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PAPER

MORPHOMETRIC ANALYSIS OF HUMAN MUSCLE BIOPSIES BY USE OF AN ELECTRONIC IMAGE ANALYSER (QUANTIMET 720)

Pierre Tankosic, Catherine Marchal, *Jean Floquet and Claude Burlet

Service Commun d'Analyse d'Images, Laboratoire d'Histologie A - *Laboratoire d'Anatomie Pathologique B, BP 184, 54505 Vandoeuvre-lès-Nancy Cedex, France

ABSTRACT

A morphometric analysis has been carried out on human muscle biopsies. In addition to an areal and number ratio of the type I and type II muscle fibres, some spatial distribution parameters have been investigated by measurement of type I and type II fibre alternations. An index of spatial distribution and an estimate of the mean size of fibre aggregates were calculated and plotted.

INTRODUCTION

In light microscopy, muscles are found to show two main fibre types which can be distinguished by a calcium-dependent ATPase reaction (Dubowitz and Pearse, 1960 ; Engel, 1962). At present, there are many morphometric studies ranging from indirect planimetry, direct measurement of diameters, counting and size distribution of muscle fibres. In the field of pathology numerous investigators have shown some characteristic deviations of these parameters. To our knowledge, there are very few quantitative studies on the grouping phenomenon of fibres of uniform histochemical type as seen in some muscle diseases. In this way, Jennekens et al. (1971) used the concept of the "enclosed fibre" defined as a fibre which is completely surrounded by fibres of its own type. In this study, other distribution parameters have been investigated allowing the characterization of this grouping statement.

MATERIAL AND METHODS

Biopsies : 25 biopsy specimens were studied and were taken to clarify a clinical problem (table 1). They were visually classified by the pathologist into two groups according to the absence (13 control biopsies) or the presence (12 pathologic biopsies) of any change in the spatial distribution of the fibre types. The site of muscle biopsy was peroneus longus (12), tibialis anterior (7) and quadriceps (6). The tissue specimens were frozen in isopentane cooled to -150°C in liquid nitrogen. Cryostat sections were cut in a transverse plane at a thickness of 10 µm and were stained to demonstrate the activity of myofibrillar ATPase at pH 9.4 after an acid preincubation at pH 4.63 according to the technique of Brooke and Kaiser (1969). With this technique, we observed strongly reactive type I fibres(F_I). The non reactive type II_A and weakly reactive type II_B fibres were considered as type II fibres (F_{II}).

Morphometry : The morphometric system consists essentially of an electronic image analyser Quantimet 720 (Cambridge Instrument), interfaced with a 9825 T Hewlet-Packard calculator. A good contrast being obtained with the histochemical staining, a grey level was set, allowing the detection of two complementary phases between the white and the black, fully detected F_I and non-detected F_{II}.

A first soft program was developed for computing the numerical density of F_I and F_{II} fibers and the F_I/F_{II} ratio. Four 207,900 µm2 unbiased counting frames (Gundersen, 1977) were measured and contained about 250 to 300 fibre-cross-sections.

A second computer program tested the spatial organization of the muscle fibres. The analytic step consisted in scanning a reference area by a standard frame of measure (S.F.M) of 50x10 image points area (62.5 µm x 12.5 µm). The counting rule was that if the detected area was higher than half of the S.F.M. area (detection > 250 image points), the center of the S.F.M. was located in a F_I. If not, the center of the S.F.M. was in a F_{II} (Fig. 1).

Four reference areas were selected and in each counting frame (751,700 µm2), 4x15 consecutive S.F.M. were measured. We obtained a succession of 240 FI-and F_{II}-events and number "S" of sequences of consecutive similar events, as in the following example :

$$S \longrightarrow \underbrace{FI}_{1}, \underbrace{F_I}_{2}, \underbrace{F_{II}}_{3}, \underbrace{F_I, F_I, F_I, F_I}_{4}, \underbrace{FII, FII}_{5}, \underbrace{F_I}_{\ldots\ldots}$$

TABLE 1 . CLINICAL AND HISTOPATHOLOGIC DIAGNOSIS ON PATIENTS

BIOPSY	Nº	AGE YEARS	SEX	CLINICAL DIAGNOSIS	HISTOPATHOLOGIC DIAGNOSIS	PRESENCE OF AGREGATES
P.L.	5	14	M	system disease ?	no significant pathologic changes	no
	10	51	M	periarteritis nodosa ?	no significant pathologic changes	no
	13	59	M	periarteritis nodosa ?	no significant pathologic changes	no
	18	38	M	neurofibromatosis	no significant pathologic changes	no
	20	58	F	dystrophia myotonica	irregularity in fibre areas	no
	27	34	M	Leber's disease	no significant pathologic changes	no
	28	29	F	weakness of leg muscles	no significant pathologic changes	no
T.A.	4	14	F	cramps	no significant pathologic changes	no
	15	51	M	cardiomyopathy	abnormal ATPase reaction within a few fibres	no
	26	26	M	no indication	slight atrophy of type I fibres ?	no
Q.F.	24	47	M	combined sclerosis	discrete neurogenic atrophy ?	no
	29	5	F	hypotonia	no significant pathologic changes	no
	30	7	M	girdle weakness	no significant pathologic changes	no
P.L.	2	40	M	no indication	tendency of fibre clustering	yes
	14	9	M	hemiparesis	predominance of type I fibres	yes
	16	60	F	periarteresis nodosa ?	clusters of type I fibres	yes
	19	27	M	cardiomyopathy ?	pathological changes in electron microscopy	yes
	25	22	F	degenerative radicular neuropathy	abnormal spatial distribution	yes
T.A.	1	70	F	lumbago	beginning neurogenic	yes
	6	40	M	consequent hypotonia	neurogenic muscular atrophy	yes
	7	23	M	no indication	type I atrophy, type II hypertrophy	yes
	17	13	M	cramps	a few type I clusters	yes
Q.F.	8	44	M	myalgia	numerous intermediate type IIB fibres	yes
	21	77	F	myalgia	possibly neurogenic atrophy	yes
	23	65	F	progressive muscular atrophy	neurogenic atrophy	yes

P.L.: Peroneus longus T.A.: Tibialis anterior Q.F.: Quadriceps femoris

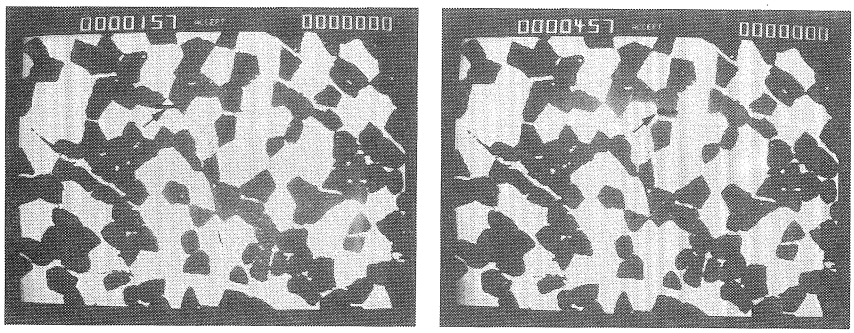

Fig. 1 . Display of the Quantimet. Detection of a F_{II} (a) and a F_I (b) muscle fibre by the S.F.M. (arrows)

Thus we observed N_I F_I-events, N_{II} F_{II}-events ($N_I+N_{II}=240$) and S/2 F_I-and F_{II}-sequences when S is an even number (If S is odd, we have (S+1)/2 and (S-1)/2 sequences).

The computing program gave :

*The areal density of F_I and F_{II}, respectively $N_I/240$ and $N_{II}/240$.

*The total number of sequences S

*The mean size of a FI sequences $X_I=N_I$:(S/2) and of a F_{II} sequence $X_{II}=N_{II}$: (S/2) in S.F.M. units (S.F.M.length=62.5µm).

*The size distribution of the F_I-and F_{II}-sequences and the histogram of the cumulative frequencies (Fig. 2).

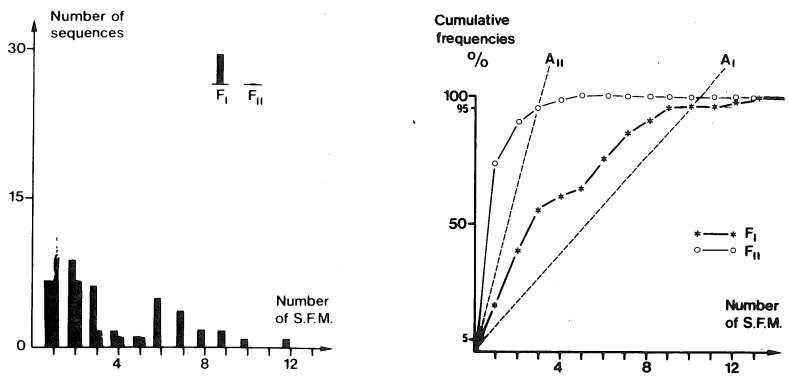

Fig. 2 . Histogram of the relative number and the cumulative frequencies of the S.F.M. sequences

In order to get a numerical value which can characterize this distribution, we have calculated the slopes A_I and A_{II} of the lines where we observed at least 5 % and 95 % of the F_I and the F_{II} sequences. These values were chosen to exclude possible isolated and non significant sequences (Telerman-Toppet and Coers, 1973). It is of evidence that the more heterogeneous is the size of the aggregates the lower the A value will be.

Statistics : The mean were compared by an analysis of variance, and the statistical differences were calculated using "t" test.

RESULTS

All the individual morphometric data, means and coefficients of variation (C.V.) are given in table 2.

Control biopsies : A slightly larger numerical percentage of F_I is observed, the F_I/F_{II} ratio ranging between 1.2 to 3.2. The data for the surface density are more or less similar, the F_I/F_{II} ratio ranging between 0.8 to 2.3. No significant differences are observed between the three studied muscles.

It is of evidence that the number of sequences S gives an information of the spatial distribution of the muscle fibres. A high value will indicate a relatively regular arrangement according to a chequered model. A low value will indicate the presence of fibre aggregates. Whatever the muscle type or the age of the patient may be, the control group shows high values for S, with low coefficients of variation.

When we consider the other parameters, the fibre type showing aggregates will be indicated by X_I and X_{II}, and the size heterogeneity of these aggregates by A_I and A_{II}. Slightly higher X values and slightly lower A values are observed for the F_I because of the higher percentage of this fibre type. No significant differences are observed, neither for the X and A values nor for their respective A/X ratios.

No statistical differences being observed between the three studied muscles for the whole parameters, and the various groups of 13 date being normally distributed, we have reported in table 3 the mean values, the coefficients of variation and the normal ranges (based on mean $\pm$ C.V.) of the calculated parameters for a single control group.

Pathologic biopsies : 10 out of the 12 biopsies show abnormal fibre type proportions, the percentage of type I fibres being either significantly raised (7 biopsies) or significantly lowered (3 biopsies). For the surface density, only one biopsy (case n° 1) shows values within normal limits.

TABLE 2 . LIST OF THE MORPHOMETRIC DATA OF THE ANALYSED BIOPSIES

BIOPSY	NUMERICAL DENSITY			SURFACE DENSITY			PARAMETERS OF SPATIAL DISTRIBUTION						
	ND%			SD%				X		A		A/X	
N°	I	II	I/II	I	II	I/II	S	I	II	I	II	I	II
P.L. 5	66	34	1.9	70	30	2.3	88	3.8	1.7	10	24	2.6	14.4
10	68	32	2.1	56	44	1.3	104	2.6	2.0	16	20	6.1	10.0
13	55	45	1.2	45	55	0.8	103	2.1	2.5	20	16	9.4	6.3
18	64	36	1.8	55	45	1.2	102	2.6	2.1	10	14	5.1	8.9
20	76	24	3.2	60	40	1.5	89	3.2	2.2	14	19	4.3	8.7
27	62	38	1.6	54	46	1.2	94	2.8	2.3	16	19	5.8	8.1
28	74	26	2.8	63	37	1.7	90	3.4	2.0	11	25	3.2	12.8
mean	**66**	**34**	**2.1**	**58**	**42**	**1.4**	**96**	**2.9**	**2.1**	**14**	**20**	**5.2**	**9.9**
C.V.	12%	21%	33%	14%	19%	33%	7%	20%	12%	27%	20%	43%	28%
T.A. 4	60	40	1.5	62	38	1.6	103	2.9	1.8	11	25	3.8	14.0
15	56	44	1.3	44	56	0.8	97	2.2	2.8	20	11	9.3	4.0
26	56	44	1.3	54	46	1.2	101	2.6	2.2	14	16	5.4	7.3
mean	**57**	**43**	**1.4**	**53**	**47**	**1.2**	**100**	**2.6**	**2.3**	**15**	**17**	**6.2**	**8.4**
C.V.	4%	5%	8%	17%	19%	33%	3%	14%	22%	31%	41%	46%	60%
Q.F. 24	74	26	2.8	56	44	1.3	108	2.5	2.0	16	20	6.4	10.2
29	52	48	1.1	58	42	1.4	98	2.8	2.0	12	24	4.3	12.0
30	51	49	1.0	53	47	1.1	100	2.7	2.2	12	19	4.4	8.6
mean	**59**	**41**	**1.6**	**56**	**44**	**1.3**	**102**	**2.7**	**2.1**	**13**	**21**	**5.0**	**10.3**
C.V.	22%	32%	62%	5%	6%	9%	5%	6%	6%	17%	13%	23%	17%
P.L. 2	84	16	5.3	74	26	2.8	62	5.7	2.0	7	24	1.2	11.8
14	81	19	4.3	75	25	3.0	73	5.0	1.6	7	32	1.4	19.8
16	90	10	9.0	84	16	5.3	42	9.6	1.8	6	33	0.6	18.3
19	75	25	3.0	63	37	1.7	70	4.3	2.5	10	14	2.3	5.6
25	36	64	0.6	45	55	0.8	60	3.6	4.4	6	9	1.7	2.0
mean	**73**	**27**	**4.4**	**68**	**32**	**2.7**	**61**	**5.6**	**2.5**	**7**	**22**	**1**	**11.5**
C.V.	29%	80%	70%	22%	47%	62%	30%	42%	46%	23%	48%	44%	67%
T.A. 1	65	35	1.9	56	44	1.3	78	3.5	2.7	10	17	2.9	6.3
6	87	13	6.7	76	24	3.2	80	4.6	1.5	9	32	2.0	21.9
7	41	59	0.7	27	73	0.4	81	1.6	4.3	33	9	20.6	2.1
17	80	20	4.0	71	29	2.4	89	3.8	1.6	10	33	2.6	20.8
mean	**68**	**32**	**3.3**	**58**	**42**	**1.8**	**82**	**3.4**	**2.5**	**16**	**23**	**7.0**	**12.8**
C.V.	30%	64%	79%	38%	52%	67%	6%	38%	52%	75%	52%	129%	79%
Q.F. 8	31	69	0.5	22	78	0.3	68	1.5	5.5	25	6	16.6	1.1
21	77	23	3.3	75	25	3.0	56	6.4	2.1	7	24	1.1	11.2
23	55	45	1.2	64	36	1.8	116	2.6	1.5	16	33	6.0	22.3
mean	**54**	**46**	**1.7**	**54**	**46**	**1.7**	**80**	**3.5**	**3.0**	**16**	**21**	**7.9**	**11.5**
C.V.	42%	50%	87%	53%	60%	80%	40%	73%	71%	56%	65%	100%	92%

P.L.: PERONEUS LONGUS T.A. TIBIALIS ANTERIOR Q.F.: QUADRICEPS FEMORIS

TABLE 3 . MEAN, COEFFICIENT OF VARIATION (C.V.) AND NORMAL RANGE OF THE ± C.V. CONTROL GROUP PARAMETERS.

	ND%			SD%			S	X		A		A/X	
	I	II	I/II	I	II	I/II		I	II	I	II	I	II
mean	63	37	1.8	56	44	1.3	98	2.8	2.1	14	19	5.4	9.6
C.V.	14%	23%	39%	12%	15%	29%	6%	17%	13%	25%	23%	39%	32%
lower limit	54	28	1.1	49	37	0.9	92	2.3	1.8	10	15	3.3	6.6
upper limit	72	46	2.5	63	51	1.7	104	3.3	2.4	18	23	7.5	12.6

Except for the case n° 23, the pathologic group is characterized by a significant decrease ($p < 0.001$ with the Student "t" test) of the S value. The presence of aggregates of one fibre type involves a high corresponding X value and a small value for A, indicating a variability in the size of these aggregates. The other fibre type generally shows a slightly lower X value than the control group and a higher A value indicating that this fibre type becomes more and more isolated. Because of these variations, we have thought that the A/X ratio would give a better estimate of the spatial distribution of the muscle fibres. The diagram (Fig. 3) of the A/X ratio (type I versus type II) shows relatively grouped control values whereas those of the diseased group are scattered.

Except for the S value, we have not been able to compare the means between the control and the pathologic groups because in this latter, the data were not normally distributed.

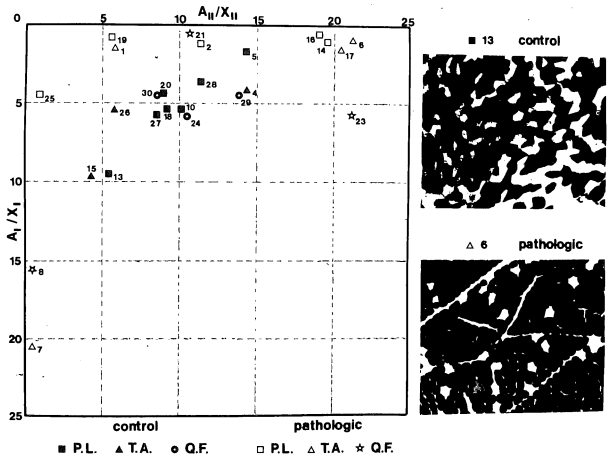

Fig.3 . Diagram of the A/X parameters : two examples of biopsies

DISCUSSION

The technique used has shown low values for the number of sequences "S" for 11 out of the 12 pathologic biopsies. Except for one case (n°1), the presence of one fibre type grouping is due to a significant increase in the numerical or the surface proportion of that fibre type. The diagram of the A/X ratios may permit the identification of 2 distinct populations of pathologic biopsies : those with F_I aggregates (biopsies 6,14,16,17 with low A_I/X_I values) and those with F_{II} aggregates (biopsies 7,8 with low A_{II}/X_{II} values). In these two cases, the other fibre type has very isolated fibres and displays a high A/X ratio. The other biopsies can be considered as intermediate cases with aggregates of the two fibre types (biopsies 1,19,25), or with aggregates of one fibre type and normal pattern of the other type (biopsies 2,21).

It can be seen that the controls are not very homogeneous. In two cases (biopsies 5,28), the calculated parameters are slightly out of the normal limits because of a high numerical or surface ratio, and seem to have a somewhat modified distribution pattern.

These data are in agreement with those of Johnson et al. (1973a) who found a random pattern for the normal spatial distribution of the fibre types in almost all human muscles. Any change in the spatial distribution will seem to presuppose either a physical or a physiological change in the innervation of the muscle (johnson et al., 1973b).

In summary we believe that the present technique enables the histopathologist to substantiate his subjective impression of the degree of spatial distribution pattern by objective and quantitative parameters.

REFERENCES

Brooke MJ, Kaiser KK. Some comments on the histochemical characterization of muscle adenosine-triphosphatase. J Histochem Cytochem 1969 ; 17 : 431-432.

Dubovitz V, Pearse AGE. A comparative histochemical study of oxidative enzyme and phosphorylase activity in skeletal muscle. Histochemie 1960 ; 2 : 105-117.

Engel WK. The essentially of histo-and cytochemical studies of skeletal muscle in the investigation of neuro-muscular diseases. Neurology 1962 ; 12 : 778-784.

Gundersen HJG. Notes on the estimation of the numerical density of arbitrary profiles : the edge effect. J Microsc 1977 ; 111 : 219-223.

Jennekens FGT, Tomlinson BE, Walton JN. Data on the distribution of fibre types in five human limb muscles. An autopsy study. J Neurol Sci 1971 ; 14 : 245-257.

Johnson MA, Polgar J, Weightman D, Appleton D. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. J Neurol Sci 1973a ; 18 : 111-129.

Johnson MA, Sideri G, Weightman D, Appleton D. A comparison of fibre size, fibre type constitution and spatial fibre type distribution in normal human muscle from cases of spinal muscular atrophy and from other neuromuscular disorders. J Neurol Sci 1973b ; 20 : 345-361.

Telerman-Toppet N, Coers C. L'histochimie des fibres musculaires normales. Ann Anat Pathol, Paris 1973 ; 18 : 35-48.