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Research Article

Circulatory matrix metalloproteinases as tissue destruction indicators for improving clinical management of pressure ulcers patients

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Abstract. Background and objectives: Pressure ulcer remains a major and debilitating health issue. Biomarkers that can evaluate the patient's status are important tools in monitoring this disease. A less studied family of circulatory biomarkers, matrix-metalloproteinases (MMPs) are gaining interest in research of pressure ulcers. Our study analyses nine circulatory MMPs evaluated in the serum of pressure ulcers patients prior to any treatment. Material and methods: Forty patients with pressure ulcers and 25 healthy subjects as control group matching ages and gender were included in the current study. The control group was chosen on purpose with an increased aged domain in order to mitigate the known healthy age-related serum MMPs increment. The following group of MMPs: MMP-1, 2, 3, 7, 8, 9, 10, 12 and 13 were quantified in the harvested serum using the multi-analyte technology xMAP array. Results: The highest serum concentration was registered for MMP-9, namely 185 times increased in patients compared to the control group, followed closely by serum MMP-1, found increased 54 times. Conclusion: Several serum MMPs could stand out as biomarkers in pressure ulcers patients.

Key Words: pressure ulcers, matrix-metalloproteinase, xMAP array, multi-analyte.

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Introduction

Pressure ulcers are deep injuries to both skin and underlying tissue and are the consequence of prolonged skin pressure. This condition develops mainly on the bony areas such as heels, ankles, hips and tailbone. The main causes are the medical conditions limiting the capability to change positions. These skin lesions develop quickly, are very difficult to treat and, more recently, were associated with post-stroke mortality (Lee et al 2015).

In this light, pressure ulcer remains a major and debilitating health issue. Extended European programs, like Pressure UlceR Programme Of reSEarch (PURPOSE) are studying patients' quality of life. Besides several studied parameters, evaluation of healing capacity is a subject of high interest. Biomarkers that can evaluate the patient's status are important tools in monitoring this disease (Nixon et al 2015) and matrix-metalloproteinases (MMPs) are gaining interest in research of pressure ulcers. MMPs are involved in pathological conditions, such as tissue destruction and tumor metastasis. Traditionally MMPs were considered to cleavage only components of the extracellular matrix, but recently other substrates were discovered (growth factors, cell receptors), outlining new biological functions (McCawley and Matrisian 2001).

Our study focused on several families of MMPs that are both enzymes in extracellular matrix remodeling but as well as destructive molecules involved in tissue degradation.

MMP-1 (interstitial collagenase), cleaves types I, II, III, VII and X collagens (Desrochers et al 1991) and its production is enhanced by inflammatory cytokines (Nissinen and Kähäri 2014). Wound healing seems to be correlated with circulatory MMP-2 and MMP-9 (Caimi et al 2015). MMP-3 and 10 is produced in chronic wounds by basal keratinocytes (Saarialho-Kere et al 1994). MMP-7 (matrilysin) cleaves collagen type I, III, IV, V, casein, and fibronectin and enhances gelatinases MMP-2 and MMP-9 expression (Quantin et al 1989; Knox et al 1997; Yokoyama et al 2008).

MMP-8 (neutrophil collagenase), a distinct collagenase, cleaves preferentially collagen type I in contrast to fibroblast collagenase which cleaves preferentially the collagen type III (Devarajan et al 1991) and MMP-8 is a feature for inflammatory cells in chronic wounds (Pirila et al 2007).

Table 1. Matrix-metalloproteinases families concomitantly detected using xMAP technology

Analyte	Spectral region	Analyte	Spectral region	Analyte	Spectral region
MMP-1	43	**MMP-7**	66	**MMP-10**	62
MMP-2	26	**MMP-8**	37	**MMP-12**	53
MMP-3	45	**MMP-9**	55	**MMP-13**	47

MMP 9 (type IV collagenase or gelatinase B) is activated by MMP-3 (van den Steen et al 2002; Nagase and Woessner 1999; Ramos-DeSimone et al 1999) and it is highly expressed in patients with chronic venous ulcers (Bigg et al 2007; Serra et al 2013).
MMP 10 (stromelysin-2) cleaves fibronectin, laminin, elastin, proteoglycan, gelatin and collagen activating MMP-9 proenzyme and MMP-7 (Madlener et al 1997; Nakamura et al 1998).
MMP 12 (macrophage metalloelastase) cleaves collagen type I and III (Belaaouaj et al 1993; Shapiro et al 1993; Shapiro and Senior 1998; Taddese et al 2010) and its expression is enhanced in chronic inflammation (Stawski et al 2014).
MMP 13 (collagenase-3) cleaves fibrillary type I, II and III collagens being a promoter of fibroblasts survival and proliferation (Toriseva et al 2007).
Taking into account the possibly to monitor circulatory MMPs as biomarkers in pressure ulcer patients we have embarked in studying serum MMPs patterns as potential tool for ulcer patients monitoring.

Material and Method

In the current study patients diagnosed with pressure ulcers and healthy volunteers with matching ages and gender were included. The study was approved by the Ethics Committee of the "Sf. Pantelimon" Emergency Hospital and all the enrolled individuals gave their informed consent regarding the samples usage, data evaluation and presentation.

Patients group

Forty patients diagnosed with pressure ulcers admitted in "Sf. Pantelimon" Emergency Hospital were enrolled. The age domain was 23 to 90 years (mean± standard deviation 69.56±12.5 years), 60% males. The enrolled patients had hip and/or coccyx pressure ulcers diagnosed in stages II and III. The etiology of pressure ulcers was grade II and III obesity.

Control group

Healthy 25 normal weight subjects with no ulcer pressure or any associated pathology, no diabetes, no cardiovascular diseases, no neoplasias and no background treatment were enrolled. The age domain was 63 to 85 years (mean± standard deviation 65.33±5 years), 45% males.

Biological samples

Serum samples were harvested upon admittance in Hospital for patients and upon volunteering for healthy subjects. Blood was taken by venipuncture and harvested on clotting tubes. Serum was immediately aliquoted and kept until investigation at -20^{0}C. Samples were not subjected to any freeze/thaw cycles and were investigated after 7-14 days upon harvest.

Multi-analyte investigation

Multi-analyte quantification using Bio - Plex® MAGPIX ™ System and the standard kit Bio-Plex Pro™ Human MMP Assays (Bio-Rad Laboratories US) was used. The principle of the test resides in xMAP technology that uses MagPlex polystiren 6.5 microns beads impregnated with iron and magnetite. These beads are labeled with 2 or 3 fluorochromes and display hence an unique spectral region. Each bead type contains also a unique monoclonal antibody that captures from the biological sample the specific antigen. We have used this technology to quantify concomitantly 9 MMPs from serum samples as presented in Table 1. Standard curves were used for quantifying all MMPs and thus expressing the results in pg mL-1.

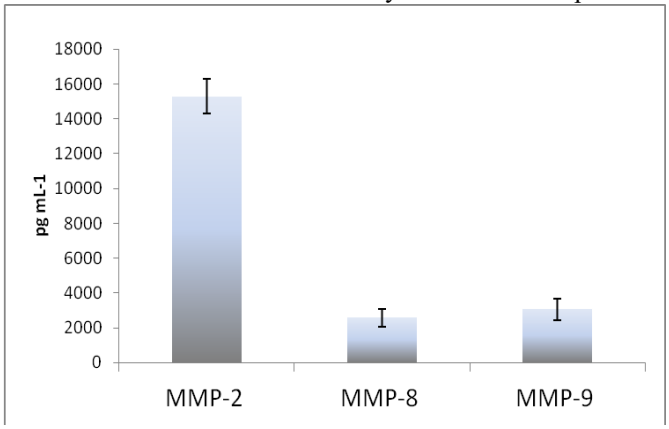
Statistical analysis

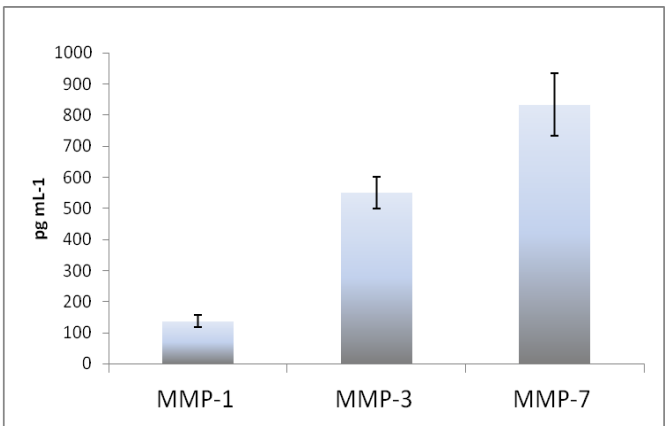
SPSS version 19.0 was used for analysis. Pearson coefficient was used to establish if there is a correlation between each individual age and the corresponding MMPs levels. Independent t-test was used to determine if there is a statistically significant difference between the means of MMPs in control group versus MMPs in patients. Two-tailed t-test with statistical significance 0.05 (5%), 95% confidence level was taken into account. Results were presented as pg mL-1 and, when indicated, as index = patient value/control value.

Results

MMPs normal serum values

In terms of statistics there was no difference between the patients age and control group. Moreover we have intentionally chosen more aged healthy subjects to mitigate the known aged-related increased values of circulatory MMPs. We can pinpoint 3 classes of serum MMPs in terms of their concentrations. The first class comprising MMP-2, 8 and 9 are in the 2000 – 15000 pg mL-1, the second one, MMP-1, 3 and 7 in the 100-900 pg mL-1 and the third one MMP-10, 12 and 13 in the lowest 10-90 pg mL-1 interval (Figure 1). The levels of MMP-8 and 9 are statistically identical while MMP-2 is statistically increased compared to 8 and 9 ($p<0.001$). MMP-1, 3 and 7 are statistically different in control serum ($p<0.001$). MMP-12 and 13 are not statistically different in control serum, while MMP-10 is statistically increased in comparison to MMP-12 and 13. MMP-1 and 10 serum concentrations are statistically different with a $p<0.05$.





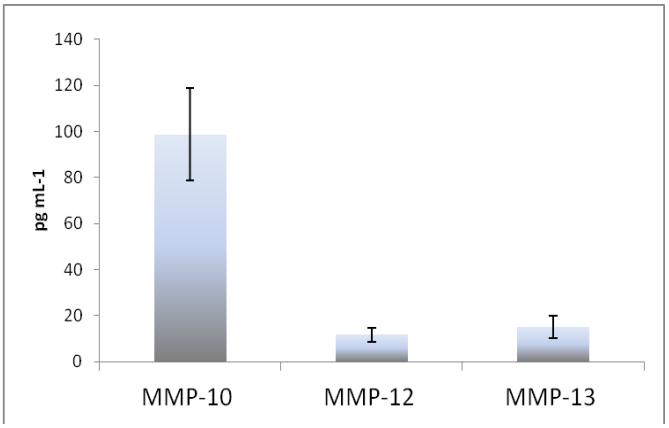
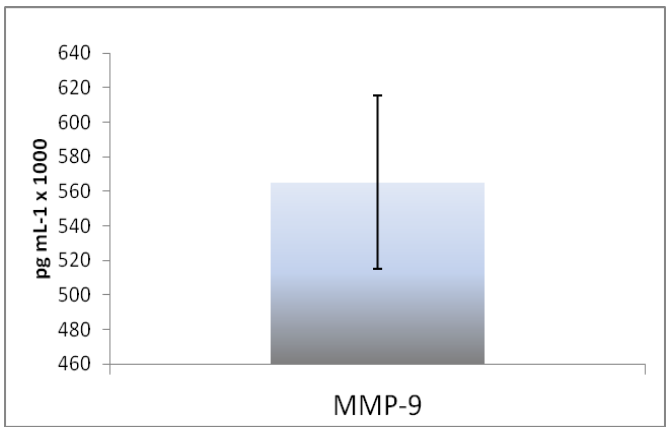


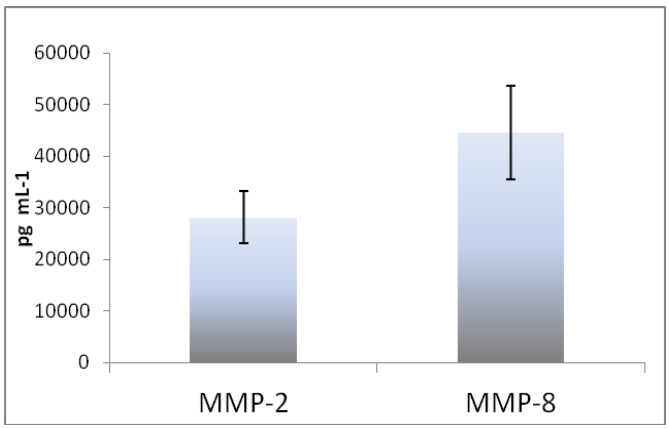
Figure 1. MMPs values detected in control group; A) MMP-2, 8 and 9; B) MMP-1, 3 and 7; C) MMP-10, 12 and 13

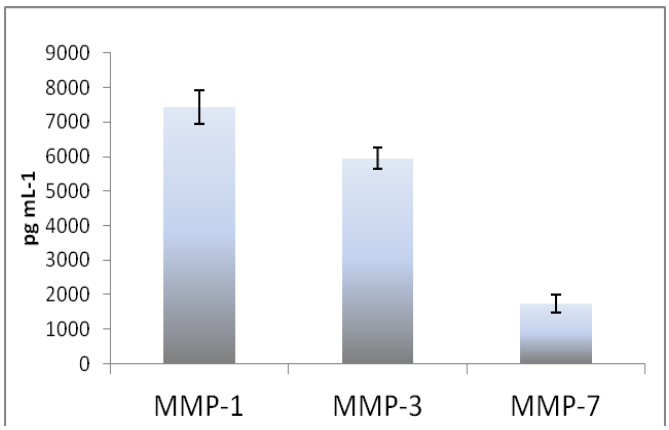
Serum MMPs in patients diagnosed with pressure ulcers

Grade II and III obese patients were admitted with hip and/or coccyx pressure ulcers. The lesions had diameters ranging from 14 to 64 cm^2 and, according to the standard scale; lesions were staged as II and III (Edsberg et al 2014). Pressure ulcers stage II had partial thickness loss of dermis showing an open ulcer, shallow in depth with a red pink wound bed. For stage III, the lesion had full thickness tissue loss, subcutaneous fat clearly visible, no bone, tendon or muscle were visible.

At hospital admittance, patients with extensive pressure ulcers were tested prior to any medication. The overall picture of the serum MMPs are significantly increased for MMP-9, 1, 8, 12, 3, 10 and 13, but no statistical difference compared to control values for MMP-7 and 2. Serum MMP-9 was found 185 times increased in comparison to control values. This MMP had the highest increment for all the investigated MMPs (Table 2). It is worth mentioning that, except for MMP-9, the same concentration ranges categories as in control group were found also in patients. Specifically, MMPs in patient's serum divide as well in three concentrations categories: high for MMP-2, and 8; medium for MMP-1, 3 and 7, and low for MMP-10, 12 and 13. Besides MMP-9, statistically increased serum values were found for MMP-1 (54 X), MMP-8 (17X), MMP-12 (11X), MMP-3 (10X) and MMP-10 (4.7X) and MMP-13 (3X). There are also two MMPs that are not statistically different in patients compared to controls (MMP-7 and 2) although their concentrations evaluated in patients, are 2X and 1.8X increased respectively.







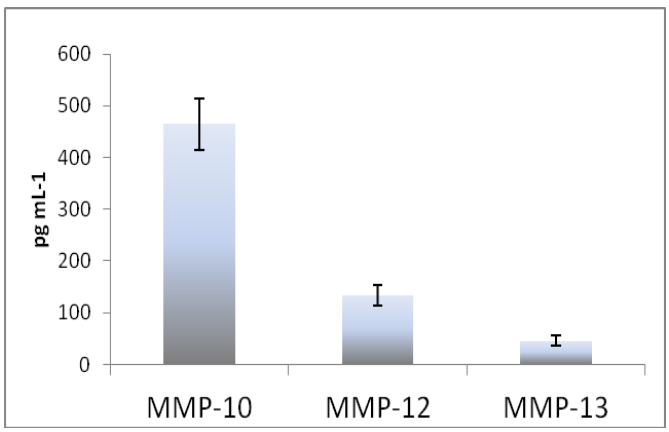


Figure 2. MMPs detected in patients serum; A) MMP-9; B) MMP-2, and 8; C) MMP-1, 3 and 7; D) MMP-10, 12 and 13

Table 2. Matrix-metalloproteinases serum concentrations in pressure ulcer patients compared to controls

Serum values pg mL-1	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8
Patients					
MEAN (n=40)	7431.37	28123.4	5949.01	1738.89	44546.53
STDEV	574.8	519.79	1307.03	476.58	12143.7
Controls					
MEAN (n=25)	137.52	15284.86	550.66	833.71	2585.05
STDEV	25.33	1002.01	113.09	265.67	125.98
Index	54.04	1.84	10.8	2.08	17.23

Serum values pg mL-1	MMP-9	MMP-10	MMP-12	MMP-13
Patients				
MEAN (n=40)	565205.2	464.32	134.16	45.81
STDEV	11206	77.01	45.28	10.39
Controls				
MEAN (n=25)	3060.58	98.67	11.76	15.12
STDEV	250.01	12.56	3.22	5.09
Index	184.67	4.71	11.41	3.03

Multiplexing technology offered the possibility to concomitantly quantify 9 MMPs in the serum of enrolled patients, thus bypassing any individual ELISA determinations that can be subjected to errors and moreover, are time consuming technique. There are reports stating that there are aged related differences while quantifying serum MMPs (Thrailkill et al 2005). This was the reasons of using aged healthy controls. We do not rule out the hypothesis that the MMPs values presented as control or reference values could have different intervals for other age groups. It was shown that MMP-2 is significantly negatively correlated with age while MMP-3 was reported as significantly positively correlated with age (Thrailkill et al 2005). In our study we did not find any significant correlation between age and the level of any particular circulatory MMP whether in controls or patients prior to any therapy.

Discussion

Literature is extremely poor regarding the normal ranges of serum MMPs (Edkins et al 2012). It seems that with normal ageing there are correlations with MMP-3, 10 (Komosinska-Vassev et al 2011) and MMP-2 (Thrailkill et al 2005). That is the reason why we had enrolled normal healthy aged individuals to mitigate normal physiological age-related alteration of circulatory MMPs.

Up to our knowledge, there are no reports showing circulatory panels of MMPs in pressure ulcers. There are although several disparate reports showing one or the other of serum MMPs as altered in other pathology like venous leg ulcers. Excessive proteolysis caused by an imbalance between matrix metalloproteinases and their tissue inhibitors is the main mechanism responsible for impaired wound healing in chronic venous leg ulcers. Wound fluids from venous leg ulcers showed significantly increased concentrations of MMP-1 and MMP-3 and decreased tissue inhibitor TIMP-1 compared with other sources of acute wound fluids, such as inflammatory fluid from skin grafts or post-mastectomy axillary drains. These MMPs are stimulated by inflammatory cytokines, such as interleukin-1 beta and tumor necrosis factor-alpha (Subramaniam et al 2008).

Wound healing in venous leg ulcers was associated with an increased collagen III turnover and enhanced MMP-1 activity, compared with both venous leg ulcers resistant to treatment and healthy controls (Meyer et al 2008).

Studies on human fibroblasts treated with MMPs show a decrease in synthesis of prostaglandin E2 by IL-1 beta (Nissinen and Kähäri 2014). This finding could be important in treatments regarding tissue repair, since prostaglandin E2 was found to be rapidly induced after injury with beneficial roles in the early inflammatory phase of wound healing (Stratton and Shiwen 2010).

Overexpression of the activated collagenase MMP-8 associated with decreased levels of tissue inhibitor TIMP-1 seems to be implicated in the resistance to wound healing in chronic ulcers (Nwomeh et al 1999). Interestingly, plasma and wound fluid of infected chronic venous ulcers showed higher levels of collagenases MMP-1 and MMP-8 associated with increased concentrations of inflammatory cytokines, such as IL-1, IL-6, IL-8, vascular endothelial growth factor and tumor necrosis factor-alpha, compared with those uninfected, where gelatinases MMP-2 and MMP-9 were predominantly expressed (Serra et al 2014). The high MMPs levels found by us could be the main pathway for resistance in wound healing, thus, studies on venous leg ulcers are showing a decreasing pattern of MMPs in the process of healing. For example, MMP-2 and MMP-9, initially found increased in bilateral venous leg ulcers, decline after a good response to alginogel therapy (Grzela et al 2014). Interestingly, MMP-10 is expressed later than MMP-1 in normal healing wounds, and it is induced by keratinocytes migrating to

the dermal matrix. The keratinocytes of inflammatory chronic diabetes and venous ulcers express higher MMP-10 levels compared with other ulcers, such as rheumatoid and pressure ulcers (decubitus ulcers) (Rechardt et al 2000). In our study circulatory MMP-10 was found 4 times increased as compared to controls. Moreover, recent identified enzyme substrates such as integrin alpha-6 subunit, cysteine-rich angiogenic inducer 61 and dermokine highlight the complex biologically involvement of MMP-10 in cell adhesion, cell invasion, proliferation and differentiation necessary for wound healing but also for cancer progression (Schlage et al 2015).

MMP-13 was initially reported in deep fibroblasts of the chronic ulcers, being important in matrix remodeling, in contrast to MMP-1 which is expressed by keratinocytes and dermal cells in the superficial wound, which is critical for re-epithelization (Vaalamo et al 1997). As our results show the circulatory levels of MMP-1 are higher than those registered for MMP-13, indicating that our patients were in the phases in which keratinocytes and dermal cells were actively involved in wound healing. Increasing the number of controls and patients can emphasize other aspects such as correlation of the MMPs circulatory levels to ageing and/or gender and/or with the extension and grade of lesions. However our study is one of the first focusing on a large panel of circulatory MMPs, enzymes involved in cutaneous regeneration/degradation and inflammatory processes. Our results indicate that serum MMPs are robust enough markers to indicate an actively on-going pressure ulcer and possible to monitor in the future the regenerative status of the cutaneous lesions.

Conclusion

An outline can be drawn regarding serum MMPs quantified in pressure ulcer patients prior to any treatment. Therefore, the MMPs portrait is dominated by very high MMP-9 concentrations, followed by a group of MMPs 1, 3, 8, 10 and 12 that are also statistically increased in this pathology, while MMP-2, 7 and 13 are not found statistically different compared to controls.

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References

Belaaouaj A, Shipley JM, Kobayashi DK, Zimonjic DB, Popescu N, Silverman GA, Shapiro SD. Human macrophage metalloelastase. Genomic organization, chromosomal location, gene linkage, and tissue-specific expression. J Biol Chem 1995;270 (24):14568–14575.

Bigg HF, Rowan AD, Barker MD, Cawston TE. Activity of matrix metalloproteinase-9 against native collagen types I and III. FEBS J 2007;274(5):1246-1255.

Caimi G, Ferrara F, Montana M, Muratori I, Amato C, Canino B, et al. Behaviour of the plasma concentration of gelatinases and their tissue inhibitors in subjects with venous leg ulcers. Clin Hemorheol Microcirc 2015;60(3): 309-316.

Desrochers PE, Jeffrey JJ, Weiss SJ. Interstitial collagenase (matrix metalloproteinase-1) expresses serpinase activity. J Clin Invest 1991;87(6):2258-2265.

Devarajan P, Mookhtiar K, Van Wart H, Berliner N. Structure and expression of the cDNA encoding human neutrophil collagenase. Blood 1991;77:2731-2738.

Edkins TJ, Alhadeff JA, Kwok V, Kalensky C, Rock MT, Vidmar TJ, Del Tito BJ Jr. Development and validation of novel enzyme activity methods to assess inhibition of matrix metalloproteinases (MMPs) in human serum by antibodies against enzyme therapeutics. J Pharm Biomed Anal 2012;70:408-414.

Edsberg LE, Langemo D, Baharestani MM, Posthauer ME, Goldberg M, Unavoidable Pressure Injury: State of the Science and Consensus Outcomes, Journal of Wound, Ostomy & Continence Nursing 2014;41(4):313–334.

Grzela T, Niderla-Bielinska J, Litwiniuk M, White R. The direct inhibition of MMP-2 and MMP-9 by an enzyme alginogel: a possible mechanism of healing support for venous leg ulcers. J Wound Care;2014. 23(5):278,280-2,284-5.

Komosinska-Vassev K, Olczyk P, Winsz-Szczotka K, Kuznik-Trocha K, Klimek K, Olczyk K. Age- and gender-dependent changes in connective tissue remodeling: physiological differences in circulatingMMP-3, MMP-10, TIMP-1 and TIMP-2 level. Gerontology 2011;57(1):44-52.

Knox JD, Boreham DR, Walker JA, Morrison DP, Matrisian LM, Nagle RB, Bowden GT. Mapping of the metalloproteinase gene matrilysin (MMP7) to human chromosome 11q21-->q22. Cytogenet Cell Gene 1997;72(2-3):179-182.

Lee SY, Chou CL, Hsu SP, Shih CC, Yeh CC, Hung CJ, Chen TL, Liao CC. Outcomes after Stroke in Patients with Previous Pressure Ulcer: A Nationwide Matched Retrospective Cohort Study. J Stroke Cerebrovasc Dis. 2015 Oct 20. pii: S1052-3057(15)00508-X. doi: 10.1016/j.jstrokecerebrovasdis.2015.09.022.

Madlener, M, Werner S. cDNA cloning and expression of the gene encoding murine stromelysin-2 (MMP-10). Gene 1997;202:75-81

McCawley LJ, Matrisian LM. Matrix metalloproteinases: they're not just for matrix anymore! Curr Opin Cell Biol 2001;13:534–540.

Meyer FJ, Burnand KG, Abisi S, Tekoppele JM, van Els B, Smith A. Effect of collagen turnover and matrix metalloproteinase activity on healing of venous leg ulcers. Br J Surg 2008;95(3):319-325.

Nagase H, Woessner JF. Matrix metalloproteinases. J Biol Chem 1999;274(31):21491-21494.

Nakamura H, Fujii Y, Ohuchi E, Yamamoto E, Okada Y. Activation of the precursor of human stromelysin 2 and its interactions with other matrix metalloproteinases. Europ J Biochem 1998;253:67-75.

Nissinen L, Kähäri V-M. Matrix metalloproteinases in inflammation. BBA 2014;1840: 2571–2580.

Nixon J, Nelson EA, Rutherford C, Coleman S, Muir D, Keen J, et al. Pressure UlceR Programme Of reSEarch (PURPOSE): using mixed methods (systematic reviews, prospective cohort, case study, consensus and psychometrics) to identify patient and organisational risk, develop a risk assessment tool and patient-reported outcome Quality of Life and Health Utility measures. Southampton (UK): IHR Journals Library;2015 Sep. Programme Grants for Applied Research.

Nwomeh BC, Liang HX, Cohen IK, Yager DR. MMP-8 is the predominant collagenase in healing wounds and nonhealingulcers. J Surg Res. 1999;81(2):189-195.

Pirila E, Korpi JT, Korkiamäki T, Jahkola T, Gutierrez-Fernandez A, Lopez-Otin C, et al. Collagenase-2 (MMP-8) and matrilysin-2 (MMP-26) expression in human wounds of different etiologies. Wound Repair Regen 2007;15(1):47-57.

Quantin B, Murphy G, Breathnach R. Pump-1 cDNA codes for a protein with characteristics similar to those of classical collagenase family members. Biochemistry 1989;28:5327-5334.

Ramos-DeSimone N, Hahn-Dantona E, Sipley J, Nagase H, French DL, Quigley JP. Activation of Matrix Metalloproteinase-9 (MMP-9) via a Converging Plasmin/Stromelysin-1 Cascade Enhances Tumor Cell Invasion. J Biol Chem 1999;274(19):13066-13076.

Rechardt O, Elomaa O, Vaalamo M, Pääkkönen K, Jahkola T, Höök-Nikanne J, et al. Stromelysin-2 is upregulated during normal wound repair and is induced by cytokines. J Invest Dermatol 2000;115(5):778-787.

Richard Stratton, Xu Shiwen. Role of prostaglandins in fibroblast activation and fibrosis. J Cell Commun Signal 2010;4(2):75–77.

Saarialho-Kere UK, Pentland AP, Birkedal-Hansen H, Parks WC, Welgus, HG. Distinct populations of basal keratinocytes express stromelysin-1 and stromelysin-2 in chronic wounds. J Clin Invest 1994;94:79-88.

Schlage P, Kockmann T, Sabino F, Kizhakkedathu JN, Auf dem Keller U. Matrix metalloproteinase 10 degradomics in keratinocytes and epidermal tissue identifies bioactive substrates with pleiotropic functions. Mol Cell Proteomics 2015.

Serra R, Buffone G, Falcone D, Molinari V, Scaramuzzino M, Gallelli L, de Franciscis S. Chronic venous leg ulcers are associated with high levels of metalloproteinases-9 and neutrophil gelatinase-associated lipocalin. Wound Repair Regen 2013;21(3):395-401.

Serra R, Grande R, Buffone G, Molinari V, Perri P, Perri A, Amato B, Colosimo M, de Franciscis S. Extracellular matrix assessment of infected chronic venous leg ulcers: role of metalloproteinases and inflammatory cytokines. Int Wound J 2014.

Shapiro SD, Kobayashi D, Ley TJ. Cloning and characterization of a unique elastolytic metalloproteinase produced by human alveolar macrophages. J Biol Chem 1993;268: 23824–23829.

Shapiro SD, Senior RM. Macrophage elastase (MMP-12). In: Parks, WC Mecham, RP. eds. Matrix Metalloproteinases. 1998. San Diego: Academic Press. 185–196.

Stawski L, Haines P, Fine A, Rudnicka L, Trojanowska M. MMP-12 deficiency attenuates angiotensin II-induced vascular injury, M2 macrophage accumulation, and skin and heart fibrosis. PLoS One 2014;9(10):e109763.

van den Steen PE, Dubois B, Nelissen I, Rudd PM, Dwek RA, Opdenakker, G. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). Crit Rev Biochem Mol Biol 2002;37(6): 375-536.

Subramaniam K, Pech CM, Stacey MC, Wallace HJ. Induction of MMP-1, MMP-3 and TIMP-1 in normal dermal fibroblasts by chronic venous leg ulcer wound fluid. Int Wound J 2008;5(1):79-86.

Taddese S, Jung MC, Ihling C, Heinz A, Neubert RH, Schmelzer CE. MMP-12 catalytic domain recognizes and cleaves at multiple sites in humanskin collagen type I and type III. Biochim Biophys Acta 2010;1804(4):731-739.

Thrailkill KM, Moreau CS, Cockrell G, Simpson P, Goel R, North P, Fowlkes JL, Bunn RC. Physiological matrix metalloproteinase concentrations in serum during childhood and adolescence, using Luminex Multiplex technology. Clin Chem Lab Med. 2005;43(12):1392-1399.

Toriseva MJ, Ala-aho R, Karvinen J, Baker AH, Marjomäki VS, Heino J, Kähäri VM. Collagenase-3 (MMP-13) enhances remodeling of three-dimensional collagen and promotes survival of human skin fibroblasts. J Invest Dermatol 2007;127(1):49-59.

Vaalamo M, Mattila L, Johansson N, Kariniemi AL, Karjalainen-Lindsberg ML, Kähäri VM, Saarialho-Kere U. Distinct populations of stromal cells express collagenase-3 (MMP-13) and collagenase-1 (MMP-1) in chronic ulcers but not in normally healing wounds. J Invest Dermatol 1997;109(1):96-101.

Yokoyama Y, Grünebach F, Schmidt SM, Heine A, Häntschel M, Stevanovic S, et al. Matrilysin (MMP-7) is a novel broadly expressed tumor antigen recognized by antigen-specific T cells. Clin Cancer Res 2008;14(17):5503–5511.

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