TITLE: Clinical variability in spinal muscular atrophy type III

Running head: *Clinical variability in type III SMA*

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Abstract

Objective: We report natural history data in a large cohort of 199 spinal muscular atrophy (SMA) type III patients assessed using the Hammersmith Functional Motor Scale Expanded (HFMSE). The aim of the study was to establish annual rate and possible patterns of progression according to a number of variables, such as age of onset, age at assessment, *SMN2* copy number and functional status.

Methods: HFMSE longitudinal changes were assessed using piecewise linear mixed-effects models. The dependency in the data due to repeated measures was accounted for by a random intercept per individual and an unstructured covariance R matrix was used as correlation structure. An additional descriptive analysis was performed for 123 patients, for a total of 375 12-month assessments.

Results: A break point at age 7 was set for the whole cohort and for SMA IIIA and IIIB. Age, SMA type and ambulatory status were significantly associated with changes in mean HFMSE score while sex and *SMN2* copy number were not.

The increase in response before the break point of age 7 is significant only for SMA IIIA (β=1.79, p<.0001). After the break point the change in the rate of HFMSE score significantly decrease for both SMA IIIA (β=-1.15, p<.0001) and IIIB (β=-0.69, p=0.002).

Interpretation: Our findings contribute to the understanding of the natural history of type III SMA and will be helpful in the interpretation of the real-world data of patients treated with commercially available drugs.

Introduction

Spinal muscular atrophy (SMA) is one of the most common autosomal recessive diseases, presenting with progressive weakness of skeletal and respiratory muscles, leading to muscle atrophy [1]. The disease is caused by mutations in the survival motor neuron 1 (*SMN1*) gene [2]. The clinical subtypes, based on age of onset and maximum motor function achieved, range from the most severe SMA type I to the mildest SMA type IV [3].

SMA has a cumulative incidence of 1/11,000 live births[4]. However, the epidemiologic burden of SMA is not equally divided over the subtypes. A recent review showed incidence rates of around 5.5, 1.9 and 1.7 per 100,000 for type I, II and III, respectively [5].

Type III is not only less represented than the other SMA types but is also quite a heterogenous conditions. Type III patients are further classified into type IIIA with onset between ages 18 months and 3 years, and IIIB with onset after the age of 3 years [6, 7]. Even if by definition independent walking is achieved in all SMA III patients, the disease course is very variable with many patients losing ambulation later in life [7]. A recent study highlights the prognostic value of age of onset in predicting loss of ambulation, which occurs in the second decade of life in patients with onset before 3 years; or at the end of the third/fourth decade in those with onset between 3-12 years, and at the end of the fifth decade when onset occurred after 12 years [8].

Over the last few years there has been increasing interest in understanding functional changes over time in type III SMA using different functional assessments [9-17].

The available data, however, is limited as type III SMA is less frequent than other SMA types, and type III patients are less represented in the natural history.

Following the commercially availability of Nusinersen for all SMA types, the first papers reporting real world data in treated patients have highlighted the need to have natural history of type III SMA for comparison. As the pivotal studies in late onset SMA did not specifically target type III patients [18] and often did not include patients above the age of 12 years, the balance between relative effectiveness and treatment costs is still a burning issue in many countries in which this drug is not available for all type III patients. It is, therefore, important to gather more information

on the natural history of SMA type III for the interpretation of the real-world data that are increasingly becoming available [19-22].

The aim of this study was to describe natural history in a large cohort of type III patients assessed longitudinally using the Hammersmith Functional Motor Scale Expanded (HFMSE) [23].

More specifically, we were interested in establishing the distribution of scores at different ages, and the possible patterns of progression in type IIIA and IIIB patients. We have also attempted to identify the possible effect of variables such as age, ambulatory status, *SMN2* copy number on functional changes in this large SMA type III cohort.

Materials and methods

The data used in this study were prospectively collected from the retrospective data of International SMA Registry (including centres in United States, Italy, UK) [24], and the Hospital Universitari Sant Joan de Deu, Barcelona (Spain) and Leuven University (Belgium).

All patients with a genetically confirmed diagnosis of SMA and a clinically confirmed diagnosis of type III SMA, in whom data were retrospectively available in the datasets were considered for inclusion. Data from patients participating in clinical trials or treated with disease modifying drugs (e.g.nusinersen, onasemnogene abeparvovec-xioi or other investigational products) were also excluded.

As part of the activities of the participating networks all participants or their guardians provided written informed consent approved by the respective institutional review boards. Type III SMA was subdivided into IIIA or IIIB according to age at symptom onset (before or after 3 years).

Hammersmith functional motor scale Expanded

The HFMSE, is a functional assessment including 33 items, developed as an expansion of the HFMS [25] which included 20 items, with additional 13 items adapted from the Gross Motor Function Measure (GMFM) to cover functional aspects in ambulant patients [23]. Each item is scored on a 3-point scale, ranging from 0 (unable to perform the task) to 2 (complete ability to perform the task without modification or adaptation), and a total score of 66, with lower scores indicating poorer motor function.

Training sessions

Training and the same procedure manual were shared by all the participating networks [14]. Individual evaluators were trained at in-person meetings in US and in Europe and established reliability on the HFMSE [26]. As part of the studies in both Europe and US, evaluators have regular annual refresher trainings with item scoring review [26, 27].

Statistical analysis

Quantitative data are presented as medians (range) or means (± standard deviation) and categorical data as absolute numbers and frequencies. Differences between SMA Type IIIA and SMA Type IIIB at baseline were assessed by Mann–Whitney U or t-test according to the distribution of the variables. For ordinal variables, Pearson's chi-square test was used.

Because we cannot assume that the natural course of the SMA functional abilities is linear, we assessed longitudinal changes of HFMSE score using piecewise linear mixed-effects models.

The dependency in the data due to repeated measures was accounted for by a random intercept per individual and an unstructured covariance R matrix was used as correlation structure. A

random slope for age and time spline were added to assess whether there were differences in rates of decline between patients (as measure of disease heterogeneity or between-patient slope variability). The default estimation method REML was used for the covariance parameters. The Kenward Roger method was used to compute the degrees-of-freedom for the tests of fixed effects. SAS 9.3 (Institute Inc., Cary, NC, USA) and R software (version 3.5.0) were used for the analysis.

An additional descriptive analysis was performed taking into account the HFMSE 12-month intervals. Only patients with at least two assessments at a 12-month interval were selected for this analysis. For patients who had data with more than 12 months follow up, multiple 12 months intervals were considered. Descriptive statistics included N, mean, median, SD, range and were used to analyze age, HFMSE at baseline and 12 months as well as 12-month changes.

As previous studies have shown that the great majority of patient variability is within +/- 2 points [10, 14, 28], and focus groups of families have reported that changes above 2 points are clinically meaningful [29], we considered three ranges of change in the HFMSE. The percentage of patients with a change < -2 points (meaningful decline), between -2 and +2 (non- meaningful variation), and >2 points (meaningful improvement) was compared across age classes.

Results

The cohort included 199 patients, 147 IIIA and 52 IIIB, 96 males and 103 females. Their age ranged between 30 months and 30 years (mean 11.53, SD 6.86). Of the 199 patients, 11 of them had spinal surgery at first visit, 7 SMA IIIA and 4 SMA IIIB. Nine additional patients had scoliosis surgery

after first visit, for a total of 20 patients, 16 SMA IIIA and 4 SMA IIIB, age at scoliosis surgery in the 20 patients ranged between 9 and 19 years (Mean: 12.55, SD: ±2.55), ranging between 9 and 19 in the IIIA (Mean: 12.07, SD: ±2.49) and between 12 and 17 in the IIIB (mean: 14.25, SD:±2.22).

Twenty-six of the 199 patients lost ambulation during follow up, 22 type IIIA and 4 type IIIB. Mean age of ambulation loss for the 26 patients was 11.77 years (SD ±6.51), 11.47 (SD ±6.59) for the type IIIA and 13.43 (SD ±3.85) for the type IIIB patients.

Of the 199 patients enrolled, 17 were excluded due to lack of follow-up. One hundred eighty-two patients were retained in the final analysis, 136 Type IIIA and 46 Type IIIB. The analysis was performed enrolling SMA type III patients followed for at least 6 months.

Patients were followed from a minimum of 0.46 years to a maximum of 13.34 years and the median number of visits was 4 (range 2-25) with a median time difference between visit of 0.54 (range 0.07-9.90) years. The median age at baseline was 10.02 years (range 2.5-28.67).

Descriptive analysis on the 182 patients at first visit, divided by SMA IIIA or IIIB is reported in Table 1.

Figure 1 provides a straightforward graphical representation of the response profiles for the entire type III patients over time with the patient's age on the x-axis and total HFMSE score on the y-axis. The black line represents the mean response over time. The grey lines depict the change in response for each individual. The spaghetti plot shows an increase in the HFMSE score in the younger age and then a decrease in the older age. The graph seems to imply that age 7 is a break point for the analyzed patients. The break point at age 7 remains also when we considered SMA type IIIA and SMA type IIIB separately (figure 1, B-C).

Model 1) Time effect before and after critical time point k, adjusting for the effect of potential confounders (SMA type, sex, ambulatory status and SMN2 copy number)

First, we assessed the relationship between possible confounders and changes in mean response profile over time (Table 2).

The estimate for slope after k is the sum of parameter estimates for time and time-spline (1.51-2.59=-1.08). Age, SMA type and ambulatory status were significantly associated with changes in mean HFMSE score while sex and number of *SMN2* copy were not.

Model 2) SMA type and ambulatory status effect on the response profile over time

The increase in response before k is significant only for SMA type IIIA (β=1.79, p<.0001), while is not significant for SMA type IIIB (β=-1.97, p=0.153). After the break point of age 7 the change in the rate of HFMSE score significantly decrease for both SMA type IIIA and SMA type IIIB (β=-1.15, p<.0001 and β=-0.69, p=0.002, respectively).

The interaction term of the slope before age 7 and after age 7 and the ambulatory status of the patients was not significant.

12-month intervals

Of the 182 patients, 123 had multiple evaluations for at least 12 months, 98 IIIA and 25 IIIB, resulting in 375 assessments at 12-month intervals. Their age at baseline ranged between 30 months and 29.60 years (mean 11.61, SD ±6.24). Of the 123 patients, 11 lost ambulation during

follow up, 10 type IIIA and 1 type IIIB. Mean age of ambulation loss for the 11 patients was 10.21 years (SD ±6.43), being 9.22 (SD ±6.47) for the type IIIA and 20.1 for the one type IIIB patient

Of the 375 12-month assessments, 278 were assessments from ambulant and 97 from non-ambulant patients, i.e. type III patients who lost independent walking. In 12 patients, one of the two performances were affected by transient pain (n=2) or intercurrent spinal surgery (n=10) that was reported to affect temporarily one of the two assessments. These assessments were excluded from the analysis.

Details on age and HFMSE scores at baseline and after 12 months for each 12-month interval, subdivided by ambulatory status, are reported in table 4.

The HFMSE scores ranged between 4 and 66 (mean 45.37, SD ±15.37) (Fig 1). The 12-month change ranged between –22 and +10 (mean -1.22, SD ±4.37).

Of the 375 assessments, 192 (51.20%) had HFMSE changes between ±2 points, 121 (32.26%) had a decrease of more than 2 points and 62 (16.53%) had an increase of more than 2 points.

Descriptive statistics and 2-point percentage range analysis are reported in supplementary table 1 and 2, subgrouped in SMA III type, age and ambulatory status. The subdivision in age groups was arbitrarily based on previous reports[14] using the age of 5 to define the youngest group, and the peak found at 7 years in the analysis performed in this paper.

Discussion

This study reports the largest cohort of type III patients followed longitudinally with a structured functional assessment. This was obtained as part of an international effort involving individual

centers or networks from 5 countries. Although this was a retrospective study, the individual data were prospectively collected as part of natural history studies in each country. All the examiners shared training and manuals, with established intereobserver reliability [26] as had been involved in clinical trials and received further training on the rating scales used in the study.

In this study we only included patients up to the age of 30 years as data from older patients were scantier with large age gaps. This was mainly due to the fact that some of the participating centres are pediatric centres and follow patients until the age of 18 years, with only few also seeing adult patients. Nevertheless, some pediatric centres keep following patients after the age of 18 years as continuation of care, and this justified the recruitment of the adult patients between 18 and 30 years.

When we analyzed the whole cohort of type III patients there was relative stability with a modest functional improvement until the age of 7, followed by a steeper decline. While both type IIIA and IIIB had a peak around this age, it should be noted that in type IIIB the scores remained relatively stable after the peak until the age of 10 years while in IIIA there was an immediate decline.

This data is different from what we had reported in type II patients, who have a peak of functional achievement around the age of 5 years, confirming previous observation of different trajectories of progression between type II and III [14]. In the whole cohort, only 7 reached the HFMSE maximum score (66), indicating that the ceiling of the HFMSE is rarely achieved even in type III SMA.

Both IIIA and IIIB showed significant changes before and after 7 years with a difference between the two subtypes (p=<0.001). Both subtypes showed some decline, that appeared to be earlier in type IIIB patients. It is of interest however that, despite the loss of many points, most type IIIB

patient by the end of the second decade still had scores above 40 and were ambulant, this reflecting the fact that they had much higher scores at baseline and a slower rate of decline.
We also tried to establish which variables were driving the differences observed. Age, SMA type and ambulatory status were significantly associated with changes in mean HFMSE score while sex and number of SMN2 copies were not.

A further analysis prompted by the need to have reference data for the increasingly available 12-month data in treated pediatric and adult patients[19, 20] looked at 12-month changes in our natural history patients. This data, even if limited by the fact that there were repeated measures from the same patients, allowed us to provide additional information on the possibility to see stability or meaningful changes within each subgroup.
At 12-month, the highest rate of improvement (more than 2 points) was found in the assessments performed before the age of 5 years (42.85%) and between 5 to 7.99 years (26.92%). It is of interest that in these age groups type IIIA patients had a very variable progression, with a loss of more than 2 points found in 14.30% below 5 years and in 17.95 % between 5 and 7.99 years.
At 12-month, the highest rate of loss of more than 2 points was in the assessments performed between 8 and 14.99 years for type IIIA (46.80%) and between 15 and 17.99 years in type IIIB (50%). No gender effect was observed in our data analysis, suggesting that the age when the growth spurt accelerates during adolescence is not a major factor in this observed decline.
In conclusion, the results of our study, even if limited by several factors, like the exclusion of patients above the age of 30 years, confirm that there is some decline in type III SMA that can be

observed after the age of 7 years. The level of decline varies according to SMA III subtype, age and ambulatory status. SMN2 copy number, in contrast did not appear to be predictive of the decline, this probably due to the fact that the great majority of our patients had 3 SMN2 copies and that in over 30 % of our patients, mainly adults, the SMN2 copies had not been previously analyzed.

Taking these variables into consideration could help to stratify patients and to identify different patterns of progression. These findings, together with the 12 month analysis, will be of use as a reference for the interpretation of the real word data of pediatric and adult type III patients treated with commercially available drugs and, more generally, will help to better understand the natural history of this form of SMA, highlighting the need to prevent the progressive functional decline that, with few exceptions, occurs even in the type IIIB patients.

A more systematic approach, using cluster analysis as recently reported in DMD [24, 30, 31], also exploring other possible variables, such as gene modifiers[32] or possible differences in standards of care, may help to categorize further different trajectories and to produce composite prognostic scores that may improve prognostic accuracy and reduce variability in outcomes.

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Author contribution:

GC, SM, MCP, SL, FB, MP, MS, LA, AF, MS, GV, ADA, EA, NG, JM, TD, ESM, BTD, EB, VAS, JD, ANO, CB, CMB, FM, DCDV, RF, EM have contributed in conception and design of the study.

GC, MCP, SM, SL, FB, MP, AP, JEE, AM, AMG, SDY, RS, RML, ESM, MC, RDS, SC, MVDH, EM have contributed in the acquisition and analysis of data.

GC, MCP, SM, SL, FB, MP, NG, JM, TD, ESM, BTD, EB, VAS, JD, ANO, CB, CMB, FM, DCDV, RF, EM have contributed in drafting the manuscript.

Potential Conflicts of Interest:

Authors have nothing to report as potential conflict of interest relative to this study. Full disclosures can be found in the ICMJE conflict of interest forms.

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Figure legend:

Figure 1. Spaghetti plot of the response profiles over time. *Key to figure: grey line= individual response; black line= mean individual response. A: Whole cohort; B: IIIA cohort, C: IIIB cohort.*

Table legends:

Table 1: Descriptive statistics of the 182 patients at first visit, reporting age, gender, SMN2 copy number, HFMSE score and scoliosis surgery age (N, mean, SD, min and max) subdivided by ambulatory status and type IIIA or B.

Table 2. Multivariate regression model of changes in mean HFMSE score over time

Table 3. Estimates table.

Table 4. Descriptive statistics on Age, HFMSE at baseline, 12 months and HFMSE 12-month changes subdivided by SMA III type and ambulatory status.

Table 1: Descriptive statistics of the 182 patients at first visit, reporting age, gender, SMN2 copy number, HFMSE score and scoliosis surgery age (N, mean, SD, min and max) subdivided by ambulatory status and type IIIA or B.

		SMA III			SMA IIIA			SMA IIIB		
		All (N:182)	Ambulant (N:130)	Non ambulant (N:52)	All (N:136)	Ambulant (N:91)	Non ambulant (N:45)	All (N:46)	Ambulant (N:39)	Non ambulant (N:7)
Age	*Mean*	11.12	10.08	13.71	9.62	7.95	12.99	15.55	15.05	18.34
	SD	6.47	6.18	6.5	5.89	5.09	5.99	6.11	5.65	8.17
	Min	2.5	2.5	2.95	2.5	2.5	2.95	3.66	5.7	3.66
	Max	28.67	28.67	27.06	27.06	26.45	27.06	28.67	28.67	25.07
GENDER	*Female*	52.20% (N:95)	54.62% (N:71)	46.15% (N:24)	55.88% (N:76)	59.34% (N:54)	48.89% (N:22)	41.30% (N:19)	43.59% (N:17)	28.57% (N:2)
	Male	47.80% (N:87)	45.38% (N:59)	53.85% (N:28)	44.12% (N:60)	40.66% (N:37)	51.11% (N:23)	58.70% (N:27)	56.41% (N:22)	71.43% (N:5)
SMN2 copy number	*2*	4.94% (N:9)	4.62% (N:6)	5.76% (N:3)	2.94% (N:4)	1.10% (N:1)	6.67% (N:3)	10.87% (N:5)	12.82% (N:5)	0.00% (N:0)
	3	42.86% (N:78)	46.15% (N:60)	34.62% (N:18)	47.06% (N:64)	52.75% (N:48)	35.55% (N:16)	30.43% (N:14)	30.77% (N:12)	28.57% (N:2)
	4	21.98% (N:40)	26.92% (N:35)	9.62% (N:5)	17.65% (N:24)	23.08% (N:21)	6.67% (N:3)	34.78% (N:16)	35.90% (N:14)	28.57% (N:2)
	Unk	30.22% (N:55)	22.31% (N:29)	50.00% (N:26)	32.35% (N:44)	23.08% (N:21)	51.11% (N:23)	23.92% (N:11)	20.51% (N:8)	42.86% (N:3)
HFMSE score	*Mean*	44.02	51.68	24.87	41.46	49.66	24.87	51.61	56.41	24.86
	SD	15.93	8.83	13.38	15.14	7.74	12.64	15.94	9.49	18.72
	Min	1	28	1	3	28	3	1	31	1
	Max	66	66	56	66	66	49	66	66	56
SCOLIOSIS SURGERY AGE	*Mean*	12	16	11	11	N/A	11	14	16	13
	SD	2	1	1	1	N/A	1	2	1	1
	Min	10	15	10	10	N/A	10	12	15	12
	Max	17	17	13	12	N/A	12	17	17	13

Table 2. Multivariate regression model of changes in mean HFMSE score over time

Effect	Estimate	Standard error	DF	t value	Pr > |t|	Alpha	95% CI	
Intercept	19.7618	4.7957	81.5	4.12	<.0001	0.05	10.2207	29.3028
Slope before k	1.5076	0.3388	36.5	4.45	<.0001	0.05	0.8209	2.1943
Slope after k	-2.5911	0.4074	49.3	-6.36	<.0001	0.05	-3.4096	-1.7725
Type IIIB	11.8076	2.1183	134	5.57	<.0001	0.05	7.6178	15.9973
Male gender	-1.6106	1.6597	83.2	-0.97	0.3347	0.05	-4.9117	1.6904
Walker	13.6029	0.8745	585	15.56	<.0001	0.05	11.8854	15.3204
SMN2 copy number	2.6950	1.4081	84.8	1.91	0.0590	0.05	-0.1047	5.4948

Table 3. Estimates table.

Effect	TYPE	walker	Estimate	Standard error	DF	t value	Pr > |t|	Alpha	95% CI	
Intercept			24.5404	4.9784	70.5	4.93	<.0001	0.05	14.6125	34.4682
Slope before k			1.8560	0.7477	78.3	2.48	0.0152	0.05	0.3675	3.3445
Slope after k			-2.9422	0.8078	91.4	-3.64	0.0004	0.05	-4.5467	-1.3376
TYPE	B		33.2711	9.1833	10.9	3.62	0.0041	0.05	13.0355	53.5067
Slope before k *TYPE	B		-3.7678	1.3512	13.7	-2.79	0.0148	0.05	-6.6722	-0.8634
Slope after k *TYPE	B		4.2276	1.4270	19.3	2.96	0.0079	0.05	1.2440	7.2113
Ambulatory status		walker	15.5700	5.1676	75.1	3.01	0.0035	0.05	5.2759	25.8640
Slope before k * Ambulatory status		walker	-0.1255	0.7780	82.5	-0.16	0.8722	0.05	-1.6730	1.4219
Slope after k * Ambulatory status		walker	-0.01057	0.8344	96.5	-0.01	0.9899	0.05	-1.6668	1.6457

Table 4. Descriptive statistics on Age, HFMSE at baseline, 12 months and HFMSE 12-month changes subdivided by SMA III type and ambulatory status.

ALL 12-MONTH ASSESSMENTS (Assessments: 375, patients: 123)															
ALL (N:375)					NON AMBULANT (N:97)					AMBULANT (N:278)					
	AGE	BASELINE	12M	CHANGES		AGE	BASELINE	12M	CHANGES		AGE	BASELINE	12M	CHANGES
MEAN	11.57	45.37	44.15	-1.22	MEAN	15.92	24.06	21.60	-2.45	MEAN	10.05	52.81	52.01	-0.79
SD	6.21	15.37	16.21	4.37	SD	6.67	11.15	10.54	4.23	SD	5.26	7.83	8.74	4.34
MIN	2.50	4.00	3.00	-22.00	MIN	3.03	4.00	3.00	-22.00	MIN	2.50	30.00	30.00	-17.00
MAX	29.60	66.00	66.00	10.00	MAX	28.43	45.00	45.00	5.00	MAX	29.60	66.00	66.00	10.00
SMA IIIA 12 MONTH-ASSESSMENTS (N:305)															
ALL (N:305)					NON AMBULANT (N:85)					AMBULANT (N:220)					
	AGE	BASELINE	12M	CHANGES		AGE	BASELINE	12M	CHANGES		AGE	BASELINE	12M	CHANGES
MEAN	10.37	43.73	42.51	-1.22	MEAN	14.73	23.76	21.20	-2.56	MEAN	8.69	51.45	50.75	-0.70
SD	5.68	15.19	16.07	4.57	SD	6.18	11.64	10.90	4.31	SD	4.46	7.33	8.26	4.57
MIN	2.50	4.00	3.00	-22.00	MIN	3.03	4.00	3.00	-22.00	MIN	2.50	30.00	30.00	-17.00
MAX	28.43	65.00	65.00	10.00	MAX	28.43	45.00	45.00	5.00	MAX	26.76	65.00	65.00	10.00
SMA IIIB 12 MONTH-ASSESSMENTS (N:70)															
ALL (N:70)					NON AMBULANT (N:12)					AMBULANT (N:58)					
	AGE	BASELINE	12M	CHANGES		AGE	BASELINE	12M	CHANGES		AGE	BASELINE	12M	CHANGES
MEAN	16.79	52.51	51.27	-1.24	MEAN	24.38	26.17	24.50	-1.67	MEAN	15.22	57.97	56.81	-1.15
SD	5.75	14.14	14.91	3.49	SD	2.73	6.66	7.20	3.70	SD	4.90	7.56	8.77	3.34
MIN	5.79	14.00	12.00	-14.00	MIN	20.19	14.00	12.00	-9.00	MIN	5.79	38.00	33.00	-14.00
MAX	29.60	66.00	66.00	5.00	MAX	28.40	33.00	36.00	3.00	MAX	29.60	66.00	66.00	5.00