Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Regensburger A. P.*, Wagner A. L.* et al. "**Multispectral Optoacoustic Tomography for non-invasive disease phenotyping in pediatric spinal muscular atrophy patients**"

Supplementary Table 1 – Physical examination assignment

< 2 years	≥ 2 years and no ability to sit	≥ 2 years and ability to sit in wheelchair	≥ 2 years and ability to sit	≥ 3 years and ability to walk
HINE Section 2	HINE Section 2	HINE Section 2		
CHOP Intend	CHOP Intend	CHOP Intend		
		(HFMSE)	HFMSE	HFMSE
		RULM	RULM	RULM
				6MWT

Supplementary Table 1 – Physical examination assignment

HINE= The Hammersmith Infant Neurological Examination, Section 2 (measures motor milestones, range 0-26; lower score represents a lower development of motor milestones),[1, 2] CHOP-Intend= The Childrens's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (measures motor skills, range 0-64, lower score represents a lower repertoire of motor skills),[3, 4] HFMSE= Hammersmith functional motor scale-expended (measures motor function, range 0-66, lower score represents lower motor function),[5-7] RULM= Revised upper Limb Module (measures upper limb function, range 0-37, lower score represents lower upper limb motor function),[8] 6-MWT=6-minute-walk-test (measures walking distance in meter within six minutes; range: 0-theoretically infinite; lower distance represents a higher degree of muscle function loss).[9] All subjects were tested by three well-trained physiotherapists (J.T., P.P., M. M.-A.) with respect to their age and physical function prior to ultrasound and MSOT imaging.

Supplementary Table 2 – Duration of examinations

Duration of examinations		
	HV (n = 10)	SMA (n = 10)
Duration of physical examination-min.	32.5±6.0	40.3±10.1
Duration of Ultrasound-min.	10.7±2.8	15.1±4.5
Duration of MSOT-min.*	32.3±6.8	40.9±4.4

Supplementary Table 2 – Duration of examinations

Min.= minutes, HV=healthy volunteers, SMA = SMA patients, mean±SD are labeled Plus-minus values. Data are shown as mean±SD. n = 20 biologically independent subjects (n = 10 HV/n = 10 SMA). * door to door time including explanations and breaks, if necessary.

Supplementary Table 3 – Physical examination of SMA patients and healthy volunteers

Physical examination of HV and SMA patients		
	HV (n = 0)	SMA (n = 3)
HINE – score	-	4.3±1.16
	HV (n = 0)	SMA (n = 2)
CHOP-Intend – score	-	25.0±5.7
	HV (n = 10)	SMA (n = 9)
HFMSE – score	65.6±1.0	25.7±21.8
RULM –score	36.8±0.4	24.0±10.5
	HV (n = 10)	SMA (n = 2)
6-MWT – meter	538.0±94.2	264.5±14.9

Supplementary Table 3 – Physical examination of SMA patients and healthy volunteers HV=healthy volunteers, SMA=SMA patients, mean ±SD are labeled Plus-minus values. Data are shown as mean±SD. n = 20 biologically independent subjects (n = 10 HV/n = 10 SMA). Physical tests were not uniformly completed. Incomplete tests were excluded for analysis. HINE= The Hammersmith Infant Neurological Examination, Section 2 (measures motor milestones, range 0-26; lower score represents a lower development of motor milestones),[1, 2] CHOP-Intend= The Childrens's Hospital of Philadelphia Infant of Neuromuscular Disorders (measures motor skills, range 0-64, lower score represents a lower repertoire of motor skills) [3, 4], HFMSE= Hammersmith functional motor scale-expended (measures motor function, range 0-66, lower score represents lower motor function) [5-7], RULM= Revised upper Limb Module (measures upper limb function, range 0-37, lower score represents lower upper limb motor function) [8], 6-MWT=6-minute-walk-test (measures walking distance within six minutes; range: 0-theoretically infinite; lower distance represents a higher degree of muscle function loss) [9].

Supplementary Table 4 – B-mode ultrasound results of independent muscle regions

	Ultrasound Scoring	HV (N=10, N=80 scans)	SMA (N=10, N=80 scans)
Echogenicity	hypo-echogenic	80 (100%)	7 (8.75%)
	echogenic	0	10 (12.5%)
	hyper-echogenic	0	63 (78.75%)
Muscle texture	coarse-granular	4 (5%)	10 (12.5%)
	medium-granular	45 (56.25%)	0
	fine-granular	31 (38.75%)	70 (87.75%)
Distribution pattern	Focal	0	0
	Inhomogeneous	80 (100%)	80 (100%)
	Homogeneous	0	0
Heckmatt scale	1	80 (100%)	13 (16.25%)
	2	0	16 (20%)
	3	0	34 (42.50%)
	4	0	17 (21.25%)
Pathological	No	80 (100%)	8 (10%)
	Yes	0	72 (90%)

Supplementary Table 4 – B-mode ultrasound results of independent muscle regions

HV=healthy volunteers, SMA=SMA patients. N=160 images (n = 80 HV/ n= 80 SMA) were evaluated for echo intensity, muscle texture, distribution pattern, Heckmatt scale, and pathological rating. The investigator (JJ) assessed echogenicity (hypoechogenic/echogenic/hyperechogenic), muscle texture (coarse-/medium-/fine-granular), distribution pattern (inhomo-/homo-geneous/focal) and Heckmatt scale (grade 1-4: 1 = normal muscle echo, 2 = increased muscle echo while bone echo is still distinct, 3 = increased muscle echo and reduced bone echo, 4 = very strong muscle echo and complete loss of bone echo) in parallel to the examination.[10, 11] Furthermore, the muscle was

evaluated by the overall impression as healthy or pathological. Categorical variables are provided as numbers and percentages. n = 160 independent muscle regions (n = 80 HV/n = 80 SMA) in n = 20 biologically independent subjects (n = 10 HV/n = 10 SMA patients).

Supplementary Table 5 – Adverse events

	HV N=10	SMA patients N=10
Reversible adverse events- no. (%)		
Coolness of Ultrasound-gel	1 (10%)	1 (10%)
Serious adverse events- no. (%)	0 (0%)	0 (0%)

Supplementary Table 5 – Adverse events

In each group one patient complained about the coolness of the ultrasound-gel. The gel was then removed. No serious adverse events occurred during the study.

References

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