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RESEARCH ARTICLE

Glial fibrillary acidic protein as a serum neuromarker of brain injury in pediatric patients with congenital heart defects undergoing cardiac surgery

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Objective: The aim of this study was to assess glial fibrillary acidic protein (GFAP) as a marker of short-term neurodevelopmental delay in pediatric patients with congenital defects (CHD) after cardiovascular surgical intervention. **Methods**: Included patients were screened by Denver Developmental Screening Test II scale a few days before and then at 4 to 6 months after the surgical intervention. Blood samples were collected preoperatory and at 24 hours after surgery; GFAP levels were assessed by enzyme-linked immunosorbent assay using commercial kit form BioVendor. **Results**: Forty children were enrolled and dichotomized into two groups based on peripheric oxygen saturation: cyanotic (<95%) and non-cyanotic (>=95%) group. 63% from our population had an abnormal neurodevelopmental outcome. Significant differences between groups were found in language domain scores preoperatory (p=0.03) and in fine motor domain postoperatory (p=0.03). In the postoperatory period, GFAP had significantly higher values (p=0.0248) in the cyanotic CHD group. Association between GFAP and NIRS were analyzed and significant differences were found in both groups with a good predicting model in the non-cyanotic CHD group (aria under curve of 0.7 for receiver operative characteristic). Higher GFAP levels from the postoperatory period correlated with neurodevelopmental impairment (mean value of: 0.66 ± 0.02ng/ml in those with good neurodevelopmental score, 0.69 ± 0.02ng/ml in those with low neurodevelopmental score, p=0.01). **Conclusions**: GFAP could be a reliable neuromarker in identifying early acute brain injury documented by NIRS monitorization during perioperatory period and it also could identify short term neurodevelopmental impairment documented by lower neurodevelopmental scores.

Keywords: congenital heart defect, glial fibrillary acidic protein, neurodevelopment

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Introduction

The most common extracardiac problem encountered in pediatric patients with congenital heart disease (CHD) is neurodevelopmental impairment, in a percent that reaches 50% in some epidemiological studies [1]. There are multiple factors that can precipitate neurodevelopmental impairment but just a few of them could be influenced like those from the surgical period (surgery duration, cardiopulmonary bypass time, aortic cross clamp time, induced hypothermia) or those from the period of intensive care unit admission (ventilation duration, associated infections, hemodynamically instability, number of days in the intensive care unit) [2]. Scientists studied factors like albumin, inotropic scores, echocardiographic parameters, neuromarkers that could be associated with neurological impairment [3,4]. The best diagnostic and prognostic role of these factors is held by neuromarkers. Some of them have already been studied in the field of CHD requiring surgery, like neuron specific enolase, protein S100B, glial fibrillary acidic protein (GFAP), activin A, adrenomedullin and others represent new fields for research, like Tau protein, myelin basic protein, neurofilaments, monocyte chemoattractant protein, intracellular adhesion molecule 5, metalloproteases 12, ubiquitin C terminal hydroxylase-L1 [5].

The aim of this study was to assess GFAP as a serum marker of neurological damage associated with short term neurodevelopmental delay in pediatric patients with CHD after cardiovascular surgical intervention.

Methods

Study design

We conducted a prospective study on forty pediatric patients requiring cardiovascular surgery due to a congenital heart defect, which were admitted to Pediatric Cardiology Department of Emergency Institute for Cardiovascular Diseases and Heart Transplant in Targu Mures, Romania. The ethical committee approval was registered under number 1161 form 10/26/2020 (George Emil Palade University of Medicine, Pharmacy, Sciences and Technology of Targu Mures). We included patients with age span from neonatal period until 6 years, which underwent surgery for correction of a CHD. We excluded patients with diseases that could affect the neurodevelopmental process like prematurity, genetic disorders, neurologic disorders, other congenital anomalies, surgical interventions before inclusion in the study. We also excluded patients which did not speak Romanian or institutionalized children because

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the neurological development had to be encouraged by a guardian. Written consent of parents or guardians was obtained before inclusion in the study.

Patients' characteristics

Forty pediatric patients diagnosed with CHD and admitted for surgery between January 2022 to February 2023 were enrolled. Patients with no complex cardiac defects were chosen and dichotomized into two groups based on peripheric oxygen saturation (SpO2): cyanotic (SpO2<95%) and non-cyanotic (SpO2>=95%) groups as shown in table I.

Table I. Types of congenital heart defects of children included in the study (number of patients)

Cyanotic defects (n=13)	Non-cyanotic defects (n=27)
Septal defects	
- Unbalanced atrioventricular septal defect (1)	- Ventricular septal defect (20) - Atrial septal defect (1)
Conal defects	
- Double outlet right ventricle with pulmonary stenosis (5) - Tetralogy of Fallot (7)	- Tetralogy of Fallot without cyanosis (3)
Others	
	- Coarctation of aorta (2) - Patent ductus arteriosus (1)

Neurodevelopmental assessment

Neurodevelopmental acquisitions were assessed first a few days before surgery and then at 4 to 6 months after the surgical intervention by Denver Developmental Screening Test II scale (DDST II) [6]. It evaluates children according to age with items grouped in four domains: personal-social behavior, fine-motor adaptive function, language and gross motor function. We used a DDST II adaptation system for children with medical complex conditions like CHD [7]. This adaptation system includes four levels of development for each child: the basal level of competence = first three successive age-corresponding items passed, all passed through level = highest item passed before failure, highest item passed level = highest item passed beyond failure and highest item passed before consistent failure level = highest item passed before three consecutive failed items. The adaptation system also includes some calculated indicators: domain-specific developmental functioning estimates (DFE) = (baseline level + highest item passed before consistent failure) /2, overall developmental functioning estimates = mean of the four domain-specific developmental functioning estimates, developmental quotient score = developmental functioning estimate / chronological age, developmental gain score = (overall DFE evaluation 2 – overall DFE evaluation 1) / total number of days between assessments. A developmental score of 1 means that the child's development has progressed at the expected rate, a score above 1 means more progress than expected and a score below 1 means delayed development.

Samples collection and assay

For every child, samples were collected first preoperatory, after anesthesia induction and secondly at 24 hours after surgery. Samples were stored at 2-4 °C for a couple of hours and allowed to clot, after which were centrifugated 5 minutes at 3000 rotation per minute. Aliquots were taken and stored at -80 °C. The aliquots were than managed by enzyme-linked immunosorbent assay (ELISA) using commercial kit form BioVendor in order to determine GFAP levels.

Near-infrared spectroscopy monitoring (NIRS

In the perioperatory period, cerebral tissue oxygen saturations monitoring was done with a NIRS probe placed on the patient's midline forehead. NIRS data was recorded and values form different operatory moments were analyzed (initial value = NIRS value before beginning of the surgery; intraoperatory NIRS values – minimum, average, maximum; final value = NIRS value at the end of the surgery). Low cerebral tissue oxygen saturations were noted if a difference >= of 20% between the maximum and minimum NIRS values was registered during perioperatory period.

Statistical analysis

For statistical analysis, Stata version 13 and GraphPad InStat were used. Normality of data was assessed by Shapiro-Wilk test. Association was assessed by parametric (t test) and non-parametric (Mann–Whitney U test, Wilcoxon matched pair test) tests. Correlation was assessed by Spearman's correlation test and receiver operating characteristic (ROC) analysis was calculated. Probability (two-tail p) value less than 0.05 was considered statistically significant.

Results

Anatomical, clinical and surgical characteristics of patients

The two groups of included patients were uniform with statistically significant difference only between clinical characteristics which are associated with cyanosis (SpO2, hemoglobin) and cyanotic-defects related surgery and postoperatory care (surgery duration, CBP duration, aortic clamp duration, mechanical ventilation duration and ICU admission period). Patients' neonatal, clinical and surgical characteristics are represented in table II.

Neurodevelopmental assessment by DDST II

Neurodevelopmental status of each child was calculated pre and postoperatory and the two groups (cyanotic and non-cyanotic) were compared based on developmental levels, domain-specific developmental functioning estimates, developmental quotient scores and developmental gain score as represented in table III and table IV. A percent of 63% from our population had abnormal neurodevelopmental outcome.

Table II. Patients' neonatal, clinical and surgical characteristics. Data is represented as mean ± SD, median, percent (%) or n (total number).

Characteristics	Non-cyanotic CHD (n=27)	Cyanotic CHD (n=13)	P value
Neonatal characteristics			
Gestational age (weeks)	38.7 ± 1.6	38.6 ± 2	0.66
Apgar score	9	9	0.11
Birth weight (g)	3118 ± 354	3442 ± 722	0.66
Birth length (cm)	51.7 ± 3.6	53.4 ± 4.5	0.23
Alimentation (breast milk/formula/mixt)	10/14/3	7/3/3	0.43
Maternal age at birth (years)	27.9 ± 5.2	27.08 ± 4.9	0.97
Paternal age at birth (years)	31.2 ± 4.7	30.5 ± 6.03	0.7
Clinical characteristics			
Sex (M/F)	17/10	7/6	0.65
Age (months)	13.5 ± 15	9.8 ± 5	0.64
Weight (kg)	7.82 ± 3	8.09 ± 1	0.27
Height (cm)	71.8 ± 12	70.69 ± 5	0.47
Head circumference (cm)	43.6 ± 3	43.9 ± 2	0.64
Saturation (%)	94 ± 17	82 ± 8	**<0.0001**
Hemoglobin (g/dl)	11.8 ± 1.3	13.55 ± 2.4	**0.046**
Albumin (g/dl)	4.64 ± 0.3	4.5 ± 0.2	0.28
Surgical characteristics			
Surgery duration (min)	200 ± 48	265.7 ± 56	**0.006**
CBP duration (min)	84.9 ± 29	131 ± 42	**0.02**
Aortic clamp duration (min)	51.9 ± 21	82 ± 35	**0.002**
Mechanical ventilation duration (hours)	11.3 ± 15	80.7 ± 110	0.01
ICU admission period (days)	4.1 ± 1.8	7 ± 4.9	**0.009**

Abbreviations: CHD= congenital heart defect; CPB=cardiopulmonary bypass; ICU=intensive care unit.

Table III. Preoperatory neurodevelopmental comparison of studied patients (non-cyanotic CHD group in normal writing, cyanotic CHD group in *italic* writing)

Level / Domain	Personal/Social	Fine Motor	Language	Gross Motor
Domain-specific Developmental Functioning Estimates	384 ± 414 *241 ± 147* P=0.52	466 ± 453 *331 ± 179* P=0.94	507 ± 489 *225 ± 132* P=0.11	379 ± 466 *197 ± 109* P=0.47
Overall Developmental Functioning Estimates	434 ± 434 *248 ± 127* P=0.38			
Domain-specific Developmental Quotient Scores	0.95 ± 0.5 *0.8 ± 0.14* P=0.36	1.34 ± 0.9 *1.16 ± 0.1* P=0.65	1.63 ± 1.9 *0.79 ± 0.3* P=0.03	0.82 ± 0.3 *0.69 ± 0.1* P=0.15
Overall Developmental Quotient Scores	1.18 ± 0.8 *0.86 ± 0.1* P=0.08			

Table IV. Postoperatory neurodevelopmental comparison of studied patients (non-cyanotic CHD group in normal writing, cyanotic CHD group in *italic* writing)

Level / Domain	Personal/Social	Fine Motor	Language	Gross Motor
Domain-specific Developmental Functioning Estimates	521 ± 394 *363 ± 117* P=0.31	563 ± 412 *382 ± 116* P=0.24	468 ± 394 *354 ± 60* P=0.57	535 ± 514 *340 ± 98* P=0.48
Overall Developmental Functioning Estimates	522 ± 412 *360 ± 83* P=0.44			
Domain-specific Developmental Quotient Scores	0.98 ± 0.2 *0.86 ± 0.2* P=0.18	1.06 ± 0.22 *0.91 ± 0.2* **P=0.03**	0.89 ± 0.3 *0.87 ± 0.2* P=0.86	0.91 ± 0.2 *0.81 ± 0.1* P=0.28
Overall Developmental Quotient Scores	0.96 ± 0.1 *0.86 ± 0.1* P=0.09			

Preoperatory, statistically significant differences between non-cyanotic and cyanotic groups were found in language domain-specific developmental quotient scores (p=0.03). Postoperatory, statistically significant differences were found in fine motor domain-specific developmental quotient scores (p=0.03).

Near-infrared spectroscopy monitoring of the patients

The cyanotic CHD group had lower NIRS values in all perioperative moments but the difference was not statistically significant. In figure 1 are represented cerebral tissue oxygen saturations measured by NIRS over the frontal cortex starting immediately after anesthesia induction and during the entire operatory period. These are classified as initial, minimum, mean, maximum and final NIRS values.

Glial fibrillary acidic protein

Preoperatory, the cyanotic CHD group had non-significantly higher values (p=0.0636) compared with the non-cyanotic CHD group. Postoperatory, the cyanotic CHD group had significantly higher values (p=0.0277) compared with the non-cyanotic CHD group.

In the postoperatory period, GFAP had significantly lower values (p=0.0248) in the non-cyanotic CHD group and non-significantly higher values (p=0.748) in the cyanotic CHD group compared with preoperatory period. Data are reported in table V and figure 2.

Glial fibrillary acidic protein and near-infrared spectroscopy monitoring

Association between GFAP and NIRS were analyzed and statistically significant differences were found in both non-cyanotic and cyanotic groups between initial NIRS values and preoperatory GFAP levels, between minimum, mean, maximum, final NIRS values and postoperatory GFAP levels as shown in table VI.

GFAP as a marker of brain injury

Patients with significant low cerebral tissue oxygen saturations were considered those in which a difference >= of 20% between the maximum and minimum NIRS values was registered during perioperatory period. Statistical tests were run in order to assess if GFAP levels are linked with NIRS values and could diagnose low cerebral tissue oxygen saturation. A good predicting model was observed with GFAP in the non-cyanotic CHD group, defined by an aria under curve of 0.7 for receiver operative characteristic as shown in figure 3.

Higher GFAP levels from the postoperatory period did correlate with neurodevelopmental impairment, with a mean value of 0.66 ± 0.02ng/ml in those with good neurodevelopmental score comparing to a mean level of 0.69 ± 0.02ng/ml in those with low neurodevelopmental score (p=0.01).

Discussions

Neurodevelopmental impairment is still a great concern in the evolution of pediatric patients which underwent surgical correction for CHD. In the populational group included in our study, approximately two thirds of patients (63%) had neurodevelopmental impairment. Early identification of these patients is important because it can lead to early inclusion in rehabilitation programs in order to correct the deficits. American Heart Association recommends

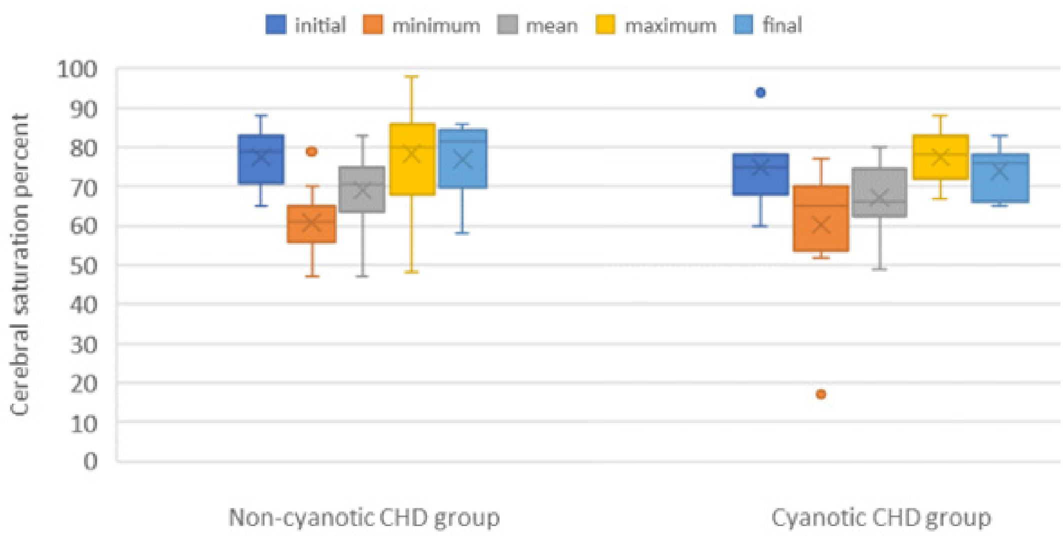

Fig. 1 Box-plot comparing cerebral oxygen saturation percent measured by NIRS in non-cyanotic and cyanotic congenital heart defect group (CHD= congenital heart defect; NIR=near-infrared spectroscopy)

Table V. Glial fibrillary acidic protein values: pre and post operatory (pg/ml)

GFAP	Preoperatory	Post operatory	P value
Non-cyanotic CHD	0.658 ± 0.05	0.649 ± 0.005	**0.0248**
Cyanotic CHD	0.680 ± 0.02	0.689 ± 0.03	0.748
P value	0.0636	**0.0277**	

Abbreviations: CHD= congenital heart defect; GFAP=glial fibrillary acidic protein.

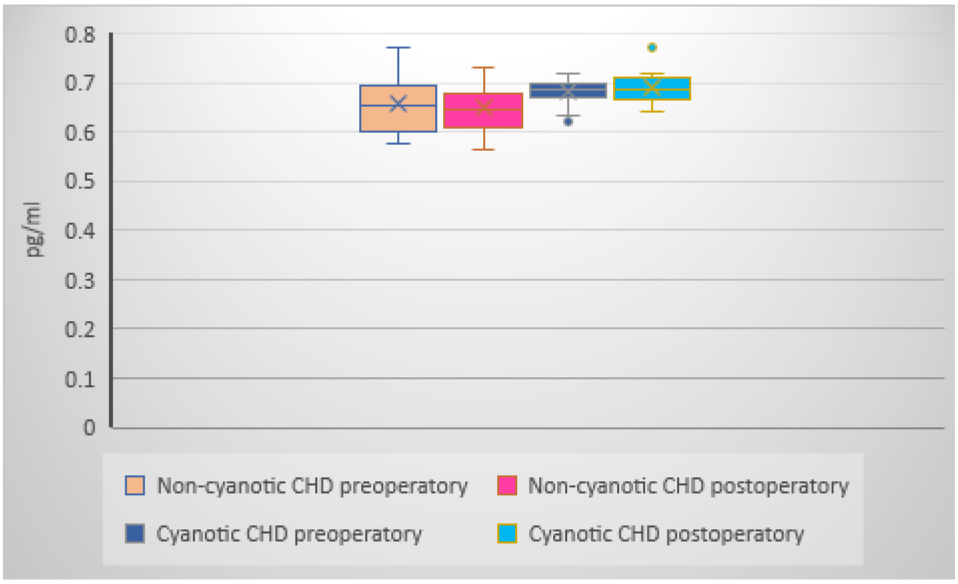

Fig. 2. Box-plot of glial fibrillary acidic protein in non-cyanotic and cyanotic CHD group, pre and postoperatory (CHD= congential heart diferect)

Table VI. GFAP and NIRS associations in the perioperatory period

Parameter	**p value for:**				
	Initial NIRS	**Minimum NIRS**	**Mean NIRS**	**Maximum NIRS**	**Final NIRS**
Preoperatory					
GFAP in non-cyanotic CHD group	**<0.0001**				
GFAP in cyanotic CHD group	**<0.0001**				
Operatory period					
GFAP in non-cyanotic CHD group		**<0.0001**	**<0.0001**	**<0.0001**	
GFAP in cyanotic CHD group		**<0.0001**	**<0.0001**	**<0.0001**	
Postoperatory					
GFAP in non-cyanotic CHD group					**<0.0001**
GFAP in cyanotic CHD group					**<0.0001**

Abbreviations: CHD=congenital heart defect; MBP=myelin basic protein; NIRS= near-infrared spectroscopy; pTau=Tau protein.

that children with CHD, simple or complex, should be periodically screened during childhood in search for neurodevelopmental impairment [2]. In our study, we used Denver Developmental Screening Test II scale (DDST II) [6] with an adaptation system for children with medical complex conditions like CHD [7] in order to quantify neurological deficits.

We compared the neurodevelopmental pattern of non-cyanotic versus cyanotic patients which were in line with literature which states that neurological damage appears in children with CHD, both in those with adequate cerebral oxygenation (non-cyanotic patients) and in those with low cerebral oxygen saturation (cyanotic patients) [8]. We demonstrated that in cyanotic patients, significant impairment was observed in language scores preoperatory and in fine motor scores postoperatory, domains with a high level of involvement of important brain structures. Language learning relay on the brain activity of forming connections for specific sounds and words. This happens early in life, in infancy, when a different cluster of neurons respond to each sound that a baby hears. In patients with CHD and low cerebral oxygen saturations, these neurons could be affected and language acquisition difficulties could arise [9]. A study published by Rowe [10] demonstrated that between 14 and 46 months, children with brain injury are slightly behind their peers without brain injury in vocabulary production. Fine motor domain was also affected in cyanotic patients. The child's brain is active during certain times in life in forming specific abilities. Altered cerebral blood flow or oxygenation affect brain dynamics during these periods, resulting in affected motor function of children with CHD as demonstrated also in a study published by Zamani [11].

NIRS is a promising tool in monitoring oxygen saturation during critical periods of time, like surgery. It can measure regional brain saturation in a non-invasive way and this information can be used in neuroprotective strategies during surgery. In our study, NIRS saturations measured in cyanotic patients were lower, although they did not reach statistical significance, than those measured in non-cyanotic patients. A study published by Sanchez-de-Toledo, concluded that patients with low NIRS values during surgery had abnormal neurodevelopmental outcomes [12]. Kussmann found that intraoperatory lower NIRS values correlate with lower neurodevelopmental scores [13].

GFAP is a protein that is expressed in central nervous system cells like astrocytes and ependymal cells [12]. Literature states that its role is to maintain astrocyte shape and mechanical strength, and it was used as a cell marker in variate studies and in different domains [14]. GFAP is involved in many important brain processes, including cell communication (astrocyte-neuron interactions) and functioning of the blood brain barrier [15]. In a study conducted at Johns Hopkins Children's Center on patients undergoing extracorporeal membrane oxygenation (ECMO), those with high levels of GFAP were 13 times more likely to die and 11 times more likely to suffer brain injury than children with normal GFAP levels [16]. In children with CHD undergoing surgical interventions, studies disagree in results concerning GFAP association with neurodevelopmental impairment mainly due to intraoperative factors that can influence its serum levels [17]. Conclusion of studies range from the fact that GFAP is not helpful in the immediate post-operative period [12], until statements like: it represents a promising early marker of abnormal long-term neuro- psychological development [18], a diagnostic mean to acutely identify perioperative brain- specific injury [19], and is associated with impaired neurodevelopment during cardiac surgery in infants [20]. In our study, postoperatory, the cyanotic CHD group had significantly higher values of serum GFAP compared with the non-cyanotic CHD group, results that are confirmed by the majority of studies mentioned above. GFAP levels were linked with NIRS values and a strong association was observed, demonstrating that GFAP could reflect low cerebral tissue oxygen saturation. A good predicting model was observed with GFAP in the non-cyanotic CHD group, defined by an aria under curve of 0.7 for receiver operative characteristic. Moreover, higher GFAP levels from the postoperatory period did associate with neurodevelopmental impairment: children with high postoperatory levels of GFAP had significant lower neurodevelopmental scores.

The limitations of this study are represented by the small number of patients (40), the absence of neuroimaging studies in order to assess brain-injury lesions, because the benefit of neuroimaging was considered smaller than the sedation and mechanical ventilation risks required for the imaging procedure and the short period at which children were reevaluated (4-6 months after surgery).

Conclusion

In conclusion, we demonstrated that GFAP could be a reliable neuromarker that could identify early acute brain injury documented by NIRS monitorization during perioperatory period and that GFAP could also identify short term neurodevelopmental impairment documented by lower scores at neurodevelopmental assessment of children with CHD requiring cardiovascular surgery.

Authors' contribution

LEC: conception and design of the work; acquisition, analysis and interpretation of data for the work; drafting the manuscript; approval of the final manuscript; accountable for all aspects of the work; obtained fundings; gathered materials for the work; collected data and processed it; responsible for the analysis and interpretation of data; review the literature; wrote the manuscript; part of laboratory work; accountable for all aspects of the work.
AH: underdone the laboratory work; critically revising the manuscript for important intellectual content; approval of the final manuscript; accountable for all aspects of the work.

Conflict of interest

The authors report there are no competing interests to declare.

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