

Radioimmunoassay of serum myoglobin in neuromuscular diseases

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SUMMARY Radioimmunoassay of serum myoglobin was performed in 85 patients with muscular symptoms. Elevated levels were found in 93% of patients with myogenic myopathy, in 54% with myasthenia gravis and in 50% with neurogenic myopathy. All 11 patients with polymyositis had elevated myoglobin concentrations. In six of seven patients with polymyositis, who were followed up with repeated determinations, a clear relationship between myoglobin levels and clinical course was found. In general serum myoglobin seemed to be a more sensitive indicator of muscle disease than creatine kinase.

Myoglobin (Mb) is a low molecular weight protein which is considered to be located solely in skeletal and myocardial muscle, and increased leakage of Mb into the circulation should be a specific indicator of muscle cell damage.

Mb was first recognised in the sera of patients with polymyositis in 1971.¹ However, following the recent introduction of radioimmunoassays for detection of Mb this protein has also been detected in low concentrations in the sera of healthy individuals.^{2,3} Radioimmunoassay of Mb has proved useful in the diagnosis of myocardial infarction^{4,5} and myopathy.⁶⁻⁸ The purpose of the present study was to investigate further the value of serum Mb assay in neuromuscular diseases.

Patients and methods

Patients The study was made on 85 patients (44 men and 41 women between 20 and 80 years of age) admitted to the Department of Neurology, Uppsala University Hospital. Eleven of them had polymyositis, eight muscular dystrophy, nine unclassified myogenic myopathy, 28 myasthenia gravis, one Eaton-Lambert's syndrome, and 12 neurogenic myopathy. The other 16 patients had muscular aching, but no convincing clinical or laboratory signs of neuromuscular disease. The diagnoses were based on the clinical picture, EMG, and serum enzyme determinations (CK, ASAT, ALAT, LD); in patients with myogenic myopathy

muscle biopsies were also performed. The criteria of Bohan and Peter⁹ were used for the diagnosis of polymyositis and the diagnosis of myasthenia gravis was confirmed by the typical case history, a positive response to edrophonium, single fibre EMG (by Stålberg¹⁰) and by determination of acetylcholine receptor antibodies.¹¹ All patients had normal serum creatinine levels and none showed clinical signs of liver disease.

Methods Serum Mb was assayed by means of a solid-phase radioimmunoassay described earlier.¹² Each serum sample was assayed in duplicate. The within-day variation was 8% (coefficient of variation). The normal values for serum Mb, in a reference material of 99 blood donors of ages 20-62 years, was 35.7 $\mu\text{g/l} \pm 20.8$ (mean ± 2 SD) for women and 50.6 $\mu\text{g/l} \pm 39.6$ (mean ± 2 SD) for men.¹² Serum CK determinations were performed at the routine laboratory. The upper normal limit ($+2$ SD) was 2.5 $\mu\text{kat/l}$ (2.5 $\mu\text{mol per second per litre, } \mu\text{mol s}^{-1} \text{ l}^{-1}$).

Results

The serum concentrations of Mb and the number of patients with elevated Mb and serum CK levels are given in fig 1 and table 1, respectively. The highest incidence of elevated Mb values was found in the group of patients with myogenic myopathy. Thus, 26 out of 28 patients (93%) with myogenic myopathy had raised Mb levels. Elevated CK values were found in 17 (61%) of these patients. The highest Mb values were found among patients with polymyositis. All 11 patients with polymyositis had elevated Mb concentrations ranging between 94 and 1972 $\mu\text{g/l}$ (mean 701). Raised CK levels were noted in seven of the

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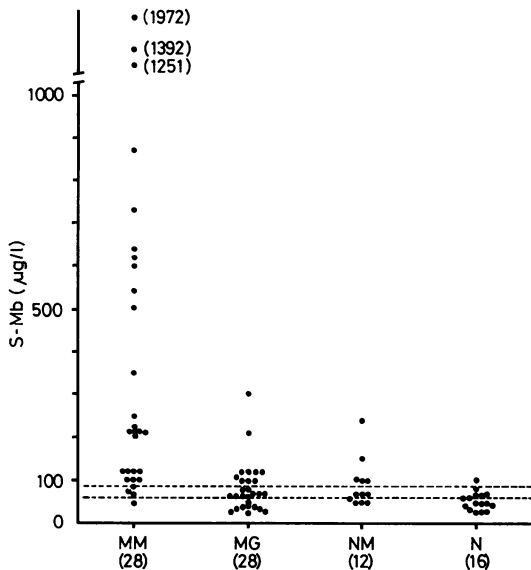


Fig 1 Serum myoglobin concentrations in patients with myogenic myopathy (MM), myasthenia gravis (MG), neurogenic myopathy (NM), and in patients with muscular ache but no evidence of neuromuscular disease (N). Upper normal limits for men and women indicated (---).

patients with polymyositis. At the start of the study three of the patients with polymyositis were being treated with steroids and these patients had normal CK levels.

Serum Mb was determined on several occasions during a period of 6–33 months in seven patients with polymyositis. In six of them there was a clear relation between Mb levels, CK levels and clinical course as exemplified in figs 2 and 3. The Mb values declined within a few weeks after institution of steroid therapy, slightly prior or parallel with the clinical improvement, and in

case of deterioration of the disease there was a rapid increase in serum Mb. In one patient with polymyositis there were only minor fluctuations of Mb and CK levels and there was no obvious relation between these fluctuations and the clinical course. However, this patient had had polymyositis for more than two years and had been treated with steroids for seven months at the start of the study, at which time he also had severe muscle atrophy.

In the group with myogenic myopathy, only two patients (one man with distal myopathy of the Welander type and one woman with an unclassified myopathy) had normal Mb levels, and these patients also had normal CK levels.

Raised Mb concentrations were noted in 15 of the 28 patients with myasthenia gravis and two of the patients with myasthenia gravis had elevated CK levels. Among the 15 myasthenia gravis patients with elevated serum Mb, six had mild generalised myasthenia (Osserman 2A). Nine of them had severe generalised myasthenia (Osserman 2B, 3, 4), although at the time of the study only four had disabling symptoms. Thymectomy had been performed in 10 of the 15 patients and thymoma had been found in five cases. In 11 of the 15 patients acetylcholine receptor antibodies (mean 5.33 arbitrary units) were demonstrated and eight patients also had antibodies against striated muscle. Eight of the patients were treated with steroids. Normal Mb levels were found in 13 of the patients with myasthenia gravis. In this group two patients had severe generalised myasthenia (Osserman 2B and 3, respectively), but only one patient had disabling symptoms at the time of the study. The other 11 patients had mild generalised myasthenia. Thymectomy had been performed in 11 of the 13 patients and thymoma had been found in one case. Acetylcholine receptor antibodies

Table 1 Incidence of elevated serum MB and CK levels in different patient groups

Condition	Number of patients	Number of patients with elevated Mb (%)	elevated CK (%)
I Myogenic myopathy	28	26 (93)	17 (61)
Polymyositis	11	11 (100)	7 (64)
Muscular dystrophy	8	7 (87)	5 (62)
Myogenic myopathy, unclassified	9	8 (89)	5 (55)
II Diseases with disturbed neuromuscular transmission	29	15 (52)	2 (7)
Myasthenia gravis	28	15 (54)	2 (7)
Eaton-Lambert's syndrome	1	0 (0)	0 (0)
III Neurogenic muscular atrophy	12	6 (50)	3 (25)
IV Patients with muscular ache but no evidence of neuromuscular disease	16	2 (12)	0 (0)

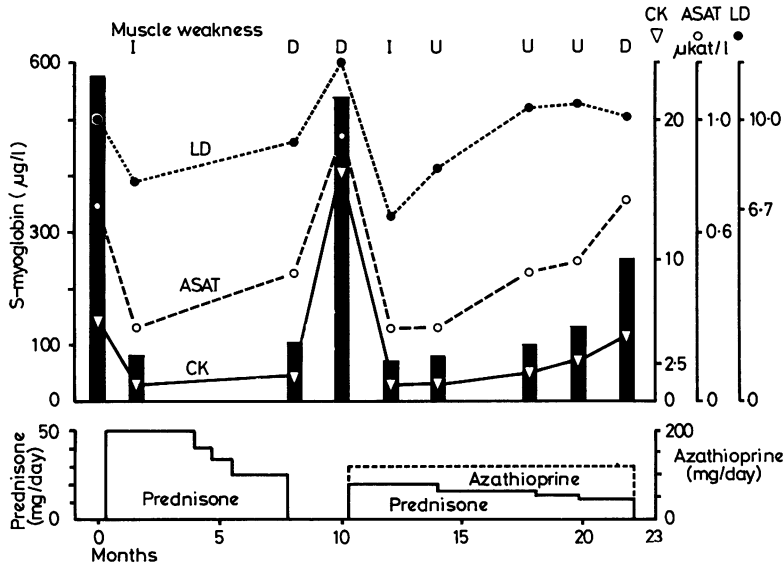


Fig 2 Relation of serum Mb, serum enzyme activity, therapy and clinical weakness (I=improvement, D=deterioration, U=unchanged) in a 64-year-old woman with polymyositis. Black bars indicate serum Mb. Upper limits of normal: CK(—)= 2.5µkat/l; ASAT (- - -)= 0.60 µkat/l; LD (· · · ·)= 6.7 µkat/l.

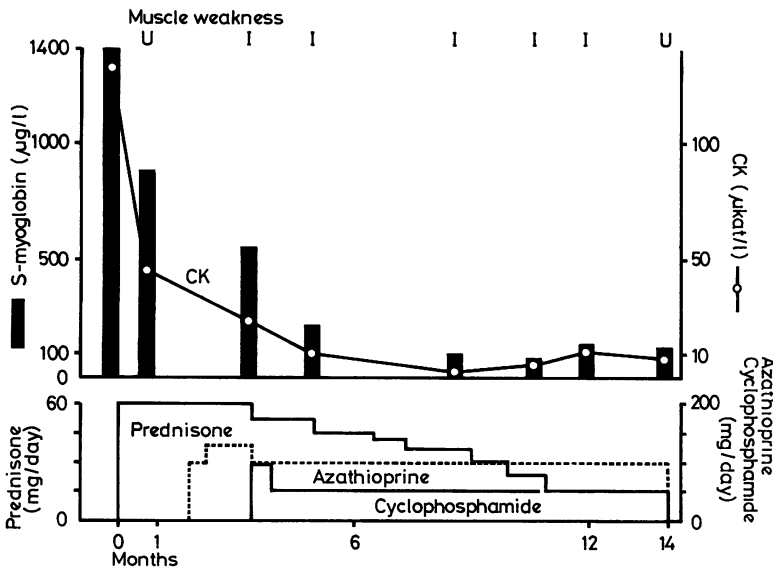


Fig 3 Relation of serum Mb, CK, therapy and clinical weakness in a 43-year-old woman with polymyositis. The same symbols as in fig 2.

(mean 2.47 arbitrary units) were demonstrated in eight patients and one patient also had antibodies against striated muscle. One patient was treated with steroids.

Six of the 12 patients with neurogenic myopathy had elevated Mb levels and three patients had elevated serum CK. The group with elevated Mb levels consisted of three patients with motor neuron disease, two with Guillain-Barré syndrome and one with Charcot-Marie-

Tooth disease. One patient with motor neuron disease, one with diabetic polyneuropathy and four with mononeuropathy had normal Mb values.

Elevated Mb concentrations were noted in two of the 16 patients who complained of muscular ache but who presented no convincing clinical or laboratory signs of neuromuscular disease. All patients in this group had normal CK levels.

The simultaneously estimated levels of Mb and

Table 2 Simultaneous determination of serum Mb and CK in 69 patients with neuromuscular diseases

	CK normal	CK elevated
Mb elevated	27	20
Mb normal	20	2

CK were compared (table 2) in all 69 patients with neuromuscular diseases (groups I-III, table 1). Mb was elevated in 47 patients and CK in 22 patients. Only in two cases were normal Mb levels found when CK was raised.

Discussion

Several serum enzymes such as aldolase, ASAT, ALAT and LD are increased in myopathies, but since these enzymes also reside in other tissues than muscle, their use in the diagnosis of myopathy is limited. Estimation of CK in serum is used widely in the diagnosis of muscle disease because of its high specificity to muscle tissue. However, CK is also present outside muscle and the serum CK level may be elevated in persons with no evidence of myopathy.¹³ Mb is considered to be located solely in skeletal and myocardial muscle, and increased serum levels of this protein, in the absence of impaired glomerular filtration and impaired liver function,¹² should be specific for disease or damage to muscle tissue. Dynamic muscular exercise seems to cause only minor variations in serum Mb levels.¹²

In our study, 26 of 28 patients (93%) with myogenic myopathy had raised Mb levels. This group included 11 patients with polymyositis and in all of these the serum Mb was increased. This finding is in accordance with the report of Miyoshi *et al*,⁷ who investigated 20 patients with myogenic myopathy (six with polymyositis and 14 with Duchenne's muscular dystrophy), and found elevated Mb levels in all of them. Hische and Van der Helm reported that the serum Mb levels were raised in 54% of patients with neuromuscular diseases.⁸ Eight of nine patients with untreated polymyositis had elevated Mb levels and such an increase was found in 74% of all the patients with myogenic myopathy. Using a solid-phase radioimmunoassay very similar to ours, Nishikai and Reichlin⁶ noted increased serum Mb in only 50% of patients with polymyositis and in 38% with muscular dystrophy. The use of different normal values of serum Mb might conceivably contribute to this discrepancy, but it is difficult to explain the difference between

their results and ours. Contrary to Nishikai and Reichlin we have not used the same normal values for men and women, as there is a significant difference in serum Mb levels between healthy men and women.¹² In polymyositis one other explanation might be a difference in the treatment of the patients at the time of the sampling.

The results of the present investigation indicate that radioimmunoassay of serum Mb is a more sensitive method for detection of muscle disease than determination of CK. Only two of 28 patients with myogenic myopathy had normal Mb levels, whereas normal serum CK was noted in 11 patients in this group. Consequently, the finding of a normal serum Mb does not exclude, but considerably reduces the probability of a myogenic myopathy.

Nishikai and Reichlin⁶ noted elevated Mb levels in five of 13 patients (38%) with myasthenia gravis and in our study 15 of 28 patients (54%) with this disease had raised Mb levels. In myasthenia gravis elevated CK levels are seldom found (in our material four of 83 patients with myasthenia gravis had elevated serum CK). This discrepancy between Mb and CK levels in patients with myasthenia gravis indicates a selective leakage of low molecular weight protein from the muscle cell. There were more patients with severe forms of myasthenia (Osserman 2B, 3, 4) in the group with elevated Mb and this group had also higher concentrations of acetylcholine receptor antibodies. We found no distinct relationship, however, between Mb levels and the clinical condition at the time of sampling.

In conclusion radioimmunoassay of serum Mb appears to be valuable as a supplementary tool to detect the presence of a myopathy and also seems useful for evaluating the status and therapeutic result in polymyositis. Serum Mb seems to be a more sensitive indicator of muscle disease than CK, and a normal serum Mb level in a patient not treated with steroids will considerably decrease the probability of a myogenic myopathy. Further study is needed to find out whether Mb determinations might also be of practical value in myasthenia gravis.

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