Cutaneous Leukocytoclastic Vasculitis: The Yield of Direct Immunofluorescence Study

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Background : *Leukocytoclastic vasculitis (LCV) is a clinico-pathological entity. Previous direct immunofluorescence study (DIF) studies of vasculitis showed positive findings mainly in the early stage of the disease.*
Objective : *To study the positive yield and patterns of DIF in patients with various stages of LCV.*
Design : *One hundred patients with LCV who attended the Department of Dermatology, Siriraj Hospital from 1997 to 2000 were enrolled in the study.*
Results : *The study showed immunoreactive deposits in blood vessel walls in 76 cases(76%). Forty seven per cent of patients showed immunoreactant deposit only in superficial blood vessel walls, 3% had deposits only in deep blood vessel walls. Superficial and deep blood vessel wall deposits were seen in 26%. Dermo-epidermal deposit in addition to blood vessel wall deposit was found in 39%. The most common immunoreactive deposit was C3 (71%), followed by IgM (35%), IgA (12%) and IgG (8%) respectively. The age of the skin lesions at the time of biopsy ranged from 1 to 7 days. 82% of patients with one day old lesions showed immunoreactive deposits in the blood vessel walls and 74% of the group with lesions aged 2-7 days at the time of biopsy showed immunoreactive deposits in the blood vessel walls.*
Conclusion : *The present study showed a 76% positive yield for DIF study in patients with LCV when biopsies were performed within one week of onset. There was a tendency for the percentage of positive DIF results to decline when the biopsy was performed on lesions that were more than 1 day old.*

Keywords : *Vasculitis, Immunofluorescence*

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Leukocytoclastic vasculitis (LCV) is usually easily diagnosed by clinical and histologic features. Pathological diagnosis includes fibrinoid necrosis of the small dermal vessels (venules), leukocytoclasis, endothelial swelling, and extravasation of red blood cells. However, the histologic diagnosis may sometimes be difficult in early or at resolving stages of vasculitis because of the dynamic changes involved in the process. Previous direct immunofluorescence (DIF) studies of vasculitis showed positive findings mainly in the early stage of disease[1-4]. In clinical practice, whenever the diagnosis of vasculitis is suspected, a biopsy of the lesion is suggested to be performed as early as possible. But in some situations, this is not possible.

The purpose of the present study was to study the positive yield of direct immunofluorescence in patients at various stages of LCV. The authors also studied the patterns and types of immunoreactant deposits in these patients.

Patients and Method

One hundred patients, who attended the Dermatology Clinic, Siriraj Hospital, Mahidol University from January 1997 to December 2000, with the clinical diagnosis of LCV confirmed by histopathologic studies and appropiate investigations were

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included in the study. All patients gave informed consent before the biopsies were done. The data were analyzed according to possible etiology of the disease, age of lesion on the day the skin biopsy was performed (informed by the patients), and DIF findings. Direct immunofluorescence studies of skin lesions were performed as previously described[5]. Briefly, skin biopsy specimens were embedded in Cryomatrix embedding medium (SHANDON, USA) and snap-frozen at -70 °C until sectioned. The cryostat tissue sections of 4 micron each were air-dried and then washed twice with phosphate-buffered saline (PBS), pH7.4, for 10 minutes before being overlaid for 30 minutes with fluorescein isothiocyanate conjugated rabbit anti-human IgG, IgA, IgM, and C3 (DAKO patt, Denmark). Thereafter, section slides were incubated in a humidified chamber at room temperature, then washed twice with PBS, pH7.4, for 10 minutes and mounted with medium before viewing with a fluorescent microscope. Direct immunofluorescent patterns were interpreted according to standard criteria[6]. A specimen was considered to be positive if granular deposits of one or more immunoreactants were found in the walls of one or more vessels. Statistical analyses were performed using SPSS for Windows version 10.0.

Results

The study consisted of one hundred patients, 67 women and 33 men. The ages of the patients ranged from 11-80 years with the mean age of 32.6 years. Biopsy specimens were taken from the legs (82 cases), arms and hands (15 cases) and other parts of the body (3 cases) for hispathologic and immunopathologic examinations.

Types of skin lesion included 85 cases of palpable purpura (85%), 11 cases of a purpuric patch (11%), 2 cases of petechiae (2%), 2 cases of vesicles and one case of a hemorrhagic bleb (1%).

The etiologies of LCV included idiopathic (56%), infection (16%), drugs (14%), Henoch-Scholein purpura (HSP) (9%), systemic lupus erythematosus (SLE) (3%), rheumatoid arthritis(RA) (1%) and Behcet's disease (1%).

The DIF study showed immunoreactive deposits in blood vessel walls in 76 cases (76%). Table 1 shows the sites of the immunoreactive deposits. Forty seven per cent of the patients showed immunoreactive deposits only in superficial blood vessel walls. Three per cent had deposits only in deep blood vessel walls. A combination of superficial and deep blood vessel wall deposits was seen in 26%. Dermo-epidermal deposits in addition to blood vessel wall deposits were seen in 39%. The most common immunoreactive deposit was C3 (71%), followed by IgM (35%), IgA(12%) and IgG (8%).

Table 2 shows details of the immunoreactive deposits in blood vessel walls. The most common patterns were C3 deposit alone and the combination of IgM and C3. IgG was usually detected in combination with other immunoreactants. Cases with HSP showed IgA deposit alone in 2%, IgA in combination with IgM and C3 in 6%, and IgA in combination with C3 in 1%.

Table 3 shows DIF findings according to the etiology of vasculitis. Deposits of mainly C3 and IgM were seen in the group with idiopathic, infection and drug etiologies.

Table 1. Sites of immunoreactant deposits

Immuno-reactants	No. of patients case (%)	SBV only %	DBV only %	SBV & DBV %	DEJ & BV %
IgG	8 (8)	4	1	3	2
IgM	35 (35)	25	2	8	12
IgA	12 (12)	9	1	2	0
C3	71 (71)	44	3	24	28
Total positive cases	76 (76)	47	3	26	39

Note: One patient could have more than one immunoreactant deposits
SBV =superficial blood vessel
DBV =deep blood vessel
DEJ =dermo-epidermal junction

Table 2. Details of immunoreactant deposit in blood vessel walls

Immunoreactants	Cases (%)
IgG	
IgG+IgA+IgM+C3	3(3%)
IgG+IgM+C3	2(2%)
IgG+C3	2(2%)
IgG+IgM	1(1%)
IgM	
IgM+C3	21(21%)
IgM+IgA+C3	6(6%)
IgM	2(2%)
IgA	
IgA only	2(2%)
IgA+C3	1(1%)
C3 only	36(36%)

Table 3. Direct immunofluorescence findings and etiology of patients with vasculitis

Etiology	No. of patients	IgG	IgM	IgA	C3
Idiopathic	56	5	18	2	43
Infection (URI=10; Others=6)	16	0	3	0	10
Drug	14	2	5	0	8
HSP	9	0	6	9	7
SLE	3	1	1	1	2
RA	1	0	1	0	1
Behcet's disease	1	0	1	0	0
Total cases	100	8	35	12	71

Note: URI =upper respiratory tract infection,
HSP = Henoch Scholein purpura,
SLE =systemic Lupus erythematosus,
RA =Rheumatoid arthritis

Table 4 compares DIF findings in the present study with previous studies performed by Grunwald and Mackel. The positive DIF yield in the present study was 76% compared with 92% in Grunwald's study and 92% in Mackel's. The most common immunoreactive deposit in all studies was C3. The next most common one was IgM in the present study and that of Mackel, while that of Grunwald was IgG.

Table 5 shows the age of skin lesions informed by the patients by the time the skin biopsies were performed. The age of skin lesions when biopsies were performed varied from 1 to 7 days. Twelve per cent of the patients could not tell for certain the age of the lesions. The group with one day old lesions showed 82% positive immunoreactant in blood vessel walls and the group with 2-7 days old had a 74% positive yield. There was a tendency for the percentage of positive DIF yields to decline when the biopsy was performed on lesions more than 1 day old. However, there was no statistically significant difference ($p > 0.05$) between the groups.

Table 6 shows the types of immunoglobulin deposited in blood vessel walls of skin lesions of various ages. IgG was found more often in the earlier specimens but IgM and C3 were found consistently irrespective of the age of the specimen.

Discussion

The frequent postulated mechanism of vasculitis is local deposition of circulating immune complexes in the blood vessel walls which then activate the complement cascade. Other participants[7] include T lymphocytes, mononuclear cells, mast cells, eosinophils and neutrophils. Endothelial cells show increased expression of intercellular cell adhesion molecule-1(ICAM-1), E-selectin and vascular cell adhesion molecule-1 (VCAM-1) in response to TNF-α. E-selectin is an adhesion molecule for neutrophils and skin-homing memory T-cells. VCAM-1 acts as an adhesion molecule for lymphocytes, monocytes and eosinophils.

Table 4. Comparison of direct immunofluorescence studies with previous studies

Blood vessel wall staining	Present study (%)	Grunwald et al (%)	Mackel et al (%)
IgG	8	28	8
IgM	35	25	72
IgA	12	17	40
C3	71	40	81
Positive DIF	76	92	92

Table 5. Age of lesions with positive DIF staining in blood vessel walls

Age of lesions (Days)	No. of cases	Positive blood vessel deposit cases (%)
1	11	9 (82)
2	24	15 (63)
3	19	14 (74)
4	10	8 (80)
5	11	8 (73)
6	-	-
7	13	12 (92)
Total	88	66 (75)

Table 6. Types of immunoreactant deposited in blood vessel walls of skin lesions of various ages

Age of lesions (Day)	No.of patients	IgG cases(%)	IgA cases(%)	IgM cases(%)	C3 cases(%)
1	9	1(11)	0(0)	3(33)	9(100)
2	15	1(7)	3(27)	5(33)	14(93)
3	14	2(14)	2(14)	7(50)	13(93)
4	8	1(13)	2(25)	4(50)	6(75)
5	8	0(0)	1(13)	4(50)	8(100)
6	-	-	-	-	-
7	12	0(0)	1(8)	7(8)	11(92)

Previous DIF studies of vasculitis showed positive findings mainly in the early stage of the disease[1-4]. Cochrane et al[8] studied the Arthus reaction in rabbits. The intradermally injected antigen localized to vessel walls within the first 7 hours, during which time the vascular inflammatory reaction increased to nearly maximal intensity. The antigen was then noted to disappear gradually, being detectable for a period of time up to 48 hours in most instances.

Cream et al[9] studied the timing and changes of appearance in immune complex deposits in the Arthus reaction in a guinea-pig sensitized to ovalbumin. Immunofluorescence showed deposits of complement and ??-globulin from 20 minutes onwards. At 18 hours, no deposits could be found.

Gower et al[3] evaluated the kinetics of immunologic and cellular changes in histamine-induced vasculitic lesions in 4 patients with active vasculitis. There was an increase in the immunoreactants which occurred 1 to 4 hours after histamine injection. In contrast to cellular changes, immunoreactants decreased at 8 and 24 hours. However, biopsies were not performed later than 24 hours in the present study.

Grunwald et al[10] studied the correlation between different histologic stages of LCV and DIF results. Early histologic stage was characterized by focal destruction of a few capillary blood vessels, very mild granulocytic infiltrate in the upper dermis and small foci of nuclear dust. The fully developed stage included more prominent damage to blood vessel walls, a dense inflammatory infiltrate and nuclear dust. The late stage was characterized by plump regenerating endothelial cells, and remnants of fibrin deposited in the blood vessel walls. The perivascular infiltrates were mild and composed predominantly of mononuclear cells. Thirty-seven out of 40 patients (92%) showed positive DIF in the blood vessel walls. Eight patients studied were in the early stages of vasculitis defined by histologic criteria, 17 were at the fully developed vasculitis, and 15 were at the late stages. They conclude that DIF examination can be used in all stages of vasculitis and not only in the early stages. However, they did not state the exact age of the lesions biopsied.

Zax et al[11] reported sequential biopsy results at 0, 24, 48 and 120 hours from a patient with a cutaneous LCV manifest as palpable purpura. At a time of 0 (a clinical age of less than 24 hours) there was severe invasion of the vessel walls by neutrophils as well as severe leukocytoclasis and hemorrhage. At 120 hours there was absence of neutrophils, leukocytoclasis, with only mild extravasation of erythrocytes. In contrast, the mononuclear infiltrate progressed from mild to moderate during this same period.

76% were positive for blood vessel immunoreactant staining in the present study. The age of the lesions when biopsies were performed varied from 1 to 7 days. The pitfall of the present study was that the authors derived the information concerning the age of the biopsy lesions mostly from patients in the out patient clinic. Some patients who had many active lesions might not remember the exact age of all the lesions. However, this is a real situation that occurs in everyday dermatological practice. Twelve per cent of the presented patients did not know the age of the lesions. When the authors compared the group that had early lesions (1 day old) and the group that had lesions that were older than 1 day, the percentage of immunoreactant deposit in blood vessel walls were 82% and 74% respectively. There was a tendency for the percentage of positive DIF yield to decline when the biopsy was performed on an older lesion. However, the positive yield was not too low. the present study supports that of Grunwald et al. Even though the present results had a relatively lower positive yield compared with previous studies of Grunwald and Mackel, this might be due to the relatively late stage at which the skin biopsies were performed.

In summary, the results of the present study suggest that in a case of LCV, if the lesion was biopied within one week, there will still be a chance to get a positive yield of direct immunofluorescence study.

References

1. Braverman IM, Yen AG. Demonstration of immune complexes in spontaneous and histamine-induced lesions and normal skin of patients with leukocytoclastic angiitis. J Invest Dermatol 1975; 64: 105-12.
2. Sams WM, Claman HN, Kohler PF, et al. Human necrotizing vasculitis: immunoglobulins and complement in vessel walls of cutaneous lesions and normal skin. J Invest Dermatol 1975; 65: 441-5.
3. Gower RG, Sams M, Thorne GE, et al. Leukocytoclastic vasculitis: sequential appearance of immunoreactants and cellular changes in serial biopsies. J Invest Dermatol 1977; 69: 477-84.
4. Mackel SE, Jordan RE. Leukocytoclastic vasculitis. Arch Dermatol 1982; 118: 296-301.
5. Beutner EH, Kumar V, Krasny DA, et al. Defined immunofluorescence immunodermatology. In: Beutner EH, Chorzelski TP, Kumar V, eds. Immuno-

pathology of the skin. 3rd ed. New York: John Wiley & Son, 1987: 3-40.
6. Valenzuela R, Bergfeld WF, Deodhar SD. Interpretation of immunofluorescent patterns in skin diseases. Chicago: American Society of Clinical Pathologists Press, 1984.
7. Soter NA. Cutaneous necrotizing venulitis. In: Fitzpatrick TB, Eisen AZ, Wolff K, et al. Dermatology in general medicine, 5 thed . New York: McGraw-Hill 1996;2044-6.
8. Cochrane CG, Weigle WO. The cutaneous reaction to soluble antigen–antibody complexes. A comparison with Arthus phenomenon. J Exp. Med 1958; 108: 591-604.
9. Cream JJ, Bryceson ADM, Ryder G. Disappearance of immunoglobulin and complement from the Arthus reaction and its relevance to studies of vasculitis in man. Br J Derm 1971; 84:106-9.
10. Grunwald MH, Avinoach I, Amichai B, et al. Leukocytoclastic vasculitis- correlation between different histologic stages and direct immunofluorescence results. Int J Dermatol 1997; 36:349-52.
11. Zax RH, Hodge HJ, Callen JP. Cutaneous leukocytoclastic vasculitis. Arch Dermatol 1990:126:69-72.

หลอดเลือดผิวหนังอักเสบ: ผลบวกของตจอิมมูนพยาธิวิทยา

กนกวลัย กุลทนันทน์, สำรวย ปิ่นแก้ว, สุขุม เจียมตน, พรรณแข มไหสวริยะ, ป่วน สุทธิพินิจธรรม

Leukocytoclastic Vasculitis (LCV) คือ การอักเสบของหลอดเลือดขนาดเล็กที่ผิวหนัง การศึกษาตจอิมมูนพยาธิวิทยา (DIF) มักให้ผลบวก เมื่อตัดชิ้นเนื้อจากรอยโรคในระยะเริ่มแรก

***วัตถุประสงค์** : เพื่อศึกษาการให้ผลบวกและรูปแบบของ DIF ในรอยโรค LCV ระยะต่างๆ*

***วิธีการศึกษา** : ผู้ป่วยที่ได้รับการวินิจฉัยว่าเป็น LCV 100 ราย ของภาควิชาตจวิทยา โรงพยาบาลศิริราช ระหว่างปี พ.ศ.2540-2543*

***ผลการศึกษา** : พบการติดของสารอิมมูนเรืองแสงที่ผนังหลอดเลือดในผู้ป่วย 76 ราย (76%) โดย 47% ติดเฉพาะหลอดเลือดที่อยู่ตื้นๆ ในชั้นหนังแท้ อีก 3% ติดเฉพาะหลอดเลือดที่อยู่ลึก อีก 26% ติดทั้งชั้นตื้น และลึก พบการติดของสารอิมมูนเรืองแสงที่รอยต่อของชั้นหนังกำพร้า และหนังแท้ร่วมด้วย 39% พบการติดของ C3 บ่อยที่สุด (71%) รองลงมาคือ IgM (35%) IgA (12%) และ IgG (8%) อายุของรอยโรคที่ทำการตัดชิ้นเนื้ออยู่ระหว่าง 1-7 วัน กลุ่มที่รอยโรคที่ตัดมีอายุ 1 วัน มีการติดสารอิมมูนเรืองแสงที่หลอดเลือด 82% ส่วนกลุ่มที่รอยโรคอายุ 2-7 วัน มีการติด 74%*

***สรุป** : การศึกษาพบว่า DIF ให้ผลบวกร้อยละ 76 ในรอยโรค LCV ที่ทำการตัดชิ้นเนื้อจากรอยโรคที่อายุ 1-7 วัน ผลบวกจะลดลงในรอยโรคที่อายุเกิน 1 วัน*