Prothymosin alpha(100-109) as a surrogate biomarker of bacterial infections

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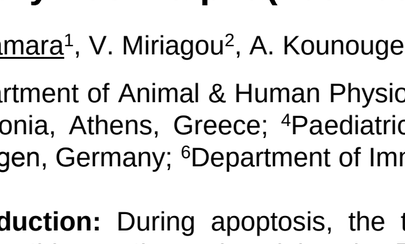
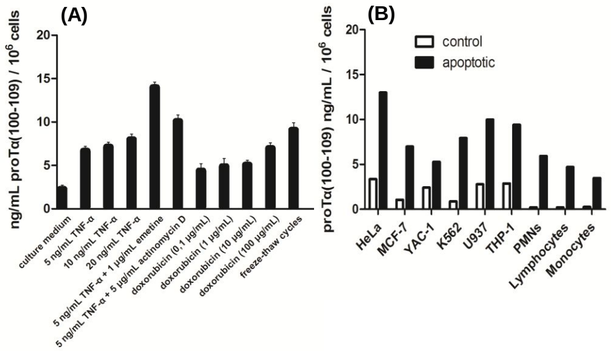
Introduction: During apoptosis, the thymic polypeptide prothymosin alpha (proTα) is cleaved by activated caspase-3, generating the C-terminal decapeptide proTα(100-109).[1] We considered that under conditions of massive cell apoptosis caused by pathogenic bacteria, the levels of proTα(100-109) in serum would be increased and correlated with induction of apoptosis. Thus, we developed a competitive immunoassay for quantitating proTα(100-109) in biological fluids, using high-affinity-purified polyclonal antibodies.[2] Our ELISA was initially validated in a *Streptococcus pyogenes* mice model.[2] Furthermore, we determined proTα(100-109) levels: (i) *in vitro*, in supernatants of cells led to apoptosis, (ii) *in vivo*, in sera of mice infected with *Klebsiella pneumoniae (K. pneumoniae)* and (iii) *ex vivo*, in human sera.

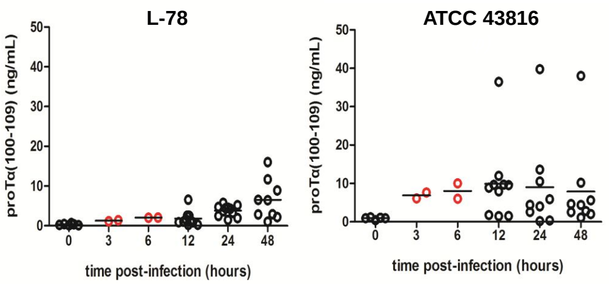


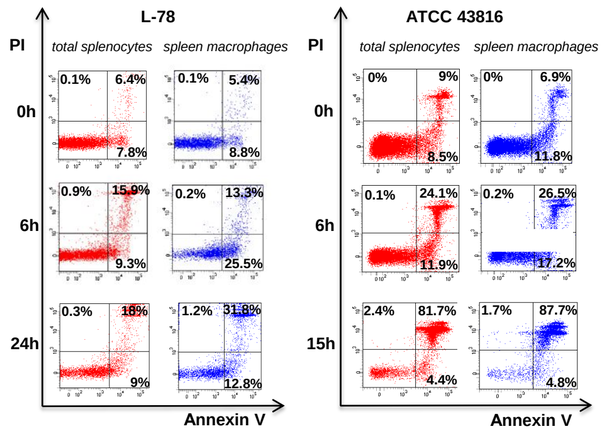
Materials/Methods: For the *in vitro* assay, human cancer cell lines were variously led to cell death and analyzed by flow cytometry. For the *in vivo* model of lethal septicaemia, CD-1 mice were intraperitoneally injected with the clinical isolate *K. pneumoniae* L-78 (endocytosed by monocytes/macrophages), or the prototype *K. pneumoniae* strain ATCC 43816 (not endocytosed).[3] Blood sera and spleens from these mice were studied. For the *ex vivo* analysis, we collected blood sera from healthy individuals, paediatric patients and patients hospitalized in intensive care units (ICU).

Results: Apoptotic **supernatants** of HeLa cells (produced upon several treatments) contain increased levels of proTα(100-109) (A). When various cell lines were led to apoptosis by TNF-α and emetine, increased proTα(100-109) levels were detected in their culture supernatants (B).

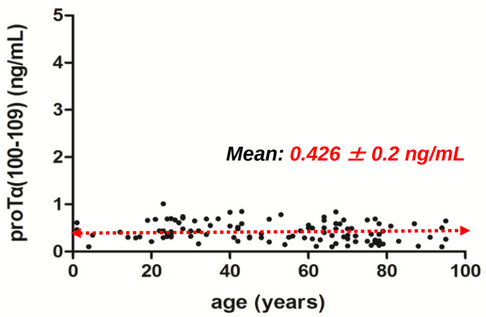


In the serum of **mice** infected with L-78, we recorded a gradual increase of proTα(100-109) concentration; with the highest levels detected 48h post-infection (pi). Conversely, in sera of mice infected with ATCC 43816, an increase in proTα(100-109) concentration was detected 3h pi and its levels remained constantly high throughout infection. Spleen macrophages from L-78-infected mice presented increased percentages of early apoptotic cells at initial time-points and late apoptotic/necrotic cells at later time-points, while spleen macrophages from ATCC 43816-infected mice were mainly necrotic.

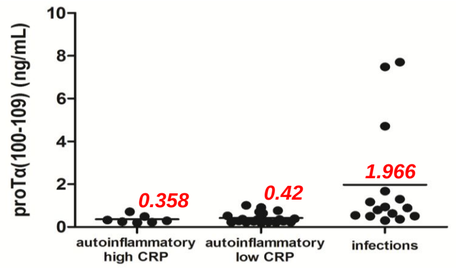




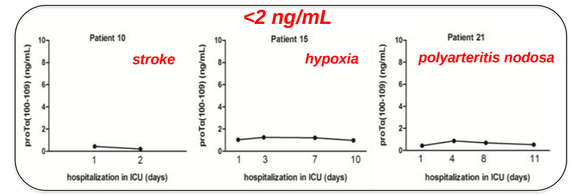
The levels of proTα(100-109) in sera of **healthy subjects** were very low and the values were not correlated with age or sex.

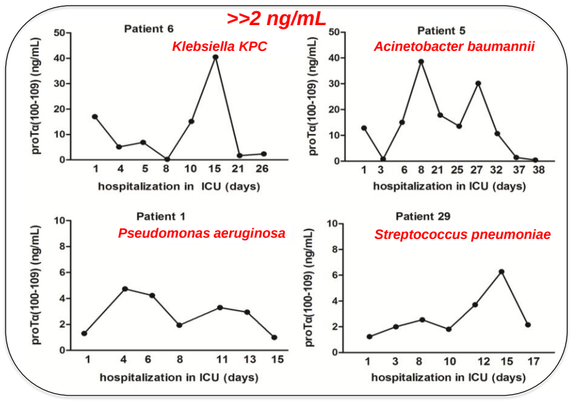


Paediatric patients hospitalized due to infection had augmented concentrations of proTα(100-109) in their blood serum compared to low concentrations in children diagnosed with autoinflammatory diseases.



Serum samples from **ICU patients** were serially collected from admission to exit. Fluctuations in proTα(100-109) levels were recorded in patients admitted with or developed severe bacterial infections. These high proTα(100-109) levels were correlated with clinical symptoms and sepsis biomarkers. In contrast, the concentration of proTα(100-109) was minimal in sera of ICU patients who did not develop an infection.





Conclusion: We developed an ELISA for the determination of proTα(100-109) in biological fluids. The levels of proTα(100-109) increased in culture supernatants of apoptotic cells, in mice infected with *K. pneumoniae* and in patients with bacterial infections. We suggest that proTα(100-109) can be used as a sepsis biomarker. The quantitation of proTα(100-109) in blood serum may be considered as a surrogate marker for the differential diagnosis between septic and aseptic inflammation.

References:

[1]*Evstafieva et al, Exp Cell Res 2003*

[2]*Samara et al, J Immunol Methods 2013*

[3]*Tzouvelekis et al, Antimicrob Agents Chemother 2013*

Funding: *IKY Fellowships of Excellence for Postgraduate Studies in Greece-Siemens Program*

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