Endothelial Dysfunction in Children with Juvenile Psoriatic Arthritis

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ABSTRACT

Background: To evaluate the presence of endothelial dysfunction in Slovak children with juvenile psoriatic arthritis in the absence of classic cardiovascular risk factors in order to assess its relationship to the disease activity and disability.

Methods: 25 juvenile psoriatic arthritis patients (JPSA) and 25 healthy controls aged 6–19 years were enrolled into this study. In all subjects vascular measurements over a period of three years (January 2013 – January 2016) were performed, in accordance with the guidelines for ultrasonographic evaluation of FMD% (flow-mediated endothelial dependent vasodilatation) of the brachial artery. The measured items were compared to the variables reflecting the disease activity and disability.

Results: Significantly lower FMD% values in patients with JPSA when compared to healthy controls {mean(SD), median, range: 5.49% (3.77), 3.55, 0.3–13.0 vs. 9.28% (1.72), 9.3, 6.4–13.1} ($p < 0.001$) have been documented. Strong correlations between FMD% values and disease duration ($p < 0.01$), non-specific inflammatory markers levels ($p < 0.001$) or functional disability ($p < 0.01$) have been observed. Significantly lower FMD% values in patients with an early disease onset (JPSA onset < 5 years of age) when compared to the rest of JPSA group {mean (SD), median, range: 4.39% (2.47), 4.45, 0.9–13.2 vs. 6.38% (1.42), 6.3, 3.2–12.1} ($p < 0.01$) have also been detected.

Conclusion: Study is the only one addressing endothelial dysfunction development in Slovak children with psoriatic arthritides. We state that endothelial dysfunction is present in these patients even during childhood and in the absence of classic cardiovascular risk factors. Its development seems to be related to an early disease onset as well as to the increased disease activity and disability. Potential genetic predictors have also been identified.

KEYWORDS

cardiovascular risk; endothelial dysfunction; psoriasis; arthritis; childhood

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INTRODUCTION

Association of adult-onset psoriatic arthritis (PsA) with relatively high risk of cardiovascular (CV) morbidity has been well established (1–14). Recent metaanalyses discovered that patients with PsA had a 43% higher risk of having (or developing) cardiovascular diseases compared to non-psoriatic individuals, while the risk of developing an incident cardiovascular event was 55% higher in PsA patients compared with the general population. Furthermore, the risk of each of the individual cardiovascular outcomes was increased, including MI (68%), cerebrovascular diseases (22%), and heart failure (31%) in PsA patients compared with the general population (3). The presence of psoriasis (PS) on the other hand largely contributes to this fact, regarding especially timeliness of its occurrence, its severity (15, 6) and in adults, frequent association with traditional CV risk factors and fully developed metabolic syndrome (17).

Moreover, the prevalence of CV manifestations in psoriatic arthritides exhibits strong correlations to the levels of non-specific inflammatory markers, as well as to the PS/PsA duration (18). On the other hand, the question arises as to whether the risk of subclinical CV manifestations in psoriatic arthritides appears to be higher even in the low age groups, ie. in the groups of young children without presence of classic CV risk factors. However, it is assumed that the release of inflammatory markers and mediators at a stage of high disease activity significantly potentiates the formation of endothelial dysfunction (ED) and thus, despite the absence of conventional CV risk factors, might predispose to atherosclerosis (ATS) as well as the resulting complications. Whereas the inflammatory genesis of ED development is (under the same conditions) also expected during childhood, by contrast, the presence of ED in juvenile psoriatic arthritides (JPSA) remains very poorly defined (worldwide). Endothelial dysfunction represents a key step in the initiation and maintenance of atherosclerosis and may serve as a marker for future risk of cardiovascular events (19, 20). It can be noninvasively measured by detecting the postoclussive flow mediated vasodilation (FMD%) of the brachial artery using high sensitive ultrasonography (B-mode) (20). This study was aimed to assess whether ED is present in children with JPSA in Slovakia (even providing the absence of classic CV risk factors) and to evaluate to what extent it is related to the disease activity and functional disability.

MATERIAL AND METHODS

The study was realized over a period of three years (January 2013 – January 2016). Children with JPSA aged 6–19 years (n = 25) and 25 healthy controls were included. Only patients fulfilling the ILAR (International League of Associations for Rheumatology) criteria for JPSA were included (21). These were as follows: 1) juvenile arthritis and psoriasis or 2) arthritis and at least two of the following a) dactylitis, b) nail pitting or onycholysis, c) positive family history of psoriasis in a first-degree relative. Patients with JPSA were sub-categorized in one of the following categories according to the characteristic patterns of joint involvement: 1) polyarticular onset, 2) monoarticular or assymetrical oligoarticular onset, 3) predominant involvement of the distal interphalangeal joints, 4) involvement of the sacroiliac joints. With regards to the ILAR exclusion criteria all included patients were rheumatoid factor (RF) negative. No family history of HLA B27 associated disease and no onset of arthritis in a male with HLA B27 antigen positivity after the age of 6th. year were detected. Patients with systemic onset of juvenile idiopathic athritis were excluded.

We aimed to eliminate the presence of traditional CV risk factors in our study by expanding the exclusion criteria such as: 1) presence of hypertension (blood pressure > 95.th percentile for given age, gender and body hight), 2) presence of diabetes mellitus (defined as fasting plasma glucose ≥ 7.0 mmol/L), 3) prediabetes (defined as borderline fasting plasma glucose ranging from 5.6 to 6.9 mmol/L), 4) dyslipidaemia (defined as total cholesterol ≥ 4.85 mmol/L, low-density lipoprotein cholesterol ≥ 3.25 mmol/L, high-density lipoprotein ≤ 0.85 mmol/L, triacylglycerides ≥ 1.50 mmol/L) 5) obesity (body mass index > 95.th percentile), 6) evident CV disease or positive history of well-defined atherosclerotic events, 7) smoking, 8) treatment with antihypertensive agents, antiaggregant drugs or estrogens. In all included subjects, the renal function values fell within the reference range.

Activity of JPSA was evaluated according to the criteria presented by Giannini et al. (22). Variables in the core set included: 1) physician global assessment of overall disease activity on visual analogue scale (VAS), range 0–10 cm, 2) parent or patient global assessment of overall well-being on visual analogue scale (range 0–10 cm), 3) assessment of functional disability (23), 4) number of joints with active arthritis, 5) number of joints with limited range of motion, and 6) markers of inflammation – erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) levels.

On the whole, the fasting plasma glucose, serum lipid profile, blood pressure values, body mass index (BMI), age at the JPSA onset, JPSA activity and disability as well as the JPSA duration were assessed. Moreover, some laboratory predictors of disease severity course (antinuclear antibodies and major histocompatibility complex class I and II) were evaluated. In all patients vascular measurements were performed, in accordance with the guidelines for FMD% ultrasonographic evaluation of brachial artery as shown by Corretti et al. (24). The measurements were performed using high-resolution Doppler ultrasound (Acuson Antares 5.0, Siemens Medical Solutions).

Informed consent was obtained from all subjects or from their parents in underaged patients with prior approval of the ethics committee in Martin, Slovakia. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

STATISTICAL ANALYSIS

The results have been analyzed using statistical program SPSS (version 14.0, SPSS Inc., Chicago, IL 60606-6412,

USA). Continuous data have been expressed as mean ± standard deviation (SD), median and range. Categorical data have been presented directly as numeric and percentage expression. We compared categorical data and proportions using the chi-square test or Fisher's exact test as indicated. FMD% values have been compared using the Mann-Whitney test. The correlation between continuous variables was assessed using Pearson's linear correlation coefficients (r). P ≤ 0.05 has expressed a statistically significant difference between the compared variables.

RESULTS

Demographic, clinical and laboratory features of children with JPSA are shown in Table 1. Most patients of the JPSA group (76%) were treated with NSAID (non-steroidal antirheumatic drugs), of that 14 (56%) children received naproxen in a dose of 10–20 mg/kg/day, 5 (20%) patients received ibuprofen in a dose of 30–40 mg/kg/day. No patient was treated with systemic corticosteroids. At the time of study, 21 (84%) patients were using Disease Modifying Antirheumatic Drugs (DMARDs). 18 (72%) were taking methotrexate in a dose of 0.3–0.5 mg/kg once a week, 2 (8%) of patients received sulphasalazine in a dose of 50 mg/kg/day. 1 (4%) patient received adalimumab (40 mg once per 14 days).

The outcome showed a significantly lower FMD% values in patients with JPSA when compared to healthy controls {mean (SD), median, range: 5.49% (3.77), 3.55, 0.3–13 vs. 9.28% (1.72), 9.3, 6.4–13.1} ($p < 0.001$). We have also detected the significant correlations between FMD% and the level of CRP or ESR values ($p < 0.001$). Likewise, in patients with JPSA the significant correlations between FMD% values and disease duration ($p < 0.01$), CHAQ (Childhood Health Assessment Questionnaire) disability index (range 1–3) ($p < 0.01$) and physician global assessment of disease activity on the visual analogue scale (VAS) ($p < 0.01$) have been observed. No significant relations have been detected between the FMD% values and values of assessed traditional CV risk factors. The interpretation of data is shown in Tables 2 and 3. We also compared some clinical and laboratory features between early-onset JPSA group with remaining JPSA patients (we used age 5 years as the cut-off based on our previous findings) (25). Significantly lower FMD% values in patients with an early disease onset when compared to the rest of JPSA group (late onset JPSA) {mean (SD), median, range: 4.39% (2.47), 4.45, 0.9–13.2 vs. 6.38% (1.42), 6.3, 3.2–12.1} ($p < 0.01$) have been detected. Similarly, in early-onset JPSA subgroup significantly higher frequency of the presence of HLA DRw8, HLA DR 5 or both ($p < 0.005$) has been documented. Moreover, higher degree of functional disability (CHAQ) and disease activity (number of swollen joints, number of painful

Tab. 1 Characteristic of JPSA patients.

Variable	Patients (n = 25)	Controls (n = 25)	P value
Age (years), mean±SD			
At time of this study	13.91 ± 2.98	14.01 ± 3.0	0.34
At the time of JPSA onset	8.43 ± 2.83		
Boys/Girls	11/14	11/14	1.00
Age < 5 years at the time of JPSA onset, n (%)	12 (48%)		
JPSA Duration (years)	5.48 ± 3.91		
JPSA Activity and disability			
No. of swollen joints	2.0 ± 1.2		
No. of painful joints	2.91 ± 1.57		
No. of joints with limited ROM	2.12 ± 1.34		
VAS 1 (0–10 cm)	2.6 ±1.4		
VAS 2 (0–10 cm)	2.8 ± 1.7		
CRP (mg/L)	12.82 ± 8.58	2.29 ± 1.4	**<0.001**
ESR (mm/hour)	17.91 ± 8.6	5.91 ± 3.43	**<0.001**
CHAQ disability index (range 1–3)	0.81 ± 0.54	0.00 ± 0.02	**<0.01**
Morning stiffness (min.)	17.5 ± 10.58		
Other JPSA characteristics			
Polyarticular onset, n (%)	5 (20%)		
Mono- or assymetrical oligoarticular onset, n (%)	20 (80%)		
Predominat impairment of DIP, n (%)	8 (32%)		
Sacroiliitis, n (%)	3 (12%)		
Nail changes, n (%),anytime during the course of disease	18 (72%)		
Dactylitis, n (%),anytime during the course of disease	13 (52%)		
Enthesitis, n (%),at the time when the examination is carried out	7 (28%)		
ANA, n (%)	13 (52%)		
HLA B27, n (%)	7 (28%)		

Values in the table are expressed as mean ±SD or number and percentage expressing.
JPSA = juvenile psoriatic arthritis, n = number of subjects, ROM = range of moving, VAS 1 = visual analogue scale 1 (physician global assessment of overall disease activity on visual analogue scale), VAS 2 = visual analogue scale 2 (parent or patient global assessment of overall well-being on visual analogue scale), CRP = C-reactive protein, ESR = erythrocyte sedimentation rate (Westergren method), CHAQ = Childhood Health Assessment Questionnaire, DIP = distal interphalangeal joints, ANA = antinuclear antibodies, HLA = Human Leukocyte Antigen

joints, number of joints with limited mobility, CRP/ESR, VAS) has been observed (p for all the above listed < 0.01) in early-onset JPSA subgroup. The interpretation of data is shown in Table 4. We also compared the FMD% values in the group of patients with JPSA treated with NSAIDs with the values in the non-NSAID group of patients with JPSA. Even though we observed higher FMD% values in the group of patients treated with NSAIDs, statistically significant differences between the compared groups were not confirmed {mean (SD), median, range, NSAID group: 5.59% (1.47), 5.55, 2.8–13.2 vs. non-NSAID group: 5.36% (1.44), 5.3, 0.8–11.1} (p = 0.81). While evaluating the conventional CV risk factors we have not observed any statistically significant differences between the group of patients with JPSA and the control group of healthy children.

Tab. 2 Evaluated traditional CV risk factors.

Variable	Patients (n = 25) Mean ± SD	Controls (n = 25) Mean ± SD	P value
Blood pressure (percentile)	59.62 ± 10.43	60.0 ± 10.1	0.78
Body mass index (percentile)	55.25 ± 7.39	55.33 ± 6.88	0.93
Total cholesterol (mmol/L)	3.85 ± 0.38	3.82 ± 0.37	0.71
HDL(mmol/L)	1.29 ± 0.27	1.37 ± 0.26	0.23
LDL (mmol/L)	2.15 ± 0.35	2.12 ± 0.31	0.48
Triglycerides (mmol/L)	0.52 ± 1.6	0.48 ± 0.18	0.88
Fasting plasma glucose (mmol/L)	4.59 ± 0.58	4.55 ± 0.48	0.80
FMD% values mean (SD), median, range	5.49% (3.77), 3.55 0.3–13	9.28% (1.72), 9.3 6.4–13.1	**<0.001**

Values in the table are expressed as mean ± SD, CV = cardiovascular, HD = high-density lipoprotein, LDL = low-density lipoprotein, FMD% = postoclussive flow-mediated vasodilation

Tab. 3 Correlation of FMD% in patients with JPSA with selected variables.

Variable	r	P value
Age at the time of study	–0.247	0.24
JPSA duration	–0.755	<0.01
CHAQ disability index (range 0–3)	–0.733	<0.01
VAS (range 0–10 cm)	–0.715	<0.01
ESR	–0.850	<0.001
CRP	–0.901	<0.001

JPSA = juvenile psoriatic arthritis, CHAQ = Childhood Health Assessment Questionnaire, VAS = visual analogue scale (physician global assessment of overall disease activity on visual analogue scale), ESR = erythrocyte sedimentation rate (Westergren method), CRP = C-reactive protein

DISCUSSION

The very existence of cutaneous psoriasis, even in the absence of joint disease, has been linked to accelerated ATS. A 2014 systematic review of 20 studies of endothelial

Tab. 4 Comparison of patients with early-onset JPSA with those with late-onset JPSA.

Variable	Early-onset N = 12	Late-onset N = 13	P value
JPSA duration, (years), mean ± SD	6.5 ± 2.4	4.8 ± 3.0	<0.01
Females, n (%)	9 (75%)	4 (30.8%)	<0.01
Age of onset (years), mean ± SD	2.3 ± 1.4	10.88 ± 3.4	<0.001
CHAQ disability index (0–3), mean ± SD	1.13 ± 0.13	0.63 ± 0.38	<0.01
ESR (mm/hour), mean ± SD	24.6 ± 8.4	15.8 ± 3.5	<0.01
CRP (mg/L), mean ± SD	15.8 ± 8.3	8.85 ± 7.5	<0.01
No. of swollen joints	3.0 ± 6.16	1.0 ± 3.3	<0.01
No. of painful joints	4.4 ± 10.2	1.4 ± 6.9	<0.01
No. of joints with limited ROM	3.3 ± 6.5	0.9 ± 3.2	<0.01
VAS (0 – 10 cm)	3.6 ± 3.2	1.6 ± 3.2	<0.01
HLA DRw8 (%)	7 (58.3%)	1 (7.6%)	<0.005
HLA DR 5 (%)	6 (50%)	1 (7,6%)	<0.005
HLA B 27 (%)*	0 (0%)	7 (53.8%)	<0.001
ANA (%)	8 (66.6%)	5 (38.4%)	<0.05
FMD% values mean (SD), median, range	4.39% (2.47), 4.45 0.9–13.2	6.38% (1.42), 6.3 3.2–12.1	<0.01

JPSA = juvenile psoriatic arthritis, CHAQ = Childhood Health Assessment Questionnaire, ESR = erythrocyte sedimentation rate (Westergren method), CRP = C-reactive protein, HLA = Human Leukocyte Antigen, ANA = antinuclear antibodies, FMD% = postoclussive flow-mediated vasodilation; *Note: Three HLA B 27-positive boys with the onset of the disease between year 5 and year 6 of their lives were included in the group

function in psoriasis patients found that FMD% was significantly impaired in the majority of studies (26). Some data suggest that the likelyhood of endothelial dysfunction is correlated with disease severity or disease duration (26). Interestingly, increased carotid intima-media thickness, a measure of subclinical ATS, has been demonstrated repeatedly in patients with PS and has been shown to correlate with FMD% (26, 27). Taken together, these data suggest that patients with psoriasis display impaired endothelial-dependent relaxation and that this may correlate with future development of ATS. On the other hand, with the concurrent presence of arthritis and psoriasis, the CV risk might be even higher. Polachek et al. assessed the magnitude of risk of cardiovascular and cerebrovascular morbidity in patients with adult form of psoriatic arthritis (PsA) compared with the general population through a systematic review and meta-analysis of observational studies. Eleven studies, comprising 32,973 patients with PsA, were evaluated. There was a 43% increased risk of cardiovascular diseases in patients with PsA compared with the general population (pooled odds ratio [OR] 1.43 [95% confidence interval (95% CI) 1.24–1.66]). The risk of incident of cardiovascular events was increased by 55% (pooled OR1.22–1.96). Morbidity risks for myocardial in-

farction, cerebrovascular diseases, and heart failure were increased by 68%, 22%, and 31%, respectively (pooled OR 1.68 [95% CI 1.31–2.15], pooled OR 1.22 [95% CI 1.05–1.41], and pooled OR 1.31 [95% CI 1.11–1.55] (3).

Juvenile psoriatic arthritis (JPSA), on the other hand, is probably the most discussed and controversial of the various forms of childhood arthritides. Part of the debate derives from its characterisation as a single diagnostic category in current classifications, despite numerous demonstrations of its heterogeneous nature (28). In JPSA, it is necessary to distinguish between at least two different clinical phenotypes. Some of the children with JPSA can certainly be included in the SEA syndrome (seronegative – enthesitis – arthritis), which we also called juvenile spondyloarthropathy in the past, but the second group, are ANA positive early-onset children with clinical signs characteristic of JIA.

Children with JPSA, however, are exposed to various proatherogenic insults, but unlike the adult-forms of PsA, prevalence of subclinical atherosclerosis remains poorly defined in these patients. The exact mechanism of the predisposition to cardiovascular disease in psoriasis and psoriatic arthritides has not been explained satisfactorily. It has been suggested that three factors contribute to the cardiovascular risk profile. First, systemic and chronic inflammation due to the persistent secretion of tumour necrosis factor alpha and other proinflammatory cytokines plays a major role in the excessively high cardiovascular risk (29, 30). Second, comorbidities, such as smoking, diabetes mellitus, hypertension, metabolic syndrome and dyslipidaemia (have been exluded in our study). The third factor is the atherogenic side effects of systemic therapy. Recent results of some meta-analyses that are assessing associations between CV events and use of TNF (tumor necrosis factor) inhibitors, methotrexate, NSAIDs and glucocorticosteroids (GCs) in adult patients with RA (rheumatoid arthritis) or PS/PsA patients show, that NSAIDs treatment increased the risk of all CV events (RR, 1.18; 95% CI 1.01 to 1.38; p = 0.04) as well as strokes. This effect is mostly driven by cyclooxygenase-2 (COX-2) inhibitors (RR, 1.36; 95% CI 1.10 to 1.67; p = 0.004) rather than non-selective NSAIDs (RR, 1.08; 95% CI 0.94 to 1.24; p = 0.28). The potential negative cardiovascular effects of corticosteroids are well known but not strongly evidence-based (31, 32). Corticosteroids may modulate the risk of cardiovascular disease in rheumatoid patients in two competing ways – by increasing the risk due to deleterious effects on lipids, glucose tolerance, weight gain and hypertension, but on the other hand, potentially decreasing the risk by exerting antiinflammatory and antiproliferative effects on vascular wall (33). Furthermore, GCs may result in two independent effects over a time: an immediate effect of current exposure and a long-term cumulative effect of past exposure (31, 34). The same authors also quote that, corticosteroids are linked to the increased risk of all CV events (RR, 1.47; 95% CI 1.34 to 1.60; p < 0.001), as well as risk of myocardial infarction, stroke and heart failure in RA patients (31, 34). On contrary to this, the systemic treatment of patients with PS/PsA was associated with a significant decrease in risk of all the CV events (RR, 0.75; 95% CI 0.63 to 0.91; p = 0.003). The new biological treatments with a specific target, such as suppression of systemic inflammation by anti TNF – therapy, tend to be associated with concomitant risk reduction of the CV events in all abovementioned diseases. However, there is a need for further research focused on the role of recent biological treatments effects on patients with CV risk profile. Overall, all PsA patients have higher prevalence of CV comorbidities, including diabetes and hypertension, than the general population (35, 36). Besides that, women's prevalence rate for CV risk is higher than men's due to a higher prevalence of metabolic syndrome and a high blood pressure. Costa et al. reported that HDL-cholesterol was increased and triacylglycerides were decreased in PsA patients treated with adalimumab (37). According to the same authors, the PsA patients treated with etanercept had decreased level of triacylglycerides (37). However other two studies did not prove a significant change (36, 38). There were no significant effects on total cholesterol, LDL-cholesterol, atherogenic index, or Apolipoprotein B : Apolipoprotein A-1 ratio (36, 38). Among children with JIA, treatment with etanercept significantly decreased total cholesterol, LDL-cholesterol, triacylglycerides, and CRP, but not HDL-cholesterol (39). However, this overall improvement in lipid profile and inflammation did not translate into improvement in atherogenic index.

Our study is the only one addressing ED development in Slovak children with juvenile psoriatic arthritides. The results are suggesting that ED development could be present even during childhood and in the absence of the traditional cardiovascular risk factors in these groups. Observation of significant relations between FMD% values and non-specific inflammatory markers levels and other variables reflecting the disease activity in our study leads us to believe that systemic inflammation can be regarded as dominant CV morbidity predictor in these JPSApatients. The vascular endothelium is known to be a target of TNF-α (tumor necrosis factor-α), which significantly participates in pathogenesis of the diseases. Circulating T-lymphocytes and monocyte-derived macrophages isolated from PSA patients produce increased amount of TNF-α in comparison with macrophages isolated from healthy patients. Futhermore, levels of TNF-α in PSA patients are elevated in the synovial tissue and skin lessions and correlate with disease activity (40, 41). On a cellular level, TNF-α induces theexpression of genes associated with inflammation, coagulation and proliferation. Decreased NO bioavailability appears to be a common and critical step linking TNF-α to endothelial dysfunction. TNF-α exerts its effects on the endothelium through its receptor (TNFR). Binding of TNFR by TNF-α leads to diminished eNOS (endothelial nitric oxide synthase) protein expression via suppression of promoter activity and destabilization of its mRNA. TNFR suppresses eNOS activity by preventing the degradation of its endogenous inhibitor ADMA (asymmetric dimethylarginine). TNFR signaling also induces the transcription factor NF-κB leading to enhanced expression of intercellular adhesion molecules (ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1) (41, 42). Moreover, the long-lasting chronic inflammation may act as a promoter of oxidative processes, insulin resistance, and dyslipidemia that definitely have to be considered in the ED development (41–47). In this context, the

early investigation of ED in active JPSA patients (even in the absence of traditional CV risk factors) might prevent severe complications during adulthood.

Physical inactivity, on the other hand, is another modifiable risk factor for premature atherosclerosis according to some authors. A study in adolescents and young adults by Edwards et al. (48) showed that higher physical activity was an independent positive predictor for lower arterial stiffness, measured as peripheral arterial distensibility and AIx (augmentation index). In this meaning, the detection of significant correlations between CHAQ disability index (functional impairrment) and FMD% values in our study suggests, that chronic inflammation, when associated with higher degree of functional disability, could significantly influence the ED development. The amplifications of above mentioned considerations is assumed in relation to the observed significant correlations between FMD% and early onset of the disease, since in our study the early JPSA onset was associated with higher degree of disease activity as well asfunctional impairment. Also, in patients with early-onset JPSA, we observed the presence of HLA DR5 and HLA DRw8 antigens. In association with the possible partial distortion of our results, we have to mention, that treatment with DMARDs might be associated with a significant decrease of inflammation, degree of disease disability as well as with reduction in diastolic and systolic blood pressure values and lipids profile (49). Methotrexate, an inhibitor of folic acid metabolism, can directly improve endothelium-dependent vasodilation in patients with inflammatory arthritides (although the data are limited) (50, 51), as it reduces the systemic inflammation and improves synovitis in patients with inflammatory arthritides. Anti-TNF-α inhibitors, on the other hand, also improve endothelium-dependent vasodilation in these patients (52, 53) and correlates with improvement in disease activity and markers of systemic inflammation (16). Last but not least, it should be mentioned that due to the low prevalence of JPSA in our country, our group of patients is relatively small. Therefore, to get more relevant results, combining outcomes of several national and international centers seems to be more beneficial.

CONCLUSIONS

It can be concluded that endothelial dysfunction is present in patients with JPSA even during childhood and in the absence of classic CV risk factors – not only in association with increased disease activity and disability, but also if JPSA is associated with an early onset or in relation to prolonged JPSA duration. It appears to be desirable, therefore, to evaluate the presence of ED in patients with JPSA. A desired objective of further research is to examine the possibility of early intervention in those children in order to prevent the future ATS development with resulting complications in adulthood.

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INFORMED CONSENT

Informed consent was obtained from all subjects with prior approval of the ethics committee in Martin, Slovakia. Informed written consent was obtained from each patient.

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Soluble Tumor Necrosis Factor (TNF)-Like Weak Inducer of Apoptosis (Tweak) Independently Predicts Subclinical Atherosclerosis in Behcet's Disease

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ABSTRACT

Background: Vasculopathy is a major cause of mortality and morbidity in Behcet's Disease (BD). Subclinical atherosclerosis can even be detected in the early stage of BD. Soluble tumor necrosis factor-like (TNF) weak inducer of apoptosis (TWEAK) is known as a good marker of the inflammation in vascular tree. The aim of this study is to examine the relationship between carotid artery intima-media thickness (cIMT) and serum TWEAK levels in patients with BD.

Materials and Methods: In line with International BD Study Group criteria, 48 BD, and 30 controls were included in our study. Disease activity was evaluated according to BD current activity form (BDCAF). C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), lipid parameters, serum TWEAK levels, and cIMT were measured.

Results: Disease activity score of BD patients was found as 2 (range 0–7). cIMT, serum TWEAK, CRP and ESR levels of BD patients were significantly higher comparing to cIMT (0.62 ± 0.13 mm vs. 0.43 ± 0.09 mm, $p < 0.001$), serum TWEAK (667.5 ± 130.6 vs. 603.4 ± 89.6 pg/ml, $p = 0.015$), CRP (3.9 ± 4.3 vs. 1.4 ± 1.0 mg/dl, $p < 0.001$) and ESR (10.2 ± 10.0 vs. 5.6 ± 3.7 mm/h, $p = 0.005$) levels of the control group. There was a positive correlation between serum TWEAK level and disease activity ($r = 0.251$, $p = 0.030$) and cIMT ($r = 0.463$, $p < 0.001$). Our study also revealed an independent correlation between cIMT and serum TWEAK levels (beta = 0.354, $p < 0.001$).

Conclusion: Increased serum TWEAK levels can play a part in the development of atherosclerotic heart disease in BD. Due to their liability to atherosclerosis, patients with BD must followed closely.

KEYWORDS

Behcet's disease; Tumor necrosis factor (TNF)-like weak inducer of apoptosis; TWEAK; carotid artery intima-media thickness; atherosclerosis

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INTRODUCTION

Behcet's disease (BD) is a complex systemic inflammatory disease associated with vascular involvement, thrombogenicity, endothelial cell damage by platelets and leukocytes, and activation of endothelial cells by pro-inflammatory cytokines and chemokines (1). Recent studies still demonstrate the importance of inflammatory cytokines and chemokines because BD affects young adults and it is characterized by remitting-relapsing course (2). Thus, the cardiac involvement is generally associated with morbidity and mortality and it is one of the most severe complications in patients with BD (3).

Endothelial damage/activation has been described as the initial lesion in the development of atherosclerosis and it has been shown to increase in the active state of disease (4). Furthermore, subclinical atherosclerosis in these patients can be easily and reliably determined by evaluating the carotid artery intima-media thickness (cIMT) (5).

Recent studies emphasize the diagnostic potential of the soluble tumor necrosis factor-like (TNF) weak inducer of apoptosis (TWEAK) as a useful biomarker in various inflammatory and non-inflammatory disorders (6). In recent decades, there has been a significant increase in the literature regarding the use of TNF inhibitors in the treatment of BD. Therefore, TWEAK has been the focus of attention in current studies (7). TWEAK is a cytokine which is mostly derived from leukocytes and belongs to the TNF family. Furthermore, various diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and multiple sclerosis occur through cellular responses which can be associated with inflammatory pathways induced by inflammatory multifunctional cytokines such as TWEAK (8).

Newly mounting evidence has suggested that TWEAK plays a major role in the pathological remodeling underlying other inflammatory diseases, namely cardiovascular diseases and obesity-associated type-2 diabetes mellitus (9–11), particularly in myocardial remodeling leading to heart failure (7). Therefore, soluble TWEAK may be used as a potential biomarker in cardiovascular diseases (12).

In this current study, our aim is to investigate the relationship between serum TWEAK levels and cIMT in patients with BD.

METHODS

PATIENT POPULATION

This cross-sectional study was conducted with outpatients who were admitted to the Rheumatology and Cardiology clinics of our University Hospital between May 2015 and October 2015. We included totally 48 male BD patients in this study. Additionally, 30 age- and sex-matched volunteers were selected to be the control group during the same period.

Patients who previously diagnosed with BD according to International BD Study Group criteria were included in this study (International study group for BD) (Evaluation of diagnostic ('classification') criteria in BD disease-towards internationally agreed criteria) (13). Disease severity in BD patients was evaluated by using BD current activity form (BDCAF) (14). Patients with concomitant systemic diseases such as diabetes mellitus, chronic obstructive lung disease, hypertension, coronary artery disease, cancer, thyroid function disorder, hematological disorders, acute or chronic liver and renal diseases, acute or chronic infections, history of smoking and alcohol consumption were excluded from the study. We did not include patients who were actively using drugs such as antihypertensive agents, steroids or statins; which can negatively or positively affect the cIMT values. Subjects with no regular use of medications, smoking history, alcohol consumption and who did not have a known disease were selected as the control group. The study was approved by the Ethics Committee of the Medical Faculty of our university and written and verbal informed consents (consistent with the Helsinki Declaration) were obtained from all participants.

SAMPLE SELECTION (PATIENTS AND CONTROLS)

Totally 60 outpatients in the Rheumatology clinics were included in this study. However, 12 patients who did not meet the inclusion criteria were excluded from the study. Three out of twelve patients excluded due to hyperlipidemia, 2 of them had a diabetes mellitus, 2 of them had a thyroid disease, 2 of them had a liver dysfunction disease, 1 had a renal disease, 1 had a coronary heart disease, and 1 of them had a hematological disease. Control patients were individuals who were admitted to the cardiology outpatient policlinic in our hospital due to the chest pain and who did not have any cardiac or other diseases as a result of their physical examinations.

cIMT MEASUREMENTS

In this study, cIMT measurements were performed by one of the authors who were unaware of patients. The Vivid 7 echocardiography device (General Electrics, Horten, Norway) was used to assess the carotid arteries with the help of 10-MHz linear probe. Playback analysis was performed by using the recorded acquired images and they were measured off-line. On both sides of the body, the common carotid artery, the carotid bulb, and internal and external carotid arteries were observed. The intima-media thickness (IMT) was measured in the distal part of the carotid artery which was located in the 15 to 20 mm proximal to the carotid bulb. Furthermore, two bright echogenic lines, which were located in the arterial wall, were determined as intima and the media. For each side of the body, totally three measurements were performed, mean values of the results were separately calculated for each measurement and findings were recorded for both right and left IMT (15).

SYSTOLIC AND DIASTOLIC BLOOD PRESSURE (BP) MEASUREMENT

All patients systolic and diastolic BP measurements obtained from their left arm by using sphygmomanometer after 15 minutes relaxing. After two additional recordings which were obtained from the same arm, waiting 5 minutes between two readings, average systolic and diastolic BP were calculated in order to get an accurate measurement.

BIOCHEMICAL ANALYSIS

Venous blood samples were collected from all participants after 10–12 h fasting. Fasting serum glucose (FPG), creatinine, alanine aminotransferase (ALT), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and C-reactive protein (CRP) levels were analyzed on Abbot Architect 16000 system with the original reagents. HDL-C levels were detected by using a direct enzymatic method without precipitation. The Friedewald formula was used in order to determine the concentrations of Low-density lipoprotein cholesterol (LDL-C). The method of laser-based flow cytometric impedance was applied to measure the complete blood counts by using an automated blood cell counter (Mindray BC-6800, Shenzhen, PR China). Furthermore, automatic ESR analyzer device was used to measure the erythrocyte sedimentation rate (ESR). On the other hand, commercial kits (Advia Centaur XP System) were used to measure the thyroid stimulating hormone (TSH) levels.

TWEAK MEASUREMENTS

Enzyme-linked immunosorbent assay kit (eBiosience, Human TWEAK Instant Elisa, Cat no: BMS2006INST) was used in order to measure the serum TWEAK concentrations (expressed as pg/mL) in patients and control individuals. The overall intra-assay coefficient of variation (CV) was calculated as 7.9%.

STATISTICAL ANALYSIS

Statistical analysis was performed by using the SPSS version 18 (Chicago IL, USA). Results were represented as means ± standard deviation or median and range depending on data distribution. Normally distributed data were analyzed by using the independent t-test. Pearson correlation test was conducted in order to examine whether or not there is a relationship between cIMT, disease activity, TWEAK levels, and other variables. Stepwise linear regression analysis was conducted in order to detect the variable(s) which mostly affected the dependent variables such as cIMT and disease activity because the numbers of independent variables were quite high. Parameters such as TWEAK levels, systolic and diastolic BP measurements, ESR, CRP, TC, TG, LDL, HDL, age, BMI, the duration of the disease, creatinine, ALT, Hb, WBC, and PLT were independent variables and they were also evaluated. Dependent variables such as cIMT and disease activity were detected by eliminating other step by step with the help of the Stepwise Linear regression analysis. A two-sided $P < 0.05$ was considered significant.

RESULTS

The mean duration of disease for patients 8.0 ± 6.3 years and the disease activity score was 2 (range 0–7). All demographic data of patients with BD were presented in Table 1. cIMT measurements and serum TWEAK levels, as well as CRP and ESR levels of patients with BD, were significantly higher comparing to cIMT measurements (0.62 ± 0.13 vs. 0.43 ± 0.09 mm, $p < 0.001$), serum TWEAK (667.5 ± 130.6 vs. 603.4 ± 89.6 pg/ml, $p = 0.015$), CRP (3.9 ± 4.3 vs. 1.4 ± 1.0 mg/dl, $p < 0.001$), and ESR (10.2 ± 10.0 vs. 5.6 ± 3.7 mm/h, $p = 0.005$) levels of the control group. Also, white blood cell (WBC) count of BD patients (7.8 ± 2.8 × 10^3/mm^3) was considerably higher than the WBC counts of control individuals (6.6 ± 1.8 × 10^3/mm^3) ($p = 0.034$). Although recordings of systolic and diastolic BP measurements of patients with BD were higher than control group, they were not statistically significant. Table 2 presents ages, BMI values, and biochemical and hematological parameters of patients with BD and the control group. TWEAK levels were presented in Figure 1.

Tab. 1 Demographics of patients with Behcet's Disease.

Findings	Behcet's Disease (n = 48)
Joint involvement n (%)	26 (54.1%)
Kidney involvement n (%)	1 (2.1%)
Eye involvement n (%)	18 (37.5%)
CNS n (%)	2 (4.2%)
Pulmonary aneurism n (%)	0
Vascular involvement n (%)	6 (12.5%)
Thrombophlebitis n (%)	8 (16.7%)
Pathergy n (%)	21 (43.8%)
Oral aphthae n (%)	47 (97.9%)
Genital ulcer n (%)	19 (39.6%)
Folliculitis n (%)	23 (47.9%)
Erythema nodosum n (%)	1 (2.1%)
GIS involvement (years) n (%)	0
Duration of the disease n (%)	8.0 ± 6.3
Disease activity score median (range) **Colchicine dose (mg)** **Azathioprine n (%)**	2 (0–7) 1.2 ± 0.5 9 (18.7%)

Abbreviations: CNS – Central Nervous System; **GIS** – Gastrointestinal System

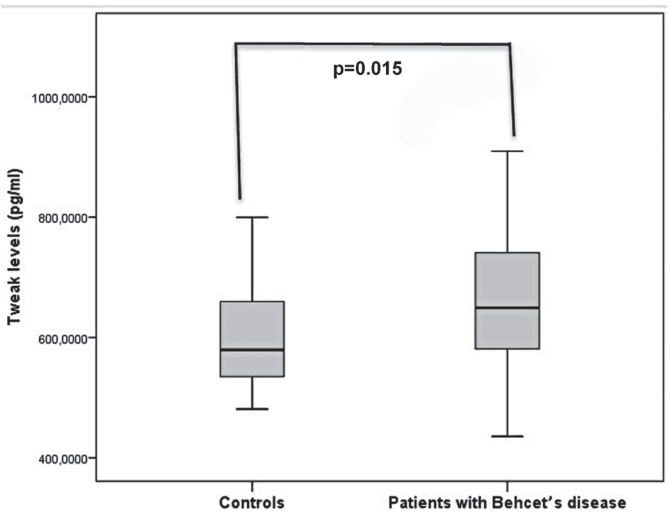

Fig. 1. Serum TWEAK levels in Behcet's disease and control groups.

Tab. 2 Ages, body mass index, carotid artery intima media thickness, and laboratory parameters of patients with Behcet's disease and the control group.

Parameters	Behcet's (n = 48) (mean ± SD)	Control (n = 30) (mean ± SD)	P value
Age (years) Gender (M/F)	37.3 ± 11.2 32/16	38.2 ± 12.6 24/6	0.758 0.155
BMI (kg/m2)	26.6 ± 5.0	25.8 ± 4.5	0.492
cIMT (mm)	0.62 ± 0.13	0.43 ± 0.09	0.001
Carotid plaque n (%)	6 (12.5)	0 (0)	0.001
SBP (mmHg)	117.6 ± 8.6	114.2 ± 7.5	0.088
DBP (mmHg)	72.5 ± 7.6	69.4 ± 5.2	0.065
TWEAK (pg/ml)	667.5 ± 130.6	603.4 ± 89.6	0.015
CRP (mg/dl)	3.9 ± 4.3	1.4 ± 1.0	0.001
ESR (mm/h)	10.2 ± 10.0	5.6 ± 3.7	0.005
TSH (mU/l)	1.6 ± 0.9	1.5 ± 1.0	0.741
FPG (mg/dl)	96.1 ± 15.1	92.9 ± 5.9	0.198
Creatinine (mg/dl)	0.79 ± 0.12	0.85 ± 0.15	0.099
ALT (IU/l)	25.2 ± 16.5	27.1 ± 17.6	0.663
TC (mmol/l)	4.4 ± 0.9	4.9 ± 0.8	0.056
TG (mmol/l)	1.7 ± 0.9	1.4 ± 0.7	0.186
HDL-C (mmol/l)	1.1 ± 0.2	1.1 ± 0.3	0.682
LDL-C (mmol/l)	2.6 ± 0.7	2.9 ± 0.8	0.096
Wbc (×10^3/mm^3)	7.8 ± 2.8	6.6 ± 1.8	0.034
Hb (g/dl)	14.3 ± 1.5	15.3 ± 1.8	0.026
PLT (×10^3/mm^3)	265.7 ± 57.3	238.8 ± 63.1	0.073

Abbreviations: BMI – Body mass index; **cIMT** – Carotid artery intima-media thickness; **SBP** – systolic blood pressure; DBP – diastolic blood pressure; **TWEAK** – Tumor necrosis factor (TNF)-like weak inducer of apoptosis; **ESR** – Erythrocyte sedimentation rate; **CRP** – C-reactive protein; **TSH** – Thyroid stimulating hormone; **FPG** – Fasting plasma glucose; **ALT** – Alanine aminotransferase; **TC** – Total cholesterol; **TG** – Triglycerides; **HDL-C** – High-density lipoprotein cholesterol; **LDL-C** – Low-density lipoprotein cholesterol; **Wbc** – White blood cells; **Hb** – Hemoglobin; **PLT** – Platelets.

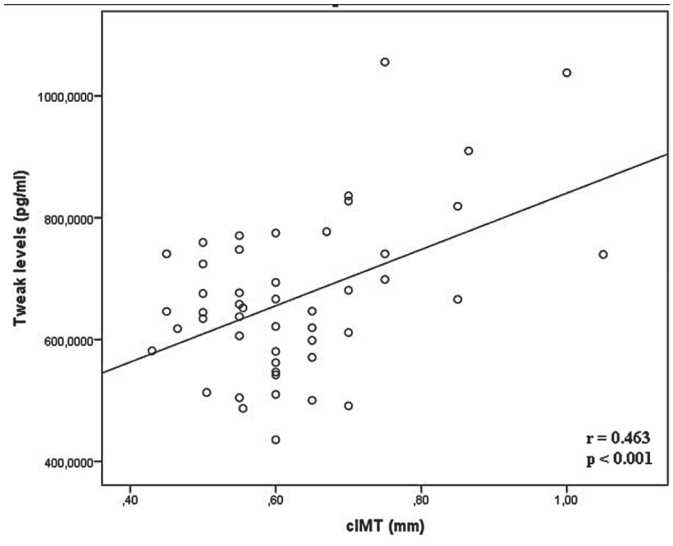

Fig. 2. Correlation between serum TWEAK level and carotid intima-media thickness.

There was positive correlation between serum TWEAK levels with disease activity (r = 0.251, p = 0.030), cIMT (r = 0.463, p < 0.001), systolic BP (r = 0.242, p = 0.036), diastolic BP recordings (r = 0.233, p = 0.045) and WBC (r = 0.294, p = 0.011). There was a positive correlation between cIMT and the disease activity (r = 0.520, p < 0.001), disease duration (r = 0.455, p < 0.001), FPG (r = 0.296, p = 0.010), ESR (r = 0.259, 0.025), systolic BP (r = 0.281, p = 0.015), and diastolic BP (r = 0.249, p = 0.031). There was positive correlation between the disease activity and ESR (r = 0.298, p = 0.008), CRP (r = 0.321, p = 0.005). The cIMT and the disease activity correlation analysis results were presented in Table 3. Correlation relation between cIMT and TWEAK was shown in Figure 2.

Tab. 3 cIMT, disease activity and correlation analysis of other factors (Pearson) in patients with BD.

	cIMT		Disease activity	
Variable	r value	p value	r value	p value
Age	0.168	0.150	0.023	0.844
BMI	0.140	0.232	0.095	0.418
Duration of disease	0.168	0.254	0.101	0.496
Disease activity	**0.520**	**0.001**		
SBP	**0.281**	**0.015**	**0.111**	**0.344**
DBP	**0.249**	**0.031**	**0.223**	**0.054**
TWEAK	**0.463**	**0.001**	**0.251**	**0.030**
ESR	**0.259**	**0.025**	**0.298**	**0.008**
CRP	0.192	0.098	**0.321**	**0.005**
FPG	**0.296**	**0.010**	0.171	0.142
TSH	0.075	0.523	0.112	0.317
TC	0.039	0.740	0.205	0.077
HDL-C	-0.119	0.311	-0.012	0.917
TG	0.118	0.314	0.048	0.682
LDL-C	0.031	0.794	0.134	0.250
Creatinine	0.037	0.751	0.180	0.123
ALT	0.173	0.137	0.038	0.748
WBC	0.128	0.275	0.144	0.219
Hb	-0.168	0.149	**-0.281**	**0.014**
Platelets	0.027	0.821	0.164	0.160

Abbreviations: cIMT – Carotid artery intima-media thickness; **BMI** – Body mass index; **SBP** – systolic blood pressure; DBP – diastolic blood pressure; **TWEAK** – Tumor necrosis factor (TNF)-like weak inducer of apoptosis; **ESR** – Erythrocyte sedimentation rate; **CRP** – C-reactive protein; **FPG** – Fasting plasma glucose; **TSH** – Thyroid stimulating hormone; **TC** – Total cholesterol; **TG** – Triglycerides; **HDL-C** – High-density lipoprotein cholesterol; **LDL-C** – Low-density lipoprotein cholesterol; **ALT** – Alanine aminotransferase; **Wbc** – White blood cells; **Hb** – Hemoglobin.

In stepwise linear regression analysis, we detected an independent relationship between cIMT and the disease activity (beta [β] = 0.431, p < 0.001) and TWEAK levels (β = 0.354, p < 0.001) when cIMT was a dependent variable. When disease activity was a dependent variable, there was an independent relationship between disease activity and CRP levels (β = 0.297, p = 0.008) and TWEAK levels (β = 0.219, p = 0.040). The stepwise regression analysis results were presented in Table 4.

Tab. 4 Determinants of carotid intima media thickness and disease activity in patients with Behcet's Disease: linear regression analysis.

Dependent variable	Independent variables	Beta regression coefficient	P value
cIMT	Disease activity	0.431	0.001
	TWEAK levels	0.354	0.001
Disease activity	CRP levels	0.297	0.008
	TWEAK levels	0.219	0.040

Abbreviations: cIMT – carotid intima media thickness; **TWEAK** –Tumor necrosis factor (TNF)-like weak inducer of apoptosis; **CRP** – C-reactive protein.

DISCUSSION

In this present study, we found that patients with BD have a higher level of cIMT comparing to control group. We also observed carotid plaques in 12.5% of patients according to their carotid ultrasound imaging. We showed that the serum TWEAK levels in BD patients were significantly higher compared to the TWEAK levels of control group individuals. There was a strong relationship between serum TWEAK levels and both cIMT and the disease activity. In regression analysis, we also detected an independent relationship between cIMT and serum TWEAK levels.

Although the etiology of BD has not been completely clarified, it is known as an auto-immune disease with chronic inflammation and multi-organ involvement (13). It was reported that auto-immune diseases such as rheumatoid arthritis, SLE, and psoriatic arthritis are characterized with high levels of TWEAK, and disease activity scores of these diseases were also associated with serum TWEAK levels (16–18). TWEAK levels are higher in SLE patients according to the controls and it has been reported that TWEAK levels are highly and positively correlated with the disease activity (19). Furthermore, particularly in SLE patients with vasculitis, serum TWEAK levels were higher compared to the patients without vasculitis (16). TWEAK levels in the synovial fluid of RA patients are also higher compared to the control individuals (20). Besides, serum TWEAK levels were positively correlated with IL-6 and TNF-α levels (17). Also, it has been shown that TWEAK causes the cutaneous vasculitis by leading to the leukocyte migration (21). Low serum TWEAK level was associated with vascular injury in systemic sclerosis which is known as an auto-immune disease (22). During BD, it was detected that pro-inflammatory cytokines such as interleukin (IL)-1β, IL-2, IL-8 and TNF-alpha (α) increased in all tissues (23). Although some studies claimed that there was a negative relationship between the serum TWEAK levels and IL-6, a number of studies reported that TWEAK simulated the NF-κβ nuclear receptor and increased the release of pro-inflammatory cytokines (24–26). In our study, the serum TWEAK levels of BD patients were quite higher compared to control individuals. The increase of the serum TWEAK level can be due to the BD which is a vasculitic disease. Our study is the first study in which the TWEAK levels are examined in BD patients. Our findings can be beneficial in terms of the role of serum TWEAK levels in BD patients and the BD etiology.

TWEAK is a member of the TNF superfamily and has an important role in pro-inflammatory cytokines release, inducing apoptosis, and simulation of cell growth (25–26). In the literature, there are contradictory findings of the relationship between TWEAK levels and atherosclerosis (27, 28). Previous studies showed a negative relationship between serum TWEAK levels and coronary artery disease, cIMT, insulin resistance, gestational diabetes and type II diabetes (29–32). However, studies cannot explain the reason why atherosclerotic process accelerated when serum TWEAK levels were low (33). Recently, an increasing number of articles has been reported that increased TWEAK levels are associated with atherosclerosis (7, 8, 12). It was detected that an increase in serum TWEAK levels resulted in an easy adherence of adhesion molecules to endothelial cells (21). Furthermore, TWEAK levels and increased smooth muscle cell proliferation and an increase in matrix metalloproteinase 3 and 9 activity were reported in experimental studies (34, 35). TWEAK was shown to cause prothrombotic events in veins due to the increased release of tissue factor and plasminogen activator in rats, which were injected with the TWEAK (36). Increased release of TWEAK leads to in an increase in pro-inflammatory cytokines and adhesion molecules which results in an increase in the monocyte and macrophage adhesion to arterial walls (37). After the migration of leukocytes, there can be deterioration in the vascular structure and inflammation and vasculitis development can be observed. On the other hand, apoptosis which is increased by TWEAK levels can lead to atherosclerotic heart disease. In other words, TWEAK can lead to vasculitis and atherosclerosis. It has been known that BD patients have an increased risk of atherosclerotic heart diseases (38). cIMT which is an important marker for subclinical atherosclerosis in BD patients was significantly higher in controls (39). In our study, cIMT and serum TWEAK levels had a strong and independent relationship. TWEAK levels can have an important role in BD etiopathogenesis and atherosclerotic heart disease development due to BD.

It is well known that hypertension is one of the major factors of atherosclerosis. Ferreira et al. showed that levels of systolic BP were an independent risk factor for cIMT even in normotensive patients (40). In our study patients with history of hypertension were excluded. Although systolic and diastolic BP measurements of patients with BD were within standard normal limits, their recordings were found to be higher compared to control group. As a matter of fact, this difference did not reach statistical significance. There was also a positive correlation between serum TWEAK levels and cIMT. No matter how we observed BP recordings of patients with BD were within standard normal limits, there was still a correlation between systolic BP levels and atherosclerosis.

In our study, there was no correlation between the CRP and cIMT whereas there was a weak correlation between ESR and cIMT. However, we found in regression analysis that TWEAK levels mostly affected cIMT. There was a strong relationship between disease activity, CRP, and TWEAK levels. Since high ESR levels in BD patients

is observed together with high CRP levels in patients with vascular involvement, uveitis, and arthritis. However high ESR levels do not reflect the disease activity in BD patients who do not experience these organ involvements (41). High CRP levels are frequently observed in male BD patients and their high CRP levels reflect the BD disease activity (42). Majority of our patients (2/3) were male and this could be the reason for the significant relationship between CRP levels and the disease activity. Therefore, serum TWEAK levels can be a better marker in BD patients compared to CRP levels in terms of disease activity and atherosclerotic processes. In our study, we detected a strong relationship between cIMT and the disease activity. Therefore, we cannot expect to use TWEAK instead of BDCAF which is a very cheap method in order to mention about the atherosclerotic process in BD patients. Our study can be a preliminary study which can show the roles of TWEAK levels in BD etiopathogenesis. In this regard, our study can direct the further studies and our findings can be beneficial for the development of new treatment protocols in BD.

LIMITATION OF STUDY

In our study, the size of our study group was small and we examined only TWEAK levels and cIMT for the detection of atherosclerosis. It could be possible to examine the relationship between increased pro-inflammatory cytokines, TWEAK levels, and other atherosclerosis markers. Furthermore, it could also be possible to have knowledge about the role of TWEAK levels in BD etiopathogenesis by detecting the relationship between BD patients and other cytokines. Our study is the first one in which TWEAK levels of BD patients were examined and there should be higher numbers of study groups in future studies.

CONCLUSION

Our study shows that there were higher serum TWEAK levels in patients with BD compared to control group which may be interpreted as an association between disease activity and atherosclerosis. Even though systolic and diastolic BP recordings of patients with BD were within standard normal limits, there was still a correlation between BP levels and subclinical atherosclerosis. Due to strong relationship between BD and atherosclerosis and patients with history of BD must follow closely.

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Evaluation of Risk Stratification Markers and Models in Acute Pulmonary Embolism: Rationale and Design of the MARS-PE (Mainz Retrospective Study of Pulmonary Embolism) Study Programme

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ABSTRACT
An acute pulmonary embolism (PE) is a crucial event in patients' life and connected with serious morbidity and mortality. Regarding a high case-fatality rate, early and accurate risk-stratification is crucial. Risk for mortality and complications are closely related to hemodynamic stability and cardiac adaptations. The currently recommended risk-stratification approach is not overall simple to use and might delay the identification of those patients, who should be monitored more closely and may treated with more aggressive treatment strategies. Additionally, some risk-stratification criteria for the imaging procedures are still imprecise. Summarized, the search for the most effective risk-stratification tools is still ongoing and some diagnostic criteria might have to be refined.
In the MAinz Retrospective Study of Pulmonary Embolism (MARS-PE), overall 182 consecutive patients with confirmed PE were retrospectively included over a 5-year period. Clinical, echocardiographic, functional and laboratory parameters were assessed. The study was designed to provide answers to some of the mentioned relevant questions.

KEYWORDS
pulmonary embolism; study design; risk stratification; symptoms; outcome

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INTRODUCTION

An acute pulmonary embolism (PE) is a crucial event in a patients' life accompanied by serious morbidity and death worldwide (1, 2). Annual PE incidence has been reported ranging between 23 and 69 cases per 100,000 people in the general population (3).

Clinical presentation of acute PE comprises a wide spectrum from asymptomatic incidental finding to typical symptoms such as chest pain, dyspnoea, hemoptysis, collapse, syncope that may accompanied by hypotension, right heart failure, right heart infarction, cardiogenic shock, resuscitation and sudden cardiac death (2, 4–6). Regarding a high case fatality rate ranging between 1% and 60% (4, 7), promptly, early and accurate risk stratification in respect to adverse outcome of patients with an acute PE event is crucial (2, 6, 8, 9).

Pathophysiologically, PE is caused by thrombotic material occluding the pulmonary arteries (10–12). If occlusion affects more than 30–50% of the pulmonary arterial bed, hemodynamically consequences of acute PE have to be expected. Right ventricle (RV) under normal conditions has a narrow range to handle an acute afterload increase. An abrupt afterload increase, seen in acute PE events, frequently results in RV overload with an elevated RV wall tension and right ventricular dysfunction (RVD) (10, 12–18). Depending on PE severity and intensity of right heart overload, RVD, coronary under-perfusion, decline of cardiac output, myocardial injury, cardiac shock, right heart failure and PE-related death could be the results (12–14, 16, 19, 20).

Risk for mortality and complications in acute PE events are closely related to (initial) hemodynamic stability as well as cardiac adaptations (12–17, 21–24). Therefore, a risk-related classification of acute PE severity has been recommended to guide risk-adjusted management of patients; the short-term adverse outcome of acute PE is dependent on PE severity status stratified by clinical findings during the acute phase, results of imaging procedures and biomarker measurements indicating for RVD, but also on the factors age and comorbidities (determining the reserve capacity of the cardio-pulmonary system) (2, 12). Haemodynamically unstable PE patients are classified as high-risk PE (2, 12) or massive PE (9), with high mortality rate (>15% in the first 30 days after PE event) (11, 12, 16, 21). Haemodynamically stable PE patients (non-high-risk or non-massive PE patients) can be subdivided into those with RVD and/or positive biomarkers, such as cardiac troponin (cTn) and/or brain natriuretic peptides (BNP), and those without both (11, 12). Haemodynamically stable PE patients with RVD or positive biomarker levels (submassive PE stadium) show an intermediate risk, with a short term mortality of 3–15% (10, 12, 16, 21). Haemodynamically stable PE patients without RVD and without an elevated biomarker levels (low-risk PE) reveal the best prognosis, with a short term mortality rate <2% (10–12, 14, 21). During the past years, recommendations for PE risk stratification were adapted, because it became obvious that laboratory biomarkers alone were not sufficient for risk stratification (12). Several risk stratification markers, algorithms and scores have been developed to identify those patients, who are at higher risk for the development of adverse events and especially mortality (2, 25).

Therefore, PE classification approach was refined and improved by the (simplified) Pulmonary Embolism Severity Index (PESI) implemented in the recommendations of the 2014 ESC guideline (2). Summarized, risk stratification comprises the factors age, gender, symptoms, clinical examination markers such as temperature, altered mental status, hypoxia, respiratory rate, blood pressure and heart rate, comorbidities, VTE risk factors, as well as biomarkers including especially markers of heart strain and myocardial injury and imaging procedures focusing on the adaptations of the heart and the burden of PE (2).

Several diagnostic tools and biomarkers are already in use for early risk stratification in non-high-risk PE patients. Especially, biomarker testing can improve inpatient management, enabling better outcomes in acute PE (21). cTn and BNP are well established markers for risk stratification in acute PE, and their elevations are closely connected with an increased mortality rate (11–18, 22, 24, 26–28). Besides these two biomarkers, several other biomarkers have been identified for prediction of poor outcome (9, 12, 21, 29).

More than 80% of the PE patients are normotensive (30, 31), and between 25% and 55% of the normotensive PE patients have an identified RVD in echocardiographic or computer-tomographic examinations (30, 32). RVD in hemodynamically stable PE patients appears to alter patients' prognosis significantly (30) and right ventricular failure (RVF) with RVD are the most common causes of death in the first 30 days after the PE index event (33). Therefore, morphologic adaptations of the heart identified in imaging procedures are important findings in risk stratification process. Especially, the prognostic value of echocardiography for risk stratification in hemodynamically stable PE patients was confirmed in several studies (30–36). Echocardiography is currently the mainstay examination for assessment of RVD in patients with PE (32, 37). Although several studies confirmed that RVD is connected with higher rates of death, recurrent PE events and complications (9, 12, 30, 32–34, 36), definitions of PE-induced RVD criteria vary markedly in different studies (30–32, 37) and recommendations for detection of RVD in the guidelines for the echocardiographic criteria for the assessment of RVD in acute PE are not precisely defined (2, 9, 12).

Another often used examination tool is the electrocardiography (ECG). In general, all patients with chest pain, dyspnoea, syncope or collapse should obtain an ECG in the emergency department immediately after first medical contact (38–40). This ECG is primarily used to exclude an acute ST-segment elevation myocardial infarction and arrhythmias (38, 39), but is also an important diagnostic tool in PE (40, 41).

Although this study focus on the in-hospital course of the PE patients and PE has traditionally been assumed as acute disease, also the long-term course of PE survivors can be complicated by recurrent venous thromboembolism events (VTE), development of post-thrombotic syndrome after accompanying deep venous thrombosis (DVT), chronic thromboembolic pulmonary hypertension (CTEPH), and treatment complications such as bleeding

events caused by anticoagulant therapy and might be accompanied by a higher risk for artherosclerotic events (42–45). Despite these established long-term sequelae, studies have consistently demonstrated, that approximately 1/2 of the patients suffer from functional limitations or decreased QoL and rare CTEPH seems to be only the most extreme expression (46).

UNANSWERED QUESTIONS IN THE ACUTE PHASE OF PE AND OBJECTIVES OF THIS STUDY

An acute PE is a frequent cause of death and serious disability (2). Risk of adverse outcome during the in-hospital phase varies widely depending on the clinical severity and presence of RVD and elevated biomarkers (2, 25).

The currently recommended risk stratification approach is in part not simple to use and might delay the resulting risk classification and identification of those patients, who should be monitored more closely and might undergo more aggressive treatment strategies such as systemic thrombolysis. Although it is widely easy to identify the high-risk PE patients, further risk classification in normotensive PE patients depends on imaging procedure, biomarkers and the sPESI or PESI according to the ESC guideline of the year 2014 (2). This approach for risk stratification in normotensive patients might delay the risk classification and is complicated for physicians in the emergency room with small time slots for each patient.

Additionally, some of the criteria, especially for the imaging procedures, remained imprecise.

Therefore, the search for the most effective risk stratification tools is still ongoing and diagnostic imaging criteria might have to be refined.

The MAinz Retrospective Study of Pulmonary Embolism (MARS-PE) study programme (Figure 1) for the evaluation of risk stratification process in acute PE enrolled consecutive patients with confirmed PE retrospectively over a 5-year period. Clinical, echocardiographic, functional and laboratory parameters were assessed. MARS-PE has been designed to provide answers to the above relevant remaining questions regarding morbidity and mortality after PE.

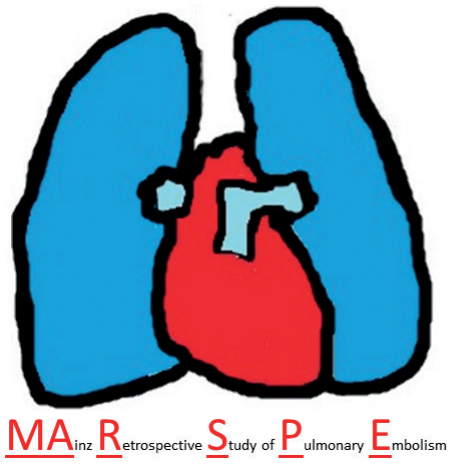

Fig. 1 MAinz Retrospective Study of Pulmonary Embolism (MARS-PE) Logo.

STUDY POPULATION

A total of 182 consecutive patients with acute, objectively diagnosed PE were retrospectively included in MARS-PE on the basis of the eligibility criteria listed in Table 1. The MARS-PE aimed to include PE all-comers with PE, irrespective of clinical severity, RVD, and size or extent of pulmonary emboli. All included patients were treated at the Department of Internal Medicine of the St. Vincenz and Elisabeth hospital of the Catholic Clinic Mainz (Mainz, Germany) between May 2006 and June 2011. The patients were found by a search of the hospital information system database for the diagnostic code of PE (ICD-Code: I26).

All CT, scintigraphic and phlebography images were analysed by experienced radiologists. If the diagnosis of PE was not confirmed by the criteria above, the patient was not included in this study.

Tab. 1 Eligibility criteria.

Inclusion Criteria	Exclusion Criteria
– Objectively confirmed diagnosis of acute PE by identified filling defect in the pulmonary artery system on a computed tomography pulmonary angiogram (CT) of the chest, a scintigraphic ventilation-perfusion (V/Q) scan read as high probability for PE or positive venous ultrasound/phlebography of an extremity consistent with deep venous thrombosis (DVT) in patients with typical symptoms of PE (chest pain or dyspnoea) and positive D-dimer – Age ≥18 years – Patients were treated in the Department of Internal Medicine in the St. Vincenz and Elisabeth hospital of the Catholic Clinic of Mainz, Mainz, Germany	– Patients in whom the diagnosis of PE was not confirmed by the examinations – Patients younger than 18 years old – Previous enrollment in this study

DEFINITIONS

MYOCARDIAL INJURY

According to the AHA scientific statement from 2011, myocardial necrosis was defined as a cardiac troponin I (cTnI) elevation >0.4 ng/ml (9).

RIGHT VENTRICULAR DYSFUNCTION (RVD)

RVD was defined according to the AHA scientific statement (9) as a quotient of right ventricular (RV) septal-lateral diameter / left ventricular (LV) septal-lateral diameter >0.9 in the four-chamber view on transthoracic echocardiography or CT (9). Moreover, the RVD was defined as RV hypokinesis and tricuspid regurgitation by echocardiography (9). For some analyses a sPAP > 30mmHg was additionally included in the RVD criteria.

HIGH-RISK PE SEVERITY STATUS

PE patients with a systolic blood pressure <90 mmHg at admission were classified as high-risk PE according to the definition from the recent and current ESC guidelines (2, 12) and the AHA scientific statement (9).

INTERMEDIATE-RISK (= SUBMASSIVE) PE SEVERITY STATUS

According to the recent, but at this time valid ESC guidelines from 2008 on the diagnosis and management of acute pulmonary embolism (12) and the AHA scientific statement for management of massive and submassive PE, iliofemoral deep vein thrombosis, and chronic thromboembolic pulmonary hypertension from 2011 (9) the non-high-risk PE patients were subdivided in 2 groups with regard to RVD and cTnI level. PE patients with RVD or pathological cTnI levels were included in the submassive PE group with intermediate risk. Patients without RVD and without elevated troponin levels were classified as non-massive PE group with low risk.

STUDY PARAMETERS

The retrospective analysis of PE patients focused on anamnesis with medical history as well as clinical, laboratory, ultrasound, echocardiographic and CT examination results.

PATIENT OUTCOMES

We analyzed the study outcome parameters in all included PE patients or in those non-high-risk PE patients, who were hemodynamically stable (normotensive) and among these, especially in those normotensive patients with an accurate transthoracic echocardiography, as appropriate.

The proposed study endpoints comprised the following: i) all-cause in-hospital mortality as well the surrogate markers ii) RVD, iii) myocardial injury and iv) PE severity status according to the ESC guidelines from 2008 (12). The study endpoints of RVD, myocardial injury as well as the PE severity status according to the ESC guidelines from 2008 (12) are established surrogate markers of poorer outcome in the acute course after the PE event and therefore, were chosen as proposed study endpoint parameters.

STATISTICAL ANALYSIS

Descriptive statistics for the relevant baseline comparisons of the respective baseline-groups were provided with mean ± standard deviation, median and interquartile range (IQR), depending on Gaussian or skewed distribution, or absolute numbers and corresponding percentages. Baseline variables of the groups were compared using the Wilcoxon-Whitney U test, the Students' T-Test or in categorical variables with Fisher's exact or Chi² test, as appropriate.

We calculated uni-variate and (if necessary to test the independence) multi-variate logistic regression models to examine the associations between risk stratification markers and the study outcome markers.

Receiver operating characteristic (ROC) curves with areas under the curves (AUC) and Youden indices were calculated to test the effectiveness of markers to predict outcome parameters in acute PE. The Wilcoxon-Mann-Whitney test was used to test the deviation of the ROC curve from the angle bisector.

Although some of the statisticians recommend to calculate a post-hoc power analysis (47), we are in accordance with the majority of the statisticians of the opinion that a post hoc power analysis is inappropriate for the evaluation of study results (48, 49). Therefore, we did not calculate such a post-hoc power calculation.

The software SPSS® (version 22.0; SPSS Inc., Chicago, Illinois), R version 2.14.1 from R Development Core Team (2011) (R Foundation for Statistical Computing, Vienna, Austria) and the commercially available software BIAS® (version 10.04; epsilon press, Frankfurt, Germany) were used for computerized analysis. Only P values of <0.05 (two-sided) were considered to be statistically significant.

ETHICAL ASPECTS

The requirement for informed consent was waived as we used only anonymized retrospective data routinely collected during the health screening process. Studies in Germany involving a retrospective analysis of diagnostic standard data do not require an ethics statement.

The study was conducted in St. Vincenz and Elisabeth Hospital Mainz (KKM).

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Next Generation Sequencing in Molecular Diagnosis of Lynch Syndrome – a Pilot Study Using New Stratification Criteria

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ABSTRACT

The development of the new technologies such as the next-generation sequencing (NGS) makes more accessible the diagnosis of genetically heterogeneous diseases such as Lynch syndrome (LS). LS is one of the most common hereditary form of colorectal cancer. This autosomal dominant inherited disorder is caused by deleterious germline mutations in one of the mismatch repair (MMR) genes – MLH1, MSH2, MSH6 or PMS2, or the deletion in the EPCAM gene. These mutations eventually result in microsatellite instability (MSI), which can be easily tested in tumor tissue. According to the actual recommendations, all patients with CRC that are suspect to have LS, should be offered the MSI testing. When the MSI is positive, these patients should be recommended to genetic counseling.

Here we report a pilot study about the application of NGS in the LS diagnosis in patients considered to have sporadic colorectal cancer. The inclusion criteria for the NGS testing were MSI positivity, BRAF V600E and MHL1 methylation negativity. We have used 5 gene amplicon based massive parallel sequencing on MiSeq platform. In one patient, we have identified a new pathogenic mutation in the exon 4 of the MSH6 gene that was previously not described in ClinVar, Human Gene Mutation Database, Ensembl and InSight databases. This mutation was confirmed by the Sanger method.

We have shown that the implementation of new criteria for colorectal patients screening are important in clinical praxis and the NGS gene panel testing is suitable for routine laboratory settings.

KEYWORDS

sporadic colorectal cancer; microsatellite instability; Lynch syndrome; MMR genes; next generation sequencing

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INTRODUCTION

Colorectal cancer (CRC) is a multifactorial disease with strong genetic background. Regarding inheritance, we differentiate between sporadic, familial and hereditary forms. One of the most common hereditary forms is Lynch syndrome (LS). LS is autosomal dominant inherited disorder caused by deleterious germline mutations in one of the mismatch repair (MMR) genes – MLH1, MSH2, MSH6, or PMS2, and the deletion within EPCAM gene or by epimutations in MLH1 gene (1, 2). Early onset of the disease and multiple tumors with microsatellite instability (MSI) are typical in the affected patients. When the patients are suspected for LS according to Amsterdam criteria II (AM) or Revised Bethesda guidelines (RBG) and are positive for microsatellite instability (MSI), testing for germline mutations in candidate genes is recommended after genetic counseling (3). However, it is widely accepted that these diagnostic criteria are suboptimal for the detection of LS. The detection of MSI and immunohistochemistry (IHC) of MMR proteins have to be used prior to genetic testing (3). In the first published study that used MSI as the primary screening method followed by IHC staining for MMR protein expression in 1066 patients, 19.5% expressed MSI and approximately 2.2% harbored mutations causing LS (4). Moreover, other authors reported that MLH1 promoter hypermethylation and BRAF V600E mutation distinguishes the hereditary non – polyposis colon cancer from sporadic MSI-H positive colon cancer (5) and can be used in patient stratification. Because LS is under-recognized in the population, a universal tumor screening (UTS) approach for LS, based on MSI testing and/or IHC for expression of MMR proteins, is recommended by several professional societies (6). Recently, the sensitivity and specificity of MSI and/or IHC testing for the identification of LS was evaluated. The authors concluded that this is an effective screening test for the identification of LS suspect patients (7). The recommendations are also included within the methodical guidelines by the Slovak Association of Medical Genetics (http://www.sslg.sk/index.php/dokumenty/metodicke-pokyny/90-metodicke-usmernenie-lynch).

The conventional molecular testing for pathogenic mutations in LS is focusing on the detection of single nucleotide variants (SNVs) and the screening for deletions or duplications by the Sanger sequencing and the multiplex ligand probe dependent amplification (MLPA), respectively. The dideoxysequencing according Sanger is based on a chain-termination using fluorescently labeled dideoxynucleotides and the capillary electrophoresis, which makes it to a robust and reliable, but also laborious and expensive method. The main limitation is the number of sequenced base pairs in the one sequencing run (8). The next-generation sequencing (NGS) based on massive parallel sequencing has revolutionized the molecular diagnostics in numerous clinical settings by their capacity to sequence several millions basepairs in one sequencing run. NGS technology substantially increased the number of sequenced genes, lowered the costs and enhanced the sensitivity of the mutation detection. A new 5-tiered nomenclature for estimation of variant's pathogenicity was introduced, which is very helpful especially in the case of missense substitutions. According this classification, the variants are defined as non-pathogenic (class 1), likely non-pathogenic (class 2), variants of uncertain clinical significance (VUS, class 3), likely pathogenic (class 4) and pathogenic (class 5) (9). Nowadays, the application of gene panels is a standard approach for the molecular diagnosis of genetically heterogeneous diseases, including LS, because all candidate genes can be sequenced in one run (10).

In order to identify the germline mutations, the massive parallel sequencing on Miseq platform was applied with the panel of mismatch repair genes – the MLH1, MSH2, MSH6, PMS2 and 3'UTR of EPCAM gene. The aim of our study was the implementation of NGS in order to identify mutations in MMR genes in patients without family history considered to have sporadic colorectal cancer, which are positive for MSI-H and negative for both, BRAF V600E and MLH1 hypermethylation.

MATERIALS AND METHODS

SPECIMENS

Blood samples were obtained from 4 patients with colorectal carcinoma suspected to have the LS. The patients were positive for MSI and negative for BRAF V600E and also negative for MLH1 methylation, as described previously (11, 12, 13). The formalin-fixed and paraffin-embedded (FFPE) sections were analyzed using IHC for the presence of the MMR protein expression (MLH1, MSH2, MSH6, and PMS2) according the standard protocol for IHC staining (13). All tested patients were recommended for a genetic counseling. The study was approved by the Ethical Committee and the patients signed informed consent. The peripheral blood cells were isolated by the DNeasy Blood and Tissue kit (Qiagen, USA), according to the manufacturer's protocol. The concertation of DNA was measured using QubitTM dsDNA BR Assay Kit on Qubit 2.0 Fluorometer (Thermo Fisher Scientific, USA) and diluted to a working concentration of 10 ng/µl.

NGS SEQUENCING ON MISEQ

NGS library preparation

For the target enrichment of coding regions and the flanking intronic sequences, all exons of MLH1, MSH2, MSH6, PMS2 and 3´UTR of EPCAM gene were amplified using HNPCC MASTR Plus kit (Multiplicom, Belgium) in five multiplex PCRs. The multiplex PCR was performed using 50 ng of genomic DNA per reaction. The amplicons (84) cover four MMR genes and 3´UTR of the EPCAM gene. The PCR products were purified with Agencourt AM Pure XP beads (Beckman Coulter, USA) and diluted 1/1000. The diluted PCR products were ligated with molecular identifiers MID from Illumina Miseq kit (1–48) (Multiplicom, Belgium). In a universal PCR, 2 µl of the ligated PCR products and 48 µl of universal master mix reaction were used. The PCR products (469bp) were analyzed with the High Sensitivity DNA 1000 kit (Agilent technologies, USA) (Fig. 1) as described previously (14). The concentration of obtained tagged amplicons was determined with Qubit™ dsDNA HS Assay kit (Thermo Fisher Scientific, USA), after repeated

purification of PCR products with Agencourt AM Pure XP beads (Beckman Courter, CA). The amplicon's libraries were diluted to 10 nM, pooled equimolar at a 2 nmol/L concentration and denatured.

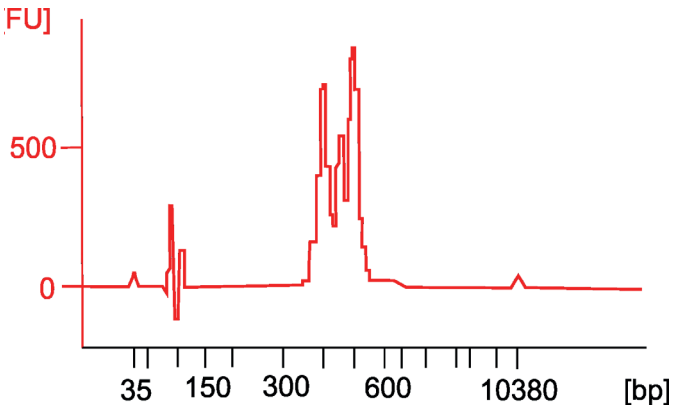

Fig. 1 Electrophoresis on the chip after universal PCR showing successful library preparation. The amplicon size after universal PCR should be between 350 to 550 bp in blood samples. Electrophoresis was prepared at Agilent 2100 Bioanalyzer (Agilent).

NGS

Amplicon pool was processed by bridge amplification and sequenced by Miseq Reagent Nano kit v2 (500 cycles) (Illumina, USA) with 500Mb capacity and the read length 2 × 250 bp on Miseq (Illumina, USA) platform. Analysis was conducted according to the manufacturer's instructions. The coverage of the region of interest (ROI) was indicated by the manufacturer as 100%.

Bioinformatic evaluation

The NGS generated for each cluster a forward and a reverse read. Read1 and Read2 are defined as read pairs. The sequencing run was valid when both Read1 and Read2 had at least 220 bp. The bioinformatic evaluation comprised of three parts – alignment of obtained reads to .bam files (Assembly GRCh37), secondary data analysis getting the variant annotation in the .vcf files and tertiary data analysis resulting in interpretation of variants. The sequence alignment was performed with MiSeq Reporter Software v2.5.1.3 (Illumina, USA) and the .vcf files were generated with Illumina Variant Studio Software v 3.0 (Illumina, USA). Sequences were annotated starting with the first nucleotide that corresponds for the first A in the first translated amino acid in coding reference cDNA. The cDNA reference sequences for MHL1, MSH2, MSH6 and PMS2 were NM_000249.3, NM_000251.2, NM_000179.2 and NM_000535.5, respectively. The potential pathogenic variant was compared with free accessible database ClinVar, Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/all.php), Ensembl (https://www.ensembl.org/Homo_sapiens/Transcript/ProtVariations/), the InSight databases (https://www.insight-group.org/variants/databases/) and published literature. The presence of pathogenic variant was confirmed by Sanger sequencing.

SANGER SEQUENCING VALIDATION

The forward primer MSH6 F and the reverse primer MSH6 R were used for the Sanger method (Table 1). The PCR products were placed into a thermal cycler with the following program: 95 °C denaturation for 10 min, cycling was repeated 30× with 95 °C for 30 sec, annealing at 60 °C for 30 sec and extension at 72c for 30 sec. The PCR products were purified using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Germany). After purification, the sequencing PCR reaction was performed using BigDye Terminator v 1.1 Cycle Sequencing Kit (TermoFisher Scientific, USA) according to the manufacturer's instructions. The sequenced fragments were purified with SigmaSpin Sequencing Reaction Clean-up (Sigma-Aldrich, USA). The purified products were denatured in deionized Hi-Di Formamid (Thermo Fisher Scientific, USA) and sequenced on the ABI 3500 Genetic Analyzer (Applied Biosystems, USA). The data were analyzed using Chromas Pro Software (Technelysium Pty Ltd, Australia).

Tab. 1 Primer sequences for the sequencing of the region of interest in the exon 4 of MSH6 gene and the size of the final PCR product.

Primer	Sequence	Annealing	Size (bp)
MSH6 F	GCACGAGTGGAACAGACTGA	60 °C	339
MSH6 R	TTGTACTGGGGGATAGTGTGC	60 °C	

RESULTS

Using the HNPCC MASTR Plus Kit (Multiplicom, Belgium) and NGS, we identified pathogenic variants in two patients. The value of the Q30 score for run was 78.46%. We identified 95.53% of reads and only 4.47% of reads remained undetermined. In the first sample, we detected a 4 bp deletion in the exon 4 of the MSH6 gene c.1627_1630AAAG (Assembly GRCh37). This mutation is within the protein coding sequence resulting into a frameshift at the protein level (p. Glu544Lysfs*26). The termination codon lies 26 nucleotide downstream of the deletion and was confirmed by the Sanger method (Fig. 2). This mutation was not recorded in any database we mentioned in the section Methods. A very similar pathogenic mutation NM_000179.2 (MSH6):c.1632_1635delAAAA (p.Lys545Argfs) was reported in the ClinVar database (assembly GRCh38), in Ensembl (rs267608064) and InSight (15, 16) and was assigned into the class 5 of the 5-tiered scale. Moreover, the sequencing of DNA obtained from the peripheral blood of patients with Muir-Torre syndrome identified a new 4 bp deletion within MSH6 gene c.1634_1637delAAGA. This mutation causes a premature stop gain 24 codons downstream, is considered pathogenic (17) and registered in HGMD, ClinVar and Ensembl. Therefore, we consider the mutation c.1627_1630AAAG (p.Glu544Lysfs) pathogenic. Our result was confirmed also by the certified commercial diagnostic laboratory, which recommended also testing of asymptomatic descendants of the patient. In the second sample, we identified a pathogenic stop gain variant in MSH2 gene c.1030C>T (Gln344Ter). We were not able to verify this by

the Sanger sequencing. According to a five-tiered classification of the International Agency for Research on Cancer (on Assembly GRCh37), this variant has high impact on the MSH2 gene and we recommended a verification by a commercial laboratory, which result is unknown to us. In other two samples tested, we detected variants, which were classified as benign.

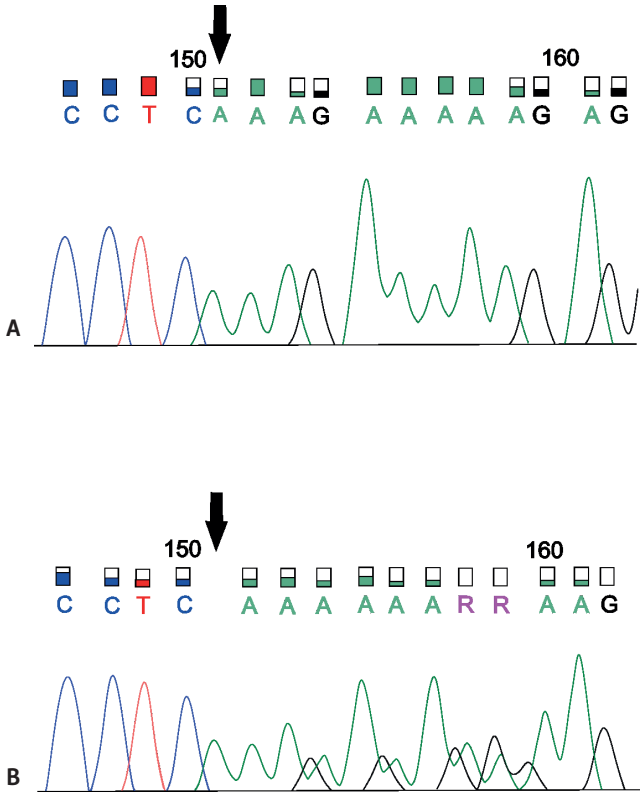

Fig. 2 A – Validation of the mutation using Sanger sequencing with deletion 1627_1630del AAAG (Glu544Lysfs*26); B – reference DNA sequence.

DISCUSSION

The development of massive parallel sequencing allowed to perform whole-genome, whole-exome and gene panel sequencing in different clinical settings. The method of choice for a lot of clinical applications including the diagnostics of LS, is the use of gene panels with the defined number of candidate genes known to be mutated in this particular disease. NGS technologies provide useful tools for the detection of single-nucleotide variants (SNV) in many genes simultaneously (10). The other advantage of NGS is that it requires, in contrast to traditional sequencing methods, very low input of DNA/RNA (18). In our study, we used 250 ng of DNA for the sequencing of 84 amplicons. Nowadays, the conventional Sanger sequencing is used mostly for the confirmation of NGS results. Here we report the application of NGS in diagnostics of patients with Lynch syndrome using the commercially available gene panel with four MMR genes (MLH1, MSH2, MSH6 and PMS2) and 3´UTR of the EPCAM gene. This work was a part of the study, which has developed a patient's stratification algorithm for LS. A similar algorithm is already recommended by the Slovak Association of Medical Genetics (http://www.sslg.sk/index.php/dokumenty/metodicke-pokyny/90-metodicke-usmernenie-lynch). We have used the gene panel included in the HNPCC MASTR Plus Kit (Multiplicom, Belgium) and MiSeq NGS platform. We identified two pathogenic variants, one of them was confirmed by Sanger method by us and also in a commercial laboratory. The identified mutation c.1627_1630AAAG (p.Glu544Lysfs) is a deletion of 4 bp resulting in a downstream termination of protein synthesis. It was not described in any database, which we used for a result interpretation. However, similar 4 bp deletion resulting in preterm stop codon was described in this gene region involving codons 543 and 544 in patients with LS (15, 16) and was classified as pathogenic (class 5) in the database of the InSight consortium (9). Another similar variant was identified in codon 544 in DNA of a patient with Muir-Torre syndrome (17). Therefore we consider this variant pathogenic.

The mutations in the MHS6 gene comprise 18% of mutations in MMR genes predisposing to LS (www.insight-group.org). Compared with pathogenic variants in MLH1 and MSH2 genes, germline variants in MSH6 gene are mostly associated with mild and variable phenotypes. The onset of the disease symptoms among mutation carriers is after age 50 and the incidence of colorectal cancer is lower than in other patients with LS. The patients often fulfill neither the AM nor RBG (19, 20). Our patient had no family history and colorectal cancer was diagnosed at age 64. One explanation of this is that MSH6 mutation could cause functional redundancy of MSH6 protein. This protein can be particularly substituted by MSH3 in the heteromeric MutSα complex (21).

The NGS gene panel testing allows rapid identification of mutations in genetically heterogeneous diseases as we have shown in our small pilot study. There are several targeted gene panels for inherited cancer syndrome that are commercially available and are best suited for the routine laboratory praxis.

CONCLUSION

Here we report the application of targeted NGS in the diagnostics of Lynch syndrome in patients considered to have sporadic CRC and stratified using MSI test, BRAF V600E determination and MLH1 methylation screening. We have identified a new pathogenic variant in a patient without family history of the disease. Moreover, we have shown the implementation of the MSI test, the evaluation of BRAF V600E and MLH1 methylation in patients considered to have sporadic CRC are important in clinical praxis. Our aim was to address the need for more clearly stratification of patients suspected for LS. We recommend utilization of the MSI screening strategy for all patients with colorectal cancer, not only for patients with positive family history, and subsequent NGS sequencing in MSI-H, BRAF V600E and MLH1 methylation negative cases. It would be also appropriate to include this mutation c.1627_1630AAAG (p.Glu544Lysfs) into further studies.

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Quality of Life after Reconstructive Surgery for Intestinal Fistulas

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ABSTRACT
Background: This retrospective clinical study would like to objectively denote a quality of life of persons afflicted by an abdominal catastrophe and managed by an extensive surgery can be almost as well conformable as those of healthy people in a similar age group.
Methods: A set of eighteen patients who were successfully surgically treated and cured enjoyed a relatively good convalescence after their surgery and returned to a satisfactory standard of life from the point of view of organ function and psychosomatic state. Statistical analysis of the data collected over a period of 1 to 6 years after this complex therapy using special questionnaire for QOL assessment SF-36 was performed.
Results: Almost half of the patients evaluated their state similarly to the rest of the population of comparable age and general health status. The remainder of the patients declared significantly worse evaluations in the majority of the observed domains of the questionnaire.
Conclusion: Therapy of these patients was and must be complex: it included preparation for surgery at a special metabolic internal site, careful diagnostics of the digestive tract state, suitable surgery and good quality care after the surgery.

KEYWORDS
intestinal fistulas; reconstructive bowel surgery; quality of life; questionnaire SF-36

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INTRODUCTION

Disintegration of the surgical wound or intestinal anastomoses complicated by intestinal fistulas can be classified as so-called abdominal catastrophe. These conditions are encountered as postoperative complications of elective as well as of urgent abdominal operations (1–3) or as a result of abdominal traumas (4). Although these surgical problems occur relatively less frequently, they endanger the life of the patient, their solution is very expensive and they always significantly influence the quality of life of the affected person. In addition to that, the treatment itself of abdominal catastrophes is associated with high morbidity and mortality. It takes a long time, is complex, and consists of several stages: stabilization of the patient, therapy of septic complications, optimisation of nutrition, special local care of the wound, diagnosis of the intestinal tract state, repair of the bowel itself and postoperative care (1, 5, 6).

Even after successful bowel repair following abdominal catastrophe, patients struggle with many problems which significantly influence the quality of their future lives. In the past, published papers concerning quality of life (QOL) in various fields of medicine have used special questionnaires, indices and scales which seek to quantify QOL in such a way that comparison can be made with other sets of patients or with healthy people. Methodology in chiropractic can be cited as an example: visual analogous scale, Oswestry pain/disability Index, Roland-Morris Low Back Pain, Disability Questionnaire, SF-36 (7). For surgical and non-surgical obesity treatment IWQOL is cited (8). The status of patients after kidney transplant or requiring dialysis due to kidney lesion has been evaluated through the perceived health status (PHS) with the use of SF-36 and SSLDQ (9, 10). For anorectal malformations paediatricians use PedsQLI (11). For analysis of the status of patients suffering inflammatory diseases of the gastrointestinal tract following radiotherapy in the pelvic area, IBDQ, SOMA, etc. have been used (12).

The quality of life of patients following surgery for entero-cutaneous fistula is determined largely by the extent of loss of intestine, with problems of metabolism and an uncomfortable personal life for the patient. There is significant weakening of the abdominal wall as a result of repeated operations prior to the actual bowel repair itself. The patients can suffer weight loss, frequent diarrhoeic stool, disorders of intestinal passage and the possibility of large ventral ruptures. All this significantly influences the quality of life in terms of physical and organ function, resulting in emotional, social and psychological difficulties.

Evaluation of patient status after bowel repair following abdominal catastrophes can be carried out tentatively by personal questionnaires according to the capabilities of the therapeutic institution, or by quantification of the overall state of the patient with the help of special methods for measuring quality of life. This can be done using techniques which quantify subjective information in various fields of life for individual patients. One such questionnaire in which the answers can be quantified is the previously-mentioned SF-36, which was used for evaluation of the general status of our patients. Several sets of patients have been processed (5, 13–15), and these can be used as a standard for comparison with findings in our patients.

The aim of this paper is to judge whether and how the states of patients affected by abdominal catastrophes followed by bowel repair differ in the main fields of quality of life from those in the general population of corresponding age and regular health state (HRQOL).

PATIENTS AND METHODS

20 patients with extensive defects of the abdominal wall with the complication of one or more intestinal fistulas underwent surgery. In all cases there was a fistula of the small intestine in the area of an abdominal wall defect. Three cases were complicated by a vast loss of intestine: in two cases the ileum together with about half of the jejunum was removed, leading to short bowel syndrome. In one case there was resection with jejuno-rectal anastomosis. The majority of patients had previously undergone surgery at other abdominal sites including repeated surgical revisions for intra-abdominal septic complications.

Therapy of these serious cases was carried out according to our internal standards and in compliance with recommended procedures. With the patients transferred to our hospital, therapy was initiated at the 3rd Department of Internal Medicine – Metabolic Care and Gerontology. The therapy was carried out in the stages described above, with emphasis on solving septic complications, improvement of the nutritional status of the patient, stabilization of the internal state and special care of the wound. Prior to each operation a detailed examination of the gastrointestinal tract had been carried out to localize the fistula precisely within the frame of the intestinal tract (x-ray using contrast medium, plus endoscopy). Bowel repair was always carried out at least three months after the occurrence of the fistula or the previous operation. Immediately after the operation the patients were returned to the 3rd Department of Internal Medicine – Metabolic Care and Gerontology, where intensive postoperative therapy was delivered in cooperation with the surgeon. It also included special care for the wound in cases of laparostoma. General rehabilitation of the patient was an integral part of the therapy.

One to six years after the surgery – the above-mentioned group of 20 patients was sent questionnaire SF-36. The set of 18 of these patients who had been examined and answered all the questions (1 patient died, 1 patient did not answer) comprised 12 men and 6 women. The average age of the patients was 58.3 years with median of 56.5 years (interval 40 to 82 years). The answers of these patients were processed according to the appropriate methodology for the SF-36 questionnaire. The questionnaire consists of 36 questions divided into 8 fields (health domains): 10 questions on PF (physical function), 4 questions on RP (role – physical), 2 questions on BP (bodily pain), 5 questions on GH (general health), 4 questions on VT (vitality), 2 questions on SF (social functioning), 3 questions on RE (role – emotional) and 5 questions on MH (mental health).

The final question is aimed at the perceived change of the state of general health, and in the evaluation is included as the 6th question in the field of GH. Answers to individual questions are translated into a score achieved in each particular domain. The worst possible score in each domain is zero, and the best is 100 points (15). As a reference group the specific age group 55–64 from sample according to Jenkinson et al. (14) was used. Means and standard deviations (men and women together) were compared with our set of patients in individual dimensions of SF-36.

The results of the questionnaire were processed by statistical methods. Clusters were identified by a cluster analysis (k–cluster), with test of the equality of their means by a multidimensional Hotteling test. Comparison with the reference group was carried out by unpaired t-test, which was preceded by a Levene's test of equality of variances. Statistically significant results are based on Bonferroni correction and family wise error rate 0.05. Confidence limits (CL) for estimate of the proportions of patients in both categories (clusters differing in their health perception) were settled as exact 95 per cent confidence limits for parameter p of the binomial distribution. The results are presented in the form of figures and tables.

This published research and its methodology comply with the guidelines for human studies and animal welfare regulations.

RESULTS

A cluster analysis on the set of patients identified two internally homogeneous groups of patients (Table 1) which were statistically significantly different in the mean values of domains investigated by the SF-36 questionnaire ($p < 0.05$). The group with the more favourable evaluation (Cluster 1) consisted of 8 patients, 44.4 per cent of the total set, with 95 per cent confidence limits from 21.5 to 69.2 per cent of patients. The second group (Cluster 2) consisted of the remaining 10 patients. The processed questionnaire data and their statistical interpretation proved that patients included in Cluster 1 did not differ statistically significantly in quality of life from the reference set according to Jenkinson et al. (14). They differed only in the GH domain which for these patients was significantly lower (worse). The patients of Cluster 2 achieved significantly lower (worse) values than the reference set in all 8 measured domains (Table 2, Figure 2).

The analysis of the set of patients as a whole can be summarized (Table 2):

a) Perception of PF ($p < 0.01$), RP ($p < 0.01$), SF ($p < 0.01$) and GH ($p < 0.01$) was significantly worse within the framework of the observed indices.

b) RE, MH, VT and BP did not differ statistically significant from the same indices in the population of men

Tab. 1 Domains of SF-36 in two groups of patients identified by cluster analysis.

	Patients: Cluster 1				Patients: Cluster 2			
	Mean	n	SD	SE	Mean	n	SD	SE
PF	69.4	8	28.84	10.20	52.0	10	29.27	9.26
RP	84.4	8	26.52	9.38	2.5	10	2.50	0.79
RE	91.6	8	23.58	8.34	49.9	10	45.14	14.27
VT	62.5	8	18.71	6.61	39.0	10	11.74	3.71
MH	81.0	8	9.26	3.27	54.8	10	13.73	4.34
SF	79.6	8	21.06	7.45	46.1	10	17.73	5.61
BP	83.1	8	23.29	8.23	56.2	10	28.61	9.05
GH	55.0	8	21.55	7.62	27.8	10	12.56	3.97

n = size of the sample, SD = standard deviation, SE = standard error of mean

Tab. 2 Domains of SF-36 in patients compared with the set of reference values according to Jenkinson (14).

	Patients HK					Reference set – Jenkinson 55–64				p-value
	Mean	n	SD	SE		Mean	n	SD	SE	
PF	59.7	18	29.58	6.97	PF	77.4	1365	22.98	0.62	0.0013
RP	38.9	18	38.00	8.96	RP	77.7	1474	36.55	0.95	0.0000
RE	68.4	18	41.97	9.89	RE	84.5	1470	31.31	0.82	0.0321
VT	49.4	18	17.74	4.18	VT	60.9	1470	20.98	0.55	0.0215
MH	66.4	18	21.61	5.09	MH	76.1	1439	18.03	0.48	0.0238
SF	61.0	18	25.32	5.97	SF	86.4	1512	22.62	0.58	0.0000
BP	68.2	18	29.02	6.84	BP	76.8	1503	24.48	0.63	0.1443
GH	39.9	18	19.01	4.48	GH	68.0	1456	22.46	0.59	0.0000

n = size of the sample, SD = standard deviation, SE = standard error of mean

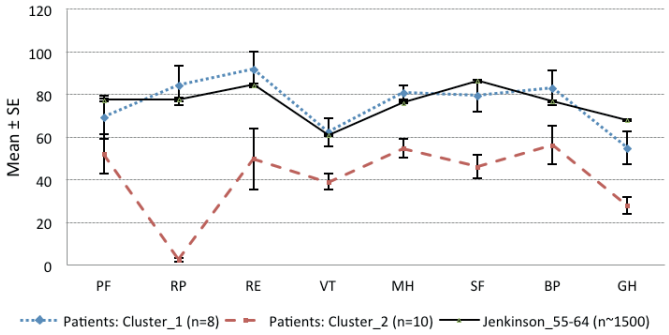

Fig. 1 SF-36: Comparison of Jenkinson's sample characteristics with identified clusters of patients.

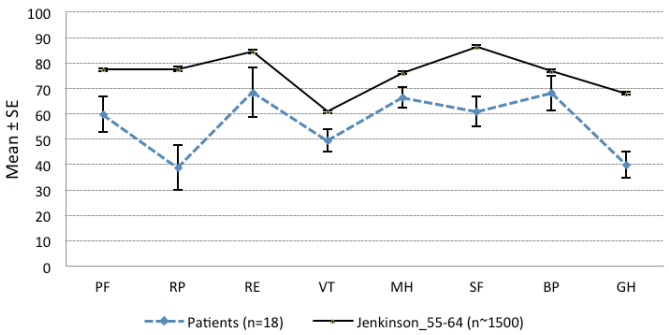

Fig. 2 SF-36: Comparison of Jenkinson's sample characteristics with patients.

and women of comparable age without serious morbidity ($p < 0.01$) as shown in Table 1 and Figure 1.

DISCUSSION

Processing of data from postoperative answers to the SF-36 questionnaire can provide certain information about the general health state of our patients in comparison with a similar population group of comparable age and without significant co-morbidity (14). It is without doubt difficult to obtain an objective view of the whole problem since questionnaires cannot be structured to elicit feelings across the whole spectrum of quality of life, nor can measurement and interpretation of responses be made entirely consistent. As discussed in the Introduction section of this paper, objective evaluation of quality of life is usually achieved through questionnaires which use direct or multiple-choice questions to assess personal perception in various fields of life.

For assessment of the quality of life of our patients we used the SF-36 questionnaire, which is widely used in various branches of medicine and is highly regarded for its ability to capture also the social dimension of life (15). Based on currently published information it is obvious that the quality of life of surgical patients can be quantified for the purpose of objective evaluation of the work of the surgeon. However, it is a matter of discussion with which subset of the population the results should be compared. There are published findings for young and healthy groups (13), or for groups suffering from certain co-morbidities (9), and the results can be also compared to the subset of population of similar age without significant co-morbidity. Such a subset has been selected for our clinical study (14).

When using statistical tests to compare individual domains of health between patients and a reference set it is desirable to reduce the level of significance of the individual tests so that the overall (familywise) level of significance (alpha) of the whole presentation is 0.05 (Table 2). Then, according to the Bonferroni correction in each test a p-level of 0.00625 should be considered, and according to the Sidak test a p-level of 0.006391 should be considered. Using these criteria the probability is below these values in domains PF, RP, SF and GH, and hence there are significant differences between the compared groups in these domains; in the other domains there is no significant difference.

Cluster analysis identified two clusters of patients with different mean characteristics as measured by the SF-36 questionnaire, and their proportions were estimated. Cluster 1 comprised 44.4 per cent (21.5–69.2 per cent) of the patients; with the exception of the GH domain their characteristics were wholly comparable with our reference set of persons of similar age and without co-morbidity. However, in the GH domain this group does score significantly worse. The remainder of the patients could all be assigned to Cluster 2, which in general differs significantly from Cluster 1 as well as from the reference set; it comprised 55.6 per cent of the total patients with interval of reliability between 30.8 and 78.5 per cent.

Several other papers have dealt in a similar way with evaluation of the quality of life of patients: obese patients looking or not looking for surgical solutions, patients with anorectal malformations (11), patients with gastrointestinal symptoms after irradiation (12), those with permanent ileostomy, and patients after operation for Crohn disease (6). It is the task of the authors to make objective assessment of both comfortable and uncomfortable phases in the life of a patient in a particular pathological state, and to search for the causes of problems; the task is thereafter to apply the results of such analyses of treated patients in order for example to show whether an amended therapeutic methodology leads to improvement in the quality of life.

CONCLUSIONS

From the surgical point of view we can claim that the functional states of the repaired bowels and abdominal walls were fully satisfactory in all 18 patients. The cosmetic impact of the operation is certainly significant, but with respect to the patient's return to a relatively active life it is less important.

Statistical processing of SF-36 questionnaire results has revealed that the general state of the patients after extensive surgery and demanding complex therapy following abdominal catastrophes endangering their lives may be either quite satisfactory or worse (see the division of the set into clusters) in comparison with the population without significant chronic disease. Physical functions and their restrictions, mental state and general health

evaluated for the whole set of patients are statistically significantly worse than for the reference set of the population of similar age without significant health difficulties. In the fields of emotional state, vitality, psychological state and pain state there is either no significant difference from the same-age group or are insignificantly worse. Within the set of patients was identified a subset of 44.4 per cent (21.5–69.2 per cent) whose mean values differ from the reference set only in the domain of GH; in the other domains there was no significant difference from the reference population of comparable age. However, a second subset of patients (55.6 per cent) (30.8–78.5 per cent) exhibited significantly worse characteristics in all measured domains than those in the reference set.

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Adult Bochdalek Hernia with Organo-Axial Gastric Volvulus: Misdiagnosed as Hydropneumothorax

Sugandha Arya, Saqib Shahab, Rahul Kumar, Pankaj Kumar Garg*

ABSTRACT

Bochdalek hernia (BH) in an adult may manifest clinically with a myriad of abdominal or chest symptoms or a combination of them. Diagnosis of an adult BH is usually delayed in view of rarity of the lesion and its varied presentation. A 30-year-old adult gentleman presented to us with a left thoracostomy which was draining pus and ingested food particles. The tube thoracostomy had been performed in another hospital for an apparent left hydropneumothorax before he arrived in our hospital. Computed tomography of Chest and abdomen revealed a left diaphragmatic defect with herniation of stomach, spleen and omentum into the chest with organo-axial volvulus of the stomach. A thoracostomy tube was seen to be traversing through the stomach with its tip located close to the left pulmonary artery. The patient underwent left thoraco-abdominal exploration with dissection and reposition of the hernial contents in the abdominal cavity. The gastric perforations and the diaphragmatic defect were repaired. This case reiterates a well-known fact that an adult type BH must find a place in the differential diagnosis of a hydropneumothorax. Though the adult BH is a rare diagnosis, unawareness or reluctance to consider the possibility of adult BH may prolong the suffering of the patient as it happened in our patient who had iatrogenic perforation of the stomach due to tube thoracostomy.

KEYWORDS

diaphragmatic hernia; Bochdalek hernia; tube thoracostomy; gastric volvulus; misdiagnosis

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INTRODUCTION

Bochdalek hernia (BH) occurs due to persistence of the pleuroperitoneal canal due to the incomplete fusion of the pleura-peritoneal folds (1). They are commonly present on the left posterolateral aspect of diaphragm. BH is usually observed in pediatric age group and rarely diagnosed in adults. The majority of the diaphragmatic hernias seen in adults are frequently secondary to trauma. Though BH in adults is usually detected incidentally, it may present with a varied clinical features including both abdominal or chest symptoms. Failure to consider BH in the differential diagnosis of left lower zone opacity with or without air-fluid level in a chest X-ray may result in misdiagnosis of pleural effusion or hydropneumothorax and a tube thoracostomy injuring the hernia contents. We present a case of BH in an adult who presented to another hospital with chest symptoms. He was erroneously diagnosed to have hydropneumothorax resulting in tube thoracostomy which perforated the stomach, the hernia content.

CASE REPORT

A 30-year-old gentleman presented to us with an in-situ tube thoracostomy which was draining the ingested food. He had developed severe pain in his abdomen in March 2016; the pain responded to nasogastric tube aspiration and proton-pup inhibitors in two days. He did not undergo further investigations that time. Two months later in May 2016, he developed persistent cough. A chest radiograph suggested left lower zone opacity in the chest with an air-fluid level (Fig. 1). He was apparently diagnosed to have left hydropneumothorax in a private hospital and a tube thoracostomy was performed. Surprisingly, the thoracostomy tube started draining the ingested food. Then, the patient was referred to our hospital. Computed tomography of the Chest and abdomen revealed a left diaphragmatic defect with herniation of stomach, spleen and omentum into the chest; there was organo-axial volvulus of the stomach. The thoracostomy tube was seen to be traversing through the stomach with its tip located close to left pulmonary artery (Fig. 2 and 3). The patient underwent left thoraco-abdominal exploration which revealed a large defect in the left posterior diaphragm; the proximal two thirds of the stomach, the spleen and the distal pancreas were present in the left hemithorax with dense adhesions.

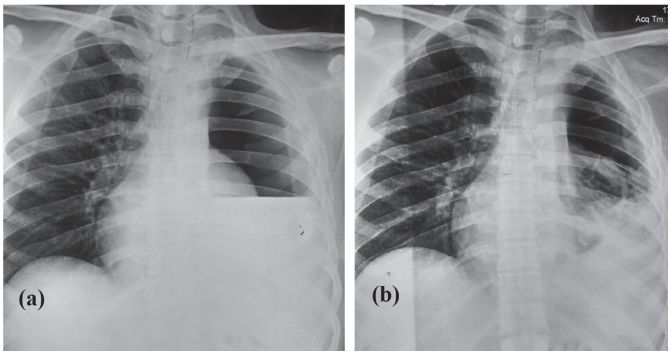

Fig. 1 (a) Pre and (b) post tube thoracostomy chest radiograph.

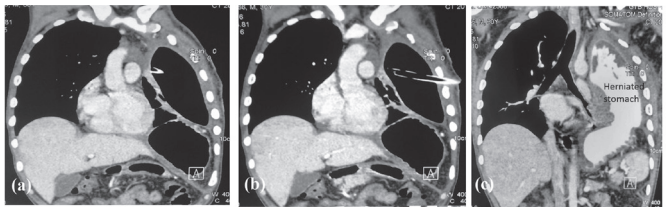

Fig. 2 (a–c) Coronal sections of computed tomography of chest and abdomen.

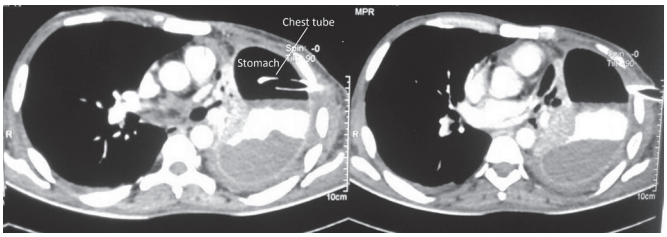

Fig. 3 (a–b) Axial sections of the computed tomography of chest.

Thoracostomy tube was traversing through the greater and lesser curvature of the stomach. There was 100 ml of pus in the left pleural cavity. The abdominal contents were meticulously dissected and positioned back in the abdominal cavity. The gastric perforations were repaired after generous resection of the devitalized edges. The diaphragmatic defect was repaired after a tube thoracostomy was performed for drainage. The postoperative period was uneventful except superficial surgical site infection. The patient is well after 8 months of follow-up.

DISCUSSION

Congenital diaphragmatic hernias were first described by a Czech anatomist and Professor, Vincent Alexander Bochdalek in 1848 (2). This condition was later known by his name as Bochdalek Hernia. Interestingly, Bochdalek hypothesized that the diaphragmatic defect was not the congenital malformation, but rather, it resulted from rupture of an intact membrane of the diaphragm referred to as the trigone of Bochdalek. John J. White argued that it would not be right to label this as BH as the hernia mostly protrudes through the pleuroperitoneal hiatus and not through the trigone of Bochdalek. However, this congenital posterolateral hernia still continues to be known as BH because of the widely appreciated belief of giving due credit to the initial observations of Bochdalek (3). A true BH, which is extremely rare, occurs due to the failure of the development of muscle in the lumbocostal triangle as it leaves the serous pleural sac above and the peritoneum below. Thus, a true BH is a congenital posterolateral hernia with a hernia sac (3).

BH in an adult may present with a myriad of abdominal or chest symptoms or a combination of them. Diagnosis of an adult BH is usually delayed in view of rarity of the lesion and its varied presentation. Moreover, incorrect diagnosis may be thought of in a significant number of patients and may result in unnecessary intervention prolonging the patient morbidity and mortality. In a re-

view of 51 cases of adult BH, Thomas et al reported that it was misdiagnosed in 38% of the patients (4). Adult BH may present with a left lower zone opacity with or without air-fluid level masquerading as a effusion or a hydropneumothorax. A clinician who is unaware of the possibility of BH may be tempted to perform a tube thoracostomy resulting in injury to the hernia contents. BH, though rare, may masquerade hydropneumothorax and a tube thoracostomy intended to manage the same may injure the hernia contents. A detailed chest examination is the key to avoid any misadventure (5). Presence of gurgling gastric or bowel sounds in the chest suggests the presence of diaphragmatic hernia. Moreover, if a chest radiograph is performed after a nasogastric tube is placed; it can delineate the position of the stomach in the chest and clinch the diagnosis. A chest ultrasonography should be done in suspected cases before a tube thoracostomy is done. There is a limited literature regarding the etiology and the management of the hydropneumothorax. A recently published study form India analyzing a cohort of 57 patients of hydropneumothorax highlighted that Tuberculosis (TB) was the etiology in 80.7% patients, acute bacterial infection in 14%, malignancy in 3.5%, and obstructive airway disease in 1.8% (6). All of these patients were managed with tube thoracostomy. The etiology of hydropneumothorax is different in developed countries where incidence of tuberculosis is low.

The definitive treatment of BH is surgical. A number of surgical approaches to address the BH have been described – abdominal, thoracic, or thoraco-abdominal. Abdominal approach is most popular and has distinct advantages over thoracic approach as it allows the inspection of the abdominal viscera and permits the performance of any resection or repairs as required by the condition of these viscera (7). The disadvantages include: (a) difficult access to dome of the diaphragm, (b) difficult reduction of the hernia contents due to negative pressure and possible adhesions within the chest, and (c) unfavorable mechanical features of a closure done on the concave rather than on the convex surface of the diaphragm. The thoracic approach has the advantage of a much more immediate access to the site of the trouble and does away with the negative pressure of the pleural cavity, and so makes reduction easier and gives a much better field for suture of the hernial opening (8). The main disadvantage of the thoracic approach is inability to repair or even to recognize serious intra-abdominal lesions through the thoracic incision alone. The combined abdominal and thoracic approach obviously affords all the advantages offered by either method alone. We utilized the thoraco-abdominal approach as it greatly facilitated the necessary steps of the operation by allowing us to meticulously dissect the adhesions in the chest, to perform the adequate thoracic lavage, and at the same time provided us the opportunity for a complete inspection of the abdominal organs including repair of the gastric perforations.

Our patient also had organo-axial gastric volvulus, though surprisingly, he did not have vomiting. The first episode of the acute pain in our patient may also be related to the gastric volvulus which got resolved spontaneously. A close look at the position of the nasogastric tube in the chest X-ray done at that time provides a subtle evidence of the gastric volvulus. We did not perform anterior gastropexy as a large part of greater curvature of the stomach, which was unhealthy sloughed out due to iatrogenic perforations caused by tube thoracostomy, was already excised.

This case reiterates a well-known fact that an adult type BH must find a place in the differential diagnosis of a hydropneumothorax. Though the adult BH is a rare diagnosis, reluctance or unawareness to consider the possibility of adult BH may prolong the suffering of the patient as happened in our patient who had iatrogenic perforation of the stomach due to tube thoracostomy.

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Polyneuropathy, Myocardial Dysfunction and Pericardial Effusion Following Duodenal Switch

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ABSTRACT
Duodenal Switch procedure is a type of bariatric surgery that was reserved for severely morbid obese people. Patients undergoing this procedure are at high risk for nutrient deficiencies. In this report we present a case of a patient who had developed polyneuropathy, generalized muscle weakness, Wernicke encephalopathy, myocardial dysfunction and pericardial effusion six years following this operation. He was treated by multivitamins and trace elements with a complete resolution of all of these disturbances. The patient was fully rehabilitated.

KEYWORDS
bariatric surgery; myocardial dysfunction; pericardial effusion; polyneuropathy

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INTRODUCTION

The rate of obesity continues to rise worldwide and is considered a major healthcare burden (1). Bariatric surgeries offer an effective treatment modality for this epidemic due to their dramatic and durable results. Bariatric surgeries are classified into three categories: Restrictive, malabsorptive, and restrictive-malabsorptive, which combines both types of procedures (2). However, these procedures have been associated with increased risk of nutritional deficiencies. Reported neurological pathologies that are related to nutritional deficiencies are variable and include encephalopathy, optic neuropathy, myelopathy, polyradiculoneuropathy, and polyneuropathy (3). Cardiac dysfunction and pericardial disease secondary to severe weight loss are not mentioned. In this report we present a patient that developed severe neurological damage, cardiac dysfunction and pericardial effusion following a bariatric surgery and recurrent vomiting.

CASE REPORT

A 42 year's old male patient suffered from severe obesity since childhood. Concurrently, he developed a binge eating disorder with forced vomiting. Six years ago as his weight reached 180 kg and his BMI was 53.2, he was referred to bariatric consultation and underwent partial gastrectomy with Roux-en-Y gastroenterostomy with biliopancreatic diversion. During the following years he lost 100 kg but continued to vomit. Additionally, he did not adhere to the recommended supplementation of essential vitamins and trace elements.

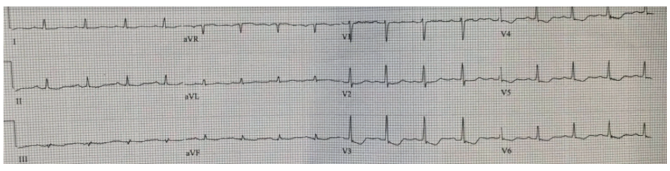
On November 2016 the patient was admitted due to progressive muscle weakness and walking disturbances. EMG demonstrated axonal neuropathy and lumbar puncture was performed with normal level of protein and without pleocytosis. He was diagnosed with atypical Guillain Barre Syndrome (GB Syndrome) and received IVIG treatment. He was then sent to a rehabilitation center where he was treated with vitamins and trace elements which resulted in clinical improvement. Two months later he was readmitted due to diplopia and vertigo. Physical examination demonstrated nystagmus. MRI of the brain was normal and he was sent home. On June 2017 he was referred to our hospital due to chest pain, confusion, blurred vision and leg weakness. On admission he was confused and non-cooperative. His blood pressure was 80/50 mmHg, and pulse rate was 100 and regular. Physical examination revealed elevated jugular venous pressure. On his neurological examination, a prominent horizontal nystagmus with an abduction paralysis of both eyes, and muscle weakness with hyporeflexia was observed. The ECG demonstrated low voltage and inversion of the T waves with ST depression in the anterior wall. An Echocardiogram demonstrated a moderate pericardial effusion with diffuse global left ventricular dysfunction. Coronary CT scan was normal without evidence of ischemia. EMG showed axonal neuropathy. Results of the lumbar puncture were normal. His laboratory results showed slight pancytopenia and decreased levels of copper, ceruloplasmin, vitamin A and D and B1. Additionally, his troponin level was elevated (Table 1). Liver function tests, Prothrombin time and thyroid functions were all normal.The patient was treated with multivitamins and trace elements intravenously and with Selenium and vitamin D.

Tab. 1 Laboratory results of the patient at admission (normal level in parentheses).

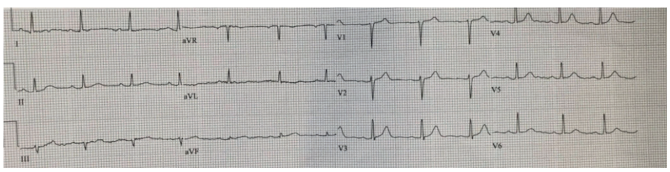
Leukocytes (4000–10800)	6040
Hemoglobin (13.5–17.7 g/dL)	10.2
Platelets (130–440000)	119000
Thiamine (66.5–200 nmol/L)	46.2
Vitamin A (0.3–0.7 mg/dL)	0.2
Copper (50–150 micgr/dL)	42
Ceruloplasmin (20–60 mg/dL)	10.2
Calcium (8.1–10.4 mg/dL)	8.4
25OH Vitamin D (deficiency <10 ng/ml)	5.1
Cholesterol (130–200 mg/dL)	76
Troponin (<0.07 micg/l)	1.05
Albumin (3.6–5.5 g/l)	2.9
CRP (0–5 mg/dL)	0.57
Iron (60–170 micg/dL)	37
Lactate (6–18 md/dL)	46

CRP – C Reactive Protein

We noticed a dramatic improvement, and after two weeks of treatment the blurred vision disappeared and the eye movements returned to normal. Muscle weakness improved substantially. Nystagmus was the last sign to disappear. Cardiac global function normalized and there was minimal residual pericardial effusion. The ECG changes disappeared as well, with normalization of the QRS voltage and correction of the ST-T changes (Figure 1–2). The patient was discharged with a recommendation to maintain a healthy diet enriched with vitamins, trace elements, copper and selenium.



Admission



Discharge

Fig. 1 ECG on admission and discharge.

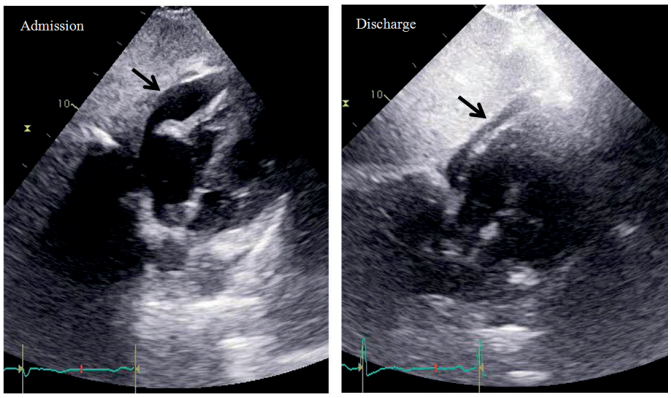


Fig. 2 Echocardiogram on admission and discharge.
The arrow signs the pericardial effusion.

DISCUSSION

Biliopancreatic diversion is an effective procedure leading to durable weight loss. It not only achieves a considerable weight loss, but has a high success rate in maintaining weight thereafter. The relatively low number of patients undergoing this procedure reflects its surgical complexity and the fear from secondary nutritional deficiencies and associated adverse effects (4).

Neurological complications following bariatric surgery can develop within a few weeks to many years afterwards (3). These complications may result from mechanical or inflammatory mechanisms but mainly from nutritional deficiencies. Rapidly progressive polyradiculoneuropathy resembling GB Syndrome has been associated with vitamin B1 deficiency (5). Other neurological complications of bariatric surgeries include Wernicke's encephalopathy, optic neuropathy, myelopathy, peripheral neuropathy and myopathy.

Another interesting and less familiar finding was pancytopenia. Copper deficiency may be the cause of this disturbance (6), and probably also contributed to some of the neurological damage. Surprisingly, ceruloplasmin level was also reduced, perhaps explained by underproduction by the liver as a response to the low copper levels.

The most interesting disturbances were pericardial effusion and cardiac dysfunction. There was no evidence of viral or inflammatory pericarditis. Pericardial effusion is described in patients suffering from anorexia nervosa (7). The pathophysiology for the development of pericardial effusion is not known, however the effusion usually remits by a concurrent increase in weight. Cardiac dysfunction and the ECG changes were difficult to explain. Coronary artery disease was excluded by normal coronary artery CT. No description of patients demonstrating such changes following weight loss or malnutrition was found in recent literature. High output cardiac failure is the classical feature of patients with cardiovascular Beri-Beri, although thiamine deficiency can rarely cause low cardiac output (8), as was the presentation of our patient. Selenium deficiency can cause dilated cardiomyopathy, a condition that was not compatible with the echocardiogram of the patient. An article published in the Br Heart J on 1951, described 30 patients suffering from malnutrition and heart disease that was not compatible with Beri-Beri (9). It is clear that at that time it was impossible to measure levels of vitamins and trace elements. The electrocardiograms that were performed, demonstrated inverted T waves in the anterior wall, compatible with the ECG changes in our patient. As with our patient, these changes normalized when the nutritional disorder was corrected. Our patient's cardiac damage is not fully understood. We suspect that combined deficiency of thiamine, selenium and copper contributed to this disorder. Copper deficiency in animals can induce enlargement of the heart and congestive heart failure (10). It is important to note that in patients with Anorexia Nervosa myocardial fibrosis was found in 23% of the patients that were evaluated by cardiac magnetic resonance imaging (7). Reduced left ventricular mass was also very common.

CONCLUSION

Neurological disturbances can appear following bariatric surgeries. Myocardial dysfunction and pericardial effusion were not described previously. The correction of the nutritional deficiencies resulted in resolution of all the symptoms.

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