Early neurodevelopmental characterization in children with cobalamin C/defect

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Abstract

Cobalamin C (cblC) defect is the most common inherited disorder of cobalamin metabolism. Developmental delay, behavioral problems and maculopathy are common but they have not been systematically investigated.

The aim of this study was to define early neurodevelopment in cblC patients and the possible contribution of different factors, such as mode of diagnosis, age at diagnosis, presence of brain lesions and epilepsy.

Methods: Children up to the age of 4 years with a visual acuity ≥1/10 were evaluated using the Griffiths' Mental Development Scales.

Results: Eighteen children were enrolled (age range 12-48 months). Four were diagnosed by newborn screening (NBS); in the others mean age at diagnosis was 3.5 months (range 0.3 – 18 months). Eight had seizures: three in the first year, and five after the second year of life. Fourteen had brain lesions on Magnetic Resonance Imaging (MRI). Neurovisual assessment evidenced low visual acuity (< 3/10) in 4/18. NBS diagnosed patients had higher general and subquotients neurodevelopmental scores, normal brain MRI and no epilepsy. The others showed a progressive reduction of the developmental quotient with age and language impairment, which was evident after 24 months of age.

Conclusions: Our findings showed a progressive neurodevelopmental deterioration and a specific fall in language development after 24 months in cblC defect. The presence of brain lesions and

epilepsy was associated with a worst neurodevelopmental outcome. NBS, avoiding major disease-related events and allowing an earlier treatment initiation, appeared to have a protective effect on the development of brain lesions and to promote a more favorable neurodevelopment.

Take-home message

Children with cobalamin C deficiency present a progressive neurodevelopmental deterioration with important language impairment from 24 months of age. Neonatal screening appears to reduce the risk of brain lesions and neurodevelopmental impairment.

General Rules

Details of the contributions of individual authors

DR, DM, GF, SL, DC, CDV, EM: Study concept and design

DR, GF, GI, DB, DC, ML G, SL: acquisition of neurovisual, epileptic and psychometric data

SL: statistical analysis

GO, DM, AD: acquisition neurometabolic profiles

DR, RB, GF, SL, SB, EM: Drafting of the manuscript

DR, GF, SL, DC, RB, EM: Analysis and interpretation of results

DR, EM, CDV, RB: Study supervision

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Competing interest statement

Daniela Ricci, Diego Martinelli, Gloria Ferrantini, Simona Lucibello, MariaLuigia Gambardella, Giorgia Olivieri, Daniela Chieffo, Domenica Battaglia, Daria Diodato, G. Iarossi, Alice Donati, Carlo Dionisi-Vici, Roberta Battini and Eugenio Mercuri declare that they have no conflict of interest.

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All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Keywords: cobalamin C, neurodevelopment, language, visual acuity, neonatal screening, children

Introduction

Combined methylmalonic aciduria and homocystinuria, cobalamin C (cblC) type (OMIM 277400), is the most common inherited metabolic disorder of cobalamin metabolism, with an incidence of about 1/85000-1/100.000 live birth. The phenotype has a wide expression, with early onset (before 1 year from birth) often associated with multisystem involvement, neurological deterioration, maculopathy, failure to thrive, cytopenias, renal and hepatic dysfunction (Rosenblatt et al 1997; Carrillo-Carrasco & Venditti, 2012; Fischer et al.2014; Huemer et al. 2019).

Brain involvement is frequent, with hydrocephalus, cerebral atrophy, white matter abnormalities and basal ganglia lesions (Rossi et al. 2001; Longo et al. 2005; Weisfeld-Adams et al. 2013; Fischer et al.2014; Huemer et al. 2019). The association between cblC and ocular disease is also well recognized. Maculopathy and nystagmus with abnormal vision are extremely common, usually occurring before school age (Ricci et al. 2005; Fischer et al. 2014; Huemer et al. 2019). Strabismus and optic atrophy are also frequent (Weisfeld-Adams et al. 2015).

In almost all children with early onset cblC defect, motor skills, including both gross and fine motor abilities, are frequently impaired (Weisfeld-Adams et al. 2013).

Developmental delay and intellectual deficits are also frequent in cblC (Biancheri et al. 2001; Fischer et al. 2014; Huemer et al. 2019). Beauchamp et al. (2009) reported intellectual decline and attentional-executive deficits in two female patients between preschool and 12 years of age.

Only few studies have systematically assessed early neurodevelopment in individuals with cblC deficiency. In 2013, Weisfeld-Adams and colleagues reported the results in 12 early onset cblC patients, aged 9 to 76 months (mean age 41 months) (Weisfeld-Adams et al. 2013). Each child was assessed using standardized parental interviews and, when possible, age- and disability-appropriate

neuropsychological batteries. All subjects showed psychomotor delay, with a specific fall in motor skills and relative preservation of language skills and socialization. Another study (Bellerose et al 2016) assessed 9 cblC patients, aged between 23 months and 24 years; 5 of the 9 were able to perform structured examinations including the Wechsler scales, while for the other 4 a questionnaire on adaptive functions was filled in by the parents. The results of the Wechsler scales showed borderline scores in 3 patients and a mild delay in the other 2. Four of the 9 had different brain lesions on MRI, including white matter changes and atrophy of the corpus callosum. All 9 had severe visual impairment secondary to retinopathy, maculopathy, and/or nystagmus (Bellerose et al 2016). Severe ocular abnormalities have also been reported in other studies (Ricci et al. 2005) and, in their presence, it is difficult to establish to which extent they contribute to the pathogenesis of intellectual disability or if developmental delay would occur independently, in the absence of overt visual defects.

The aim of this study was to define the early neurodevelopment in cblC patients, excluding children with severe visual impairment. We also tried to establish the influence of different factors which included i) age at evaluation; ii) age at diagnosis and method of diagnosis (i.e. newborn screening (NBS) vs. clinical diagnosis); iii) presence of brain lesions and iv) presence of epilepsy.

Methods

A retrospective study was performed on children affected by cblC deficit regularly followed at the at the Metabolic Disease Units of Bambino Gesù Children's Hospital and the Department of Inherited Neuro-Metabolic Disorders, A. Meyer Children Hospital referred to the Pediatric Neurology Unit of Gemelli Hospital for neurovisual evaluation between 2007 and 2014.

Inclusion criteria for those children selected and enrolled in the study were the following: a) genetically confirmed cblC defect; b) visual acuity ≥1/10; c) ongoing therapy and stable biochemical parameters in the 3 months before the neurodevelopmental assessment; d) stable clinical situation; e) presence of a complete neurodevelopmental assessment in the first 4 years from birth; f) availability of at least one brain magnetic resonance imaging (MRI).

Children that could not complete the assessment were excluded by the analysis.

As we wished to verify a possible influence on neurodevelopmental outcome according to age at diagnosis, we subdivided the cohort into three groups according to age of introduction of treatment: birth (diagnosed by NBS), before and after 1 month. The day of introduction of treatment corresponds to the day of biochemical diagnosis.

Brain MRI of all children enrolled in the study were evaluated in relation to the observed site of lesions: white matter (WM), basal ganglia (BG). Other abnormalities, when present, were also noted.

Ethical approval for the study was obtained from the Centers involved in the study and all parents signed patient consent.

Neurovisual assessment

The neurovisual evaluation included visual acuity, by means of the Visual Teller Cards methods or of the LEA Symbols, according to age and cognitive level. Visual acuity $\geq$ 1/10 was considered sufficient to perform the neurodevelopmental assessment.

Neurodevelopmental assessment

Neurodevelopment was assessed using the Griffiths' Mental Development Scales (GMDS – Huntley M, 1996), that includes 5 domains (locomotor, personal-social, hearing and language, eye

and hand coordination, and performance) and provide 5 subquotients and a general quotient (GQ). Neurodevelopmental outcome for each domain was classified as normal when the GQ was ≥85 and abnormal when it was <85.

The assessments have been divided in 3 age subgroups: Group 1, age <24 months; Group 2, age between 24 and 36 months; Group 3, age >36 months. If children had follow-up assessments belonging to different age subgroups, both assessment were included.

Metabolic profile and pharmacological treatment

The metabolic profile included the mean levels of plasma total homocysteine (normal range: 4-19 µmol/L), of plasma methionine (normal range: 10-50 µmol/L), and of methylmalonic acid measured in plasma (normal value < 0.1 µmol/L) or in urine.

Statistical analysis

Results were analyzed according to age at the assessment, method of diagnosis (NBS versus symptomatic diagnosis), presence of epilepsy, presence of brain lesions. Descriptive statistics were computed for variables of interest and included mean values and standard deviations of continuous variables. The ANOVA test with post-hoc of Bonferroni was used to compare differences in GMDS among the three study groups classified on the basis of age at assessment (<24 months, between 24 and 36 months and >36 months), age of diagnosis (newborn screening; before 1 month of life; between 1 and 3 months and after 3 months from birth), presence and onset of epilepsy (no epilepsy, onset of epilepsy before 1 year of life; onset of epilepsy after 1 year of life) and of metabolic profile of each subject at the time of the evaluation.

T-test was used to compare the distribution of variables between the two groups divided by presence of brain lesions and newborn screening.

Pearson test was used to verify a correlation between metabolic profile and GQ and between the dosage of treatment and GQ.

The level of significance was set at $p<0.05$.

Results

Detailed clinical, neuroradiological, biochemical and genetic data of the study population are reported in Table 1.

Eighteen children fulfilled the inclusion criteria. Their age ranged between 12 and 48 months. Four of the eighteen children were diagnosed by NBS, with one being already symptomatic. In the remaining 14 the diagnosis was established at a mean age of 3.5 months (range 0.3 – 18 months). Nine were diagnosed within the first month of life, 2 within the first 3 months, and 3 between the age of 6 to 18 months.

After biochemical diagnosis, all patients were treated on the same day with parenteral hydroxocobalamine (OH-B12), betaine, folic/folinic acid and carnitine, according to current guidelines for remethylation disorders (Huemer et al. 2017). Doses of parenteral OH-Cbl and perioral betaine, are reported in Table 1. Average dose for OH-Cbl was 0.06 ± 0.03 mg/kg/day; average dose for betaine was 204.39 ±64.14 mg/kg/day. All patients showed good compliance to the prescribed therapy.

Ten infants did not present with epilepsy at follow-up, three had seizures in the first year (infantile spasms), and five after the second year of life (1 had epileptic status, 1 generalized seizures and 3 focal seizures). All epileptic patients required anti-epileptic polytherapy.

Fourteen children had brain lesions on MRI (Table 1).

Low visual acuity (< 3/10) was found in 4 of the 18 (Table 1).

Neurodevelopmental assessment

Details of the assessments are reported in Table 1.

Eighteen children completed the neurodevelopmental assessment and 5 of them had a second assessment falling in a different age subgroup, for a total of 23 assessments.

Eight assessments were performed between the age of 12 and 24 months, 10 between the age of 25 and 36 months, and 5 after 36 months.

In the age Group 1 (<24 months), the mean GQ was 74 (SD 16.54), with the following mean subquotients: scale A 73.85 (SD 21.94), scale B 71.14 (SD 17.60), scale C 69.85 (SD 18.04), scale D 65.85 (SD 13.23), scale E 72.42 (SD 19.27).

In the age Group 2 (24 – 36 months), the mean GQ was 76.04 (SD 11.62), with the following mean subquotients: scale A 84.08 (SD 16.38), scale B 79.66 (SD 17.30), scale C 60.58 (SD 13.18), scale D 74.58 (SD 13.01), scale E 81.83 (SD 13.37). The mean Scale C subquotient (Hearing & Speech) was the lowest compared to the others. Three of the 10 children in this group were diagnosed by NBS.

In the age Group 3 (>36 months), the mean GQ was 49.84 (SD 31.95), with the following mean subquotients: scale A 56.2 (SD 31.22), scale B 54.6 (SD 38.75), scale C 42 (SD 26.80), scale D 45.8 (SD 35.49), scale E 50.6 (SD 33.56). Similar to age Group 2, the lowest subquotient value was observed in Scale C (Hearing & Speech). One of the 5 children in this group were diagnosed by NBS.

Correlation between neurodevelopment and age subgroups

A difference was found for GQ and all subquotients between the oldest age Group 3 and both Group 1 and Group 2 ($p<0.05$), but not between Group 1 and Group 2 (Figure 1).

Correlation between neurodevelopment vs age at diagnosis

A correlation was found between age at diagnosis and both GQ and all subquotients, with the exception of scale D, in all age groups (*p <0.05*). Children diagnosed by newborn screening had higher GQ and subquotients (Figure 2).

Correlation between neurodevelopment vs epilepsy

A strong correlation was found between age at onset of epilepsy and both GQ and all subquotients, in all age groups (*p<0.01*). Children with onset of epilepsy in the first year of life had the lowest GQ and subquotients (Figure 3).

Correlation between neurodevelopment vs brain lesions

A correlation was found between presence of brain lesions and both GQ and all subquotients with the exception of scale D, in all age ranges ($p<0.05$). Children with normal brain MRI had highest GQ and subquotients (Figure S1).

Correlation between neurodevelopment vs metabolic profiles and treatment modalities

No correlation was found between the biomarkers of metabolic profile and GQ or subquotients (Plasma Homocysteine *p=0,174*; Plasma Methionine *p=0,938*). No correlation was also found between the dose of OH-B12 (*p=0,740*) and of betaine with GQ (*p=0.928*).

Discussion

Neurodevelopmental delay is frequent in children with cblC defect and its severity is not always related to the level of toxic metabolites that are supposed to be associated with retinal and brain toxicity in this diseases (Heumer et al, 2017).

Structured assessments in these children are not always easily obtainable because of a combination of factors, including behavioral and attention deficits and severe visual defects, that hamper the possibility to perform a structured assessment. In order to reduce the possible bias due to visual impairment, in this study we excluded children with a severe visual acuity defect.

Our data showed that, even when excluding children with severe visual impairment, there was a significant neurodevelopmental delay in most infants with cblC defects, with an overall reduction of the GQ with increasing age. The reduction was more obvious in the oldest age group.

Interestingly, we found that between the first and the second age subgroup all the neurodevelopmental aspects explored had a mild improvement, but there was a progressive reduction of the subquotient related to language development. Our data showed that in the first 2 years of life, language development was similar to other abilities (probably because in the first year most items assessed in the test are related to comprehension rather than to expressive language), but after the age of 24 months, a specific slowdown of this domain became evident. These difficulties persisted after the age of 3 years. Although it is known that language can be impaired in these children, phonological difficulty encountered has so far mainly been considered as related to the overall developmental delay (Whitaker AM et al 2018). Although an overall intellectual decline has already been reported (Beauchamp et al. 2009), a specific progressive language impairment in early onset cblC patients has never been reported. The limited number of our cohort and the lack of longitudinal data does not allow to define a language profile but the observed trend evidences a

specific impairment of this skill. Language impairment in our cohort was more marked in language production. There was a specific susceptibility of this domain, as demonstrated by the fact that at Griffith's Scales the language subscale had consistently the lowest scores compared to all the other examined domains even in patients with an overall global delay. An early audiological assessment could be advised in cblC defect, since it may help to screen peripheral auditory component and hearing perception, although language impairment could also depend on the disturbed processing of auditory information or, more specifically, from mechanisms implied in verbal production.

A relevant finding of our study was the observation that the four children identified by newborn screening were the only ones with a DQ within normal range, with a normal brain MRI and lacking epilepsy. This supports previous studies suggesting that an early diagnosis using NBS can allow a better outcome (Huemer et al. 2019; Keller et al. 2019), as it reduces the exposition to offending metabolites and prevents the occurrence of major disease-related events (e.g. hydrocephalus, hemolytic uremic syndrome, pulmonary hypertension) that greatly contribute to long-term disabilities in cblC defect. It is of interest that children with symptomatic diagnosis in the neonatal age, who had a relatively short exposure to toxic metabolites, still had a lower level of development in all examined abilities when compared to those identified by NBS. This can be explained by the fact that patients with a very-early onset in the first days of life, may have the most aggressive form of the disease, as also shown by the finding of brain lesions on MRI and of epilepsy. All these findings strongly indicate the importance of NBS in cblC defect, as a presymptomatic diagnosis allows to start the specific therapy soon after birth, improving the natural disease history and the long-term prognosis. Interestingly, we found that children diagnosed by NBS also had an uneven profile with language skills and eye-hand coordination showing relatively lower scores when

compared to the other abilities. The subquotients were within the normal range in all areas except for language and fine motor coordination.

These findings suggest that verbal skills and eye-hand coordination may be more easily affected by a chronic exposition to offending metabolites even in the absence of structural brain lesions on imaging. These findings are in agreement with previous studies reporting a possible correlation between chronic toxicity and abnormal development of both visual dorsal pathway (which is responsible for attention and fine coordination) (Braddick and Atkinson, 2011), and ventral pathway, that is more related to the early development of language (Brauer et al., 2013).

Another factor analyzed in our study was the association between epilepsy and early neurodevelopment: it is well known that early-onset epilepsy plays a role in slowing the maturation of skills or in reducing brain plasticity (Barone al., 2009; Luciano et al., 2007, Biancheri et al.2002). Our data confirmed that GQ is significantly correlated with the presence of epileptic seizures. More specifically, children who did not present seizures showed the highest GQ in all areas, while all children who developed epilepsy in the first year presented a severe delay.

The relatively small number of patients and the heterogeneity of brain lesions in our cohort did not allow performing a meaningful multivariate analysis but some patterns of associations between GQ, brain lesions and epilepsy could be observed. Not surprisingly, the neurodevelopment of children with normal MRI was better than the one of those with brain lesions. At the other end of the spectrum, patients with lower GQ, had a combination of brain lesions and epilepsy. It is of interest that in the six children with brain lesions involving white matter or basal ganglia but who did not had epilepsy had better outcome than those with epilepsy. The onset of epileptic seizures, especially

in the first year, could therefore be considered a sign of greater severity, irrespective of the severity of brain lesions at MRI.

Although previous studies reported that site and size of brain lesions can influence different aspects of development (Ricci et al, 2005; Fischer et al, 2014), our data did not show significant differences according to type of brain damage; this could be related to the relatively low number of patients in our cohort or to the heterogeneity of imaging findings within each subgroup.

Conclusions

Our study showed the progressive deterioration of the neurodevelopment in a large cohort of children with cblC defect. In addition to fine coordination skills impairment, a specific fall in language development was observed after the age of 24 months.

Our findings strongly suggests that NBS, by avoiding major disease-related events and allowing an early initiation of specific treatment, has a protective effect on the development of brain lesions and promote a more favorable neurodevelopment in cblC defect children.

Further natural history studies on this rare neurometabolic disorder in a larger cohort are needed to allow more meaningful analysis for a better understanding of the pathogenetic mechanisms leading to developmental disability in cblC.

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Table 1. Clinical details of the study sample

Patients	Genotype	Age at diagnosis	Epilepsy	Brain MRI abnormalities	Age at evaluation (months)	DQ	Visual acuity	Plasma Homocysteine*	Plasma Methionine*	OH-B12	Betaine
1	c.271dupA/c.271dupA	NBS	No	None	28	92	7.0/10	38	17	0.08	220
2	c.271dupA/c.271dupA	NBS	No	None	34	97	5.0/10	46	20	0.07	206
3	c.271dupA/c.331C>T	NBS	No	None	20	98	4.5/10	47	16	0.09	196
II assessment					31	86	5.0/10	50	20	0.07	196
4	c.271dupA/c.271dupA	NBS	No	WM	44	85	7.0/10	48	18	0.07	190
5	c.271dupA/c.271dupA	10 days	No	WM and BG	20	81	2.0/10	49	54	0.03	139
6	c.271dupA/c.271dupA	10 days	<1year	WM and BG	22	47	1.0/10	62	17	0.05	204
7	c.271dupA/c.666C>A	15 days	>1year	WM	20	58	4.5/10	60	23	0.02	280
II assessment					29	61	5.5/10	56	14	0.02	180
8	c.271dupA/c.271dupA	20 days	<1year	WM and BG	48	16	5.5/10	54	18	0.03	230
9	c.271dupA/c.271dupA	30 days	No	WM	17	77	4.5/10	41	56	0.10	208
II assessment					24	77	5.5/10	55	32	0.09	302
10	c.271dupA/c.271dupA	30 days	>1 year	WM and BG	33	75	7.0/10	45	17	0.04	160
11	c.271dupA/c.271dupA	30 days	No	WM	15	77	2.5/10	49	19	0.02	240
12	c.271dupA/c.331C>T	30 days	No	WM	16	81	5/10	71	19	0.03	290
13	c.565delC/c.440G>A	30 days	>1year	WM	22	73	1.6/10	51	17	0.04	80
II assessment					28	79	1.6/10	42	16	0.07	160
14	c.271dupA/c.271dupA	40 days	No	None	31	58	1.0/10	39	20	0.10	120
15	c.271dupA/c.467delCT4	2 months	No	WM and BG	30	67	4.0/10	60	34	0.07	380
16	c.271dupA/c.481C>T	6 months	>1 year	WM and BG	26	72	7.0/10	36	17	0.06	200
II assessment					42	83	7.0/10	51	20	0.04	180

17	c.271dupA/c.394C>T	15 months	<1year	WM	42	29	5.5/10	55	28	0.10	180
18	c.666C>A/c.3G>A	18 months	>1 year	WM	43	36	5.5/10	49	25	0.08	160

DQ, Developmental General Quotient; NBS, newborn screening; BG, basal ganglia; WM, white matter; *columns report average total homocysteine in plasma (normal values 4-19 μmol/) and average methionine in plasma (normal values 10-50 μmol/L) in the three months before neuropsychological assessment; OHB12, hydroxocobalamin, mg/kg/day (parenteral administration); betaine, mg/kg/day (perioral administration).

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Caption of Figures

Figure 1. Correlation between neurodevelopment and age at the assessment in cblC children

Figure 1 shows the distribution of GQ and Griffith's subscale values in cblC childred divided according the age of the assessment: before 24 months (7 observations), between 24 and 36 months (12 observations), after 36 months (5 observations)

The legend for the figure is: A= locomotor scale; B= personal and social scale; C= speech and language scale; D= eye-hand coordination scale; E= performance scale; GQ= general quotient; <24 months = age at the assessment < 24 months; 24-36 months= age at the assessment between 24 and 36 months; >36months = age at the assessment > 36 months.

Figure 2. Correlation between neurodevelopment vs age at diagnosis

Figure 2 shows the distribution of DQ and Griffith's subscale values in cblC childred divided according the age at diagnosis: 5 observations refer to 4 patients that received the diagnosis via NBS; 5 observations refer to 4 patients that received the diagnosis before 1 month of life (range age of diagnosis: 10-30 days); 14 observations refer to 10 patients that received the diagnosis after 1 month of life (range age of diagnosis:40-180 days)

The legend for the figure is: A= locomotor scale; B= personal and social scale; C= speech and language scale; D= eye-hand coordination scale; E= performance scale; DQ= developmental quotient.

NBS: newborn screening. Age at diagnosis is subdivided into three groups: newborn screening, earlier than 1 month and later than 1 month.

Figure 3. Correlation between neurodevelopment and epilepsy in cblC children

Figure 3 shows the distribution of DQ and Griffith's subscale values in cblC childred divided according the presence and onset of epilepsy:12 observation refer to 10 patients without epilepsy; 3 observations refer to 3 patients that presented epilepsy onset before 1 year; 9 observation refer to 5 patient that presented epilepsy onset after 1 year.

The legend for the figure is: A= locomotor scale; B= personal and social scale; C= speech and language scale; D= eye-hand coordination scale; E= performance scale; DQ= developmental quotient

Figure 1

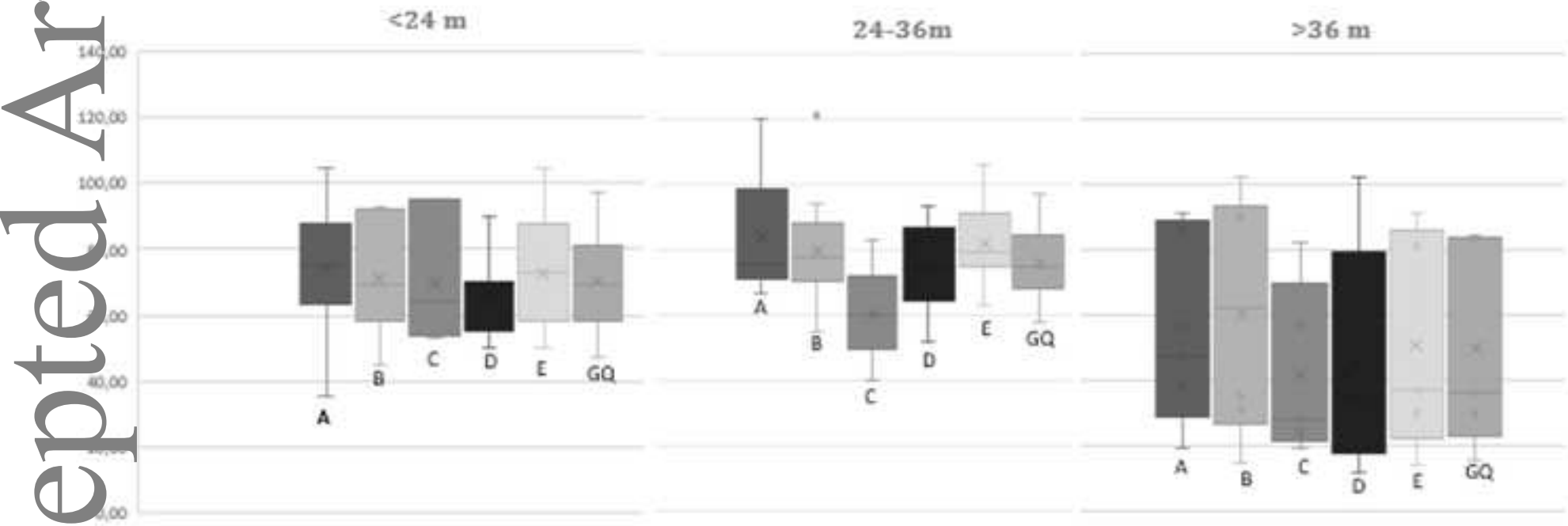

Figure 2

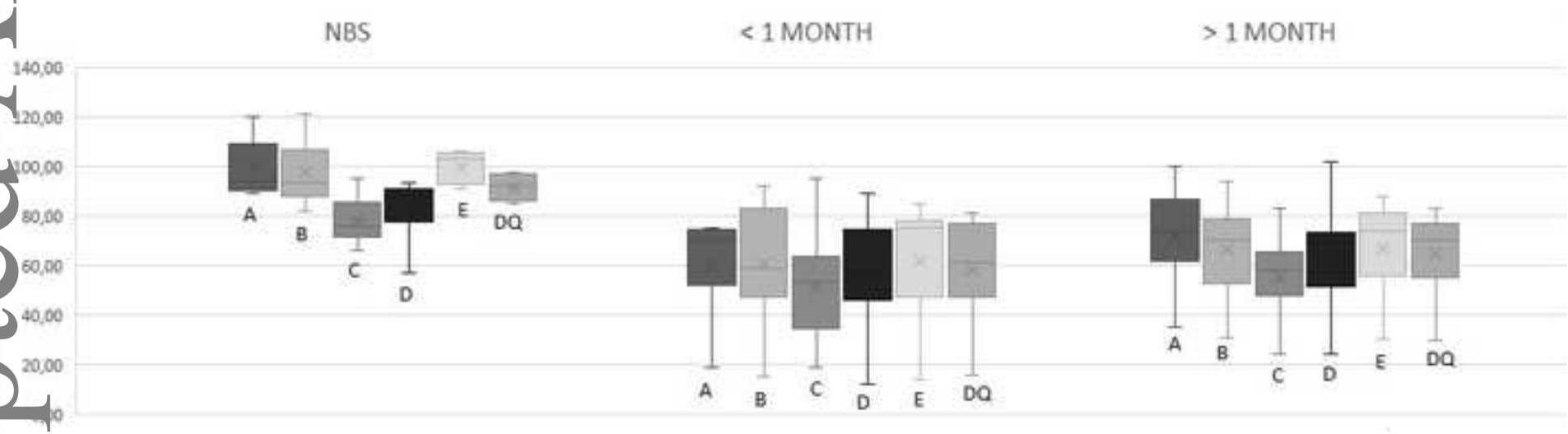

Figure 3