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### **Enzyme replacement therapy with agalsidase alfa in a cohort of Italian patients with Anderson-Fabry disease: testing the effects with**

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## Short Report

# Enzyme replacement therapy with agalsidase alfa in a cohort of Italian patients with Anderson–Fabry disease: testing the effects with the Mainz Severity Score Index

Parini R, Rigoldi M, Santus F, Furlan F, De Lorenzo P, Valsecchi G, Concolino D, Strisciuglio P, Feriozzi S, Di Vito R, Ravaglia R, Ricci R, Morrone A. Enzyme replacement therapy with agalsidase alfa in a cohort of Italian patients with Anderson–Fabry disease: testing the effects with the Mainz Severity Score Index. Clin Genet 2008. © Blackwell Munksgaard, 2008

Anderson–Fabry disease (AFD) is a rare X-linked disorder caused by lysosomal storage of several glycosphingolipids, affecting virtually all organs and systems. Enzyme replacement therapy (ERT) for AFD has been available since 2001. Due to the highly variable nature of clinical manifestations in patients with AFD, it is very difficult to assess disease progression and the effects of therapy. We used the Mainz Severity Score Index (MSSI) as a measure of disease severity to study the effects of ERT in a population of 30 patients treated with agalsidase alfa for a median of 2.9 years (range, 1.0–6.2 years). Our data show that the MSSI captures the correlation between disease severity and both gender and age (1 – males performing worse than females at baseline and 2 – severity of diseases progresses with age in both sex). Furthermore, after at least 1 year of ERT, total MSSI scores were significantly lower than those at baseline ( $p < 0.001$ ), suggesting a marked clinical improvement under ERT. In conclusion, the MSSI is a sensitive and useful tool for monitoring disease progression and assessing the effects of ERT in a population of patients from different treatment centres.

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Key words: agalsidase alfa – Anderson–Fabry disease – enzyme replacement therapy – Mainz Severity Score Index

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Anderson–Fabry disease (AFD, OMIM 301500) is an X-linked lysosomal storage disease that affects virtually all organs and systems. It is caused by mutations in the gene encoding  $\alpha$ -galactosidase

A, *GLA* (OMIM 300644), that maps to the Xq22 region of the X chromosome. The deficiency of  $\alpha$ -galactosidase A results in widespread lysosomal storage of glycosphingolipids, mainly

globotriaosylceramide (Gb3) (1). AFD affects both males and females (2, 3). The first signs and symptoms in infancy and adolescence are neuropathic and abdominal pain, cornea verticillata, hypohidrosis, angiokeratomas, fatigue, tinnitus and vertigo. In adulthood, vital organs such as the kidney, heart and brain become clinically involved, resulting in a reduced lifespan (2, 3).

Enzyme replacement therapy (ERT) became available for AFD a few years ago, bringing about a need for sensitive markers to the detection of the effects of therapy. Unfortunately, at present, no universally accepted biochemical surrogate marker has been developed for this purpose (4–6); therefore, careful and detailed clinical and biochemical monitoring of each sign and symptom is necessary to understand disease progression and the effects of treatment. Whybra et al. (7) have developed a severity scoring system – the Mainz Severity Score Index (MSSI) – that is specific for AFD and sensitive enough to allow monitoring of disease progression and patients' responses to ERT. Although the authors concede that 'the MSSI probably does not always reflect true morbidity and cannot be relied upon totally', it appears to be a promising tool for objectively monitoring a wide range of signs and symptoms in patients with AFD. To uniformly assess the overall effects of ERT in patients with AFD, we have calculated MSSI scores for 30 Italian patients (23 males and 7 females) at baseline and after at least 1 year of ERT.

## Patients and methods

### Patients

This study was performed in four different centres (Milano/Monza, Roma, Ortona, and Catanzaro) and involved 30 patients with AFD, 23 males [median age, 28.5 years (range, 4.1–47.7 years)] and 7 females [median age, 47.7 years (range, 39.1–64.3 years)], from 16 Italian families (Table 1). Five of 30 patients were boys between 4 and 18 years of age. A diagnosis of AFD was confirmed by enzymatic and/or molecular analysis in all subjects. ERT with agalsidase alfa (Replagal®; Shire Human Genetic Therapies, Danderyd, Sweden) was initiated 1–40 years after the onset of symptoms [median chronological age, 34.3 years (range, 4.1–64.3 years)]. Agalsidase alfa was administered intravenously over a period of 40 min at the standard dosage of 0.2 mg/kg every other week. No serious adverse events related to drug infusion were reported. Four male patients had a total of 11 episodes of fever and shivering during the infusion, occurring between

the 8th and the 18th infusion. Subsequent infusion reactions were easily prevented by pre-treatment with paracetamol.

Ten patients were taking non-steroidal anti-inflammatory drugs (NSAIDs) or other medications for neuropathic pain at baseline. Other concomitant treatments at baseline included medications for abdominal pain (antacids,  $n = 5$ ), angiotensin-converting enzyme inhibitors ( $n = 7$ ), calcium antagonists ( $n = 1$ ), diuretics ( $n = 2$ ), beta-blockers ( $n = 4$ ) and antidepressants ( $n = 2$ ). Patients continued to receive these medications during ERT. Only one patient stopped taking NSAIDs and antacids 6 months after starting ERT.

### Mainz Severity Score Index

The MSSI was used to assess overall disease severity in each patient by recording which organs or systems were affected (7). A weighting is applied to each affected organ depending on the anticipated impact of the organ's involvement on the overall health of the patient. For example, involvement of the kidneys, heart and cerebrovascular system has a greater impact on the total MSSI score than involvement of the skin. The MSSI is divided into four sections: general, neurological, cardiovascular and renal. The sum of these four scores gives the final total MSSI score. Total MSSI scores are divided into severity bands reflecting the clinical spectrum of the disease (<20, mild; 20–40, moderate; and >40, severe).

For each patient, disease severity was independently assessed by two different clinicians immediately before starting treatment and at least 1 year after treatment had begun [median duration of treatment, 2.9 years (range, 1.0–6.2 years)]. Neurological, cardiovascular and renal involvements were assessed by the relevant specialist. Total MSSI scores at baseline and after at least 1 year of ERT were correlated with each patient's age (<35 and  $\geq 35$  years) and sex. Changes in MSSI scores for individual components were also analysed.

### Statistics

MSSI scores at baseline were compared with those at follow-up by means of the Wilcoxon rank sum test. Analyses were two-tailed, and comparisons were considered statistically significant if  $p$  values were below 0.05. The correlation between MSSI score at baseline and age was investigated by means of a linear regression model, fitted in males and females separately.  $R^2$  was calculated as a measure of goodness of fit.

Table 1. Demographic data for Italian patients with Anderson-Fabry disease<sup>a</sup>

Centre	Patient number	Enzyme activity (%)	Molecular analysis	Gender	Years on treatment	Age at treatment start (years)	CV MSSl		Renal MSSl		Total MSSl	
							Baseline	After ERT	Baseline	After ERT	Baseline	After ERT
1	1	<1.0	Y86C	M	1.8	4.0	0	0	0	0	4	6
1	2	<1.0	No mutation detected	M	2.6	11.5	0	0	0	0	9	6
1	3	10.0	W236C	M	5.2	11.0	0	0	0	0	6	8
1	4	10.0	c124-125delAT	M	5.2	12.0	0	0	4	0	11	8
1	5	15.0	C172Y	M	3.5	17.5	0	0	0	0	15	11
4	6	4.0	C172Y	M	1.5	20.5	0	0	4	0	27	6
1	7	1.0	R342X	M	6.2	20.0	0	0	0	0	10	6
4	8	4.0	C172Y	M	1.5	23.5	2	3	4	0	22	12
4	9	3.0	C172Y	M	1.5	26.5	1	1	4	0	22	8
2	10	2.5	R227Q	M	5.0	25.0	2	1	0	0	14	14
2	11	3.0	c126-127insCATG	M	5.0	25.0	1	0	0	0	4	0
2	12	4.0	R227Q	M	2.6	29.4	1	0	0	0	6	3
4	13	3.0	C172Y	M	1.5	31.5	0	0	4	0	29	12
3	14	—	L167P	M	4.0	31.0	8	8	18	18	41	41
3	15	<1.0	S78X	M	1.5	35.5	8	7	4	4	27	20
2	16	2.1	P40L	M	5.0	34.0	8	3	0	0	27	18
2	17	4.4	c126-127insCATG	M	5.5	34.5	8	7	18	18	26	29
2	18	11.9	N215S	M	2.8	36.2	2	0	0	0	6	2
1	19	45.0	W236C	F	1.5	39.0	0	0	4	4	14	8
2	20	9.7	IVS3+1G>A	M	5.0	37.0	6	0	4	8	14	12
3	21	<1.0	S78X	M	3.0	39.0	8	5	4	4	24	14
1	22	<1.0	C52Y	M	1.0	41.0	8	8	4	4	31	31
3	23	<1.0	S78X	M	4.0	39.0	8	8	12	12	36	36
3	24	—	S78X	F	1.0	43.0	8	8	4	4	16	15
1	25	16.0	c124-125delAT	F	1.1	47.0	1	2	4	4	8	10
1	26	50.0	R342X	F	4.2	47.0	11	16	4	0	16	22
1	27	<1.0	R342X	M	4.2	48.0	16	16	0	0	29	26
2	28	30.0	R227Q	F	2.7	52.3	9	8	4	0	18	11
2	29	25.0	N215S	F	2.8	53.2	9	1	4	4	22	10
3	30	63.0	S78X	F	2.0	64.0	8	5	4	4	24	17

CV, cardiovascular; ERT, enzyme replacement therapy; MSSl, Mainz Severity Score Index.

<sup>a</sup>Individual MSSl scores are shown for CV and renal components before and after ERT. Total MSSl scores are also shown. Treatment centres: 1, Milano/Monza; 2, Roma; 3, Ortona; and 4, Catanzaro.

**Results**

Baseline data

Total MSSSI scores in the seven females ranged from 8 to 24 (median, 16), indicating mild-to-moderate disease involvement. In the 23 males, scores ranged from 4 to 41 (median, 22), demonstrating mild involvement in 11 patients, moderate severity in 11 patients and severe disease in 1 patient (total MSSSI score, 41). A clear correlation was found between total MSSSI scores before ERT and age in both males and females (Fig. 1). As expected, females had lower baseline scores at a given age than males. In male patients (plotted by age), no correlation was found between the severity of mutations (missense or nonsense) and the severity of the disease.

Effects of ERT

Figure 2 shows the correlation between total MSSSI scores at baseline and after at least 1 year of ERT (duration of treatment, 1–5 years) in patients grouped by sex (Fig. 2a) and by age (Fig. 2b). Patients younger than 35 years of age had a median change in MSSSI total score of –4, as did patients equal to or older than 35 years of age, although a more restricted range of values was observed in the older patient group (Fig. 3).

Overall, a significant reduction in total MSSSI scores was observed across the patient cohort following ERT (Table 2); 22 patients improved, 4 patients had an unmodified score and 4 patients had higher total MSSSI scores after treatment. Among the four patients with increased MSSSI

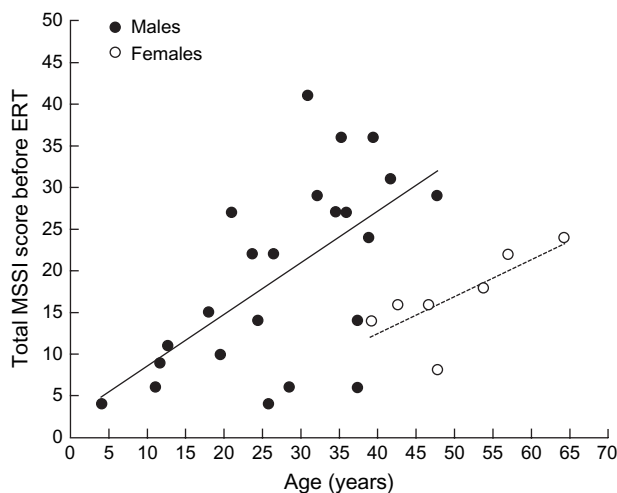


Fig. 1. Total MSSSI scores in males and females before treatment vs age at start of treatment. Correlation was determined using a linear regression model ( $R^2$  in males, 0.34 and  $R^2$  in females, 0.44). ERT, enzyme replacement therapy; MSSSI, Mainz Severity Score Index.

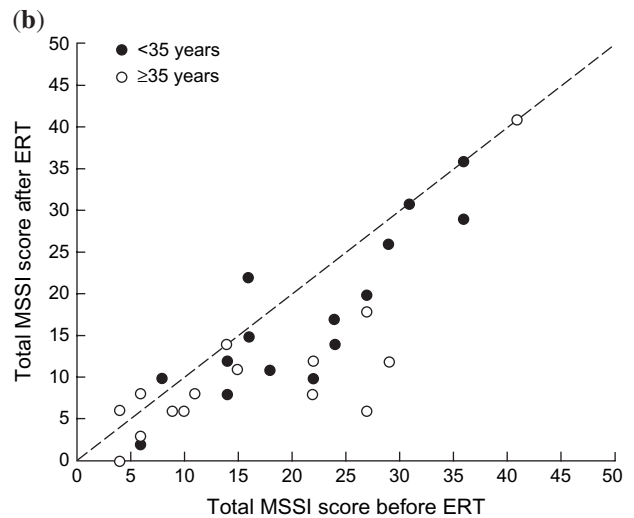
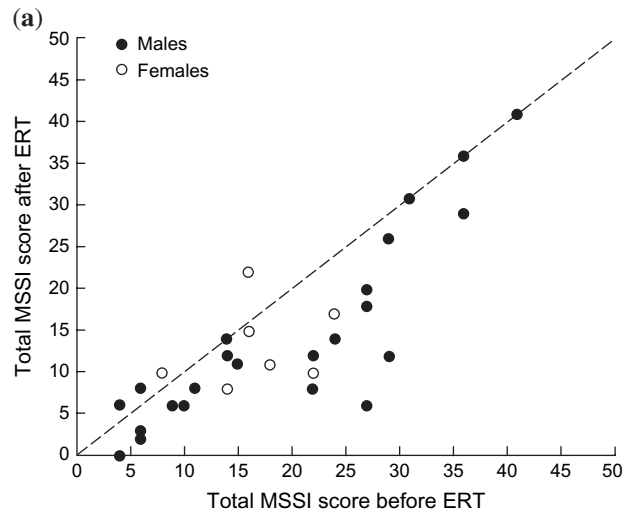


Fig. 2. Total MSSSI scores after ERT vs total MSSSI scores before ERT in (a) males and females and (b) patients younger than 35 years of age and patients equal to or older than 35 years of age. ERT, enzyme replacement therapy; MSSSI, Mainz Severity Score Index.

scores, two boys (5 and 16 years of age) and one woman (50 years of age) had low scores at baseline that increased to values that were still lower than 10 at follow-up (patients 1, 3 and 25, respectively; Table 1). The remaining patient was a 53-year-old woman treated for 4 years who had heart surgery after 2 years of ERT (patient 26; Table 1). The four patients who had an unmodified score (patients 10, 14, 22 and 23; Table 1) following treatment were all males, three of whom were older than 35 years and had severe renal or neurological symptoms.

Table 2 shows changes in MSSSI scores for the individual components (general, neurological, cardiovascular and renal) for all 30 patients. MSSSI scores following ERT were significantly different from those at baseline for all components, except

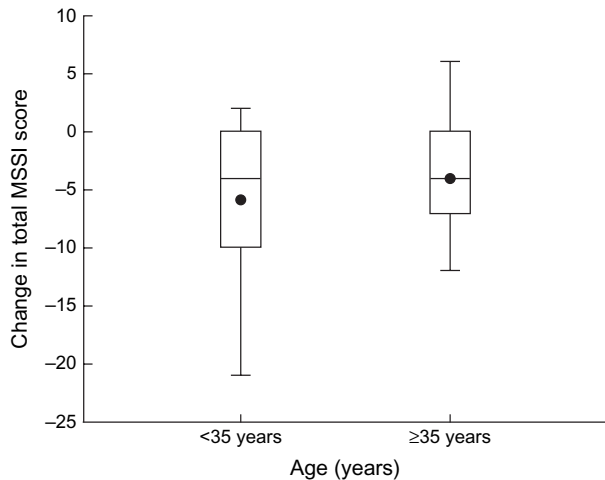


Fig. 3. Change in total MSSI scores after at least 1 year of enzyme replacement therapy in patients younger than 35 years of age and patients equal to or older than 35 years of age. The box plots are drawn by age at the start of treatment and show the mean (dot), median (rule), interquartile range (box), and minimum and maximum values (whiskers). MSSI, Mainz Severity Score Index.

renal scores. Detailed data on cardiovascular and renal scores are shown in Table 1. Twenty-one of 30 patients had a cardiovascular MSSI score greater than zero at baseline, decreasing to 17 patients after at least 1 year of ERT. Twelve patients showed improved cardiovascular MSSI scores [median change,  $-1$  (range,  $-1$  to  $-8$ )], three worsened [median change,  $+1$  (range,  $+1$  to  $+5$ )], and six remained stable. At baseline, 19 of 30 patients had renal symptoms (renal MSSI score,  $>0$ ), 16 had microalbuminuria (albumin excretion of  $30$ – $300$  mg/day) or proteinuria (albumin excretion  $>300$  mg/day) (renal MSSI score,  $4$ ), while 3 male patients (2 renal transplanted and 1 on dialysis) had a more severe renal involvement (renal MSSI score,  $12$ – $18$ ). Following ERT, renal involvement was evident in only 12 patients. Among the 16 patients with pathologic albumin excretion at baseline, 1 patient worsened (renal MSSI score,  $8$ ), 8 patients remained stable (renal MSSI score,  $4$ ), and the 7 patients who had microalbuminuria improved (renal MSSI score,  $0$ ).

Renal involvement in the three patients with more severe renal damage remained unchanged following ERT.

## Discussion

We report on the effects of treatment with agalsidase alfa in 30 Italian patients with AFD from four different treatment centres, as assessed by the MSSI (7). The MSSI was chosen as an easy instrument for evaluating and comparing the clinical effects of ERT in patients treated in different centres. The MSSI, although not formally validated, has already been shown to be a very useful tool for assessing disease severity in patients with AFD (8). It has previously been shown to correlate with age in children (9) and with serum levels of metalloproteinase 9, which, in turn, correlates with the severity of heart disease in patients with AFD (10). An adapted form of the MSSI has also been used to assess disease severity in patients enrolled in the Fabry Outcome Survey (FOS). Using this adaptation (FOS-MSSI), a significant correlation with age was recently found in 262 patients, and furthermore, patients with cutaneous vascular lesions (CVL) were shown to have higher scores than those without CVL (11, 12). This was expected as the presence of CVL indicates generalized vascular endothelial involvement.

In this study, we found a clear correlation between total MSSI scores and age, both in males and in females, with results very similar to those reported by Whybra et al. (7). As a group, females with AFD show the same manifestations as males, although they typically manifest about 10 years later (3). This is well reflected in this study in which total MSSI scores in females parallel those of the males who are 10–15 years younger (Fig. 1). The reason why most heterozygous females are symptomatic is still under discussion (13) and cannot simply be explained by skewed X-inactivation or by the need of an elevated production of enzyme in this disease. The recent finding of increased concentrations of deacylated Gb3 (lyso-globotriaosylsphingosine, lysoGb3) in plasma of

Table 2. Change in total and individual component MSSI scores after at least 1 year of enzyme replacement therapy<sup>a</sup>

	<i>n</i>	Total MSSI	General MSSI	Neurological MSSI	Cardiovascular MSSI	Renal MSSI
Change from baseline	30	$-4$ ( $-21$ to $+6$ )	$-1$ ( $-5$ to $+3$ )	$-1$ ( $-13$ to $+5$ )	$0$ ( $-8$ to $+5$ )	$0$ ( $-4$ to $+4$ )
<i>p</i> Value	—	$<0.0001$	$0.0005$	$0.0056$	$0.0226$	$0.0703$

MSSI, Mainz Severity Score Index.

<sup>a</sup>Results are given as median (range). The *p* values were determined by Wilcoxon signed-rank sum test.

AFD male and female patients probably helps to clarify this issue (14). LysoGb3 in fact is a potent inhibitor of  $\alpha$ -galactosidase A and also when it is uptaken into cells, it is probably reacylated to Gb3, thus increasing the extralysosomal pool of Gb3, which is not accessible to endogenous or exogenous (ERT)  $\alpha$ -galactosidase A. In heterozygous females then, circulating lysoGb3 may ultimately vanish the effects of  $\alpha$ -galactosidase produced by enzyme-competent cells. It has also been shown that lysoGb3 is probably directly implicated in the development of the disease as it has an effect of stimulation of vascular remodelling (14).

Overall, a significant reduction in total MSSSI scores was observed across the patient cohort following ERT (Table 2). This is in agreement with the literature as the clinical benefit of ERT has been demonstrated in both placebo-controlled trials and open-label observational studies (15–20). A certain number of patients treated with agalsidase alfa or agalsidase beta have previously been reported to show no response to ERT (15, 16, 20). In some patients, this may be attributed to excessively advanced disease, although not in all cases. Improvements in total MSSSI scores in our group of patients were mainly due to changes in general and neurological components, while heart and renal involvement showed milder improvements. Detailed analysis of our data shows that 21 of 30 patients had heart involvement and 19 had renal involvement at baseline. The vast majority of these patients exhibited an improvement or stabilization of cardiac or renal involvement after ERT. Given the progressive nature of AFD, stabilization can be seen as a positive outcome following treatment. The fact that, prior to ERT, MSSSI scores for heart and renal components were 0 for 9 and 11 patients, respectively, may explain why the differences between pre- and post-treatment scores are weakly significant for cardiovascular involvement and not significant for renal manifestations.

The benefits of ERT with agalsidase alfa are clear in the majority of our patients who showed reduction of pre-existing pain, hypohidrosis, auditory and gastrointestinal symptoms, and an improvement or stabilization of renal and cardiac signs. While we can confirm that treatment with agalsidase alfa was beneficial in most of our patients, our data show how unpredictable the evolution of the disease can be in individual patients receiving treatment.

There are reasons for a critical use of MSSSI because it certainly does not completely reflect true morbidity: firstly, it was developed when not all signs and symptoms of the disease (e.g. haematuria and hearing deficiency) had been fully

investigated and secondly, it is surely a more convenient instrument for the classic Fabry patient presenting the whole spectrum of symptoms than for mono- or oligosymptomatic females or children who do not have major organ involvement. So, on this basis, minor modifications of the MSSSI in the near future may be conceivable. Nonetheless, the MSSSI as it is at present is a useful instrument to test patients with AFD and sensitive enough to show the expected increase in severity by age, the differences in severity by gender and the improvement of symptoms due to at least 1 year of ERT. In our population of 30 patients, it allowed us to objectively and homogeneously measure the clinical status of patients from four different centres.

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