
- Reproducible and increased prognostic value compared to other known markers[4,11]

suPARnostic®

by
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suPARnostic® -Reliable and Reproducible Results

The suPARnostic® ELISA is a simplified double monoclonal antibody sandwich assay whereby samples and peroxidase-conjugated anti-suPAR are first mixed together and then incubated in anti-suPAR pre-coated micro wells. The recombinant suPAR standards of the kit are calibrated against healthy human blood donor samples. The kit provides all reagents for measurement of 41 samples in doublets and a 5-point-standard curve for accurate and reproducible results between batches.

Find out more www.suparnostic.com or email info@virogates.com for more information.

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