Acta Derm Venereol 2009; 89: 607–611

INVESTIGATIVE REPORT

Epidemiology of Chronic Wound Patients and Relation to Serum Levels of Mannan-binding Lectin

Mikael BITSCH[1]*, Inga LAURSEN[2]*, Anne-Marie ENGEL[2], Michael CHRISTIANSEN[2], Severin OLESEN LARSEN[2], Line IVERSEN[1], Per E HOLSTEIN[1] and Tonny KARLSMARK[1]

*[1]Copenhagen Wound Healing Center, University of Copenhagen, Bispebjerg Hospital, and [2]Statens Serum Institute, Copenhagen, Denmark. *Both these authors contributed equally to this paper and should be considered as first authors.*

The aim of this study was to describe the epidemiology of chronic wounds in a large cohort of patients from a tertiary hospital out-patient clinic, and examine the significance of serum mannan-binding lectin for the occurrence and clinical presentation of such wounds. The study comprised 489 consecutive patients with chronic foot and leg ulcers. A clinical classification of wound-aetiology was performed, and mannan-binding lectin was measured in the sera of patients and healthy controls. The patients presented with 639 wounds altogether; diabetic foot ulcers (309), venous leg ulcers (188), arterial ulcers (109), and vasculitis (33). The mannan-binding lectin levels of patients with venous leg ulcer, alone or in combination with other types of wounds, differed significantly from the control group, and the frequency of values < 100 ng/ml was significantly higher. In diabetic and arterial ulcer patients the frequency of values ≥3000 ng/ml was significantly higher than that of the control group. This suggests a role for the innate immunity in the pathology of venous leg ulcers, and indicates different roles for mannan-binding lectin in the development of ulcers with different aetiologies; it further suggests that mannan-binding lectin substitution should be tested in a controlled clinical trial. ***Key words: diabetic foot ulcer; venous leg ulcer; vasculitis; chronic wound; peripheral arterial disease; epidemiology; mannan-binding lectin.***

(Accepted June 22, 2009.)

Acta Derm Venereol 2009; 89: 607–611.

Mikael Bitsch, Copenhagen Wound Healing Center (CWHC), University Hospital of Copenhagen, Bispebjerg Hospital, 23 Bispebjerg Bakke, DK-2400 Copenhagen, Denmark. E-mail: mibi@dadlnet.dk

Foot and leg ulcer is a common disorder, and approximately 1% of the European population suffers from such chronic and recurrent ulceration (1, 2). Clinically, chronic wounds may be caused by external pressure, trauma, venous insufficiency, neuropathy (diabetes), arterial (ischaemic) disease, or vasculitis. Wound infections also constitute a serious complication in chronic foot and leg ulcer. Frequent wound infections may reflect an insufficient immune competence of the patients, and thereby an impaired ability to eliminate the colonizing pathogens; or an inefficient systemic response to local infection or inflammation.

In persons with a normal immune defence, this will rapidly respond to pathogens threatening an exposed wound, especially the innate immune system. If the immune response fails to clear the invading pathogens, this can result in persistent tissue injury and chronic inflammation.

Mannan-binding lectin (MBL) is an important component of the humoral innate immune system, and MBL possesses several characteristics indicating that it may play an essential role in wound healing; i.e. modulating inflammation and contributing to the clearance of micro-organisms and apoptotic cells (3, 4). Deficiency in MBL might therefore contribute to prolonged healing.

MBL is a liver-derived serum protein, and an acute phase reactant which exhibits a 2–3-fold increase in response to infections (4). MBL binds to patterns of carbohydrate ligands exposed on the surfaces of a variety of pathogenic micro-organisms; these are subsequently eliminated through opsonophagocytosis or complement-mediated killing (5). Furthermore, MBL has been shown to modulate the cytokine response to infections and inflammatory conditions (3). Structural and promoter variants of the MBL gene are common and influence functional activity and expression, which is reflected in the broad range of MBL concentrations. Persons carrying the MBL wild-type genotype show ranges from 1000 to 5000 ng/ml serum. Approximately 10–15% of Caucasians carry genotypes associated with concentrations below 100 ng/ml, these persons are defined as being MBL-deficient (6). Furthermore, persons with low MBL levels appear to be at risk of severe and recurrent infections; most prominently in childhood before maturation of the adaptive immune system, and under immune-compromised conditions (7). Comprehensive research has associated MBL deficiency with several clinical conditions, e.g. autoimmune diseases (7, 8), septicaemia (9), and endocarditis (10). The potential role of MBL in the pathogenesis of chronic wounds is underscored by the recent successful treatment of an MBL-deficient leg ulcer patient with MBL substitution therapy (11).

The aims of the present study were to describe the epidemiology of a large cohort of patients with chro-

 doi: 10.2340/00015555-0730
 ISSN 0001-5555

nic foot and leg ulcer of different aetiologies, and to examine the significance of serum MBL levels for the occurrence and clinical presentation of the ulcer.

MATERIALS AND METHODS

Patient characteristics

Between February 2003 and November 2005 a cohort of 489 patients with chronic ulcers (169 women and 320 men, mean age 65.3 years, age range 30–92 years) were enrolled at Copenhagen Wound Healing Center, a tertiary hospital out-patient clinic. The patients presented a total of 639 foot and leg ulcers with varying aetiologies, and were classified into four mutually overlapping diagnostic wound-groups: diabetic foot ulcer, venous leg ulcer, vasculitis (immunological ulcers), and arterial (ischemic) leg ulcer. Some patients having multiple ulcers with different aetiologies, e.g. diabetic foot ulcer and venous leg ulcer, were recorded in more than one group. Patients with pressure ulcers were included if they also presented a wound belonging to one of the groups mentioned. Diabetic patients were analysed as one group; the majority of this group suffered from type 2 diabetes.

All patients in this study were defined as having prolonged wound healing, i.e. they had not responded to standard treatment regimens for their particular type of ulcer within 3 months. However, all patients were capable of unassisted walking and lived in their own homes.

Patients were not included if the impaired wound healing was due to malignancy, radiation damage, severe critical ischaemia, end-stage nephropathy with dialysis or renal transplantation, if they received long-term systemic steroids with dosage > 5 mg prednisolone per day, or had foreign body implants.

Wound diagnostic and standard treatment

Most ulcers have a mixed aetiological background. A venous ulcer often has a component of deteriorated peripheral circulation, and most neuropathic ulcers in diabetics also have an ischaemic element. However, all wounds were classified according to their main aetiology; and all chronic ulcers received the relevant standard examinations and treatments.

The appropriate treatment for diabetic foot ulcer was pressure relief, immobilization, debridement of devitalized tissue, and antibiotic therapy. Ulcers with an immunological background were registered as vasculitis. A standard treatment for all types of vasculitis has not been established. The patients in this study received different local and systemic treatments including short-term aggressive therapy with glucocorticoids and immuno-suppressive agents (12).

Evaluation of peripheral circulation was performed in all patients. Arterial perfusion was evaluated in both legs using ankle and toe systolic blood pressure measurement. Arterial disease was defined as an ankle/brachial index less than 90% and absent pedal pulse (13). The main treatment for peripheral arterial disease was vascular surgery or introducing an artificial graft for the by-pass or alternatively as percutaneous transluminal angiography to eliminate the arterial stenosis by dilatation.

Venous insufficiency was defined as retrograde flow more than 0.5 sec or thrombus in the deep vein system (14). Venous leg ulcers received compression therapy, 70% of leg ulcers have been reported to heal within 3 months by this therapy (1, 15, 16).

Cases with wound infections were treated with antibiotics according to guidelines (17, 18).

Healthy control group

The control group comprised 234 healthy adult persons (133 men and 103 women, mean age 40.9 years, age range 18–65 years).

MBL analysis

At the first visit to the wound centre a blood sample was taken from all patients, and serum drawn for analysis. MBL concentrations were measured by a sandwich enzyme-linked immunoassay (ELISA) performed essentially as described previously (19). Briefly, MBL was quantified by a semi-automated time-resolved immuno-fluorescence assay using the monoclonal antibody Hyb 131-01 (Statens Serum Institut, Denmark) as catcher, biotinylated Hyb 131-01 as detector, and streptavidin-Eu-chelate for quantification. The assay was run on an AutoDelfia platform (Perkin Elmer, Shelton, CT, USA). The lower and upper quantification levels of the assay were 10 ng/ml and 3000 ng/ml, respectively. Consequently lower and higher MBL levels are referred as < 10 ng/ml and > 3000 ng/ml. For statistical data evaluation results of ≤ 10 ng/ml and ≥ 3000 ng/ml were included as 10 ng/ml and 3000 ng/ml, respectively.

Statistical analyses

χ^2 tests were used to compare frequencies and χ^2 test for trend to compare distributions of MBL levels in various groups (20).

RESULTS

The MBL serum levels of the enrolled 489 consecutive patients presenting a total of 639 wounds were analysed. The leg and foot ulcers were classified into four groups according to aetiology. Diabetic foot ulcers were most frequent, followed by venous leg ulcers, arterial ulcers, and vasculitis. In Table I the MBL values of all wounds, the four groups and the normal controls are presented as the respective quartiles. No significant difference is observed between the MBL levels of all patients and the control group, however, a marked inter-group variation appears. Interestingly, the median values of venous leg ulcer and vasculitis patients are below that of the healthy controls, whereas the median of diabetic ulcer and arterial ulcer patients is higher, reaching 3000 ng/ml. Levels ≥ 3000 ng/ml might either

Table I. *Serum mannan-binding lectin levels (ng/ml) correlated with wound diagnosis*

	All cases $n=489$	Diabetic foot ulcer $n=309$	Venous leg ulcer $n=188$	Arterial ulcer $n=109$	Vasculitis $n=33$	Healthy controls $n=234$
25% quartile	423	557	174	471	224	580
Median	2260	3000[1]	1357	3000[1]	1148	1992
75% quartile	3000[1]	3000[1]	3000[1]	3000[1]	3000[1]	3000[1]

[1]The upper quantification level of the MBL assay.

reflect an acute phase response from ongoing inflammations in the ulcer, or that the patients' basic levels are in the upper normal range.

To further explore a possible association between wound aetiology and MBL levels, the vasculitis group, including 5 patients with vasculitis only, was omitted in the subsequent data evaluation. The remaining 484 patients comprising 606 ulcers were further classified either as representing wounds belonging or not to the three main aetiological groups, i.e. diabetic foot ulcer (D), arterial ulcer (A), and venous leg ulcer (V) or as presenting more than one type of ulcer as combinations of those three groups. A total of seven subgroups were established. In Table II, the distribution of MBL levels, divided into five concentration ranges, of these seven subgroups and the healthy control group is shown. It can be seen that patients with venous leg ulcer only, and venous leg ulcer in combination with either diabetic foot ulcer or arterial ulcer (subgroups V + D and V + A) express relatively low MBL levels. A test for trend shows that MBL levels of patients with venous ulcers alone or in combination with other wound types (total V) differ significant from the healthy controls, with *p*-values of 0.012 and 0.023, respectively. Furthermore, the frequency of MBL levels < 100 ng/ml (L) of venous leg ulcer alone, subgroup V + D, and total V is significantly higher than in the control group, with *p*-values of 0.003, 0.035, and 0.001, respectively. On the other hand when focusing on the upper area of the MBL range, approximately one-half of patients with diabetic foot ulcers alone, arterial leg ulcer alone, and patients presenting both types of wounds (subgroup D + A) have MBL values ≥ 3000 ng/ml. A test for trend shows that only one group, diabetic foot ulcer alone differed significant from the healthy controls, with a *p*-value of 0.050. However, when focusing on high vs. low range with regards to serum MBL, the frequency of MBL levels ≥ 3000 ng/ml (H) of diabetic foot ulcer, subgroup D + A, total D, and total A is significantly higher than that of the control group, all with a *p*-value of < 0.001. The subgroups with mainly low or high expressing MBL patients are complementary, except for the combination of arterial and venous leg ulcer. It appears that the underlying cause of venous leg ulcers – alone or in combination with the other types of ulcer – may be linked to MBL deficiency.

DISCUSSION

This is the first study in which serum MBL has been determined in a cohort of patients with chronic foot and leg ulcer, and the relationship between MBL levels and the occurrence and aetiologies of the wounds have been investigated. We focused specifically on a possible role of MBL deficiency on healing complications, based on the facts that MBL deficiency is the most common immune disorder, and that a common causality for prolonged healing of these ulcers is infection or colonization by bacteria. Several studies have revealed a range of bacteria as targets for MBL, which are commonly found in ulcers, e.g. *Staphylococcus aureus*, β-haemolytic group A *Streptococcus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (21–23). Although no previous study has focused on MBL deficiency in relation to chronic wounds, a study comprising patients undergoing surgery for cancer of the gastrointestinal tract showed an association between postoperative infections and significantly reduced MBL levels (24).

Our first observation was that two of the four aetiological groups, venous leg ulcer and vasculitis, expressed markedly lower median MBL values than the others and the controls; although, when the entire cohort was compared with the healthy control group there was no significant difference. We then focused on the three main groups: diabetic foot ulcer, venous leg ulcer, and arterial ulcer, from which seven subgroups were combined; and their distribution of MBL levels into

Table II. *Distribution of mannan-binding lectin (MBL) levels in patient groups and subgroups*

	MBL ng/ml						*p*-values[a]	
Group	< 100 *n* (%)	100–499 *n* (%)	500–999 *n* (%)	1000–2999 *n* (%)	≥ 3000 *n* (%)	Total	Trend	L or H[b]
Controls	26 (11.1)	25 (10.7)	31 (13.2)	82 (35.0)	70 (29.9)	234		
Diabetic (D)	23 (11.1)	23 (11.1)	14 (6.8)	44 (21.3)	103 (49.8)	207	0.050	H < 0.001
Arterial (A)	2 (9.5)	4 (19.1)	2 (9.5)	2 (9.5)	11 (52.4)	21	0.637	H 0.061
D + A	6 (8.8)	11 (16.2)	5 (7.4)	9 (13.2)	37 (54.4)	68	0.155	H < 0.001
Venous (V)	32 (23.2)	16 (11.6)	15 (10.9)	37 (26.8)	38 (27.5)	138	0.012	L 0.003
V + D	8 (26.7)	3 (10.0)	2 (6.7)	6 (20.0)	11 (36.7)	30	0.224	L 0.035
V + A	4 (25.0)	2 (12.5)	2 (12.5)	0 (0.0)	8 (50.0)	16	NA	NA
V + D + A	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (100.0)	4	NA	NA
Total patients	75 (15.5)	59 (12.2)	40 (8.3)	98 (20.2)	212 (43.8)	484	0.815	H 0.001
Total D	37 (12.0)	37 (12.0)	21 (6.8)	59 (19.1)	155 (50.2)	309	0.075	H < 0.001
Total A	12 (11.0)	17 (15.6)	9 (8.3)	11 (10.1)	60 (55.0)	109	0.195	H < 0.001
Total V	44 (23.4)	21 (11.2)	19 (10.1)	43 (22.9)	61 (32.4)	188	0.023	L 0.001

[a]Comparison with controls.

[b]L: frequency of MBL < 100; H: frequency of MBL ≥ 3000.

NA: Not applicable: patients numbers too low to justify statistical analysis.

five concentration intervals was studied. This revealed that around one-quarter of patients presenting with venous leg ulcer alone or in combination with other aetiologies expressed MBL values < 100 ng/ml. When compared with the control group the frequency of MBL deficiency was significantly associated with venous leg ulcers (comprising subgroups V, V + D, and total V), which indicates a role for this innate immune component in the aetiology of the venous leg ulcers. In contrast, approximately half of the patients suffering from diabetic foot ulcer and arterial ulcer alone or combined expressed MBL values ≥ 3000 ng/ml. This also accounted for the combined venous leg and arterial ulcer subgroup, apparently reflecting patients with different dominating ulcers. Compared with the controls, the frequency of high MBL levels was significantly greater in the subgroups D, D + A, total D, and total A. The results may reflect different underlying causalities for chronic diabetic foot ulcer and arterial ulcer compared with venous leg ulcer.

Whether MBL plays a direct role in prevention or reduction of the "bio-burden" of chronic wounds is unclear. Chronic wounds colonised with bacteria are often neither infected nor inflamed, and when infection does appear antibiotic treatment will be initiated immediately – or may even have been given prophylactic. This indicates that MBL – shown to be associated with susceptibility and severity of infections – primarily functions upstreams to the manifestation of the chronic leg ulcer as different mechanisms appear to initiate and maintain the leg ulcer. In addition to combating pathogens, other functional roles of MBL, i.e. elimination of apoptotic cells or local activation of coagulation through the action of MBL-associated serine protease 1 might contribute to healing (4, 25). The potential role of MBL in the pathogenesis of chronic leg ulcer is, as mentioned, underscored by the successful treatment of an MBL-deficient patient with MBL therapy (11).

Like other immune components MBL may act as a double-edged sword; in some clinical contexts MBL-deficiency may be advantageous as protection against complement-mediated tissue injury. Studies of patients with type 1 diabetes have shown significantly elevated levels of MBL to be positively correlated with markers of renal complications and nephropathy (26, 27), possibly indicating that MBL may play a pathogenetic role or be a risk factor in type 1 diabetes. Diabetic foot ulcer is most frequently presented by type 2 diabetics, who also constitute the majority of patients in the present study. A follow-up study of Danish type 2 diabetics showed their risk of dying to be significantly correlated to high MBL, indicating an implication in diabetic vascular complication (28). Whether such MBL-associated vascular complications also contribute to the development of chronic foot ulcer in diabetic patients needs further investigation.

In conclusion, in a cohort of chronic foot and leg ulcer patients with different aetiological backgrounds, those with ulcers due to venous insufficiency, alone or in combination with other aetiologies, expressed significantly lower MBL concentrations than the healthy controls. The inverse pattern was seen in diabetic and arterial ulcer patients, who expressed significantly higher MBL levels. This indicates different roles for MBL in the development of ulcers in the different groups of patients; and the significant correlation of MBL deficiency to venous leg ulcer suggests that MBL substitution might be a relevant therapy for this group of patients.

ACKNOWLEDGEMENT

Inga Laursen works at Statens Serum Institute which holds a patent for the production of plasma-derived MBL.

REFERENCES

1. Nelzen O, Bergqvist D, Lindhagen A. (a) The prevalence of chronic lower-limb ulceration has been underestimated: results of a validated population questionnaire. Br J Surg 1996; 83: 255–258.
2. Nelzen O, Bergqvist D, Lindhagen A. (b) Leg ulcer etiology – across sectional population study. J Vasc Surg 1991; 14: 1–12.
3. Fraser DA, Bohlson SS, Jasinskiene N, Rawal N, Palmarini, Ruiz S, et al. C1q and MBL, components of the innate immune system, influence monocyte cytokine expression. J Leukc Biol 2006; 80: 107–116.
4. Dommett RM, Klein N, Turner MW. Mannose-binding lectin in innate immunity: past, present and future. Tissue Antigens 2006; 68: 193–209.
5. Super M, Lu J, Thiel S, Levinsky, RT, Turner MW. Association of low levels of mannan-binding protein with a common defect of opsonisation. Lancet 1989; 334: 1236–1239.
6. Madsen HO, Garred P, Thiel S, Kurtzhals JA, Lamm LU, Ryder LP, Svejgaard A. Interplay between promotor and structural gene variants control basal serum level of mannan-binding protein. J Immunol 1995; 155: 3013–3020.
7. Kilpatrick DC. Mannan-binding lectin: clinical significance and applications. Biochim Biophys Acta 2002; 1572: 401–413.
8. Saevarsdottir S, Kristjansdottir H, Grondal G, Vikingsdottir T, Gudnason V, Valdimarsson H. Mannan-binding lectin and complement C4A in Icelandic multicase families with systemic lupus erythematosus. Ann Rheum Dis 2006; 65: 1462–1467.
9. Eisen DP, Dean MM, Thomas P, Marshall P, Gerns N, Heatley S, et al. Low mannose-binding lectin function is associated with sepsis in adult patients. FEMS Immunol Med Microbiol 2006; 48: 274–282.
10. Tran CT, Kjeldsen K, Haunsø S, Hoiby N, Johansen HK, Christiansen M. Mannan binding lectin is a determinant of survival in infective endocarditis. Clin Exp Immunol 2007; 148: 101–105.
11. Bitsch M, Christiansen M, Laursen I, Engel AM, Holstein PE, Jørgensen B, et al. Is there a connection between MBL and chronic ulcers. Acta Derm Venereol 2009; 89: 307–308.
12. Fauci AS. The vasculitis syndromes. In: Fauci AS, Braunwald E, Kasper DL, Hauser SL, et al, editors. Harrison's Principles of Internal Medicine, 17th edition. New York: McGraw-Hill Co.; 2007, ch. 319.

13. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG, et al. Inter-Society consensus for the management of peripheral arterial disease (TASC II). Eur J Vasc Endovasc Surg 2007; 33 Suppl 1: 1–75.
14. Nicolaides AN, Vasdekis SN, Christopoulos D. In: Bernstrin EF, editor. Vascular diagnosis, detection and quantification of venous reflux with duplex scanning. 4th edn. St Louis: Mosby; 1993.
15. Mayberry JC, Moeta GL, Taylor LM, Porter JM. Fifteen-year results of ambulatory compression therapy for chronic venous ulcers. Surgery 1991; 109: 575–581.
16. Ghauri ASK, Nyamekye I, Grabs AJ, Whyman MR, Poskitt KR. Influence of a specialised leg ulcer service and venous surgery on the outcome of venous leg ulcers. Eur J Endovasc Surg 1998; 16: 238–244.
17. Hansson C, Hoborn J, Möller A, Swanbeck G. The microbial flora in venous leg ulcers without clinical signs of infection. Repeated culture using a validated standardised microbiological technique. Acta Derm Venereol 1995; 75: 24–30.
18. Apelqvist J, Bakker K, van Houtum WH, Nabuurs-Franssen MH, Schaper NC. International consensus and practical guidelines on the management and the prevention of the diabetic foot. International Working Group on the Diabetic Foot. Diabetes Metab Res Rev 2000; 16 Suppl 1: 84–92.
19. Bergmann J, Christiansen M, Laursen I, Bang P, Hansen NE, Ellegaard J, et al. Low levels of mannose-binding lectin do not affect occurrence of severe infections or duration of fever in acute myeloid leukaemia during remission induction therapy. Eur J Haematol 2003; 70: 91–97.
20. Armitage P. Trend in proportions. Statistical methods in medical research. Oxford: Blackwell Scientific Publications; 1971, ch. 12.2.
21. Wagner FW. The dysvascular foot: a system for diagnosis and treatment. Foot Ankle 1981; 2: 64.
22. Jack DL, Turner MW. Anti-microbial activities of mannose-binding lectin. Biochem Soc Trans 2003; 31: 753–757.
23. Møller-Kristensen M, Ip WKE, Shi L, Gowda LD, Hamblin MR, Thiel S, et al. Deficiency of mannose-binding lectin greatly increases susceptibility to postburn infection with pseudomonas aeruginosa. J Immunol 2006; 176: 1769–1775.
24. Siassi M, Riese J, Steffensen R, Meisner M, Thiel S, Hohenberger W, et al. Mannan-binding lectin and procalcitonin measurement for prediction of postoperative infection. Crit Care 2005; 9: 483–489.
25. Krarup A, Gulla KC, Hajela K, Sim RB. The action of MBL-associated serine protease 1 (MASP1) on factor XIII and fibrinogen. Biochim Biophys Acta 2008; 1784: 1294–1300.
26. Hansen, TK, Thiel S, Knudsen ST, Gravholt CH, Christiansen JS, Mogensen CE, et al. Elevated levels of MBL in patients with type 1 diabetes. J Clin Endocrinol Metab 2003; 88: 4857–4861.
27. Saraheimo M, Forsblom C, Hansen TK, Teppo A-M, Fagerudd J, Pettersson-Fernholm K, et al. Increased levels of mannan-binding lectin in type 1 diabetic patients with incipient and overt nephropathy. Diabetologia 2005; 48: 198–202.
28. Hansen TK, Galle MA, Tarnow L, Thiel S, Stehouwer CD, Schalkwijk C, et al. Mannose-binding lectin and mortality in type 2 diabetes. Arch Intern Med 2006; 166: 2007–2013.