Inflammatory Markers in Leg Ulcer Fluid from Chronic Venous Insufficiency

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Endothelial dysfunction represents an important factor in the pathogenesis of chronic venous insufficiency (CVI) that is a common invalidating disease. The blood stasis that is often associated with CVI leads to ischemia and inflammatory mediators' secretion, factors that actively contribute to a local inflammatory degradative pattern.

The purpose of our study was to evaluate soluble matrixmetalloproteinases (MMPs) and their inhibitor (TIMP1) associated to leg ulcer wound fluid in comparison with the tissue localized ones. We aimed to develop an easy-to-perform protocol for the quantification of soluble MMPs / TIMP and associate the obtained levels with the disease stage and prognostic.

The main substrates of MMPs are type IV collagen and gelatin. The following MMPs are presented: MMP-2 (72 kDa gelatinase, gelatinase-A) MMP-9 (92 kDa gelatinase, gelatinase-B), MMP-1 (interstitial collagenase). Overall, all MMPs are inhibited by TIMPs once they are activated. The balance between activated and inhibited MMPs contributes to the degradative pattern of the tissue.

Methods

Soluble MMPs detection - Luminex IS 200 System, xMAP technology using Fluorokine_ MAP multiplex kit Human MMP for the detection of MMPs: 1, 2, 3, 7, 8, 9, 12 and 13.

Soluble TIMP-1 - ELISA using Quantikine Human TIMP-1 Immunoassay.

Protein profiling of the samples was performed using LabChip microfluidic technology (BioRad) and Experion automated electrophoresis system. Experion™ Pro260 Analysis Kit was used in this system for protein ranging from 10 to 260 kDa in mass.

Controls – normal human sera from 25 volunteers with matching ages were used according to the technical specification of each method.

Patients - 18 patients with chronic venous insufficiency, aged 32-60yr., 40% men, were diagnosed by clinical examination, standard blood analysis and venous 2D Color echography. After informed consent, during their standard operation, fluid was harvested and subjected to the protocol. Sera from patients were as well subjected to MMP, TIMP1.

In parallel imunohistochemistry for vein biopsies were performed for MMP-1,2 with TIMP-1 expression in tissue samples.

Chronic Venous Disease
J.J. Bergan,
G. W. Schmid-Schönbein,
P.D. Coleridge Smith,
A. N. Nicolaides,
M. R. Boisseau, B.Eklof,
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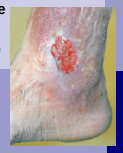

Harvest in sterile conditions fluid on RPMI1640
(200µl, no fetal calf serum, no phenol red)

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Vortex
(high speed 1-2 min.)

↓

Centrifuge at 250g, 30min., 4⁰C
(cells are removed)

↓

Centrifuge at 2000g, 30min., 4⁰C
(Repeat centrifugation until fluid is clear)

↓

Total protein concentration testing

↓

Appropriate dilution matching human sera
(for each type of test, dilution should be performed in specific calibrators)

↓

ELISA — **TIMP1** / **Protein profile** — **Experion**

↓

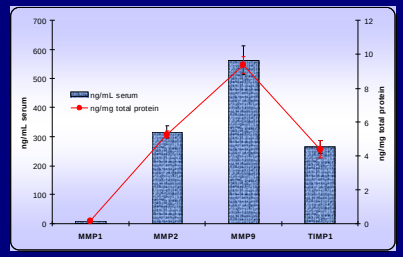
MMP

↓

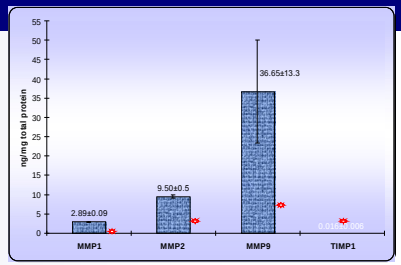
LUMINEX

↓

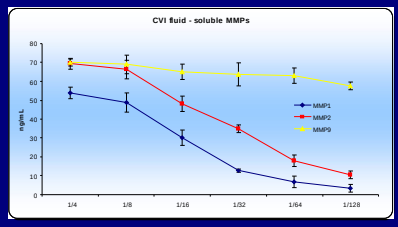
Results

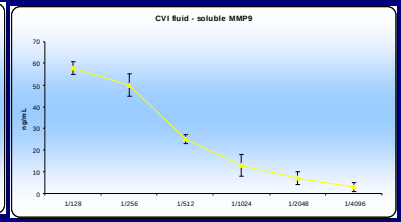


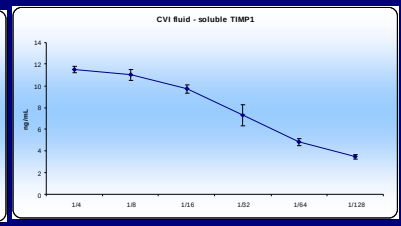
Normal seric values of MMPs and TIMP1 (multiplex data for MMPs, ELISA data for TIMP1). Data are presented as mean ± SD



Fluid values of MMPs and TIMP1 (multiplex data for MMPs, ELISA data for TIMP1). Data are presented as mean ± SD and the red stars represent normal seric values.

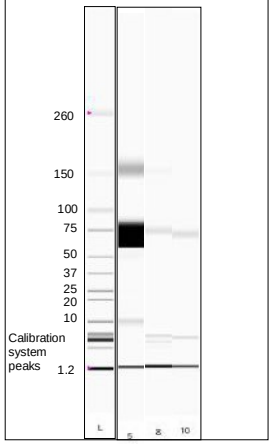






Quantification of soluble MMPs and TIMP1 in CVI fluid (multiplex data for MMPs, ELISA data for TIMP1). Presented data are titration triplicates obtained from one patient fluid. Total protein concentration related to the titration was 1/4=38mg/mL…1/128 =1mg/mL

Electropherogram of various fluid samples (5,8,10) compared to the Experion Pro260 ladder (relative positions of the upper and lower markers, system peaks, and nine Pro260 ladder peaks)



Conclusion

The used methodology can be developed for *simultaneous* detection of multiple soluble inflammatory markers in specific tissue sites.

Results

- When detection of MMPs from CVI leg ulcer fluid is aimed, samples need to be used in higher than recommended serum dilution, especially when MMP9 is to be detected;
- Due to the individual protein pattern of the harvested fluids, specific MMP or inhibitor activities need to be related to the total protein concentration of the fluid sample;
- Comparing the values obtained from normal sera (at the same protein concentration) in the CVI fluids ***MMP9*** values are 4 x more elevated, ***MMP2*** 2x and ***MMP1*** 19x, while their inhibitor ***TIMP1*** is 200x reduced;
- Higher values of soluble MMPs and lower TIMP1 were directly associated with disease recurrence;
- Various patterns of soluble MMPs and TIMP-1 were associated with the disease progression, but overall higher MMPs value and lower TIMP1 indicated a poor healing score;
- The studied patients did not present any abnormal seric values of MMPs and TIMP1;

European Biomarkers Summit

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