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ORIGINAL PAPER

In spina bifida aperta, muscle ultrasound can quantify the "second hit of damage"

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Abstract
Purpose In spina bifida aperta (SBA), the "second-hit hypothesis" addresses consequences by delayed neurological damage superimposed upon the congenital myelomeningocele (MMC). This secondary damage is postulated to underlie the disappearance of leg movements shortly after birth. Innovative fetal surgery might prevent this, but results are methodologically hard to prove in small and heterogeneous treatment groups. We reasoned that delayed postnatal alterations in muscle ultrasound density (MUD = muscle echogenicity) could quantitatively reflect consequences by "the second hit" of damage. In the present study, we investigated whether delayed postnatal leg-MUD alterations are associated with postnatal muscle function loss.
Methods We cross-sectionally assessed leg-MUD in 16 postnatally operated SBA children (MMC-L5; at 0, 6, and 12 months; in n=11/16; 11/16, and 15/16 children, respectively) and compared outcomes with 13 healthy control children. Additionally, we assessed SBA MUD *caudal* and *cranial* to the MMC and calculated MMC-L5 impact by: $dMUD_{(MMC\text{-}L5)} = [MUD_{calf\ muscle/S1-2}] - [MUD_{quadriceps\ muscle/L2-4}]$ and associated outcomes with leg muscle function caudal to the MMC.
Results At 0 month, clinically discernible dMUD was more often increased in SBA than in control newborns (p<.05), but a relationship between absolute quantitative differences and leg muscle dysfunction was still lacking. At 6–12 months, additionally increased dMUD outcomes coincided with SBA leg muscle dysfunction (p<.05).
Conclusions In post-neonatal SBA, secondarily increased dMUD (i.e., MMC impact) coincides with leg muscle dysfunction. This may implicate that muscle ultrasound could provide a quantitative tool to assess the neuromuscular impact by the second hit of damage.

Keywords Spina bifida · Myelomeningocele · Muscle ultrasound density · Second-hit hypothesis · Fetal surgery · Muscle damage

Introduction

In spina bifida aperta (SBA), leg movements caudal to the myelomeningocele (MMC) are transiently present before birth but disappear shortly thereafter [24–26]. This phenomenon has been attributed to the "second hit of damage" involving delayed, secondary neurological damage (for instance by the consequences of spinal bleedings, neurotoxicity, and mechanical trauma) superimposed upon the congenital MMC [5, 8, 12, 13, 18–20, 24, 27]. Innovative fetal therapies may reduce the consequences by the second hit of damage and (partly) preserve motor function [1, 6, 7, 14]. Although the randomized controlled, multicenter *Management of Myelomeningocele Study* (MOMS) trial [1, 7] has proven this gain, smaller European studies may still warrant more explicit quantitative parameters for

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neuromuscular validation [29]. Especially regarding inter-individual heterogeneity concerning underlying spinal and cerebral defects and iatrogenic morbidity, transparent and objective quantitative parameters are needed [2, 4, 24–26].

The muscle ultrasound technique may provide a quantitative tool for neuromuscular assessment after the second hit of damage [9, 10, 15–17, 21, 22]. In SBA fetuses, we have shown that the parameter "muscle ultrasound density" (MUD) can reflect histological muscle impairment (involving muscle fibrosis, fat deposition, and atrophy [28]). After the "second hit" upon the neuromuscular condition, histological muscle alterations would be expected after a latent time interval of weeks to months. In this perspective, we hypothesized that SBA newborns would reveal relatively small *congenital* MUD increases, whereas *secondarily* increased MUD in association with leg muscle dysfunction would be expected later. If so, leg-MUD could provide a quantitative tool to assess and compare the consequences by the second hit of damage between treatment strategies.

Patients and methods

The medical ethical committee of the University Medical Center Groningen approved the study. With informed parental consent, we retrospectively compared leg-MUD parameters between 16 SBA (MMC at L5 (range L4–S1)) and 13 control infants. SBA infants were born at 38 (35–40) weeks and controls at 40 (38–41) weeks gestational age (medians (ranges)). In all SBA infants, the MMC was closed during the first postnatal week. All SBA infants, except one, revealed Chiari II malformations. Delivery mode involved vaginal delivery or cesarean section (11 vs 12 and 5 vs 1; SBA vs controls, respectively). Cesarean section was either performed electively (two vs zero) or after the initiation of labor (three vs one; SBA vs controls, respectively). Clinical data are indicated in Table 1. All control infants were delivered after an uneventful pregnancy in the absence of perinatal complications or neurological abnormalities.

Muscle ultrasound and neurological assessments

In SBA and healthy control children, we assessed leg-MUD at 0, 6, and 12 months postnatal age (11, 11, and 15 vs 13 children; SBA vs controls, respectively). Since assessments were not possible in all SBA children at all time points, we performed cross-sectional comparison between SBA and healthy control children at 0, 6, and 12 months postnatal age. We determined segmental MMC levels by spinal MRI. By deliberately selective inclusion of MMC levels at L5 (MMC range L4–S1), MUD *caudal* to the MMC was uniformly standardized as MUD of calf muscle (innervated by S1–2, i.e., caudal to the MMC_{L5}) and MUD *cranial* to the MMC were uniformly standardized as MUD of the quadriceps muscle (innervated by L2–4, i.e., cranial to the MMC_{L5}),

Table 1 Clinical data of included SBA infants

Infant no.	AS at 1 and 5 min	Upper level MMC	Shunt dependency	No. of shunt dysfunctions at 6/12 months	Other cerebral pathology	Other spinal pathology
1	*x*/10	L4	+	2/2	CCA	–
2	7/9	L4	–[a]	–[a]	H	Syrinx
3	9/10	L4	+	0/1	CCA, HT	Syrinx
4	*x*/*x*	L4	+	2/2	–	Syrinx, TC
5	8/9	L5	+	3/3	–	Syrinx
6	9/10	L5	+	2/2	CCA, H	Syrinx
7	5/9	L5	+	2/2	CCA, H	Syrinx
8	8/10	L5	+	0/2	CCA	TC
9	4/8	L5	+	4/4	H	Syrinx, TC
10	6/9	L5	+	1/1	HT	–
11	7/9	L5	+	2/3	HT	Syrinx
12	9/10	S1	+	4/4	CCA	–
13	9/10	S1	+	–	–	TC
14	*x*/*x*	S1	+	4/4	–	Syrinx
15	9/10	S1	–	–	–	TC
16	6/8	S1	–	–	–	Syrinx, TC

SBA spina bifida aperta, *AS* apgar score, *x* missing data, *MMC* myelomeningocele, *L* lumbar, *S* sacral, + present, − absent, *CCA* corpus callosum agenesis, *H* hemorrhage, *HT* heterotopies, *TC* tethered cord

[a]Perinatal decease due to cerebral/cardiopulmonary instability

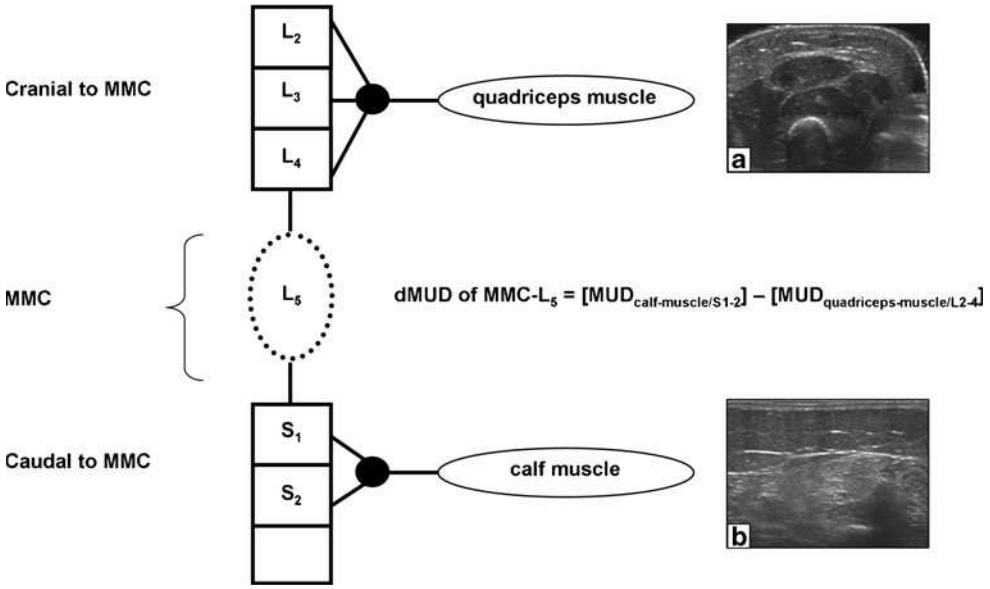

Fig. 1 Schematic representation of muscle involvement cranial and caudal to the MMC. In SBA-MMC_{L5} infants, the quadriceps muscle (L2–4) is innervated cranial to the MMC and the calf muscle (S1–2) is innervated caudal to the MMC. To derive the impact by the MMC, we calculated the intra-individual difference between MUD caudal and cranial to the MMC by: $dMUD_{(MMC\text{-}L5)}=[MUD_{calf\ muscle/S1–2}]-[MUD_{quadriceps\ muscle/L2–4}]$. The images on the right side of the figure represent examples of the quadriceps (*a*) and calf muscles (*b*) in MMC_{L5} infants. *SBA* spina bifida aperta, *MMC* myelomeningocele, *L* lumbar, *S* sacral, *MUD* muscle ultrasound density, *dMUD* intra-individual difference in muscle ultrasound density

Fig. 1. The *intra-individual* neuromuscular impact by the MMC (dMUD) can thus be calculated as: dMUD of MMC-L5=$[MUD_{calf\ muscle/S1–2}]-[MUD_{quadriceps\ muscle/L2–4}]$, Fig. 1. Subsequently, we compared dMUD between SBA and control children.

Neurological assessment of leg muscle function was performed by an independent clinical pediatric neurologist who was not involved in the study. For the association between MUD calf muscle and calf muscle function, we subdivided all SBA infants into two groups with either dysfunctional or functional calf muscle contractions (i.e., muscle force grade <3 and grade >3, respectively).

All muscle ultrasound recordings were performed under standardized conditions by an independent technician using *General Electric Healthcare LOGIQ 9* ultrasound equipment (Jiangsu, China) with a 14-MHz linear probe [16, 17]. According to standardized reference points, the technician recorded transverse ultrasound images of the quadriceps muscle in supine (probe placed halfway between trochanter major and lateral knee joint cleft) and of the calf muscle in prone position (probe placed at position of maximum circumference). All muscles were recorded during muscle relaxation [16, 17]. MUD assessment was performed (off line) by an independent investigator who was neither informed about the clinical condition of the child or nor about the muscle ultrasound recording. For digital quantification, the assessor determined five muscle ultrasound images per muscle and excluded the highest and lowest values and calculated the mean of the three remaining MUD values.

Since dMUD needs to be substantial enough to be clinically relevant (i.e., needs to represent outcomes that can be clinically visualized by the "naked" eye), we applied a MUD cutoff point for clinical relevance. In a previous study concerning the visual detection by 20 observers of 100 SBA MUD image sets, we have previously shown that a dMUD difference of 10–15 gray values can be visualized with a sensitivity of 86–92 % (i.e., representing the cutoff point for a sensitivity exceeding 80 %) [3].

Statistical analysis

We performed statistical analysis by PASW Statistics version 18.0 (SPSS, Chicago, IL). We compared the frequency of visibly discernible dMUD outcomes between SBA and controls by Chi-square test (cutoff point of 10–15 gray values). Since MUD values were not normally distributed (according to Q-Q plots and the Shapiro–Wilk test), we used the nonparametric Mann–Whitney *U* test for MUD and dMUD comparison with calf muscle function. Statistical significance was set at $\alpha=.05$.

Results

The impact by the MMC (dMUD) at 0, 6, and 12 months postnatal age

At all three time points (0, 6, and 12 months), dMUD was more often clinically significantly increased in SBA than in controls ($p<.05$, Chi-square test). However, especially at 0 months, SBA and control dMUD revealed a quantitative overlap, with only small differences between the means, outside the visually detectable range (Fig. 2)[1]. At 6 and 12 months, SBA leg-dMUD outcomes increased within the clinically significantly detectable range, which was contrasted by control outcomes.

Association between the MMC impact (dMUD) and muscle dysfunction caudal to MMC

At 0 month, SBA dMUD subdivision in accordance with newborn functional and dysfunctional calf muscles revealed no significant differences (Fig. 3). At 6 and 12 months, SBA dMUD was higher in the subgroup with dysfunctional than with functional calf muscles ($p<.05$, Mann–Whitney); Fig. 3a. All children with dysfunctional calf muscles revealed dMUD outcomes at, or above, visual detection levels [6 months, 31 (16–40) vs 6 (−6–25) and 12 months, 27 (10–39) vs 5 (1–24); medians (ranges); absent vs present plantar flexion, respectively; both $p<.05$], Fig. 3a.

Association between muscle ultrasound density and muscle function caudal to the MMC

At 0 month, SBA MUD caudal to the MMC (represented by MUD calf muscle) was not associated with muscle dysfunction caudal to the MMC (i.e., calf muscle dysfunction). At 6 and 12 months, SBA MUD was higher in the subgroup with dysfunctional than with functional calf muscles [6 months, 120 (94–133) vs 89 (82–104) and 12 months, 118 (72–145) vs 96 (70–113); medians (ranges); functional vs dysfunctional plantar flexion, respectively; both $p<.05$], Fig. 3b.

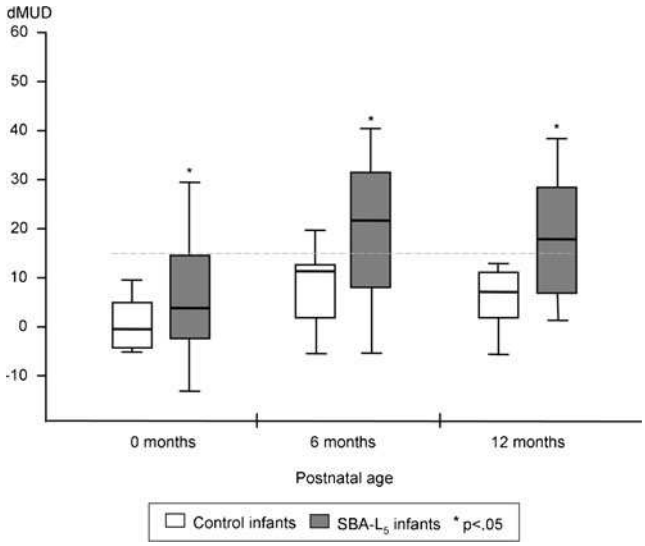

Fig. 2 Age-related comparison of dMUD between SBA and control infants. The *x*-axis indicates 0, 6, and 12 months postnatal time intervals. The *y*-axis indicates the intra-individual difference in muscle ultrasound density between leg myotomes S1 and L2–4 (i.e., for MMC_{L5} caudal and cranial to the MMC, respectively). The *broken line* demarcates the upper level for clinical relevance (at 10–15 gray values). The intra-individual difference in muscle ultrasound density is calculated by: $dMUD=[MUD_{calf\ muscle}]-[MUD_{quadriceps\ muscle}]$. At all three time points (0, 6, and 12 months), dMUD was more often clinically significantly increased in SBA than in controls ($p<.05$, chi-square test). However, only at 6 and 12 months, SBA leg-dMUD outcomes increased within the clinically significantly detectable range, which was contrasted by control outcomes. *dMUD* intra-individual difference in muscle ultrasound density, *SBA* spina bifida aperta

Discussion

In SBA, the randomized MOMS trial has shown a significant neuroprotective treatment effect [1, 7]. However, quantitatively objective neuromuscular parameters are still warranted to evaluate the impact by the second hit of damage in smaller European studies. Directly after birth, nonparametric testing revealed a clinically detectable impact by the MMC upon muscle ultrasound density (dMUD) in the SBA group. However, newborn quantitative dMUD comparison revealed only small differences which did not differentiate between functional and dysfunctional leg muscles. At 6 and 12 months, dMUD increased within clinically visible detection limits, in the majority of SBA children. At the same time points, subanalysis showed an association between dMUD outcomes and leg muscle dysfunction. Regarding the hypothesis, it is therefore tempting to speculate that subtle neonatal differences could refer to the "first hit" of damage, whereas subsequently increased dMUD alterations in association with leg muscle dysfunction are more likely to refer to the "second hit" of damage [1, 11, 14, 29].

Despite selective inclusion criteria involving MMC-L5, we still observed a considerable variation in SBA dMUD outcome parameters. This may be explained by inter-individual heterogeneity of SBA lesions. Since

[1] Leg-dMUD in SBA vs controls [0 months, 3 (−15 to 29) vs 0 (−5 to 10); 6 months, 22 (−6 to 40) vs 11 (−6 to 19); and 12 months, 18 (1–39) vs 8 (−6–13); medians (ranges)]

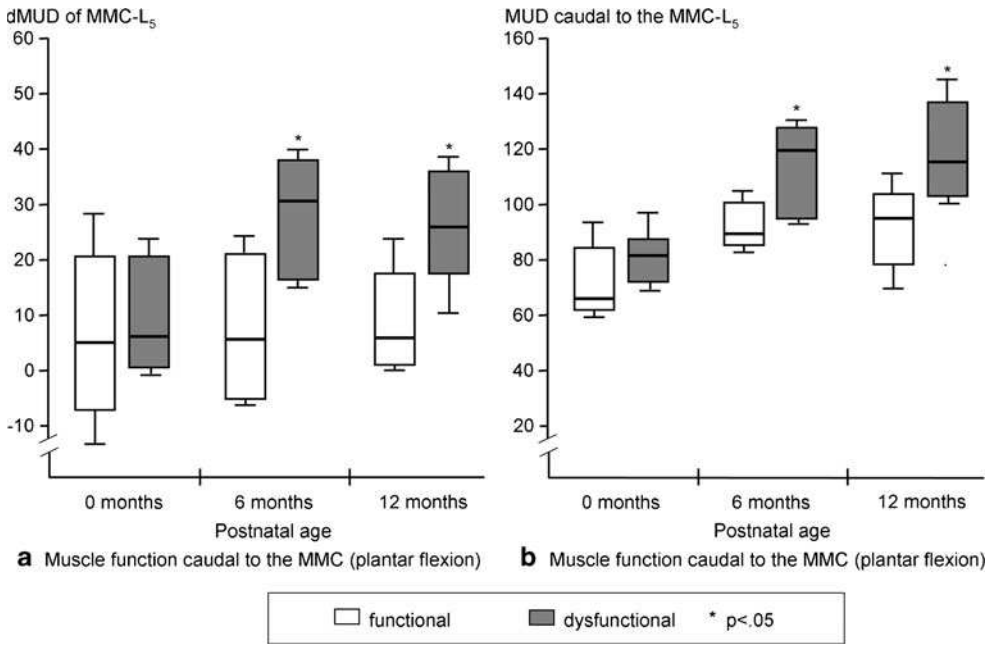

Fig. 3 Association between the MMC impact (dMUD) and muscle dysfunction caudal to MMC. **a** Relationship between dMUD and plantar flexion of the foot. The *x*-axis indicates postnatal time intervals. The *y*-axis indicates the intra-individual difference in muscle ultrasound density between leg myotomes caudal and cranial to the MMC (represented by: dMUD at MMC_{L5}=[$MUD_{calf\ muscle}$]−[$MUD_{quadriceps\ muscle}$]). The *white* and *gray boxes* indicate "functional" and "dysfunctional" plantar flexion of the foot. After the neonatal period, i.e., at 6 and 12 months postnatal age, dMUD is associated with dysfunctional plantar flexion ($p<.05$). **b** Relationship between MUD calf muscle and muscle function (foot plantar flexion) caudal to the MMC. The *x*-axis indicates postnatal age groups. The *y*-axis indicates MUD caudal to the MMC (i.e., MUD of the calf muscle). The *white* and *gray boxes* indicate "functional" and "dysfunctional" plantar flexion, respectively. After the neonatal period, i.e., at 6 and 12 months postnatal age, MUD caudal to the MMC is associated with calf muscle dysfunction ($p<.05$). *dMUD* intra-individual difference in muscle ultrasound density, *MUD* muscle ultrasound density, *MMC* myelomeningocele, *SBA* spina bifida aperta

dMUD outcomes are calculated as the intra-individual MUD difference between myotomes cranial and caudal to the MMC, large cerebral defects could falsely reduce dMUD outcomes. For clinical application, this may implicate that usage of dMUD parameters should either be reserved for very carefully matched pairs and/or for intra-individual longitudinal assessments. However, at this moment, we still have to await longitudinal results before we can decide whether this parameter is applicable for surveillance purposes (such as for detection of tethering).

We recognize that there are several weaknesses to this study. Firstly, we studied a small number of SBA children with MMC-L5 levels, only. We had deliberately chosen for this selective inclusion, so that potential bias by MUD assessment in heterogeneous muscles was avoided. However, if we would compare dMUD in infants with different segmental MMC levels, we would expect similar results, provided that dMUD assessment is adapted to appropriate segmental levels cranial and caudal to the MMC. Regarding significant outcomes in this well-selected MMC-L5 group, we would therefore suggest that results may be regarded as indicative. Furthermore, we had clinically characterized muscle contractions as "functional" and "dysfunctional." Although this was deliberately chosen for clinical relevance, we recognize that SBA children may also reveal more subtle graduations of muscle dysfunction. Finally, we accepted a small age range between the 0, 6, and 12 months age groups. However, since MUD in healthy control children is age-independent [23], we would not expect that this could have substantially influenced these results.

Conclusion

In SBA infants during the first year of life, postnatal SBA leg-MUD parameters can quantitatively reflect delayed neuromuscular consequences by the second hit of damage.

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Conflict of interest The authors declare that they have no conflict of interest.

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