

## Folate and homocysteine status in children with neurogenic bladder due to meningocele

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### ABSTRACT

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**Introduction:** Meningocele (MMC) is the most severe form of spina bifida caused by both genetic and environmental components. It is well known that status of folate plays an important role in the risk of neural tube defects. High homocysteine (Hc) level may be associated with disturbed sensory and motor peripheral nerve function and is lowering after folic acid (FA) fortification.

**Purpose:** To explore possible links of FA (folic acid) and Hc (Homocysteine) and to correlate them with renal and bladder function (based on urodynamics) as well as physical activity in patients with NB (neurogenic bladder) after MMC (myelomeningocele).

**Materials and methods:** The investigation was conducted on two groups: group 1- 30 children with neurogenic bladder, group 2 - 20 healthy children with no abnormalities in urinary and nervous systems. The Hc concentration in urine

and serum was estimated using the ELISA set-and FA was measured in serum using electrochemiluminescence method. FA/Hc ratio was calculated in all children.

**Results:** The median serum and urine Hc were higher compared with reference group. Median FA/Hc ratio was statistically significantly lower in MMC group compared to reference group. There were no differences in serum FA between studied groups. We found statistically significant correlations between urodynamics parameters and FA and Hc.

**Conclusions:** Hyperhomocysteinemia and hyperhomocysteinuria could be considered as factors influencing bladder function in MMC patients.

Although serum FA level was in normal range in MMC patients it does not exclude disturbed folic acid status.

**Key words:** folic acid, homocysteine, neurogenic bladder, urodynamic investigation,

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Received: 07.09.2014

Accepted: 08.11.2014

Progress in Health Sciences

Vol. 4(2) 2014 pp 37-44

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## INTRODUCTION

Meningocele (MMC) is the most severe form of spina bifida resulting from lack of closure of the neural tube during embryologic development with a worldwide incidence ranging from 1.0 to 10.0 per 1,000 births [1]. Neural tube defects (NTDs) are caused by both genetic and environmental components [2]. Folate status plays an important role in the risk of NTD, and folate supplementation and/or food fortification [3] decrease the risk of giving birth to a child with NTD but do not completely abolish it. These facts suggest that there are other than maternal factors responsible for MMC. Sociodemographic and epidemiological findings correlate well with higher risk of NTD [1]. Parental age (mother over 40 or under 19 years), African Ancestry [4], paternal occupational exposure (pesticides) [5], hyperthermia during early pregnancy and medication usage are suspected of being NTD risk factors. Some observations suggest genetic risk factors [2,6,7]. O'Byrne et al. [6] described for the first time an association between the polymorphism of the folate receptor gene (FOLR3) and the folate carrier gene (SLC19A1) with MMC. Higher prevalence of NTDs within families [7] state that folate metabolizing genes (e.g. methylenetetrahydrofolate reductase (MTHFR) are important not only as a NTD cause but are also critical to memory functions, urogenital malformations and limb reductions [8,9]. There are some studies which link folate/homocysteine levels with cognitive functions [10] and suggest that higher folic acid (FA) and lower homocysteine (Hc) levels improve them. The role of Hc and FA has been studied by Ebessun et al. [11], who stated that increased plasma Hc and decreased FA levels are responsible for a higher risk of depression in adults. Elevated Hc levels may increase risk of bone loss and hip fractures [12], can be responsible for preeclampsia in woman of African Ancestry [13], and may play an important role in chronic renal [14] and heart diseases [15-17] in children and adolescents. Based on the 3-year follow-up study of 60-year old and older patients, Leishear et al. [18], concluded that high Hc may be associated with impaired sensory and motor peripheral nerve function and may have important implications for disability in older adults caused by lower strength and physical performance. There are no such studies in children. Patients with multi-systems dysfunction caused by inappropriate innervations after MMC may present some abnormalities in kidney and urinary tract with most frequent neurogenic bladder and vesicoureteral refluxes, and are at risk for urinary function deterioration [19].

In this study we evaluated possible links between FA, Hc and renal and bladder function (based on urodynamics) as well as physical activity

in patients with neurogenic bladder (NB) after MMC.

## MATERIALS AND METHODS

The investigation was conducted on 50 children, divided into two groups. The study group included 30 children, whose median age was 9 years (2.6-18). They underwent surgical treatment of MMC in the first days after birth and neurogenic bladder was diagnosed based on urodynamics. All of them were under the care of the Department of Pediatrics and Nephrology and Outpatient Clinic at University Children's Hospital in Bialystok, Poland. The control group consisted of 20 healthy children with median age 10.5 years (3.5-17) without any abnormalities in the urinary or nervous systems. The samples of healthy children were obtained from healthy pupils from the OLAF study entitled: "Elaboration of reference blood pressure ranges for Polish children and adolescents" PL0080 OLAF"[20]. Samples from subjects aged 3-6 years old were obtained from healthy children attending day care or nursery school.

**Inclusion criteria:** 1. patients aged 1-18 years with neurogenic bladder due to myelomeningocele with voiding dysfunction for at least one year prior to screening, 2. cystometry performed in all MMC patients, 3. uroflowmetry performed in not catheterized patients and in healthy children, 4. normal renal function (GFR >90ml/min/1.73m<sup>2</sup> and normal serum creatinine).

**Exclusion criteria:** 1. urinary tract infections within last 3 months, 2. presence of other infections.

We analyzed all participants' medical charts to determine age, sex, previous treatment, measurements of height, weight, BMI, physical examination and activity assessment using the Hoffer' scale (1-moving without problems, 2-moving with a little help, 3-moving with difficulties, 4-wheelchair-dependent patient) [21]. We estimated serum creatinine (measured by Jaffe Gen. 2 - compensated method for serum, Cobas Integra 800, Roche), uric acid and glomerular filtration rate (ml/min/1.73m<sup>2</sup>) estimated by the Counahan-Barratt Equation (eGFR):  $GFR=0.43 \times L \text{ (cm)}/Scr \text{ (mg/dl)}$ , L – length, Scr – serum creatinine level. In urodynamics we assessed: in uroflowmetry: time to max flow, flow and voiding time, maximum and average flow rate, voided volume, residual urine (calculated by USG immediately after micturition); in cystometry: detrusor pressure at overactivity (Pdet overact), detrusor pressure at cystometric capacity (PdetCC) and compliance of the bladder wall (Comp); in uroflowmetry. Infusion liquid volume was enumerated as the average volume of urine obtained from clean intermittent catheterization (CIC) to

estimate the bladder function with close approximation to everyday filling and emptying of bladder in natural conditions. In healthy children, only uroflowmetry was performed and, additionally, uroflowmetry was performed in uncatheterized patients (5 children) with NB. In all children, mean values of 3 measurements were analyzed. Full bladder wall thickness was measured by ultrasonography in both studied groups.

The first morning sample of urine was aseptically collected between 7 and 8 am from all studied patients, frozen directly after spinning, to measure the Hc level. Additionally 24-hour urine samples were taken from all enrolled subjects and kept at a temperature of +4°C, frozen immediately after 24 hours and stored at a temperature of -80°C. The completeness of the 24-hour urine collections was evaluated by comparing total creatinine in the sample with the predicted creatinine, according to patient sex and weight. Urinary tract infections were excluded on the basis of urinary testing and urine culture. A negative C-reactive protein (CRP) excluded current infection.

Hc concentration in urine and serum of both studied groups of children was estimated using the Hc EIA Reagent kit (Axis-Shield, Scotland). The investigation was conducted according to the manual instructions. Total Hc urinary concentration was standardized to gram of creatinine and the result was expressed as hc/creatinine ratio (umol/gcrea). FA concentration was measured in serum from the morning blood sample using the electrochemi-luminescence method (immunoanalyzer cobas e-411, Roche). Additionally, we calculated the FA/Hc ratio in all the studied children.

Data analysis was performed using Statistica ver. 10.0 (StatSoft Inc., Tulsa, OK, USA). The Shapiro-Wilk W test confirmed data was not normally distributed and therefore non-parametric statistics were used. The ANOVA analysis and Mann-Whitney tests were used for comparisons, and the Spearman test was used to assess correlations between the studied parameters. A p-value less than 0.05 was considered statistically significant.

The study was approved by the Ethics Committee of the Medical University of Bialystok in accordance with the Declaration of Helsinki. The OLAF study was approved by The Children's Memorial Health Institute Ethics Committee. Written informed consent was obtained from parents or legal caregivers of all the enrolled subjects, subsequent to receiving full information about the study.

## RESULTS

The characteristics of the studied children are presented in Table 1. There were no differences in the age, sex, weight and BMI between the studied groups. Statistically significant differences in body length between NB patients and the reference group resulted from the disease (shorter vertebral dimension due to spina bifida and malformations of the bone structure).

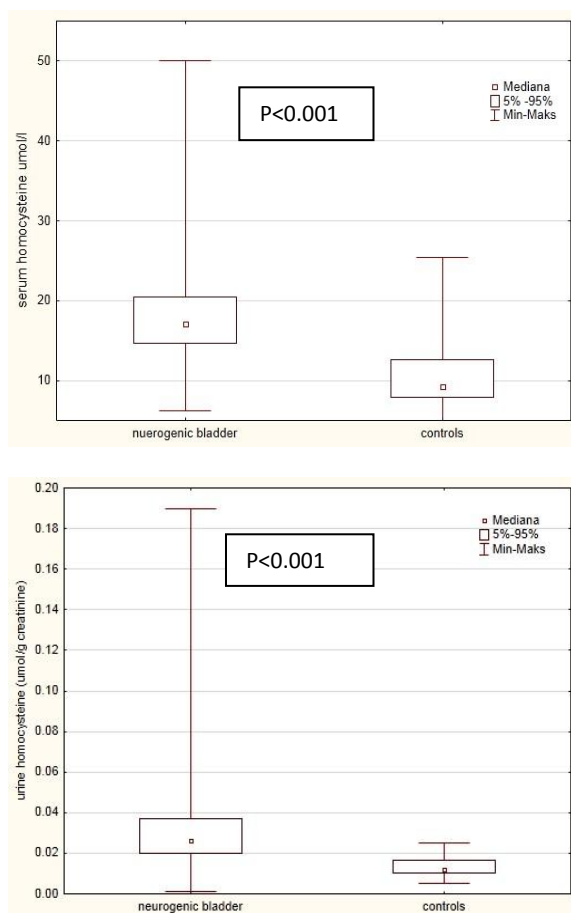
In the MMC group, bladder wall thickness values was higher than in the reference group (Tab.1) and we found statistically significant negative correlations with the Hoffer scale in this group; patients with no limitations in movement had a thinner bladder wall ( $r = -0.396, p < 0.001$ ).

**Table 1.** Demographic, clinical and biochemical characteristic of MMC patients and reference group, \*  $p < 0.05$ . CIC-clean intermittent catheterization.

	MMC median (range)	Reference group median	P value
Number	30	20	
Age/years	9 (2.6-18)	10.5 (3.5-17)	0.664
Height/cm	133 (87-164)	148 (100-175)	0.018*
Body weight /kg	31 (8-95)	33.5 (12-65)	0.579
BMI	17.36 (9.6-35.32)	15.23 (13.02-21.22)	0.12
Time of follow up/years	6 (0.5-14)		
Thickness of bladder wall CIC /day	3 (2.5-10) 4(0-5)	3 (1-4)	0.007*
Anticholinergic therapy	mg/day5 (1.25-7.75) mg/kg bw 0.17 (0.05- 0.42)		
Serum creatinine (mg/dl)	0.37 (0.19-4.7)	0.47 (0.2-0.68)	0.116
Urine creatinine (mg/dl)	51.36 (14.33-223.03)	107.9 (63.56-244)	<0.001*
GFR ml/min/1.73m <sup>2</sup>	149.8 (33.63-285.95)	134.46 (98.01-236.50)	0.499
Uric acid (mg/dl)	3.91 (1.32-7.6)	3.52 (2.38-5.41)	0.188
Urine osmolality	613 (391-1135)	0.383	0.383
Serum protein (g/dl)	7.32 (6.37- 7.92)	7.56 (6.75-8.14)	0.19

Additionally, we analyzed kidney function parameters. The results are shown in Table 1. We found statistically significant differences in urine creatinine concentration but not in serum creatinine, GFR and uric acid, although the maximum value of uric acid concentration was one and half times higher in NB than in the reference group. We also found a statistically significant negative correlation between physical activity measured by the Hoffer scale with GFR in the MMC group ( $r = -0.415$ ,  $p < 0.05$ ).

Serum and urine Hc values in MMC group (17 (6.22-50), 0.026 (0.001-0.19), respectively) were higher compared with the reference group (9.2 (4.1-25.5), 0.012 (0.005-0.025), respectively) and differences are shown in Fig 1. Additionally, urine Hc level was lower in non-catheterized patients when compared to catheterized ( $p = 0.005$ ), and there were no differences when compared with the reference group ( $p = 0.144$ ). Urine Hc level in catheterized patients was higher compared with the reference group ( $p < 0.001$ ).



**Figure 1.** Comparisons between Hc levels in MMC patients and healthy controls in serum (A) and in urine (B).

There were no differences in serum FA between the MMC children and the reference

group. All FA values in both groups were in the normal range. FA/Hc ratio values were statistically significantly lower in the MMC group compared with the reference group (0.698 (0.297-1.791); 1.051 (0.311-3.704) respectively,  $p = 0.019$ ).

In further analysis, we evaluated uroflowmetry parameters in patients with MMC, in those who were able to empty their bladder themselves. All patients were diagnosed with dysfunctional voiding based on prolonged delay time (median 7.5 (3.4-20) s), flow time (median 23.4 (8-38.8) s), voiding time (median 30.4 (10-95.6) s), decreased average flow rate (median 4.25 (1.84-7.2) ml/s), maximum flow rate (median 8.8 (4.64-14.5) ml/s) and high amount of residual urine exceeding 38 % (median 43.3 (5-117.5) ml). The median voided volume was 111.4 (30-143) ml in MMC patients. Uroflowmetry parameters were normal in the reference group: delay time was median 2 (1-3) s, flow time was median 19 (10-28) s, voiding time was median 22 (11-30) s, average flow rate was median 17.4 (12.1-27) ml/s, maximum flow rate was median 24 (13.7-35) ml/s, residual urine was median 0 (0-3) ml and median voided volume was 217 (104-456) ml. We found statistically significant differences in all uroflowmetry parameters between MMC patients and the reference group.

T max flow ( $r = 0.941$ ,  $p < 0.05$ ), Flow time ( $r = 0.9417$ ,  $p < 0.05$ ), and voiding time ( $r = 0.9417$ ,  $p < 0.05$ ) correlated positively with serum Hc in MMC but not in the reference group. Max flow rate, average flow rate and voided volume correlated negatively with urine Hc (umol/gcrea) in MMC group ( $r = -0.941$ ,  $r = -0.942$ ,  $r = -0.824$ , respectively) and results were statistically significant ( $p < 0.05$ ). There were no such correlations in reference group. No correlations between uroflowmetry parameters and FA/Hc ratio in both studied groups were found.

We assessed P det overact (median 40 (4-58) cm H<sub>2</sub>O), Pdet CC (median 13.5 (2-59) cmH<sub>2</sub>O) and CC (median 156.5 (38-287) ml) in the cystometry test. Bladder wall compliance was lower than the normal range and was median 10.5 (3-70) ml/ cmH<sub>2</sub>O. We did not find any differences in serum and urine Hc and serum FA levels between MMC patients with and without detrusor overactivity (serum Hc: median 16.09 (6.22-22.31) and 18.71 (10-58-50) umol/ml,  $p = 0.102$ ; urine Hc: 0.025 (0.004-0.04) and 0.026 (0.001- 0.19) umol/gcrea respectively,  $p = 0.2686047$ ; FA: 11.59 (8.09-18.07) and 9.5 (5.03-14.5) ng/ml respectively,  $p = 0.113$ ).

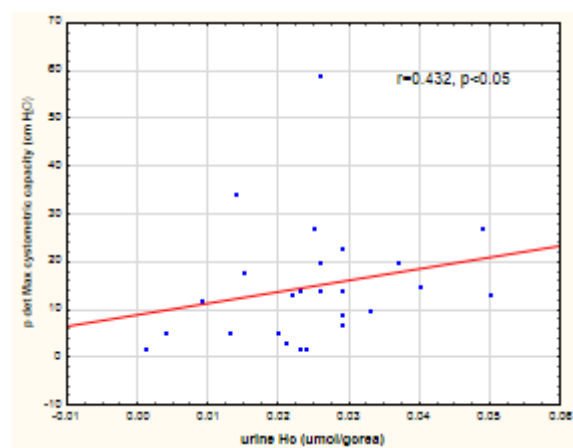
Correlations between cystometric parameters and age, height, body weight, BMI, serum and urine Hc, FA, FA/Hc ratio, and the time of follow up in the clinic are shown in Table 2.

**Table 2.** Correlations between cystometric parameters and age, height, body weight, body mass index (BMI), time of follow up, serum and urine homocysteine (Hc). CC-cystometric capacity. Compl- bladder wall compliance, P det-detrusor pressure\* p<0.05

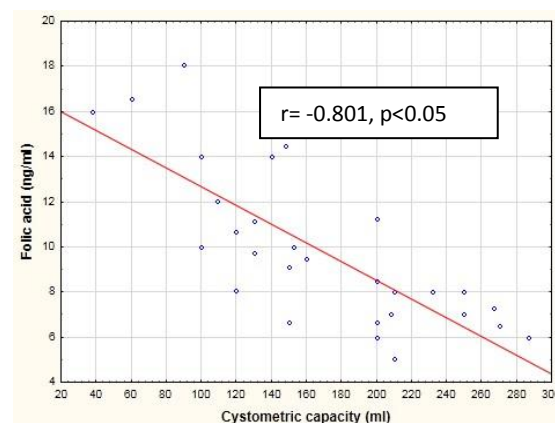
Variable	R	P value	R	P value
	P det overact (cm H <sub>2</sub> O)	P det CC (cm H <sub>2</sub> O)	CC (ml)	Compl (cm H <sub>2</sub> O/ml)
Age (years)	-0.341	0.059	0.672*	0.388*
Height(cm)	-0.289	0.05	0.612*	0.285
Body weight (kg)	-0.243	0.12	0.665*	0.169
BMI	0.001	0.168	0.609*	0.06
Time of follow up (years)	-0.341	0.021	0.326	0.307
Oxybutynin mg/day (mg)	-0.108	0.299	0.415*	-0.17
Serum Hc (umol/ml)	-0.081	0.072	0.24	0.148
Urine Hc (umol/gcrea)	-0.165	0.439*	0.128	0.134
Serum Folic acid	0.463	0.007	-0.706*	-0.243
FA/Hc ratio (ng/umol)	0.9*	0.08	-.681*	-0.278

Additionally, we analyzed serum Hc and FA concentration, urine Hc and the FA/Hc ratio between patients treated with oxybutynin and untreated patients and we did not find any differences in these parameters (p=0.54, p=0.22, p=0.59,0.427respectively). No correlations between Hc (serum and urine), FA and FA/Hc ratio and doses of oxybutynin (daily and per kg body mass) were found.

We found statistically significant negative correlations between serum FA and age (r=-0.822), height (r= -0.791) and weight (r=-0.702) in MMC patients. No correlations in these parameters were found in the controls. Correlations between urine Hc and PdetCC and between serum FA and CC are presented in Fig. 2 and Fig. 3 respectively.



**Figure 2.** Correlation between urine Hc and detrusor pressure at cystometric capacity (P det CC) in MMC patients.



**Figure 3.** Correlation between serum folic acid (FA) levels and cystometric capacity in MMC patients.

## DISCUSSION

Our study was performed on homogeneous group of patients with neurogenic bladder (NB) caused by MMC.

The results of our study show that children with neurogenic bladder after MMC have significantly higher serum and urine Hc levels compared with the reference group. The normal serum Hc range is 5-15 umol/l, our patients presented elevated levels of serum Hc. Median serum Hc in the reference group was in the normal range and achieved the desirable value of 10 umol/l [22]. The studied parameters did not differ between girls and boys. Hasegawa et al. [23] obtained different results in patients with mental diseases, who demonstrated decreased urine Hc level compared to healthy controls and a negative correlation between serum and urine Hc levels. Authors speculated that reduced urinary Hc levels

(due to decreased filtration rate) can cause increased Hc serum levels. In our study, we found positive correlations between these parameters, but they were not statistically significant. We can suspect that high serum Hc level can result in higher Hc excretion because of hyperfiltration which is observed in MMC patients.

All correlations, both uroflowmetry and cystometry parameters with Hc, FA and FA/Hc ratio which were demonstrated in our study, may suggest that Hc as well as FA can be considered as factors influencing bladder function. Detrusor activity can be modified by oxybutynin treatment and our final results may be improperly interpreted: most of our patients have been treated with oxybutynin and need long-term anticholinergic treatment. We realize that to make an optimal assessment of FA and Hc influence on bladder function we should measure these parameters twice before starting and twice during treatment. On the other hand, we did not find any differences between patients treated with oxybutynin and untreated patients in serum Hc and FA, urinary Hc and FA/Hc ratio.

High Hc levels can result in thrombosis, particularly when the level is fluctuating around 20  $\mu\text{mol/l}$  [24]. In our patients, the median level of serum Hc was close to this value and in combination with the patients' young age constitutes a very high risk of this complication. Hyperhomocysteinemia has also been recognized as a risk factor for cardiovascular diseases caused by atherosclerosis in patients with spina bifida even in the absence of obesity [25]. Serum Hc level depends on serum protein concentration. There were no differences in serum protein between both studied groups and we can say that protein concentrations did not influence serum Hc levels in patients. Elevated serum Hc level, very low activity (only 10 patients can move without any support and 11 were wheelchair dependent) are independent risk factors for cardiovascular diseases, and a large-scale study to assess the above-mentioned risk factors could be useful.

A negative correlation between bladder thickness and the Hoffer' scale means that patients with no limitation in physical activity have a thinner bladder wall, which can suggest also better bladder function. A thickened bladder wall can suggest inappropriate bladder function caused by detrusor hyperactivity or inflammation. We found negative correlations between bladder wall thickness and compliance, but the results were not statistically significant.

Statistically significant negative correlation between GFR and the Hoffer' scale assessing physical activity can result from hyperfiltration, which we observed in patients with dysfunctional voiding. These complications are

similar to obstructive nephropathy. All patients with MMC had higher median GFR than reference group, although the difference was not statistically significant. It is also possible that increased levels of urine Hc in patients with MMC were caused by impaired reabsorption in the renal tubules [26, 27]. In normal conditions, there are trace amounts of Hc in human urine [28]. Renal tubules function deterioration in MMC patients can also be caused by dysfunctional voiding. Renal failure is one of the most important causes of death at all ages, and renal function deterioration can occur even with minor neural tube defects [29]. All patients in our study had stable renal function and we speculate that it is caused by a relatively long follow up time, good compliance with recommendations resulting from a correct diagnosis, and good cooperation between patients and doctors. Preservation of renal function in this group of children is good preparation for adulthood, which is often neglected in developmental age and also poorly researched. We realized it was our duty to assess renal function more precisely in this group of children based on such molecules as microalbuminuria or retinol binding protein, which are early detectors of tubular proteinuria, and we have started such a study already.

Our patients had normal FA levels and did not differ compared with the reference group. It is well known that patients homozygous for the MTHFR mutation could require an increased daily folate dose compared with healthy individuals, although their folate levels are within the normal range [30]. Patients suspected to have MTHFR mutation require meticulous diagnostics in this area. FA negatively correlated with parameters of physical development (age, weight and length) in MMC but not in the reference group. Negative correlations between FA and FA/Hc ratio and CC and strongly positive correlations between FA/Hc and p det urg in MMC but not in controls can suggest some links and the influence of these factors on bladder function.

Treatment of hyperhomocysteinemia is very important because of a clear association with serious complications, mostly cardiovascular system disorders. Some reports have shown that FA levels correlate with serum Hc levels [29]. Low serum Hc is quite easily achieved by vitamin supplementation, where FA plays an important role [31]. Should our patients with MMC and elevated levels of Hc be treated by FA supplementation, and can this treatment result in bladder function improvement? Further research is needed to answer this question.

This survey has some limitations. Firstly, we were not able to get information about the use of FA by the mothers of our children. Secondly, we did not obtain very detailed data about our patients'

diets. We are certain of one thing - no FA supplementation was given to all enrolled subjects. Another limitation of our study is the small study group. The last one is concerning oxybutynin treatment. As it was mentioned above, oxybutynin can modify bladder function and the final results, however, there were no differences between the treated and the untreated patients

## CONCLUSIONS

Serum and urine homocysteine are elevated in MMC patients and a link with bladder function is possible. Serum FA level was in the normal range in MMC patients. FA/Hc ratio is decreased in MMC patients and correlates with cystometric parameters.

## Acknowledgements

The study was supported by a grant from the Medical University of Białystok, Poland. We would like to thank for the opportunity to use urine samples from the OLAF study.

## Conflicts of interest

The authors have no conflict of interest to disclose.

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