ACTA STEREOL 1983; 2: 368-372
STEREOLOGY AND MORPHOMETRY IN PATHOLOGY
Proc 2nd Symp Morphometry in Morphol Diagnosis
Collan Y. et al., eds.
Kuopio University Press, Kuopio 1983

Stereologic analysis of muscle capillaries in Inclusion Body Myositis

Marcelino G. Reyes, Steven M. Malkin and Moris J. Danon*

Department of Pathology, Mount Sinai Hospital, and
*Department of Neurology, University of Illinois Hospital, Chicago, Illinois USA 60608

Abstract

Muscle capillaries of plastic-embedded sections of biopsies of seven patients with Inclusion Body Myositis (IBM) and of seven control patients were quantitatively estimated by point and line intersection counting technics of stereology. Volume, surface and numerical densities of capillaries and numerical ratio of capillaries to muscle fibers of IBM did not differ from controls. In contrast, estimates from selected atrophic areas in the biopsies of six patients with IBM were significantly higher than estimates randomly obtained from the same biopsies. Our findings suggest that muscle capillary hypervascularity in IBM results from atrophy of muscle fibers.

Introduction

Inclusion Body Myositis (IBM) is a chronic inflammatory myopathy that does not respond to steroid treatment (Danon *et al*, 1982). Pathologically, muscle biopsies show variable inflammation, vacuolar degeneration of muscle fibers, groups of atrophic muscle fibers resembling denervation atrophy, an increase in muscle capillaries, mitochondrial abnormalities and tubulofilamentous inclusions in the cytoplasm or sarcolemmal nuclei of muscle fibers. The presence of these tubulofilamentous inclusions (which are seen with the electron microscope) is a *sine qua non* in the diagnosis of IBM (Carpenter *et al*, 1978; Danon *et al*, 1982).

The significance of the various histopathologic and ultrastructural findings in IBM particularly the increase of muscle capillaries (Carpenter *et al*, 1978) is not clear. Because our subjective histologic estimates of muscle capillaries in IBM was inconclusive (Danon *et al*, 1982), we decided to extend our studies with technics of stereology to quantitatively estimate the capillary network in this disorder. Our findings show a patchy increase in muscle capillaries and suggest that muscle capillary hypervascularity is a secondary phenomenon in the pathogenesis of IBM.

Materials and methods

Seven patients with IBM and seven control patients with suspected but unproved neuromuscular disorders comprised the study. The clinical and muscle biopsy data are listed in Table 1.

Table 1

Clinical and muscle biopsy data

#	Age/sex (yrs)	Muscle	Diagnosis / reason for biopsy
Inclusion Body Myositis (n = 7)			
1	38/m	Biceps	Inclusion Body Myositis
2	68/m	Biceps	Inclusion Body Myositis
3	72/m	Vastus lateralis	Inclusion Body Myositis
4	47/f	Brachioradialis	Inclusion Body Myositis
5	63/m	Biceps	Inclusion Body Myositis
6	33/f	Deltoid	Inclusion Body Myositis
7	74/f	Calf	Inclusion Body Myositis
Controls (n = 7)			
8	44/f	Deltoid	Muscle aches
9	40/m	Biceps	Carrier, acid maltase deficiency
10	66/m	Deltoid	R/O collagen disease
11	27/f	Thigh	Muscle cramps
12	22/f	Quadriceps	R/O lupus vasculitis
13	62/f	Quadriceps	Unexplained creatine kinase elevation
14	53/f	Deltoid	Chronic fatigue

Details of the clinical, electrodiagnostic and histopathologic findings of the patients with IBM have been reported (Danon *et al*, 1982). Patients used as controls had normal neurologic examination. Their muscle biopsies showed no morphologic, enzyme histochemical or ultrastructural abnormalities. Furthermore, estimates of atrophy and hypertrophy factors of transverse diameters of type 1 and type 2 fibers were normal (Brooke and Engel,1969). The muscle fiber diameters were measured by MGR on fresh frozen sections stained by myosin adenosine triphosphatase at pH 9.4 using technics previously described (Reyes and Chokroverty, 1980).

Counts for stereologic analysis were done by SMM on 1 μm thick sections of muscle which were obtained at biopsy, fixed in glutaraldehyde, post-fixed in osmium, dehydrated and embedded in Epon. Transverse sections were cut in series or in steps from one to four blocks of muscle, mounted on glass slides and stained with toluidine blue. The sections were then numbered on the slide, viewed in the microscope with the 40X objective and sampled with a grid in the eyepiece by moving the stage of the microscope. Figure shows a tracing of muscle capillaries, muscle fibers and interstitial tissue with the test grid superimposed.

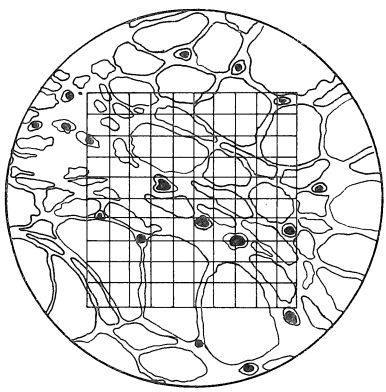

Figure: Tracing of a field sampled by grid in the eyepiece of the microscope at 40X objective. Capillaries are oval or circular outlines between muscle fibers. Many contain dark profiles of red blood cells. Note atrophic muscle fibers in the middle of the grid. Side of squares in the grid is 25 μm in this diagram.

The upper right corner of the squares in the grid was used to count the points hitting muscle capillary wall and lumina, muscle fibers and interstitial tissue. The horizontal lines formed by the top sides of the squares in the grid were used to count the number of intersections of the line and inner boundary of the profiles of the capillaries. The number of capillaries and of muscle fibers within the grid and the number of those touching the frame of the grid were then counted separately. Twenty-five fields were sampled from as many sections as possible by randomly moving the stage of the microscope. Because there appeared to be more capillaries among the atrophic muscle fibers, counts were also done on at least seven selected fields of groups of atrophic muscle fibers in six of the seven patients with IBM.

Volume density of capillaries was estimated using a derivation of Delesse's equation $V/V = P/P$ where P/P is the point fraction or the total points hitting the capillaries divided by the total points hitting the section. Surface density of capillaries was estimated as $S/V = 2I/L$ were I is the number of intersections of capillaries and the horizontal lines of the grid and L is the total line length. Numerical density of capillaries was estimated as N/A = adjusted number/transverse area of muscle fibers. The latter was equal to the total points hitting muscle fibers times the square of the length of the side of the grid squares or 25 μm^2. Adjusted number of capillaries was estimated from the equation $N/A = (N^1 + N^2/2 - \hat{p})/A$ (Weibel and Bolender, 1973) where N^1 is the number of capillaries within the frame of the grid and N^2 is the number of capillaries touching the sides of the frame of the grid, $\hat{p}$ is the point fraction P/P of capillaries and A is the total points of the section multiplied by 25 μm^2. The adjusted number of muscle fibers was also estimated with the above equation. Numerical ratio of capillaries to muscle fibers was estimated from their adjusted numbers. In addition, transverse area of a muscle fiber was estimated by dividing the total transverse area of muscle fibers by the adjusted number of muscle fibers. Volume density of muscle fibers and interstitial tissue was also estimated from their point fractions.

Because we adjusted the grid size so that the capillaries were smaller than the squares in the grid, we considered the points hitting the capillaries to be independent of each other and to be binomially distributed. In this distribution, the standard error of volume density is SE = $\sqrt{[\hat{p} \times (1-\hat{p})/n]}$ where $\hat{p}$ is the point fraction P/P and n is the total points. Similarly, we considered the distribution of the intersections of capillaries with the horizontal lines of the grid to be Poisson. In this distribution SE of surface density was estimated as SE = $2\sqrt{I}/L$. For the other estimates, the mean and standard error of the mean of the fields sampled were estimated with standard statistical tests. Results were analyzed with standard statistical tests programmed on an Apple II Plus computer.

Results

Volume, surface and numerical densities of capillaries and numerical ratios of capillaries to muscle fibers are listed in Table 2.

Table 2

Stereologic estimates of capillaries

	Inclusion body myositis		Controls	
	Random areas (n = 7)	Atrophic areas (n = 6)		(n = 7)
Volume density (*mm³/100 mm³ muscle tissue ± se*)				
1	0.32 ± 0.11	0.70 ± 0.35	8	0.81 ± 0.20
2	0.48 ± 0.15	0.17 ± 0.17	9	0.04 ± 0.04
3	0.80 ± 0.18	0.83 ± 0.17	10	0.54 ± 0.14
4	0.12 ± 0.07	0.57 ± 0.28	11	0.49 ± 0.19
5	0.27 ± 0.11	0.47 ± 0.27	12	0.18 ± 0.12
6	0.32 ± 0.11	0.44 ± 0.25	13	0.53 ± 0.19
7	0.58 ± 0.17	—	14	0.38 ± 0.09
Mean ± se	0.41 ± 0.09	0.53 ± 0.09		0.41 ± 0.10
Surface density (*mm²/100 mm³ muscle tissue ± se*)				
1	342 ± 33	674 ± 98	8	628 ± 49
2	572 ± 47	797 ± 103	9	288 ± 30
3	905 ± 54	931 ± 125	10	179 ± 23
4	394 ± 35	332 ± 62	11	434 ± 50
5	230 ± 29	524 ± 81	12	477 ± 57
6	518 ± 41	822 ± 98	13	768 ± 64
7	585 ± 50	—	14	618 ± 62
Mean ± se	507 ± 82 #	678 ± 90 #		487 ± 77
Numerical density (*number/mm² myofiber ± se*)				
1	222 ± 14	553 ± 115	8	386 ± 15
2	298 ± 26	407 ± 34	9	207 ± 12
3	568 ± 19	750 ± 75	10	119 ± 9
4	196 ± 16	262 ± 28	11	237 ± 12
5	191 ± 15	298 ± 21	12	281 ± 24
6	339 ± 17	514 ± 42	13	503 ± 16
7	344 ± 12	—	14	553 ± 59
Mean ± se	310 ± 51 †	464 ± 74 †		327 ± 60
Numerical ratio (*number of capillary/100 muscle fibers ± se*)				
1	27 ± 2	34 ± 3	8	43 ± 2
2	42 ± 3	52 ± 5	9	38 ± 2
3	107 ± 3	118 ± 10	10	50 ± 4
4	47 ± 5	45 ± 4	11	50 ± 4
5	44 ± 4	54 ± 4	12	53 ± 4
6	76 ± 4	96 ± 10	13	87 ± 4
7	81 ± 3	—	14	65 ± 4
Mean ± se	61 ± 11 *	67 ± 13 *		51 ± 8

*paired t-test: #p <0.05 †p <0.01 *p <0.005*

Table 2 shows that the volume, surface and numerical densities of capillaries and the numerical ratio of capillaries to muscle fibers of IBM did not differ from controls. However, in six patients with IBM the surface and numerical densities and numerical ratio of capillaries to muscle fibers were higher in the atrophic areas compared to the randomly sampled areas of the same muscle biopsy. Volume density of capillaries was also higher in the atrophic areas but the difference was not significant. Compared with controls, the groups of atrophic muscle fibers of IBM had higher volume, surface and numerical densities and lower mean transverse area of a muscle fiber, but the difference was not significant.

Table 3 lists the volume density of muscle fibers and interstitial tissue and the mean transverse area of a muscle fiber in the atrophic and randomly sampled areas of muscle biopsies of IBM and in controls.

Table 3

Muscle fibers and interstitial tissue

	Inclusion body myositis		Controls
	Random (n = 7)	Atrophic (n = 6)	(n = 7)
Volume density (*mm³/100 mm³ muscle tissue*)			
Muscle fibers			
Mean ± se	81 ± 1	80 ± 4	85 ± 2
Range, median	76 - 85, 81	66 - 90, 77	80 - 91, 86
Interstitial tissue			
Mean ± se	18 ± 1	19 ± 4	15 ± 1
Range, median	15 - 24, 17	10 - 34, 15	10 - 20, 13
Transverse area / myofiber (*um²*)			
Mean ± se	1999 ± 172*	1515 ± 181*	1699 ± 131
Range, median	1340 - 2370, 2265	730 - 1890, 1590	1130 - 2090, 1830

**paired t-test p <0.005*

Transverse area of a muscle fiber was smaller in the atrophic areas of IBM compared to the randomly sampled areas of the same muscle biopsy. Compared with controls, transverse area of a muscle fiber was less in the atrophic areas and more in the random areas of IBM, but the difference was not significant. Volume density of interstitial tissue was higher in the atrophic and random areas of IBM while volume density of muscle fibers was lower compared to controls, but again the differences were not significant.

Discussion

The cause of IBM is not known. A recent report of isolation of an adenovirus in the muscle of a patient with IBM (Mikol *et al*, 1982) is highly suggestive of viral infection of muscle. Such an infection can explain the vacuolar degeneration of muscle fibers, inflammation and possibly the tubulofilamentous inclusions themselves, although the latter do not resemble any known virus. The groups of atrophic muscle fibers which resemble denervation atrophy, however, are not easily explained by a viral infection of muscle fibers. It is also uncertain whether or not the increase in muscle capillaries directly results from viral infection.

An increase in muscle capillaries is known to accompany atrophy or a diminution in size of muscle fibers in various conditions. Parizkova *et al* (1971) found that the numerical ratio of capillaries to muscle fibers increased while the size of the muscle fibers decreased in sedentary but healthy old men. We have noted an increase in the relative volume and surface area of muscle capillaries in type 2 atrophy of skeletal muscle (Reyes and Yastrow, 1982). Carpenter and Karpati (1982) reported an increase in the numerical density on area of muscle capillaries in denervation atrophy of skeletal muscle which occurred in spite of a concomitant loss of muscle capillaries through necrosis.

Our findings in IBM showed an increase of muscle capillaries only among the atrophic muscle fibers. Carpenter *et al* (1978) who found higher density of muscle capillaries in their patients who had low mean transverse area of a muscle fiber suggested that the increase in muscle capillaries could have resulted from excessive demand of oxygen by muscle fibers, particularly those with mitochondrial abnormalities. We believe, however,

that the increase in muscle capillaries is more likely the result of atrophy of muscle fibers. Whether this atrophy is the result of denervation or of direct viral infection of muscle remains to be proved.

We conclude that the increase of muscle capillaries is a secondary phenomenon in IBM that probably results from atrophy of muscle fibers.

References

Brooke MH, Engel WK. Histographic analysis of muscle biopsies with regard to fiber types. 3. Myotonias, myasthenia gravis and hypokalemic periodic paralysis. *Neurology* 1969;**19**:469-477.

Carpenter S, Karpati G, Heller I, Eisen A. Inclusion Body Myositis: A distinct variety of idiopathic inflammatory myopathy. *Neurology* 1978;**28**:8-17.

Danon MJ, Reyes MG, Perurena OH, Masdeu JC, Manaligod JR. Inclusion Body Myositis: A corticosteroid-resistant idiopathic inflammatory myopathy. *Arch Neurol* 1982;**39**:760-764.

Mikol J, Felten-Papaiconomou A, Ferchal F, Perol Y, Gautier B, Haguenau M, Pepin B. Inclusion-Body Myositis: Clinicopathologic studies and isolation of an adenovirus type 2 from muscle biopsy specimen. *Annal Neurol* 1982;**11**:576-581.

Parizkova J, Eiselt E, Sprynarova S, Wachtlova M. Body composition, aerobic capacity and density of muscle capillaries in young and old men. *J Appl Physiol* 1971;**31**:323-325.

Reyes MG, Chokroverty S. Distribution and size of myofibers in quadriceps muscle of Syrian hamster. *Acta Neuropath* 1980:**51**;241-243.

Reyes MG, Yastrow ES. Stereologic analysis of muscle capillaries in type 2 fiber atrophy. *Neurology* 1982;**32**(2):A201 (Abstract).

Weibel ER, Bolender RP. Stereological techniques for electron microscopic morphometry. In: Hayat MA, ed. *Principles and Techniques of Electron Microscopy*. New York, Van Nostrand-Rheinhold Publish Co., 1973;**3**:237-296.

Presented in part at the annual meeting of the American Association of Neuropathologists in St. Louis, Missouri on June 11, 1983.

We acknowledge Dr. Thomas Tosch for his help in the statistical analysis, and Mr. Joseph Hallissey for typesetting the manuscript.

This study was supported by grants from the Mount Sinai and the Edward Weinstein Foundations.

Reprint requests to MGR, Department of Pathology, Mount Sinai Hospital, Chicago, Illinois USA 60608