Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases in Sera and Tissue of Patients with Dupuytren's Disease

Dietmar Ulrich, M.D., Klaus Hrynyschyn, M.D., and Norbert Pallua, M.D., Ph.D.

Aachen and Trier, Germany

Dupuytren's contracture is a fibroproliferative disorder characterized by progressive deposition of mature collagen fibers. In other fibrotic diseases affecting organs such as the liver, lung, heart, and skin, matrix metalloproteinases (MMPs) and their natural inhibitors, the tissue inhibitors of metalloproteinases (TIMPs), play an important role. In this study, serum concentrations of MMP-1, MMP-2, MMP-9, TIMP-1, and TIMP-2 were determined in 22 patients (five women and 17 men; average age, 67 ± 11 years) with Dupuytren's disease using an enzyme-linked immunosorbent assay. Tissue samples were obtained for standard histological and immunohistochemical analyses. Sera and samples of palmar fascia from 20 patients (13 women and seven men; average age, 60 ± 15 years) who had undergone hand surgery for carpal tunnel syndrome were used as the control group. Statistical analysis was performed using the Mann-Whitney test. Patients with Dupuytren's contracture presented with a TIMP-1 concentration of 437 ± 160 ng/ml, a significantly higher TIMP-1 concentration than that seen in the control patients, who had a concentration of 321 ± 70 ng/ml ($p < 0.05$). Patients with a proliferative active disease ($n = 14$) had a significantly higher TIMP-1 concentration (525 ± 136 ng/ml) than patients ($n = 8$) with a contracture in the late involutional and residual phase (286 ± 41 ng/ml; $p < 0.05$). There were no significant differences in the TIMP-2, MMP-1, MMP-2, and MMP-9 serum concentrations between patients with palmar fibromatosis and the control group. Patients with Dupuytren's disease had a significantly lower MMP-to-TIMP ratio (1.1 ± 0.3; $p < 0.05$) than the control group (1.5 ± 0.35). Patients with an active palmar fibromatosis presented a significantly ($p < 0.05$) reduced ratio (1 ± 0.2) compared with those in later phases (1.4 ± 0.3). TIMP-1 and TIMP-2 could be detected in tissue of patients with Dupuytren's contracture, with an accumulation in proliferative areas. MMPs could be detected locally in Dupuytren's tissue in a few patients, with less positive staining than for TIMPs. In the control group, there was just little or no staining for TIMPs and MMPs. The data indicate that the physiological balance between MMPs and their natural inhibitors is disturbed in patients with a proliferative active Dupuytren's disease. The decrease in the systemic MMP-to-TIMP ratio can cause increased synthesis and deposition of collagen, leading to palmar fibromatosis. (*Plast. Reconstr. Surg.* 112: 1279, 2003.)

Dupuytren's contracture is a fibroproliferative disorder characterized by progressive deposition of mature collagen fibers, resulting in a single or an ill-defined conglomerate of multiple aponeurotic scar nodules with an irreversible contracture.[1,2] Many epidemiological studies have suggested correlations with diabetes, epilepsy, and alcoholism, with or without cirrhosis.[3,4] Also, a genetic predisposition has been discussed.[5] Since the initial description of the myofibroblast in Dupuytren's tissue, various other studies have contributed to a more detailed understanding of the histological appearance.[6] Some authors have demonstrated the presence and the involvement of growth factors such as transforming growth factor-β and basic fibroblast growth factor in palmar fibromatosis, suggesting the possibility that contracture may be the result of a complex and an abnormal regulation of cellular proliferation.[7–9] Nevertheless, the etiopathogenesis of Dupuytren's disease is still unknown.[6] Whereas the fibrosis of the palmar fascia is not life-threatening, its pathogenesis is likely to be fairly similar to that of other fibrotic diseases affecting organs such as the liver, lung, heart, and kidney.[10] In these diseases associated with unbalanced degradation of the extracellular

matrix, matrix metalloproteinases (MMPs) and their natural inhibitors, the tissue inhibitors of metalloproteinases (TIMPs), play an important role.[11,12] The MMPs are zinc-dependent endopeptidases produced by a number of cell types involved in wound repair, including fibroblasts, macrophages, endothelial cells, and keratinocytes.[12] MMP-1 (interstitial collagenase), MMP-2 (72-kDa type IV collagenase, gelatinase A), and MMP-9 (92-kDa type IV collagenase, gelatinase B) have been implicated in various aspects of tissue maintenance and wound repair.[13,14] The activity of MMPs is carefully regulated by controlling their conversion from proenzymes to the catalytic form and by TIMPs.[15] Until now, four distinct members of the TIMP gene family have been described.[15] TIMP-1 is the most important inhibitor.[15]

In a series of 12 patients with advanced inoperable gastric carcinoma who had treatment with the synthetic MMP inhibitor Marimastat (British Biotech Ltd., Oxford, United Kingdom), with a dosage of 25 to 100 mg/day for more than 1 month, six patients developed a frozen shoulder or a condition resembling Dupuytren's disease.[16] Unfortunately, no biopsies were performed for analysis. The authors postulated that the development of the frozen shoulder and the Dupuytren-like condition in their patients was caused by the decrease in the systemic MMP-to-TIMP ratio, which might cause increased synthesis and deposition of collagen and connective tissue.[16] Until now, no further studies concerning the concentration of MMPs and TIMPs in sera and tissue samples of patients with Dupuytren's disease have been performed.

In the present study, serum concentrations of MMP-1, MMP-2, MMP-9, TIMP-1, and TIMP-2 were determined in patients with proliferative active and inactive Dupuytren's contracture for the first time. Furthermore, surgical samples for standard histological and immunohistochemical analyses were obtained from the patients.

PATIENTS AND METHODS

Patients with Dupuytren's Disease

The study included 22 patients (five women and 17 men; average age, 67 ± 11 years) with Dupuytren's disease. The duration of the disease ranged from 7 months to 30 years. In five patients, the left hand was involved, and in six, the right hand. Eleven patients presented a bilateral involvement. One patient had a plantar fibromatosis and three patients had knuckle pads. Ten patients had a family history of Dupuytren's disease. Diabetes was seen in one patient and alcoholism was seen in three patients. Treatment consisted of regional fasciectomy with complete excision of the diseased fascia in the palm and digits ($n = 12$) or radical fasciectomy for patients with extensive disease ($n = 10$). Tissue samples for standard histological and immunohistochemical analyses were obtained from all patients. The tissue obtained after fasciectomy normally consisted of the cord and/or nodule excised in its entirety. The size of the tissue varied both in cross-section and in length from patient to patient.

Control Group

Sera and samples of palmar fascia from 20 patients (13 women and seven men; average age, 60 ± 15 years) who had undergone hand surgery for carpal tunnel syndrome were used as the control group. Informed consent was obtained from all subjects. Patients with other fibroproliferative disorders were excluded because they might present pathological serum concentrations of the analyzed parameters.[12,13,15]

Serum Analysis

Blood samples of all patients were drawn immediately before operation. The samples were immediately centrifuged at 1200 g for 10 minutes within 15 minutes after collection. The sera were aliquoted and stored at $-80°C$ before analysis. MMP-1, MMP-2, MMP-9, TIMP-1, and TIMP-2 concentrations in sera were measured using a specific enzyme-linked immunosorbent assay designed as a monoclonal sandwich assay (R&D Systems, Europe Ltd., Abingdon, United Kingdom).

Histology and Immunohistochemistry

Tissue samples obtained from patients undergoing palmar fasciectomy for treatment of Dupuytren's disease or from patients who had undergone hand surgery for carpal tunnel syndrome were snap-frozen in liquid nitrogen immediately after surgical removal. Four-micrometer sections of frozen tissue samples were cut on a cryostat at $-20°C$ and stained with hematoxylin and eosin for standard histological examination. Serial sections were cut, air-dried for 20 minutes, fixed in cold acetone for 10 minutes at room temperature, and then air-dried for 10 minutes for immunohisto-

chemical analysis. Subsequently, the remaining tissues were filled in 10% buffered formaldehyde for 12 hours, embedded in paraffin, and stained with hematoxylin and eosin.

Immunohistochemical staining was performed using the alkaline phosphatase monoclonal antialkaline phosphatase method with antisera or monoclonal antibodies with different specificities: anti–MMP-1, anti–MMP-2, anti–MMP-9, anti–TIMP-1, and anti–TIMP-2 (Dianova, Hamburg, Germany). Three blinded examiners independently assessed the histological sections. When there were differences between the assessments, the mean value was calculated.

Statistical Analysis

Data were expressed as mean value and SD. Statistical analysis was performed using the Mann-Whitney test. The level of significance was considered to be $p < 0.05$.

RESULTS

Histology

Fourteen patients with Dupuytren's contracture presented a proliferative active disease (proliferative and early involutional phase), according to the criteria set forward by Luck.[17] Tissue samples of eight patients complied with criteria of the late involutional and residual phase.

Serum Analysis

TIMP-1. Figure 1, *above,* shows the results of the TIMP-1 determination in sera of patients with Dupuytren's disease and the control group. Patients with Dupuytren's contracture presented with a TIMP-1 concentration of 437 ± 160 ng/ml, a significantly higher TIMP-1 concentration than that seen in the control patients, who had a concentration of 321 ± 70 ng/ml ($p < 0.05$). Patients with a proliferative active disease ($n = 14$) had a TIMP-1 concentration of 525 ± 136 ng/ml, a significantly higher concentration than that seen in patients with later-phase disease ($n = 8$; 286 ± 41 ng/ml) ($p < 0.05$; Fig. 1, *center*). Compared with the control group, patients with an inactive disease had no significant difference in serum TIMP-1.

TIMP-2. There was no significant difference ($p < 0.05$) in the TIMP-2 serum concentration between patients with palmar fibromatosis (41 ± 7.5 ng/ml) and the control group (42 ± 8.6 ng/ml; Fig. 1, *below*). Furthermore, no
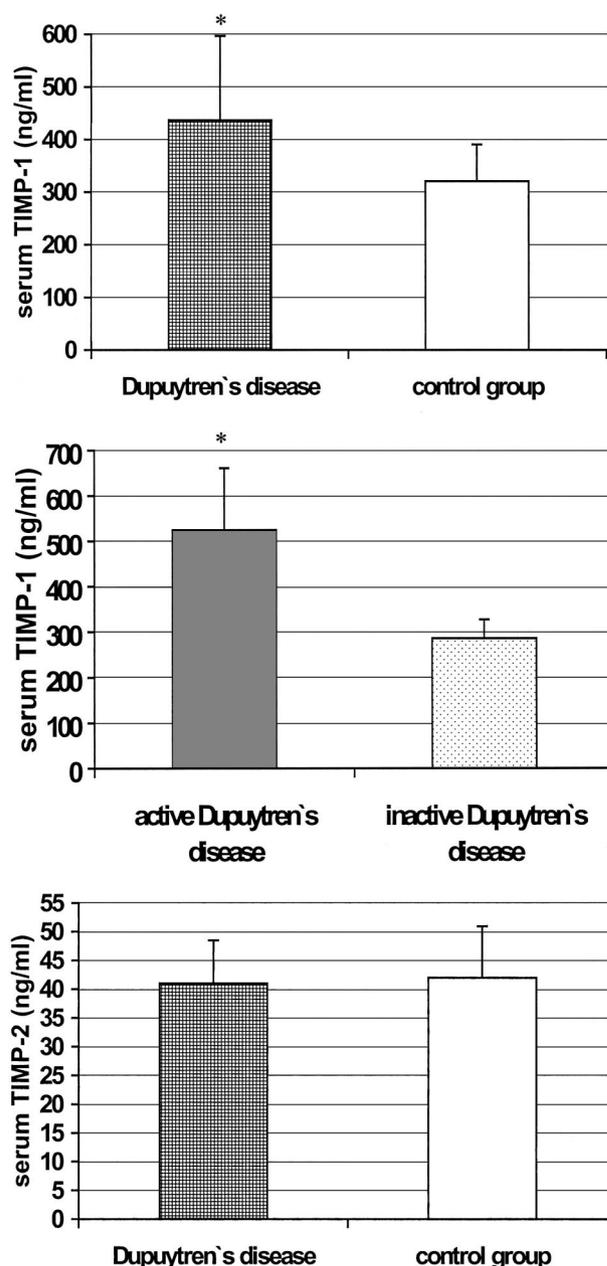

FIG. 1. (*Above*) TIMP-1 in sera of patients with Dupuytren's disease ($n = 22$) and control group ($n = 20$; mean ± SD). (*Center*) TIMP-1 in sera of patients with active ($n = 14$) and inactive ($n = 8$) Dupuytren's disease (mean ± SD). (*Below*) TIMP-2 in sera of patients with Dupuytren's disease and control group (mean ± SD).

significant difference could be observed between patients with active disease and those with inactive disease.

MMP-1. Figure 2, *above,* shows the results of the MMP-1 determination in sera of patients with Dupuytren's disease and the control group. Patients with Dupuytren's disease and the control patients presented no remarkable difference in MMP-1 serum concentration (5.2
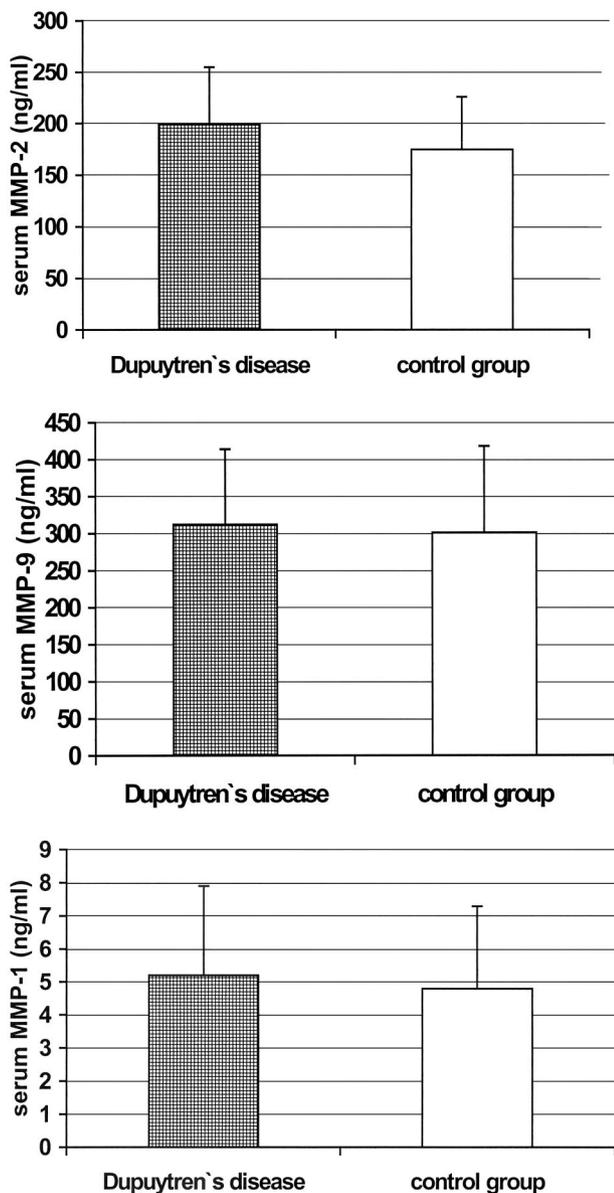
FIG. 2. (*Above*) MMP-1 in sera of patients with Dupuytren's disease and control group (mean ± SD). (*Center*) MMP-2 in sera of patients with Dupuytren's disease and control group (mean ± SD). (*Below*) MMP-9 in sera of patients with Dupuytren's disease and control group (mean ± SD).

± 2.7 ng/ml and 4.8 ± 2.5 ng/ml, respectively). In comparison to patients with a proliferative active disease, patients with inactive palmar fibromatosis showed no significant difference.

MMP-2. There was no significant difference ($p < 0.05$) in MMP-2 serum concentration between patients with palmar fibromatosis (199 ± 56 ng/ml) and the control group (175 ± 51 ng/ml; Fig. 2, *center*). Even patients with active and inactive Dupuytren's disease showed no remarkable difference.

MMP-9. Patients with Dupuytren's contracture and the control patients presented no significant difference in MMP-9 serum concentration (312 ± 102 ng/ml and 301 ± 117 ng/ml, respectively; Fig. 2, *below*). Furthermore, there was no significant difference between patients with proliferative active disease and those with inactive disease.

MMP-to-TIMP ratio. The MMP-to-TIMP ratio included the serum concentrations of MMP-1, MMP-2, MMP-9, TIMP-1, and TIMP-2. Figure 3, *above*, shows the MMP-to-TIMP ratio for patients with Dupuytren's disease and the control group.

Patients with palmar fibromatosis had a significantly lower MMP-to-TIMP ratio (1.1 ± 0.3; $p < 0.05$) than the control group (1.5 ± 0.35). Patients with active palmar fibromatosis presented a significantly ($p < 0.05$) reduced ratio (1 ± 0.2) compared with those in later phases (1.4 ± 0.3; Fig. 3, *below*). Compared with the control group, patients with inactive disease showed no significant difference in the MMP-to-TIMP ratio.
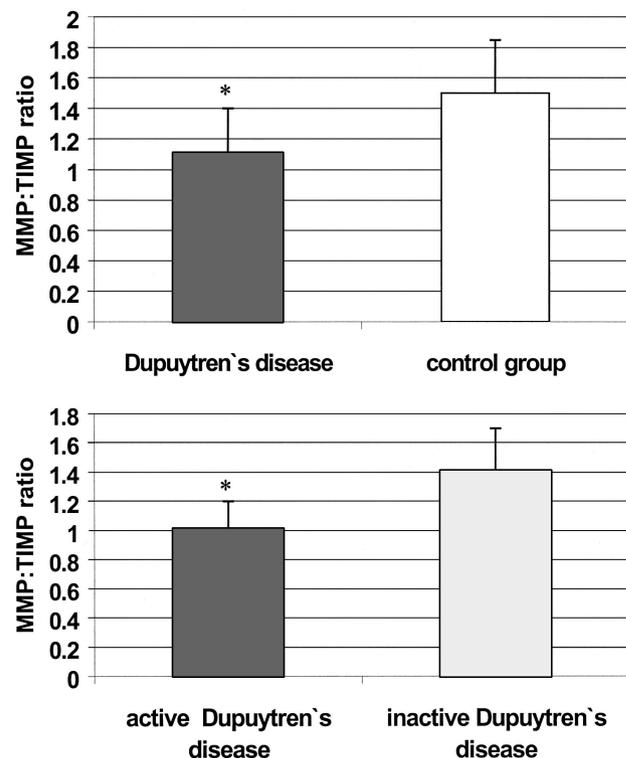

FIG. 3. (*Above*) MMP-to-TIMP ratio in sera of patients with Dupuytren's disease and control group. (*Below*) MMP-to-TIMP ratio in sera of patients with active and inactive Dupuytren's disease.

Immunohistochemistry

TIMP-1 showed an accumulation in proliferative areas of palmar aponeurosis among patients with Dupuytren's contracture (Fig. 4, *above*). In four samples from inactive disease, TIMP-1 could also be detected (Fig. 4, *center*), whereas there was no staining in the control tissue of patients who had undergone hand surgery for carpal tunnel syndrome (Fig. 4, *below*). TIMP-2 showed a reaction in proliferative areas of samples from most patients with Dupuytren's disease as well (Fig. 5, *above*). In the control group, there was no staining for TIMP-2 (Fig. 5, *below*). MMP-1 showed a local reaction in 12 samples of patients with Dupuytren's disease, but the reaction for TIMPs was stronger and more extensive than the reaction with MMP-1 (Fig. 6, *above*). In the control group, MMP-1 was not detectable (Fig. 6, *below*). MMP-2 could be detected in proliferative areas of two tissue samples from patients with palmar fibromatosis, and MMP-9 could be detected in one sample. In the control group, no samples stained positively for MMP-2 or MMP-9.

DISCUSSION

It has recently become clear that progressive fibrotic disorders such as liver cirrhosis, glomerulonephritis, atherosclerosis, and fibrotic lung disease are complex processes involving a cascade of molecular and cellular events, with several growth factors playing a pivotal role in the final common pathway.[18–21] Obviously, the MMP-to-TIMP ratio is of great importance in these diseases associated with an unbalanced degradation of extracellular matrix also.[13,22,23] Whereas patients with chronic ulcers present a lack of TIMPs with an uncontrolled activity of MMPs,[24] an elevated systemic TIMP-1 concentration can be observed in severe fibroproliferative disorders.[10,11] Recently, we have described a disturbed physiological balance between MMPs and their natural antagonist in patients after severe burn trauma, with an elevated systemic TIMP-1 concentration contributing to pathological dermal scar formation.[12] In our present study, MMPs and TIMPs were determined in sera and tissue samples of patients with a proliferative active and inactive Dupuytren's disease for the first time. Our results are nearly identical to the observations attained in patients with severe skin fibrosis and other fibroproliferative disorders.
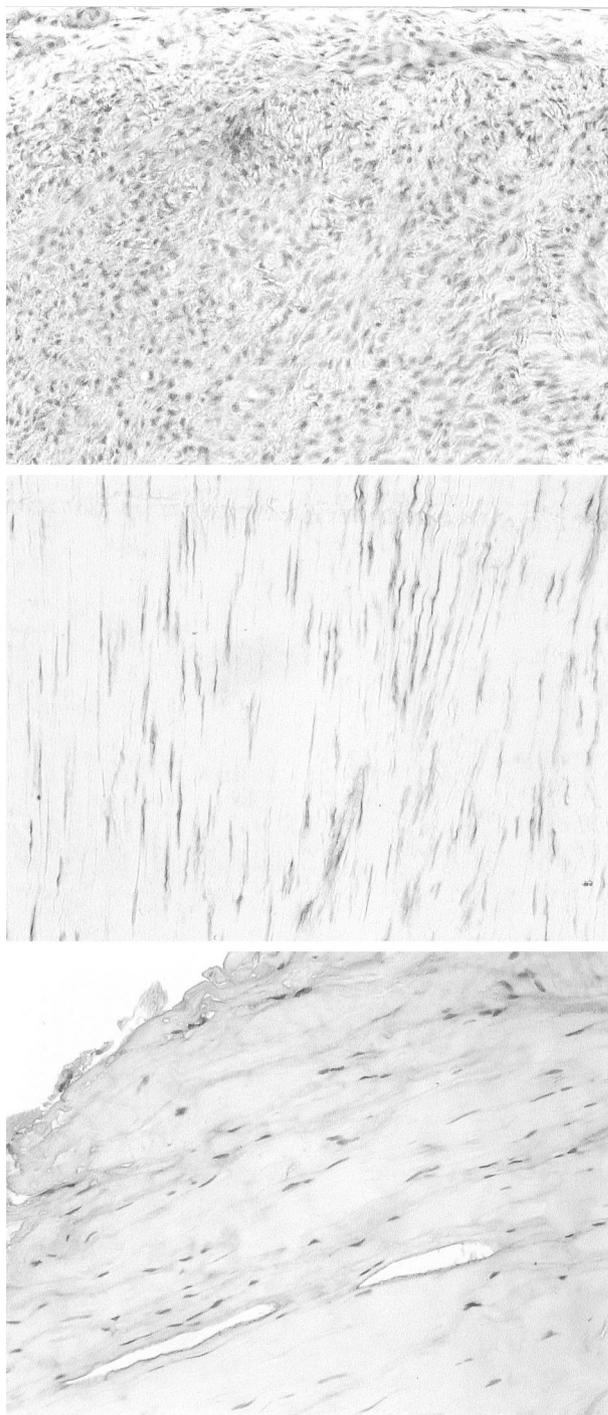

FIG. 4. (*Above*) Immunostaining for TIMP-1 in palmar aponeurosis from patients with active Dupuytren's disease (hematoxylin and eosin stain; original magnification, ×20). (*Center*) Immunostaining for TIMP-1 in palmar aponeurosis from patients with inactive Dupuytren's disease (hematoxylin and eosin stain; original magnification, ×20). (*Below*) Immunostaining for TIMP-1 in palmar aponeurosis from control patients (hematoxylin and eosin stain; original magnification, ×20).

In several carcinomas, there is an overexpression of MMPs by tumor cells leading to an increase in the breakdown of connective tissue
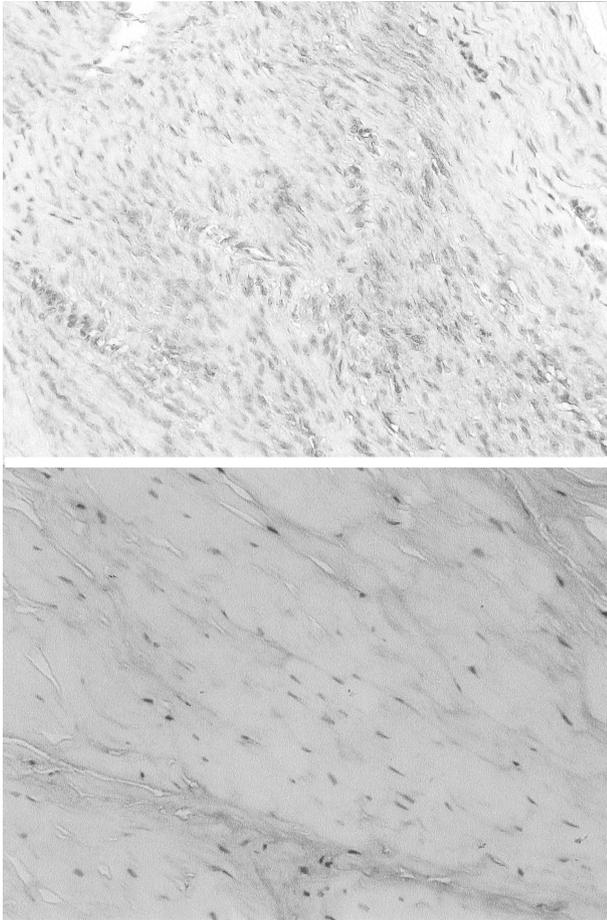
FIG. 5. (*Above*) Immunostaining for TIMP-2 in palmar aponeurosis from patients with Dupuytren's disease (hematoxylin and eosin stain; original magnification, ×20). (*Below*) Immunostaining for TIMP-2 in palmar aponeurosis from control patients (hematoxylin and eosin stain; original magnification, ×20).
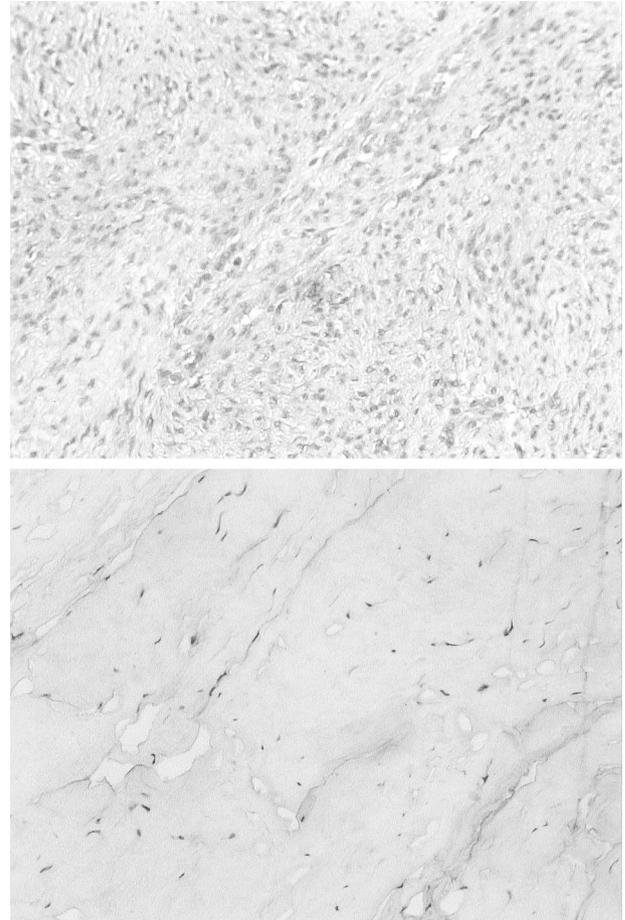
FIG. 6. (*Above*) Immunostaining for MMP-1 in palmar aponeurosis from patients with Dupuytren's disease (hematoxylin and eosin stain; original magnification, ×20). (*Below*) Immunostaining for MMP-1 in palmar aponeurosis from control group (hematoxylin and eosin stain; original magnification, ×20).

and a possible enhancement of tumor invasion and entry into blood and lymphatic vessels.[16] In gastric cancer patients, studies with a synthetic broad-spectrum MMP inhibitor have been performed.[25] In a recent phase II study of the effect of such an inhibitor, Marimastat, Hutchinson et al.[16] recruited 24 patients with inoperable advanced primary or recurrent gastric adenocarcinoma. Twelve of the patients were judged to have had a favorable response to this treatment and continued to receive it beyond an initial 1-month assessment period. In the further course, three of these patients developed clinical features of a Dupuytren's contracture, with palmar cords involving middle, ring, and little fingers ($n = 2$) and palmar nodules ($n = 1$) and a frozen shoulder, a Dupuytren-like disease.[26] Three additional patients developed symptoms of a frozen shoul-

der alone. When treatment with Marimastat was temporarily discontinued, the symptoms of the frozen shoulder improved, whereas Dupuytren's contracture remained. Unfortunately, no excision of the cords and nodules was performed. Hutchinson et al. postulated that the development of the Dupuytren-like condition in their tumor patients was because of alterations in MMP activity caused by the systemic increase of the synthetic MMP inhibitor.[16]

Until now, MMPs have been analyzed only in surgical samples of patients with Dupuytren's disease during a continuous elongation technique described by Messina and Messina.[27] This technique, in which fingers are slowly straightened on an external adjustable frame over a period of between 2 and 3 weeks, was developed for patients with such

severe contractures of their fingers that amputation was the only alternative.[27] Bailey et al.[28] demonstrated increased levels of MMP-1, MMP-2, and MMP-9 in tissue removed at fasciectomy following 2 to 3 weeks of the Messina technique. However, it was not possible to show whether the increase of the metalloproteinases was proportional to the mechanical stimulus or whether it was mediated by inflammatory cells. Using an in vitro model of controlled extensions in which inflammatory involvement was absent, Tarlton et al.[29] have shown a correlation between the load applied to the tissue samples and the release of MMP-2 and MMP-9. The subsequent degradation of the collagen results in a loss of mechanical strength.[29] Nevertheless, studies concerning the balance between metalloproteinases and their natural inhibitors in patients with Dupuytren's contracture are still lacking.

In our study, we have shown a significantly elevated level of TIMP-1 in sera of patients with active palmar fibromatosis, whereas TIMP-2, MMP-1, MMP-2, and MMP-9 presented no remarkable difference compared with a control group. TIMP-1 was significantly higher in sera of patients with fibromatous nodules in the proliferative or early involutional phase than in sera of patients in the late involutional or residual phase. Altogether, the MMP-to-TIMP ratio of patients with active Dupuytren's disease was significantly reduced. Immunohistochemically, samples of patients with palmar fibrosis stained intensively positive for TIMP-1 and TIMP-2, whereas there was no or less reaction with MMP-specific antibodies.

This study indicates that the physiological balance between MMPs and their endogenous antagonists is disturbed in patients with an active Dupuytren's disease. The elevated systemic TIMP-1 concentration with a lack of degradation of extracellular matrix components might be a pathway in the pathogenesis of this fibroproliferative disorder.

Dietmar Ulrich, M.D.
Department of Plastic Surgery and Hand Surgery,
*   Burn Center*
University Hospital
Aachen University of Technology
Pauwelsstraβe 30
52057 Aachen, Germany
dietmar.ulrich@post.rwth-aachen.de

REFERENCES

1. Chiu, H. F., and McFarlane, R. M. Pathogenesis of Dupuytren's contracture: A correlative clinical-pathological study. *J. Hand Surg. (Am.)* 3: 1, 1978.
2. Badalamente, M. A., Stern, L., and Hurst, L. C. The pathogenesis of Dupuytren's contracture: Contractile mechanisms of the myofibroblasts. *J. Hand Surg. (Am.)* 8: 235, 1983.
3. Murrell, G. A. C., and Hueston, J. T. Aetiology of Dupuytren's contracture. *Aust. N. Z. J. Surg.* 60: 247, 1990.
4. Burge, P., Hoy, G., Regan, P., and Milne, R. Smoking, alcohol and the risk of Dupuytren's contracture. *J. Bone Joint Surg. (Br.)* 79: 206, 1997.
5. Ling, R. S. M. The genetic factors in Dupuytren's disease. *J. Bone Joint Surg. (Br.)* 45: 709, 1963.
6. Kloen, P. New insights in the development of Dupuytren's contracture: A review. *Br. J. Plast. Surg.* 52: 629, 1999.
7. Baird, K. S., Crossan, J. F., and Ralston, S. H. Abnormal growth factor and cytokine expression in Dupuytren's contracture. *J. Clin. Pathol.* 46: 425, 1993.
8. Hueston, J. T., and Murrell, G. A. Cell-controlling factors in Dupuytren's contracture. *Ann. Chir. Main Memb. Super.* 9: 135, 1990.
9. McFarlane, R. M. The current status of Dupuytren's disease. *J. Hand Ther.* 8: 181, 1995.
10. Liu, B., and Connolly, M. K. The pathogenesis of cutaneus fibrosis. *Semin. Cutan. Med. Surg.* 17: 3, 1998.
11. Reynolds, J. J. Collagenases and tissue inhibitors of metalloproteinases: A functional balance in tissue degradation. *Oral Dis.* 2: 70, 1996.
12. Ulrich, D., Noah, E. M., von Heimburg, D., and Pallua, N. TIMP-1, MMP-2, -9, and PIIINP as serum markers for skin fibrosis in patients following severe burn trauma. *Plast. Reconstr. Surg.* 111: 1423, 2003.
13. Massova, I., Kotra, L. P., Fridman, R., and Mobashery, S. Matrix metalloproteinases: Structures, evolution, and diversification. *F.A.S.E.B. J.* 12: 1075, 1998.
14. Ravanti, L., and Kahari, V. M. Matrix metalloproteinases in wound repair. *Int. J. Mol. Med.* 6: 391, 2000.
15. Brew, K., Dinakarpandian, D., and Nagase, H. Tissue inhibitors of metalloproteinases: Evolution, structure and function. *Biochim. Biophys. Acta* 1477: 267, 2002.
16. Hutchinson, J. W., Tierney, G. M., Parsons, S. L., and Davis, T. R. C. Dupuytren's disease and frozen shoulder induced by treatment with a matrix metalloproteinase inhibitor. *J. Bone Joint Surg. (Br.)* 80: 907, 1998.
17. Luck, J. V. Dupuytren's contracture. *J. Bone Joint Surg. (Am.)* 41: 635, 1959.
18. Rumalla, V. K., and Borah, G. L. Cytokines, growth factors, and plastic surgery. *Plast. Reconstr. Surg.* 108: 719, 2001.
19. Ramadori, G., Knittel, T., and Saile, B. Fibrosis and altered matrix synthesis. *Digestion* 59: 372, 1998.
20. Arthur, M. J. P. Fibrosis and altered matrix degradation. *Digestion* 59: 376, 1998.
21. Eddy, A. A. Molecular basis of renal fibrosis. *Pediatr. Nephrol.* 15: 290, 2000.
22. Lichtinghagen, R., Michels, D., Heberkorn, C. I., et al.

Matrix metalloproteinase MMP-2, MMP-7, and tissue inhibitor of metalloproteinase are closely related to fibroproliferative process in the liver during chronic hepatitis C. *J. Hepatol.* 34: 239, 2001.

23. Jackson, D. S. Development of fibrosis: Cell proliferation and collagen-biosynthesis. *Ann. Rheum. Dis. 36* (Suppl.): 2, 1977.

24. Saito, S., Trovato, M., You, R., et al. Role of matrix metalloproteinases 1, 2, and 9 and tissue inhibitor of matrix metalloproteinase 1 in chronic venous insufficiency. *J. Vasc. Surg.* 34: 930, 2001.

25. Belotti, D., Paganoni, P., and Giavazzi, R. MMP inhibitors: Experimental and clinical studies. *Int. J. Biol. Markers* 14: 232, 1999.

26. Bunker, T. D., and Anthony, P. P. The pathology of frozen shoulder: A Dupuytren-like disease. *J. Bone Joint Surg. (Br.)* 77: 677, 1995.

27. Messina, A., and Messina, J. The continuous elongation treatment by the TEC device for severe Dupuytren's contracture of the fingers. *Plast. Reconstr. Surg.* 92: 84, 1995.

28. Bailey, A. J., Tarlton, J. F., Van der Steppen, J., Sims, T. J., and Messina, A. The continuous elongation technique for severe Dupuytren's disease: A biochemical mechanism. *J. Hand Surg. (Br.)* 19: 522, 1994.

29. Tarlton, J. F., Meagher, P., Brown, R. A., McGrouther, D. A., Bailey, A. J., and Afoke, A. Mechanical stress in vitro induces increased expression of MMPs 2 and 9 in excised Dupuytren's disease tissue. *J. Hand Surg. (Br.)* 23: 297, 1998.