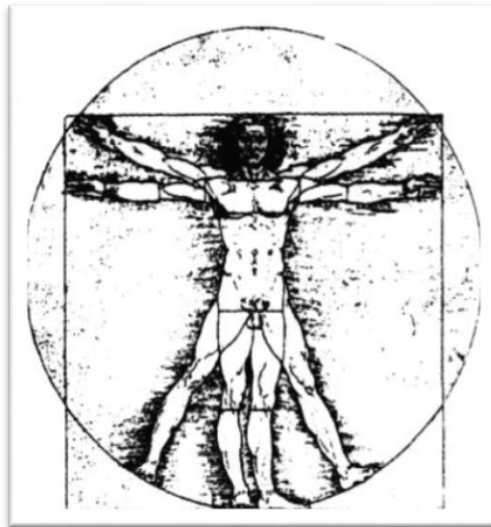


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ORIGINAL ARTICLE

**OZONE TREATMENT OF STAGE II/ III OF BISPHOSPHONATE-RELATED  
OSTEONECROSIS OF THE JAWS (BRONJ)**

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**ABSTRACT**

Bisphosphonate-related osteonecrosis of the jaws (BRONJ) is defined as exposed jawbone (part of the jawbone) in the oral cavity that persists for more than eight weeks despite the treatment, and patients haven't received radiotherapy and there is no history of bone metastases. This is a serious complication in patients receiving bisphosphonate therapy, especially in those who have received it intravenously. Bisphosphonates are used as potent inhibitors of bone resorption in various diseases and conditions of the bones, such as malignancies, osteoporosis, and bone metastases. There are many studies related to the impact of medical ozone on wound healing in bisphosphonate osteonecrosis. Several authors show the effect of ozone therapy on the wound healing in the osteonecrosis area in patients receiving bisphosphonate therapy. The aim of this study was to show the influence of ozone therapy on wound healing in stage II/III of bisphosphonate-related osteonecrosis of the jaws. This study included 25 male and female patients who received bisphosphonate therapy, and in whom II or III clinical stage of bisphosphonate-related osteonecrosis of the jaws was diagnosed. After receiving standard antibiotic therapy determined with an antibiogram, patients received medical ozone therapy for dental use in the form of a gas (ozone therapy device, Ozone DTA-Dental ozone generator of Apoza Enterprise). Patients were treated with conservative treatment or surgical treatment (sequestrectomy). In patients with clinical stage II / III, the positive effect of this therapy was observed after the application of ozone, as well as after treatment with an antibiotic agent, with a greater tendency of decline in patients with ozone therapy. Reduction of pain as well as reduction of bleeding and secretion (reduction of fetor - bad smell in oral cavity) are the benefits of the treatment with local application of ozone gas in patients with bisphosphonate-related osteonecrosis of the jaws. Ozone therapy destroys bacteria, or reduces their effect on wound healing. It also affects the improvement or maintenance of the clinical stage of osteonecrosis of the jaws.

**Keywords:** ozone therapy, bisphosphonates, pain, osteonecrosis, wound healing.

## INTRODUCTION

Bisphosphonate-related osteonecrosis of the jaws (BRONJ) was described for the first time by Marx (2003) [1] (as exposed jawbone, part of the jawbone in the oral cavity that persists for more than eight weeks despite the treatment, and patients haven't received radiotherapy and there is no history of bone metastases), which appears after dental procedures in patients that have received or still have been receiving bisphosphonates for inhibition of the bone resorption. This is a serious complication in patients receiving bisphosphonate therapy, especially in those who have received it intravenously [2]. Bisphosphonates are used as potent inhibitors of bone resorption in various diseases and conditions of the bones, such as malignancies, osteoporosis, and bone metastases [3]. There are many hypotheses about the cause of BRONJ occurrence, but very often the trigger is tooth extraction. The hypotheses of osteonecrosis are based on the inhibitory effect of bisphosphonates on the osteoclastic activity of bone cells, as well as their toxic effects on soft tissue and their anti-angiogenic effect. The influence of bisphosphonates on the oral microflora, as well as the creation of biofilm (microbiota) at the site of osteonecrosis, is one of the possible reasons for its occurrence. Biofilm is actually a set of bacterial colonies that are interconnected with fibronectin. They cover the necrotic bone and cause frequent and recurrent infections in these patients [4].

According to the American Association of Oral and Maxillofacial Surgeons (AAOMS, 2009) [5] there are several clinical stages of BRONJ.

- Patients at risk – patients have been receiving or had received bisphosphonate therapy, where tooth extraction or other oral surgery should be performed.
- Clinical stage 0 - unspecified clinical findings and symptoms (unpleasant continuous pain with low intensity, slow wound healing in the region where tooth was pulled out, patients complain of bad smell in the mouth), but there is not an exposed or necrotic bone in the oral cavity.
- Clinical stage I – clinical findings are: exposed or necrotic jawbone without signs of infection (erythema), and patients complain of persistent, not very severe pain and unpleasant smell from the oral cavity as a result of local accumulation of deposits at the site of the exposed bone
- Clinical stage II – clinical findings are: an exposed bone, but also signs of infection, pain and erythema, as well as an unpleasant smell from the oral cavity (feter).
- Clinical stage III - in addition to the exposed jawbone, there are signs of infection, bad smell (feter) and pain, as well as the possibility of pathological fracture, extraoral or intraoral fistula, and the possibility of creating oroantral communication or osteolysis of the jaw bones.

There are many studies related to the impact of medical ozone on wound healing in bisphosphonate osteonecrosis. Several authors show the effect of ozone therapy on the wound healing in the osteonecrosis area in patients receiving bisphosphonate therapy. The influence of ozone is due to its antibacterial [6, 7], antiviral [8, 9] and antifungal effect [10], improving tissue oxygenation, as well as its impact on epithelialization of the wound [11], and stimulation of local immunity. Basic forms of application of ozone in the oral cavity are: ozone gas, ozonated oil and ozonated water [12].

Ripamonti *et al.* [13] in their study presented treatment of osteonecrosis of jaws with lesions greater than > 2.5 cm, by using ozone (O<sub>3</sub>) as a gas insufflation in the area of the lesion.

They explained the effect of ozone gas with obvious results in wound healing, such as demarcation and sequestration of osteonecrotic bone from healthy bone and subsequent healing of the wound.

Agrillo *et al.* [14], in a five-year-research used ozone gas insufflations as a conservative treatment or as supplemental therapy in minimal sequestrectomy in patients with bisphosphonate-related osteonecrosis of the jaws. They also described reduction of pain and reduction of osteonecrotic lesion, as well as secretion in osteonecrotic area.

Also, medical ozone has impact on the stimulation or suppression of the immune system and oxidative influence when it is used in small concentrations [15].

In everyday dental practice use of antibiotics for treatment of BRONJ can be followed by additional ozone therapy [16], which should be applied during 15 days, then followed by two ozone insufflations during surgical treatment (sequestrectomy). Their research explains the impact of ozone in reducing pain, as well as reduction of the bad smell (fedor) in the mouth after treatment. The topical use of ozone on infected wound in oral cavity gives an obvious result in patients who have received a high dose of radiotherapy (in the treatment of primary disease) [17].

The positive effects of ozone and its application as an ozonized oil can be used for different treatments in the oral cavity, which was done by Nogales [18] in the treatment of alveolitis sicca. He compared the effectiveness between antibiotic therapy and ozone therapy. Patients were more satisfied with ozone therapy, and they felt no pain and other additional unpleasant symptoms.

In *in vitro* evaluation of wound healing in rats Borges *et al.* [19] showed the antimicrobial potential of ozone therapy, as well as its antifungal action.

In the literature, there are data for application of ozone therapy for preventive purposes as protection against postoperative infection with the application of ozone before, during and after extraction of the teeth. Filipovic *et al.* [20] used ozone for preventive purposes during extraction of the impacted third molars. They also examined postoperative pain and bad smell (fedor) in the oral cavity in these patients.

For that purpose Calvo *et al.* [21] applied ozone therapy in certain concentrations for wound healing and indicated its analgesic effect.

Besides antibiotic and ozone therapy, Passaretti *et al.* [22] also used low-frequency laser therapy in the treatment of BRONJ. They applied ozone gas three times a week (eight treatments). The concomitant use of ozone therapy and low-frequency laser were also used by Moraes *et al.* [23] The future will show which therapeutic method is more effective, but in any case they both show obvious results.

The aim of this study was to show the influence of ozone therapy on wound healing in stage II/III of bisphosphonate-related osteonecrosis of the jaws.

## **MATERIALS AND METHODS**

This study included 25 male and female patients who received bisphosphonate therapy, and where II or III clinical stage of bisphosphonate-related osteonecrosis of the jaws was diagnosed. After receiving standard antibiotic therapy determined with an antibiogram, patients received medical ozone therapy for dental use in the form of a gas (ozone therapy device, Ozone DTA-Dental ozone generator of Apoza Enterprise). The protocol applied to patients with clinical stage II and III of BRONJ consisted of: an antibiotic treatment (determined with antibiogram) for 7-10 days and it continued with an additional daily local application of ozone gas for 7-10 days (depending on the clinical symptoms). If sequestrectomy was performed after this treatment, then two ozone insufflations were applied during surgery, and another ozone insufflation was applied during sutures removal. In those patients conservative or surgical treatment (sequestrectomy) was performed. Regular check-ups were performed after 2-3 weeks of initial treatment. Sometimes there was a need for possible retreatment with ozone gas or antibiotic therapy determined with antibiogram. Patients were monitored for a period of six months or until complete wound healing. The efficacy of the therapy was followed by predetermined clinical parameters:

### **1. Wound healing as:**

- a. Complete wound healing with sequestration of necrotic tissue or sequestrectomy
- b. Partial wound healing by sequestration of necrotic tissue or sequestrectomy
- c. No healing of the wound, but the condition in the same clinical stage of the disease was maintained
- d. Progression of osteonecrosis to surrounding tissues and progression of clinical stage of disease

### **2. Local hyperemia or bleeding from the wound (both together)**

- a. Local hyperemia with spontaneous bleeding from the wound
- b. Local hyperemia with bleeding which was provoked after irritation
- c. Local hyperemia without bleeding
- d. There is no hyperemia or bleeding

### **3. Exudation from the wound or exposed bone**

- a. Serous exudate
- b. Purulent exudate
- c. No secretion

### **4. Pain using a visual analogue scale (VAS)**

### **5. The presence of bad smell (fedor) was measured by Halimeter (Tanita Corporation, HC-212S)**

All clinical parameters were monitored on three occasions:

- Before initiation of antibiotic therapy in the patient
- Before the start of ozone gas therapy
- After completing the wound treatment protocol

Patients were monitored for a six-month-period, followed by improvement of the treatment, or progression of the clinical stage of disease.



## RESULTS

Serous exudate was registered in 3.8% of these patients before treatment, in 76.91% after antibiotic therapy and in 26.9% after ozone therapy. Purulent exudate was registered before therapy in 96.2%, in 23.1% after antibiotic therapy and in 7.7% of patients after ozone therapy. No exudate in this group was registered only after ozone in 65.4% of patients (Table 1).

**Table 1.** Exudation before and after antibiotic and ozone therapy

Exudate/therapy	Befor Th		After antibiotic Th		After ozone Th	
	N	%	N	%	N	%
Purulent exudate	25	96.2	6	23.1	2	7.7
Serous exudate	1	3.8	20	76.9	7	26.9
No secretion	0		0		17	65.4
Summary	26	100.0	26	100.0	26	100.0

Local hyperemia with spontaneous bleeding before treatment was found in 61.5% of patients, and hyperemia with provoked bleeding in 38.5%. Local hyperemia after antibiotic therapy was registered in 19.2% of patients, hyperemia with spontaneous bleeding and, hyperemia with provoked bleeding was found in 38.5%, and hyperemia in 42.3% of patients. Local hyperemia after ozone therapy was registered in 23.1% of patients, hyperemia with spontaneous bleeding in 3.8%; and hyperemia with provoked bleeding was recorded in 23.1% of patients (Table 2).

**Table 2.** Local hyperemia before and after antibiotic and ozone therapy

Local hyperemia/group before therapy	N	%
No hyperemia	0	
Spontaneous bleeding	16	61.5
Bleeding provoked with irritation	10	38.5
No bleeding	0	
Summary	26	100.0
<b>After antibiotic therapy</b>		
No hyperemia	0	
Spontaneous bleeding	5	19.2
Bleeding provoked with irritation	10	38.5
No bleeding	11	42.3
Summary	26	100.0
<b>After ozone therapy</b>		
No hyperemia	13	50.0
Spontaneous bleeding	1	3.8
Bleeding provoked with irritation	6	23.1
No bleeding	6	23.1

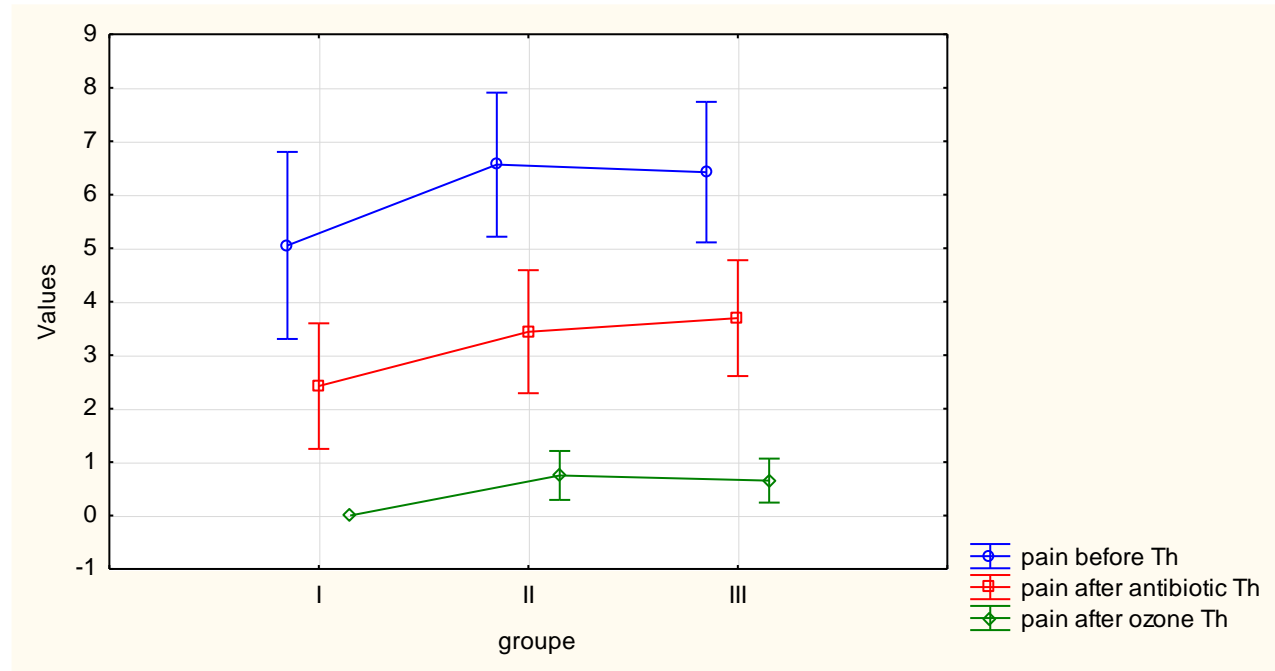
Wound healing before therapy was registered in all patients with disease progression (100.0%). Wound healing after antibiotic therapy was registered in 3.8%, partial healing in 23.1%, in 38.5% at the stage of the disease and in 34.6% with disease progression. Wound healing after ozone therapy was registered in 42.3% of patients, partial healing in 19.2%, in 23.1% at the stage of the disease and in 15.4% with disease progression. According to the dynamics index, the pace of declining progression at the stage of the wound disease between pre-therapy and after ozone therapy was registered in 84.6% of patients (Table 3).

**Table 3.** Wound healing before and after ozone therapy

<b>Wound healing/group</b>	<b>N</b>	<b>%</b>
<b>Before therapy</b>		
Complete wound healing	0	
Partial healing of the wound	0	
Same clinical stage	0	
Progression of osteonecrosis	26	100.0
<b>After antibiotic therapy</b>		
Complete wound healing	1	3.8
Partial healing of the wound	6	23.1
Same clinical stage	10	38.5
Progression of osteonecrosis	9	34.6
<b>After ozone therapy</b>		
Complete wound healing	11	42.3
Partial healing of the wound	5	19.2
Same clinical stage	6	23.1
Progression of osteonecrosis	4	15.4

According to Analysis of Variance U, the difference between the median pain values according to the VAS scale in the second group between pre-treatment values, after antibiotic therapy and after ozone therapy was statistically significant for  $p < 0.05$  ( $p = 0.000000$ ).

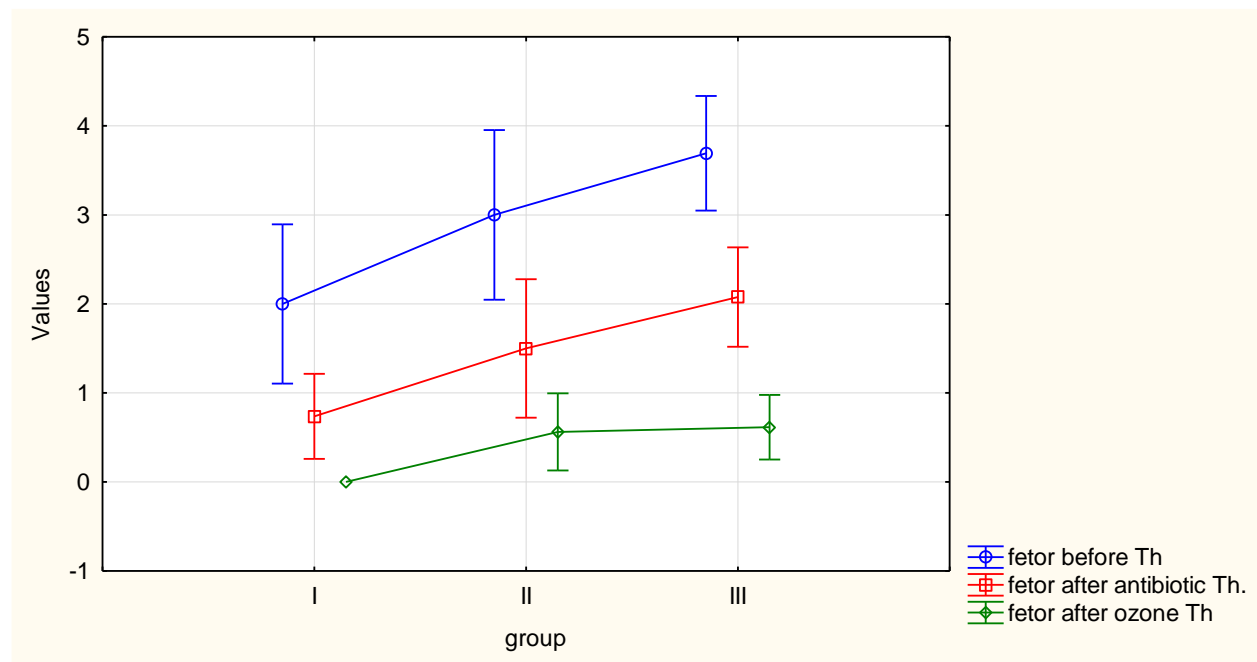
According to the Post hoc Tukey HSD test, the difference is due to a statistically significant difference between pre-therapy after antibiotic therapy prior to ozone therapy after antibiotic therapy *versus* ozone therapy ( $p = 0.000275$ ,  $p = 0.000129$ ,  $p = 0.001222$ ) (Figure 1).



**Fig. 1.** Pain before and after antibiotic and ozone therapy

According to Analysis of Variance U, the difference between the average Hamiltonian scale in the first group between pre-treatment values, after antibiotic therapy, and after ozone therapy was statistically significant for  $p < 0.05$  ( $p = 0.000022$ ).

According to the Post hoc Tukey HSD test difference is due to a statistically significant difference between pre-therapy after antibiotic therapy prior to ozone therapy ( $p = 0.006422$ ,  $p = 0.000132$ ) (Figure 2).



**Fig. 2.** Fotor before and after antibiotic and ozone therapy

## DISCUSSION

Bisphosphonates are the most commonly used drugs in the treatment of bone metastases, as well as hypercalcemia in cancer-related conditions. They perform suppression of osteoclastic differentiation and activity of cells in bones, leading to their apoptosis (programmed cell death). The dental interventions, such as tooth extraction, in these patients using bisphosphonates may increase the risk of BRONJ [23].

This study was intended to illustrate the clinical effects of ozone therapy in patients with bisphosphonate osteonecrosis.

In patients with clinical stage II / III, the positive effect of this therapy was observed after the application of ozone, as well as after treatment with an antibiotic agent, with a greater tendency of decline in patients with ozone therapy.

Agrillo *et al.* [15], in a clinical study performed on 131 patients, described the reduction of pain, as well as the reduction of the lesion in the area of osteonecrosis. Our results have shown the same effect of ozone therapy as in their study, with a statistical significance in pain reduction.

Ozone therapy can also be used as ozonated water or ozone oil in osteonecrotic lesions [12].

The positive effects of ozone therapy have also been explained in the research of Ripamonti *et al.* [13], who used ozone gas insufflations in the treatment of bisphosphonate osteonecrosis, and in some cases the necrotic part was sequestered and rejected or it was removed by surgery.

Reduction of pain as well as reduction of bleeding and secretion (reduction of fotor - bad smell in oral cavity) are the benefits of treatment with local application of ozone gas in patients with bisphosphonate-related osteonecrosis of the jaws. In our study, there was a significant decrease in the secretion, as well as bleeding from the wound in the osteonecrotic area. Ozone therapy for preventive purposes against postoperative infection was used by Filipovic *et al.* [21].

They did the application of ozone before, during and after the extraction of the impacted third molars. They also examined postoperative pain and bad smell (fedor) in the oral cavity in these patients, and they showed the positive effect of the ozone therapy.

In the treatment of BRONJ, besides antibiotic therapy, some authors like Moraes *et al.* [23], used ozone oil as well as laser therapy in its management that gave positive effects.

Ozone therapy, with its antibacterial action, destroys bacteria or reduces its effect, thus improving the wound healing in the osteonecrosis region, as has been shown with the results obtained in this study.

## CONCLUSION

Ozone therapy has a positive impact on the reduction of pain and bad smell (fedor) in the oral cavity, destroys bacteria, or reduces their effect on wound healing. It also affects the improvement or maintenance of the clinical stage of osteonecrosis of the jaws.

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ORIGINAL ARTICLE

**LOWER SERUM POTASSIUM LEVEL IS ASSOCIATED WITH MORTALITY AS  
CONFOUNDING EFFECT OF MALNUTRITION IN DIALYSIS PATIENTS**

Trajceska Lada, Selim Gj, Pavleska Kuzmanovska S, Pusevski V, Sikole A  
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**ABSTRACT**

**Introduction:** Obtaining normal serum potassium level is an important goal in maintenance hemodialysis patients. Hyperkalemia is known to be associated with mortality. In this study we aimed to assess the relationship between pre-dialysis potassium level, nutritional status and survival in dialysis patients.

**Materials and methods:** This study used annual cohorts of hemodialysis patients with 36 months of follow-up. To determine the impact of potassium level on mortality, patients were followed from the first potassium measurement until death or a censoring event; hypokalemia was defined by potassium levels below median level - 5.5 mmol/l and albumin level below 35g/l was considered as an index for undernourished. Time-dependent Cox proportional hazards modeling was used to estimate the association between potassium level and mortality.

**Results:** A total of 199 patients were included in the study. Mean age was approximately 56 years, about 59% were men and 23% had end-stage renal disease caused by diabetes. Albumin below 35 g/l was observed in 26 (13%) patients. In the follow-up period 53 (26%) patients died, consisting of 31 (31%) of the 101 hypokalemic and 22 (22%) of 98 hyperkalemic patients. The Kaplan-Meier survival rate was significantly better in the hyperkalemic population ( $34.30 \pm 0.71$  vs.  $31.06 \pm 1.16$ ,  $p=0.051$ ). Hypokalemia, when defined as serum potassium  $\leq 5.5$  mmol/l, was associated with all-cause mortality (hazards ratio (HR) 1.857, 95% CI 0.986-3.496,  $p = 0.051$ ). The significance was lost in the model after adjustment for albumin level. Only albumin level determined mortality ( $p=0.03$ ).

**Conclusion:** Lower potassium level was associated with all-cause mortality, but only as a confounding effect of malnutrition in dialysis patients.

**INTRODUCTION**

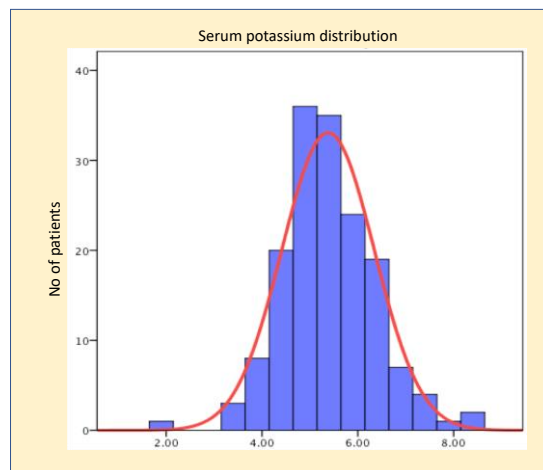
Dyskalemia is a frequent electrolyte imbalance observed among maintenance hemodialysis patients. Hyperkalemia is recognized as a silent and a potential life threatening factor. In contrast, much less attention has been paid to hypokalemia [1]. Sudden cardiac death and arrhythmias are contributed by rapid electrolyte shifts during dialysis, especially in patients with pre-dialysis high potassium level [2-4]. Serum albumin level is a predictor of nutritional status in dialysis patients [5]. Association between serum albumin and mortality in dialysis patients is partly explained by inflammation and nutrition [6]. Severe hypokalemia in hemodialysis patients usually is a result from low potassium intake (malnutrition), chronic diarrhea, etc. [1]. In this study we sought to access the relationship between pre-dialysis potassium level, nutritional status and survival in dialysis patients.

## MATERIALS AND METHODS

The cohort of 199 dialysis patients from one dialysis centre was divided into two groups according to the presence of low (below median) or high (above median) potassium serum level before dialysis. Patients with dialysis vintage more than three months and age above 18 years were included in the study. Those with active malignancy were excluded. Blood samples were drawn during routine dialysis sessions. Measurements were obtained within 24 hours in a central laboratory. The dialysis schedule was defined as Monday-Wednesday-Friday and Tuesday-Thursday-Saturday. Blood was drawn on Mondays and Tuesdays after the long interdialytic interval. Dialysis prescription consisted of minimum three times weekly, four to five hours hemodialysis sessions with low flux synthetic membranes. The dialysis concentrate consisted of 2.0 mmol/L potassium. Demographic and clinical variables were obtained from medical histories. Variables included sex, age, body mass index (BMI), dialysis vintage, and presence of diabetes. Also, serum sodium, calcium, hemoglobin, uric acid, blood urea nitrogen (BUN), creatinine, cholesterol, phosphorous, CRP, and albumin were monitored. Dialysis adequacy was calculated by kt/V formula. Patients were followed for 36 months until death, or other censoring event. Statistical analysis was performed with SPSS 16.0 for Windows: Continuous variables are shown as mean values and categorical as percentages. Kaplan-Meier survival curves were applied to estimate difference in survival in patients with low (below median), or high (above median) potassium level. The median albumin level was obtained for survival correction by nutritional status.

## RESULTS

The mean age of all patients was approximately 56 years, about 59% were men and 23% had end-stage renal disease caused by diabetes. Albumin below 35 g/l was observed in 26 (13%) patients. On average, patients were dialyzed for more than 8 years and the vast majority achieved good dialysis adequacy (mean Kt/V  $1.57 \pm 0.29$ ). Renal anemia was treated with erythropoietin and the mean hemoglobin level of the population was well – 116 g/L. The nutritional indices as albumin and BMI were in target ranges ( $38.91 \pm 4.8$ ,  $23.63 \pm 4.61$ , respectively). The values of C-reactive protein (CRP) as a marker of inflammation showed distinct variations between subjects:  $9.8 \pm 22.89$ , range 0.1-200 mg/l. The distribution of potassium level was bell-shaped (Figure 1). The mean value was  $5.4 \pm 0.94$  and median level 5.5 mmol/L with range 1.9-8.3 mmol/L. Only 2.6% of patients had potassium levels below 3.6 mmol/L and 3.5% above 7.0 mmol/L.



**Fig. 1.** Bell-shaped curve of potassium levels distribution



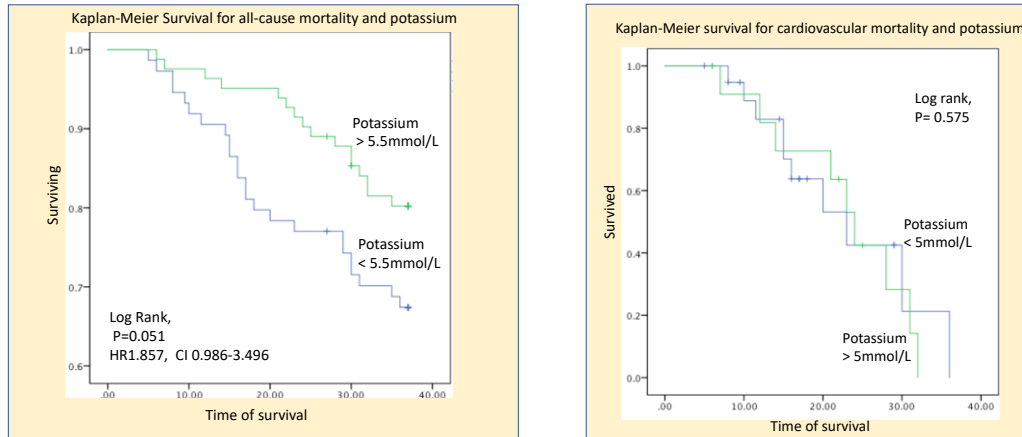
The comparative analysis of the two study groups on demographic, clinical and dialysis indices is shown in Table 1. The only significant difference between the two groups was in albumin and phosphorous levels 37.73±4.34 vs. 39.98±5.04, p=0.03; 1.29±0.47 vs. 1.49±0.53, p= 0.024, respectively. BUN showed a borderline significant difference and higher values in hyperkalemic patients (24.70±6.24 vs. 26.61±6.89, p=0.066).

**Table 1.** Comparative analysis of the two study groups on demographic, clinical and dialysis indices.

	Potassium ≤ 5.4mmol/L N=101	Potassium ≥ 5.5mmol/L N=98	p-value
Men (%)	63(63%)	66 (67%)	0.473
Age (years)	57.14±13.78	55.16±12.44	0.334
Time of HD session (hours)	4.11±0.31	4.10±0.34	0.898
Kt/V	1.56±0.34	1.57±0.26	0.902
Hemoglobin (g/L)	115.81±15.13	116.61±16.29	0.751
BUN (mmol/L)	24.70±6.24	26.61±6.89	0.066
Creatinine (µmol/L)	826.39±263.18	849.27±290.38	0.606
Sodium (mmol/L)	136.50±3.06	136.12±3.26	0.457
Calcium (mmol/L)	2.12±0.36	2.14±0.23	0.695
Phosphorous (mmol/L)	1.29±0.47	1.49±0.53	0.024
Albumin (g/L)	37.73±4.34	39.98±5.04	0.003
CRP (mg/L)	9.54±18.19	10.39±27.02	0.821
Uric acid (mmol/L)	411.22±116.17	400.81±79.58	0.528
BMI (Kg/m <sup>2</sup> )	23.69±4.41	23.65±4.86	0.961

CRP- C - Reactive Protein, BUN – Blood Urea Nitrogen, BMI – Body Mass Index

In the follow-up period 53 (27%) patients died, of those 38 (72%) were cardio-vascular deaths (11 cerebrovascular deaths, 6 acute myocardial infarctions, 3 patients with sudden cardiac death). The same number of patients had sepsis or cirrhosis as a cause of death (3), one died of malignancy and five were diagnosed with malnutrition. In the high potassium level group, 22 (22.4%) patients died and in the low potassium group 31 (30.7%). When patient's all-cause mortality and survival were analyzed in relation to high or low potassium level, the difference was borderline significant (34.30 ± 0.71 months vs. 33.7 ± 1.16 months, p= 0.051). The number of cardiovascular deaths was similar in both potassium groups [19 (18.8%) vs. 19 (19.3%), p>0.05, respectively], and there was no significant difference in survival (p=0.575), as shown in Figure 2.



**Fig. 2.** Kaplan-Meier estimates of survival with regard to all-cause and cardiovascular mortality in relation to low (below median), or high (above median) level of potassium

In Cox-regression analysis, hypokalemia defined as serum potassium  $\leq 5.5$  mmol/l, was associated with all-cause mortality (hazards ratio (HR) 1.857, 95% CI 0.986-3.496,  $p = 0.051$ ). The significance was lost in the model after adjustment for albumin level. Only albumin level determined mortality ( $p=0.03$ ).

## DISCUSSION

A large prospective study on 81.000 dialysis patients found that potassium correlated with nutritional markers and patients with potassium below 4 and above 6.3 mmol/l had worse survival when compared to patients whose serum potassium level was between 4.6 to 5.3 mmol/l [4]. In our prevalent dialysis patients, the mean value of potassium was  $5.4 \pm 0.94$ , median level of 5.5 mmol/L, with range 1.9-8.3mmol/L. Only 2.6% of patients had potassium levels below 3.6 mmol/L and 3.5% above 7.0 mmol/L. The all-cause mortality was higher in patients with lower potassium and albumin level. But, in our study we did not find higher potassium level connected to worse survival, as it was the case in other studies [3,7,8]. The malnutrition overruled the effect of potassium. Also, all our patients were dialyzed with 2.0 mmol/l potassium dialysate bath. The difference in electrolyte shifts and serum to dialysate gradient could not be taken into account, as were already seen to be predictive for death [3, 9, 10]. In our study there were only 3 (1.5%) sudden cardiac deaths, which might explain the limitation of the study in exploring the outcome and association with hyperkalemia. The presence of sudden cardiac death in other studies varied from 6-18% [11,12]. Multiple studies have shown a strong correlation between low levels of serum albumin and an increased risk of morbidity and mortality in ESRD patients [13-16]. This strong association was confirmed in our study as well. The group of patients with lower potassium level also had lower phosphorous and BUN, which are recognized as nutritional markers. Other studies have also reported that serum albumin could be a marker of overall health status rather than simply a nutritional marker, taking into account its complex physiology [17]. The interplay between nutrition, electrolytes and comorbidities defines outcomes in dialysis patients.

New options for better management of chronic hyperkalemia [18] and improving nutritional status are to be further investigated.

In conclusion, lower potassium level was associated with all-cause mortality, but only as a confounding effect of malnutrition in dialysis patients.

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ORIGINAL ARTICLE

**IMPLEMENTATION AND VALIDATION OF THE NEW AO CLASSIFICATION OF THORACOLUMBAR FRACTURES**

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**ABSTRACT**

**Introduction:** Development of a widely accepted, comprehensive and yet simple TL classification system with clinically acceptable intra- and interobserver reliability for use in clinical practice and researches, is necessity. AO Spine- thoracolumbar spine injury classification system was developed using international consensus process, making it a simple and reproducible system which consists of a morphologic classification of the fracture, a grading system for the neurological status, and description of relevant patient-specific modifiers.

**Objective:** The aims of this study were to demonstrate the AO Spine thoracolumbar spine injury classification system can be reliably applied by a group of surgeons and to detect those injury types which are difficult for spine surgeons to classify reliably.

**Materials and methods:** AO Spine Thoracolumbar Spine Injury Classification system consists of a morphologic classification of the fracture, a grading system for the neurologic status and relevant patient-specific modifiers was applied to 40 cases by 6 spinal surgeons from Traumatology department, twice independently, in grading sessions 1 month apart. The results were analyzed for classification reliability using the Kappa coefficient ( $\kappa$ ).

**Results:** Kappa coefficient for all 40 cases was 0.59, which represents moderate reliability. For type A injuries, Kappa values describing interobserver agreement were 0.71, for type B injuries 0.50 and 0.61 for type C injuries. All representing substantial reliability. Fracture subtype A4 was with the lowest level of agreement (Kappa = 0.19). Interobserver analysis demonstrated overall average Kappa statistic for subtype grading of 0.55 also representing moderate reproducibility.

**Conclusion:** Study demonstrated moderate interobserver and substantial interobserver reliability. These results suggest that most spine surgeons can reliably apply this system (AO Spine Thoracolumbar Spine Injury Classification System) to spine trauma patients as more reliably than previously described systems

**Keywords:** Implementation, Validation, AO Spine TLSIS.

**INTRODUCTION**

Fracture of the thoracolumbar segment of the spine is a fracture of the vertebrae, in the segment of the hollow part of the spine in height between the tenth bony vertebrae and the second lumbar vertebrae. It occurs as a result of the force effect along the axial axis of the spinal column and flexion. The most common cause of injury to this segment is a drop-in height, traffic accident, in the multi and polytrauma. This type of vertebrae fractures account for about 45 per cent of all fracture fractures [1-7]. But despite their frequency, there is still a significant dilemma regarding the choice of treatment, conservative versus surgical treatment.

These controversies are supported by studies in which, some prefer the advantage of surgical treatment that emphasizes patient satisfaction in terms of pain relief, better and faster rehabilitation and better functional outcomes, as well as socioeconomic advantage. But there are also studies that demonstrate the advantage of conservative treatment with good functional results and a small percentage of morbidity [8-14, 19-24]. The development of a widely accepted, comprehensive and simple classification system of thoracolumbar spinal fractures with clinically acceptable intra- and interobserver reliability for use in clinical practice and research is a necessity. Megrel's classification and thoracolumbar injury classification (TLICS) are well-known, but the TL classification system did not achieve a universal and international application [15-18]. The lack of consensus among doctors / researchers complicates studies on these injuries and the development of algorithms for their treatment. The AO Spine-thoracolumbar fractures classification system (AO Spine TL) has been developed using an international consensus, making it a simple and reproducible system consisting of a morphological classification of the fracture, a neurological assessment system and a description of the relevant specific patient modifiers [25].

### **Objectives**

The purpose of this research is to investigate whether the classification system of thoracolumbar fractures on the spine TL spine TL can be reliably applied by surgeons at the Traumatology Clinic and to detect those types of injuries that are difficult for surgeons to classify them reliably.

### **MATERIALS AND METHODS**

The study is intended to be an internal observational study of reliability and applicability, as well as evaluating the reproducibility of the AO Spine TL classification. The research is retrospectively prospectively, non-randomized, to a controlled group of patients before and after injury, by the researcher and 6 surgeons from the Traumatology Clinic twice a month, individually independently of each other. The survey was conducted at the Clinic for Traumatology and includes 40 patients with fractures of the thoracolumbar segment of the spinal column, in the period from January 2015 to September 2018. The reliability and applicability of Spine TL classification will be a labeled using the Kappa Fliesh coefficient (according to inclusion and exclusion criteria, patients who will meet the same criteria will be included or excluded in the research. The data was entered in an electronic database and analyzed with software for statistical analysis.

### **RESULTS AND DISCUSSION**

The researcher believes that the results will demonstrate that the Spine TL classification is a reliable and usable in our conditions. It generates information that provides an excellent basis for forming a decision-making pathway for the proper treatment of these injuries, as well as our compliance with the appropriate treatment choice.

**Table 1.** Epidemiological statistics

Gender prevalence	Average age
Male	22 +/- 52 years.
Female	18 +/- 58

The light prevalence of male sex in relation to the female sex. The average age in both groups shows 52 years for males and 58 for females (Table 1).

**Table 2.** Mechanism of injury

Direct fall	10 patients
Fall from height	15 patients
Car accident	15 patients

According to the mechanism of injury, fall from height and traffic accidents account for 75% of the causes of these injuries (Table 2).

**Table 3.** Neurological status

Intact	Deficit
34 patients	6 patients

In 6 patients clinically confirmed the presence of some neurological deficit (Table 3).

**Table 4.** Radiological investigations of RTG, CT, MRI,

Time of Investigation	RTG	C. Tomography	M. Resonance
<24 hours	40 patients	31 patients	7 patients
<48 hours	/	9 patients	15 patients
<72 hours	/	/	18 patients

Complete radiological investigation, (RTG, CT and MRI), in conditions of our clinic, necessary for the application of AO Spine classification of thoracolumbar fractures in all 40 patients, was done during the first 72 hours of the hospitalization (Table 4).

Results of AO Spine classification of thoracolumbar fractures analyzed with Fleiss Kappa coefficient for intra and inter observing reliability (Table 5).

The agreement, or degree of compliance, can be considered as follows: if a certain number of people assign numerical values to a certain number of references, then the Kappa coefficient will give value for how consistent they are. The cap coefficient can be defined as a binary / dichotomous agreement from the same sample between two observers. The latter represents a measure / value of an agreement between partners (independent assessors) for categorical (nominal or scale) scales when there is a minimum of two assessors in which the data from the response are related to the same observation.

The value of 1 indicates the perfect deal and 0 indicates that there is no contract, or is no better than what can be obtained by chance.

**Table 5.** Interpretation of the values of the coefficient Kappa.

</ = 0	No agreement
0.0 - 0.2	Weak or poor agreement
0.21 - 0.4	Fair/Acceptable agreement
0.41 - 0.6	Moderate agreement
0.61 - 0.8	Significant agreement
0.81 – 1	Very good / Excellent deal

Results of classification of the fractures by basic classification groups (Table 6):

**Table 6.** Type A (compression), Type B (distraction), Type C (translational)- Interobserver agreement

Type A (Compressive Fractures)	Type B (Distraction Fractures)	Type C (Transition Fractures)
0.71	0.50	0.61

The internal observational analysis of the main classification groups shows (Table 6);

- significant reliability and certainty for Type A,
- moderate reliability and safety for Type B,
- significant reliability and reliability for Type C.

**Table 7.** Under the types of classification group Type A; Interobserver agreement

A0	A1	A2	A3	A4
0.80	0.76	0.69	0.53	0.19

Internal observational analysis of subgroups of type A fractures shows (Table 7);

- for the types A0, A1, A2 significant reliability and reliability,
- for type A3 moderate reliability,
- for under type A4 internal observation analysis showed that it is with the lowest value of reliability and reliability, the lowest mutual agreement (weak agreement).

**Table 8.** Under the types of classification group Type B; Interobserver agreement

B1	B2	B3
0.61	0.27	0.59

Internal observational analysis of subgroups of type B fractures shows (Table 8);

-for the modes B1 and B3, reliability and reliability,

-for type B2 internal observation analysis showed that it is the lowest value of the coef. Kappa 0,25 with acceptable reliability and security (acceptable agreement).

The comparative internal observation analysis of the subtypes showed the following results;

**Table 9.** Subtypes Compatibility

A4	B2
0.19	0.25

The A4 and B2 are the lowest values, the lowest mutual agreement (Table 9).

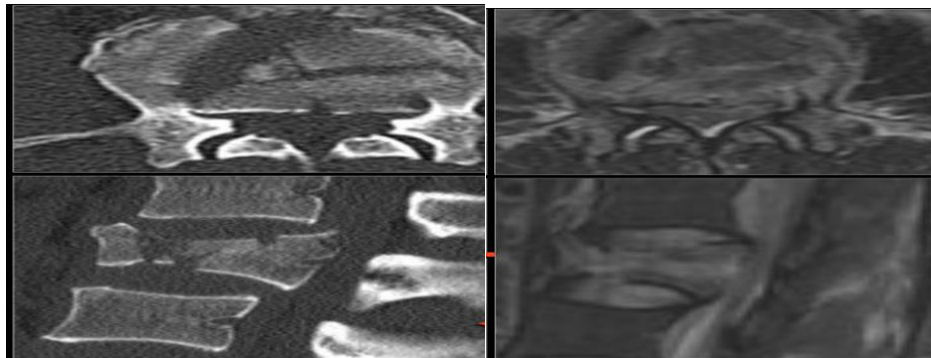
For example, case no. 11 (P.B. 56 yrs., female, fall from height)The question is what type of fracture is seen on the x-ray.Type A or Type B? Under type A3, A4? Sub type B2 is associated with A3, A4? (Image 1)



**Fig. 1.** RTG of thoracolumbar spine



CT scan and MRI are necessary for additional information and classification of the fracture, (Image 2-3)



**Fig. 2 & 3.** CT and MRI of thoracolumbar spine

Classification Type A compressive fracture, type A4.

Next case, no. 30 (A.C. 50 yrs., male, car accident). The question is what type of fracture is seen on the x-ray. Type A or Type B? Under type A3, A4? Sub type B2 is associated with A3, A4 (Image 4)



**Fig. 4.** RTG of thoracolumbar spine

CT scan and MRI are necessary for additional information and classification of the fracture (Image 5)



**Fig. 5.** MRI of thoracolumbar spine

Classification Type B2 distraction fracture with associated compressive fracture, type A3.

## CONCLUSION

Summarized results of an internal observational study of reliability and implementation of the new AO classification of thoracolumbar fractures;

- Kappa coefficient ( $\kappa$ ) for all 40 cases is 0.59, which is a moderate reliability.
- Kappa coefficient ( $\kappa$ ), for type A injuries, is 0.71, which represents significant reliability and safety,
- Kappa coefficient ( $\kappa$ ), for type B injuries is 0.50, and which represents moderate reliability and safety,
- Kappa coefficient ( $\kappa$ ), for injuries of type C is 0.61, and which represents moderate reliability and safety,
- Kappa coefficient ( $\kappa$ ), for injuries under type A4 is 0,19, and is with the lowest level of agreement
- Internal observational analysis showed the total average value of ( $\kappa$ ). for the subtypes of 0.55, which represents moderate reliability and reproducibility.

Having in itself all the necessary data from the analysis of the morphological characteristics of the fracture, the neurological status of the injured, specific modifiers for the patient, the results of this paper show that the new AO classification of thoracolumbar fractures gives a sufficiently large number of data, making it useful, reliable and safe for use in the daily work of the Traumatology Clinic.

It generates information that provides an excellent basis for forming a decision-making pathway for the proper treatment of these injuries, as well as our compliance with the appropriate treatment choice [27].

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ORIGINAL ARTICLE

**VERTICAL IRREGULARITIES INFLUENCE OVER THE SIZE, FORM AND SHAPE OF THE SYMPHYSIS**

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**ABSTRACT**

Modern orthodontics is a creation for the best possible balance between occlusal relations, dental and facial esthetics, result stability and their maintenance as well as teeth restoration. Regular or irregular vertical development of the facial skeleton is connected to multiple skeletal groups: nasomaxillary complex, alveolar processes and mandible. According to Beckmann, a significant correlation exists between the incisal rates and maxillary and mandibular dentoalveolar height, symphysis size, maxillary and mandibular surface. He concluded that long face cases had longer mandibular alveolar height, which was connected to a tight form instead of to the increased symphysis volume. Also, a connection exists between the size of the mandibular symphysis, the chin and the vertical dimension and morphological and dentoalveolar structure of both jaw systems. Determination of this connection can be useful in predicting the treatment success in overbite problems Haskel. The goal of our study is to show the symphysis shape, size and form in respondent groups. Depending on the vertical incisal rate characteristics - overbite, the respondents were divided in three groups: first group was consisted of respondents with open bite, meaning the overbite smaller or equal to -1 mm, the second group was consisted respondents with deep bite, meaning the overbite is over +4 mm, and the third control group consisted of respondents with normal overlap, meaning the overbite is more than +1 mm, but lower or equal to +4 mm. Height of symphysis in respondents with open bite was largest, and smallest in respondents with deep bite, compared to the control group. Depth of symphysis is the largest in the deep bite group, and the smallest in the open bite group.

The data obtained for symphysis height and depth can be used to anticipate treatment success in open and deep bites.

**Key words:** overbite, deep bite, height of symphysis, depth of symphysis.

**INTRODUCTION**

Examining the factors that influence facial harmony and disharmony, it is proven that facial components are inherited regardless of one another, and not as a complex that leads to different facial configuration creations. The facial configuration and facial expression depend primarily on the constitutional build of the skeleton, facial bones position and alignment, the upper and lower jaw position, bite type, soft-tissue components covering the facial base, as well as nose, lip and chin size.

Modern orthodontics goal represents the best possible balance between occlusal relations, dental and facial esthetics, result stability and their maintenance as well as teeth restoration [1]. Regular or irregular vertical development of the facial skeleton is connected to multiple skeletal groups: nasomaxillary complex, alveolar processes and mandible. There is connection between the structure of the front part of the maxilla and mandible and the lower part of the face, so in the case of open or deep bite, the dentoalveolar development can be insufficient to compensate the oversized or undersized detachment of the jaw system. According to Beckmann et al. [2, 3] a significant correlation exists between the incisal rates and maxillary and mandibular dentoalveolar height, symphysis size, maxillary and mandibular surface. The authors concluded that long face cases had longer mandibular alveolar height, which was connected to a tight form instead of the increased symphysis volume. They also analyzed the contribution of the alveolar process structures and the basal bone in relation to lower face height and ascertained that longer lower face was connected to a larger maxillary and mandibular alveolar process area and basal bone, and that a lower face was connected to a lower maxillary and mandibular frontal alveolar process area and basal bone. Beckmann et al. [2, 3] conducted measurements of 460 cephalograms of untreated patients and proved that the cases with short facial structure had a lower area and wider and shorter symphysis form. Even though cephalograms were two-dimensional, they showed that symphysis volume was smaller in cases with open bites and larger in cases with deep bites. Open bites could cause symphysis enlargement and elongation as well as shortening their form [4]. According Haskell [5, 6], a connection exists between the size of the mandibular symphysis, the chin, the overbite and morphological and dentoalveolar structure of both jaw systems. Sassouni [7] and Schudy [8] designated two different types of face forms in literature known as: skeletal open bites or hyperdivergent and skeletal deep bites or hypodivergent face type. Determination of this connection can be useful in predicting the treatment success in overbite problems [9].

The goal of our study is to show the symphysis shape, size and form in groups with open and deep bite.

## **MATERIALS AND METHOD**

For the realization of the set goal, examinations were conducted on 90 individuals from both sexes, aged 13-15, randomly chosen from the Clinic of orthodontics at PHO – Dental Clinical Centre “St. Pantelejmon” in Skopje.

Selecting the respondents taking part in realizing the set goal was based on the following criteria: individuals that had not previously underwent orthodontic treatment, with no great craniofacial disorders and with complete dentition.

In relation to the characteristics of the vertical incisal rate, the respondents were divided in three groups and classified as:

- The first group consisted of respondents with open bite, where the vertical incisal rate is lower or equal to -1 mm,
- The second consisted of respondents with deep bite, where the vertical incisal rate is over +4 mm, and
- The third group consisted of respondents with normal incisal overlap, where the vertical incisal rate is more than +1 mm, but lower or equal to +4 mm. This group was also the control group.

Every group consisted of 30 respondents, 15 female and 15 male, that came in the period from 2009-2015.

In the respondents from the research groups, standardized clinical and diagnostic procedures were conducted with x-ray cranial imaging in a standardized way in norma lateralis.

The linear parameters used in the research are:

- Symphysis height (SH mm). The distance between the points Infradentale and Menton, (highest point of the alveolar point on the mandible and the point where the shadow of the mandible base and the shadow of the chin profile meet)

- Symphysis depth (SD mm). The distance between the most prominent point of the chin profile (Pogonion) and the most prominent point of the symphysis posterior wall.

The statistical data analysis was conducted in SPSS for Windows 17,0 program.

- For data testing we used Shapiro-Wilk`s W test.
- For data depiction descriptive statistics was used.
- For comparison of the analyzed parameters between the three analyzed groups, we used One way Anova, and for inter-group differences we used Tukey test.
- For comparison of the analyzed parameters in relation to gender the Student "t" test was used.

- The levels of probability for achieving null hypothesis, concordant to international standards for bio-medical sciences were 0.05 and 0.01

## RESULTS

Average value of the symphysis height parameter SH in the three analyzed groups (open, deep and normal bite) is 33,9±3,2mm, 25,7±3,8mm and 29,65±2,0mm respectably. The tested difference in average symphysis height between the three analyzed respondent groups is significant. (F=35,2 p<0,01). Intergroup difference shows that this parameters have significantly different average values between the three comparison pairs: open bite to deep and normal bite, as well as deep bite to normal bite. (Table 1)

**Table 1. Symphysis height SH in groups with open, deep and normal bite**

Group	Symphysis Height SH		
	mean±SD	min-max	median
<b>Open bite</b>	33,9±3,2	25-39	34
<b>Deep bite</b>	25,7±3,8	20-32	24,5
<b>Normal bite</b>	29,65±2,0	26-32	30
tested differences	F=35,2 p<0,01 post hoc open vs deep p<0,01 open vs normal p<0,01 deep vs normal p<0,01		

Gender influence over SH size confirmed as significant only in the deep bite group (t=5,246 p<0,01), in this group the male respondents present significantly larger SH parameter compared to female respondents (28,6±2,7mm vs 22,8±2,1mm). In the open bite and normal bite group, the differences in average SH parameter size between male and female respondents is insignificant to be presented as significant (p>0.05). (Table 2)

**Table 2. Sex differences for symphysis height SH in groups with open, deep and normal bite**

Group	Sex	Symphysis Height SH			tested differences
		mean±SD	min-max	median	
<b>Open bite</b>	Male	35,1±2,4	30-39	35	t=1,794 p>0,05 ns
	Female	32,7±3,5	25-37	33,5	
<b>Deep bite</b>	Male	28,6±2,7	23-32	30	t=5,246 p<0,01 sig
	Female	22,8±2,1	20-27	23	
<b>Normal bite</b>	Male	30,4±1,5	28-32	30,5	t=1,761 p>0,05 ns
	Female	28,9±2,2	26-32	28	

Statistics analysis shows that for the value  $F=48,2$  ( $p<0,01$ ), a significant difference exists in average parameter size for symphysis depth SD between the three analyzed groups. The significant difference is based on the considerably shorter average parameter length in the open bite group, compared to the deep bite group ( $12,75\pm1,3\text{mm}$  vs  $17,1\pm1,5\text{mm}$ ), as well as compared to the control group ( $12,75\pm1,3\text{mm}$  vs  $14,3\pm1,5\text{mm}$ ), furthermore a considerably shorter average parameter length is present in the control group compared to the deep bite group ( $14,3\pm1,5\text{mm}$  vs  $17,1\pm1,5\text{mm}$ ). (Table 3)

**Table 3. Symphysis depth SD in groups with open, deep and normal bite**

Group	Symphysis Depth SD		
	mean±SD	min-max	median
<b>Open bite</b>	12,75±1,3	10-15	13
<b>Deep bite</b>	17,1±1,5	14-19	17
<b>Normal bite</b>	14,3±1,5	12-17	14
tested differences	F=48,2 p<0,01 post hoc open vs deep p<0,01 open vs normal p<0,01 deep vs normal p<0,01		

Average symphysis depth in male and female respondents in the open bite groups is  $12,8\pm1,0\text{mm}$  and  $12,7\pm1,6\text{mm}$  respectively, in the deep bite group it is  $17,2\text{mm}\pm1,6\text{mm}$  and  $17,05\pm1,5\text{mm}$  respectively, and in the control group it is  $14,6\text{mm}\pm1,5\text{mm}$  i  $14,6\pm1,5\text{mm}$  respectively. Average symphysis depth parameter size is not significantly dependent on the gender in none of the analyzed groups. (Table 4)

**Table 4. Sex differences for symphysis depth SD in groups with open, deep and normal bite**

Group	Sex	Symphysis Depth SD			tested differences
		mean±SD	min-max	median	
<b>Open bite</b>	Male	12,8±1,0	10-14	13	t=0,168 p>0,05 ns
	Female	12,7±1,6	10-15	12,5	
<b>Deep bite</b>	Male	17,2±1,6	14-19	17	t=0,219 p>0,05 ns
	Female	17,05±1,5	15-19	17,5	
<b>Normal bite</b>	Male	14,6±1,5	12-17	14,5	t=0,896 p>0,05 ns
	Female	14±1,5	12-17	14	



## **DISCUSSION**

The tested difference in average symphysis height between the three analyzed groups is significant. Our results correspond to the results of Beckmann [2, 3] and Ceylan [10, 11]. In the open bite and normal bite group, the difference in average SH parameter size is insignificant to be presented as significant ( $p>0.05$ ). Gender influence over SH size confirmed as significant only in the deep bite group ( $t=5,246$   $p<0,01$ ), in this group the male respondents presented significantly larger SH parameter compared to female respondents ( $28,6\pm 2,7\text{mm}$  vs  $22,8\pm 2,1\text{mm}$ ).

In studies done by Beckmann [2, 3], it was postulated that symphysis size was determined by a characteristic factor deduced from the factors that control lower face height. The studies clearly show that symphysis height is connected to the vertical incisal rate. The control factor can be a genetic factor that determines the overlap.

Descriptive statistics for symphysis depth shows that the average values in the open bite group is concordant to the Ceylan [10, 11] findings, however the deep bite average values are larger than the findings in the same one. In all three respondent groups, a statistically significant difference in gender does not exist.

## **CONCLUSION**

Symphysis height in respondents with open bite was largest, and smallest in respondents with deep bite, compared to the control group. SH analysis in relation to gender shows no statistically significant difference in open and normal bite groups, however in the deep bite group is ( $p<0.01$ ), meaning that height of symphysis is larger in male respondents compared to female respondents.

Symphysis depth is the largest in the deep bite group, and the smallest in the open bite group. No differences were noticed in relation to the gender in all three respondent groups ( $p>0.05$ ).

The data obtained for symphysis height and depth can be used to anticipate treatment success in open and deep bites.

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CASE REPORT

**PERINEO-VAGINAL RECONSTRUCTION WITH IPAP PROPELLER FLAP  
A CASE REPORT**

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**ABSTRACT**

**Background:** Perineal defects occur due to infections, trauma or after tumor resection in the region. There are many options for reconstructions, from spontaneous healing to free flaps. However, usage of local flaps seems most reasonable and feasible. Since perineum bears several orifices, this can lead to infection and reconstructive failure. That is why, these defects are really challenging for plastic surgeons.

**Case report:** We present a case of reconstruction of vagino-perineal defect after resection of proliferative vulvar planocellular carcinoma, by using internal pudental artery perforator flap (iPAP) designed in a propeller fashion.

**Conclusion:** Propeller perforator flaps are novel reconstructive methods that can be used successfully for perineal reconstruction, resulting in good functional and aesthetic outcome with minimal donor site morbidity.

**Key words:** perineum, reconstruction, propeller, perforator, flap.

**INTRODUCTION**

Perineo-genital defects can occur due to infections, trauma or mainly, after resection of tumors in this region. There are many options for reconstruction of these defects, from spontaneous closure, skin grafting, vacuum assisted closure, up to reconstruction with free flaps. Selection depends on the size of the missing tissue, among others [1].

Perforator flaps are local flaps nourished by a known perforator vessel that is directly skeletonized intraoperative or preoperatively detected with pencil Doppler, as a freestyle perforator flap [2]. As we know from Taylor's anatomic study, there are more than 350 perforators in a human body [3]. Perforator flaps are safe and dependable methods in plastic surgery that reconstruct alike tissue. When perforator flap is rotated around the vessel which is a pivot point of rotation, it becomes propeller perforator flap [4]. The concept was born in 1991, by Hyakusoku, who gave the term in order to describe an adipose-dermal flap that has been rotated for 90° around a centrally – based subcutaneous perforator [5]. Hallock in 2006 used an eccentrically based skeletonized perforator and managed to rotate the flap for 180° around it [6]. A clear definition of propeller flap was given in 2009 by the advisory panel of the first Tokyo meeting on perforator and propeller flaps, who defined it as an "island flap that reaches the recipient site through an axial rotation" [7]. The difference between a propeller flap and other pedicled flaps is that movement here is "axial", meaning that the flaps turn around a pivot that is the pedicle in fact.

According to the Gent consensus on perforator flaps, perforator propeller flaps should be named after their nutrient vessels [8].

Perineal region has a rich vascularization arriving from branches of femoral and iliac arteries. It comprises the skin boundaries of the perineum, divided in anterior and posterior part. There are many perforators coming out from superficial circumflex iliac, superficial inferior epigastric, superficial external pudendal, deep external pudendal, obturator, perineal and internal pudendal artery. These perforators can be the basis for flap's design [9]. Internal pudendal artery perforators (iPAP) are found in dense fatty tissue of ischioanal fossa, identified as a vascular triangle connecting three points: ischial tuberosity, the anus and lower vaginal orificium (lower scrotal raphe). One perforator has a wide vascular territory and flaps with dimensions up to 20 cm length can be harvested [10].

We present a case where bilateral iPAP propeller flaps were used in reconstruction of vulvo-perineal defect after excision of massive vulvar carcinoma.

### **CASE REPORT**

A 67 years old lady was referred to gynecologist for treatment of a proliferative neoplasm in vulvar-perineal region.



**Fig. 1.** Local appearance of the vulvar-perineal carcinoma

It started to grow as a nodule on the labia majora many years ago. She had denied doctor's evaluation due to embarrassment. Lately, patient had difficulties when voiding, defecating and walking and also, moderate pain was present. Biopsy was made, and planocellular carcinoma was revealed. Ultrasonography examination of inguinal regions and CT imaging of pelvis, showed enlarged lymph nodes in the right inguinal region, with no progression in the minor pelvis. Colposcopy examination found an infiltration of vaginal introitus and its distal portion. Treatment plan was radical carcinoma removal and reconstruction of the defect that will follow. Bilateral groin dissections were also planned to be done. Those were the reasons why plastic surgeon took place. Reconstruction comprised bilateral freestyle iPAP propeller flaps.

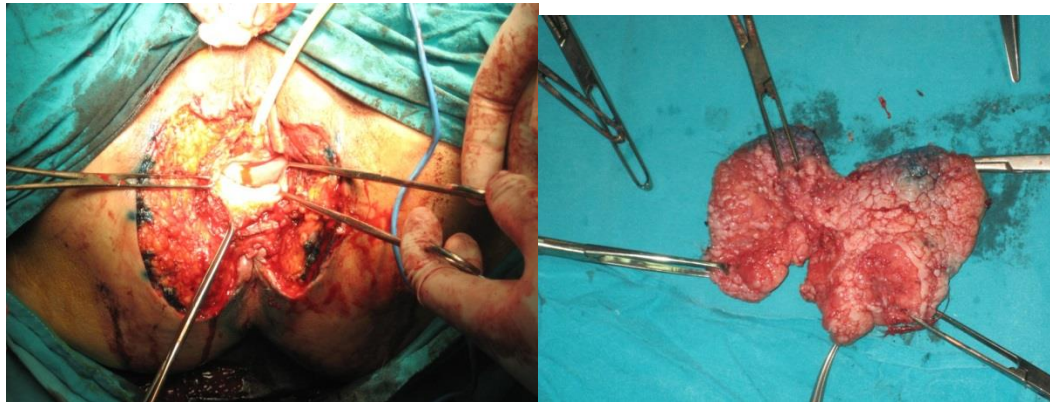
**OPERATIVE TECHNIQUE:**

In general anesthesia, patient was set in a lithotomy position and Foley catheter was put. Using pencil hand-held Doppler probe, perforators of internal pudental artery were found in the vascular triangle of ishiorectal fossa. The upper most perforator was marked as a pivot point as the plan was a propeller flap. The flap was marked in the gluteal fold.



**Fig. 2.** Preoperative markings for flap harvesting – detected perforators are dotted

Gynecologist removed the carcinoma with resection of the distal part of the vagina and whole perineum down to healthy tissue.



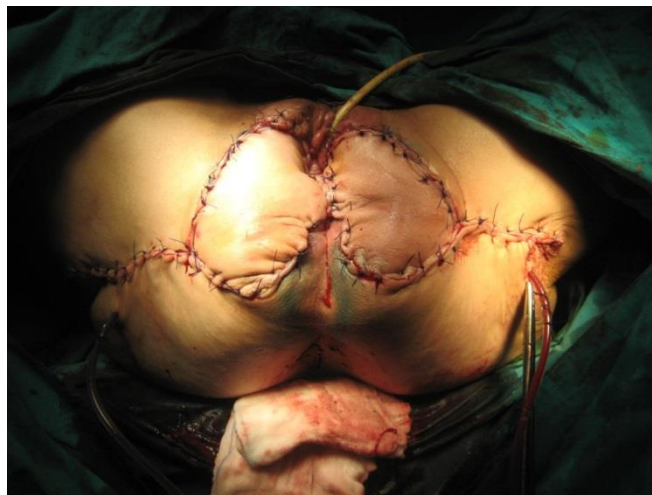
**Fig. 3.** Defect after resection of the carcinoma

After resection, reconstruction followed. Harvesting started from distally upwards, using 3x magnification loops. In thick fatty tissue, with no fascia, it is difficult to skeletonize the perforator totally. That is why a part of the tissue was left around the perforator, but small enough for a 100° rotation to be made around. The flap was then rotated in vaginal direction and its distal part (now upper) sutured to the remnant vagina and adjacent tissue.



**Fig. 4.** Flaps' elevation

After one side reconstruction, another iPAP was raised from the opposite side in the same way and placed similarly as the previous one. In the midline of the perineum, both flaps were sutured together.



**Fig. 5.** Final reconstruction with bilateral flaps

Penrose vacuum drains were introduced in donor sides, bilaterally. When reconstruction finished, radical inguinal lymphadenectomy was made bilaterally, where vacuum drains were set in as well.

After operation, among other therapy, antibiotics and blood components were administrated. Low molecular weight heparin was included as patient was advised to rest several days restraining eating solid food. Flaps had good color, adequate capillary refill and temperature with no vascular suffering. Flap edema and imbibition were obvious at the beginning, but it settled down after several days. Drains were removed on day 5 and patient discharged home on day 10. Healing was uneventful regarding reconstruction. There was seroma collection in the right iniquinim with some lymphorrhea, which stopped after few aspirations.

Pathology sampling showed invasive plano - cellular carcinoma with moderate differentiation, metastasis in two lymph nodes right, and in one lymph node left. As resection was very close to tumor margins, patient was sent to radiologist where brachytherapy followed after 3 months post op. Patient felt good with no progression of the tumor, neither locally nor regionally. Three years after operations, she is still under follow-up. Reconstruction was sufficient with minimal donor side morbidity, no restrictions in patient's daily life and great satisfaction.



**Fig. 6.** Result several months after operation

## **DISCUSSION**

Plastic surgeons are often part of multidisciplinary teams when dermatologists, urologists, gynecologists, abdominal surgeons, orthopedics etc., ask for their expertise in reconstructive field. The aim of reconstruction is to rebuild the lost tissue with similar tissue leading to sufficient functional and aesthetic results with minimal morbidity at once. Additionally, the aim of reconstruction of the perineal region is to maintain its excretory and sexual function. This region is challenging for plastic surgeons due to its intimacy to several orifices, which on other side, due to these orifices, is prone to infections and reconstructive failures [1, 9].

Defect in perineal region can be treated according to the reconstructive ladder: at the easiest way, it can be left to close spontaneously by second intention or can be skin grafted [1]. However, these approaches in larger defects lack good functional and aesthetic results. Myocutaneous flaps, as tensor fascia lata or rectus femoris, have high donor site morbidity and are very bulky. Free flaps can be applied as well, especially for bigger defects, but they bare the high morbidity again and higher failure risk [1,2]. When the options for local reconstructions are gone, especially in cases of radiotherapy, present fistulas or many previous operations, or when defects are larger, free or distant flaps come in as an alternative. However, due to rich vascular supply of perineum, approach with local flaps reconstruction seems most reasonable as it reconstruct with alike tissue, resembling good results [9,10].

Internal pudental artery is a branch of internal iliac artery, running inside the sacrotuberous ligament, giving several perforators in ischiorectal fossa, where lastly, it emerges as perineal artery. Pedicled flaps based on these perforators have been reported for perineal reconstruction, as transtpositional, V-Y advancement, gluteal fold, iPAP thigh flap and iPAP propeller flaps [1,10]. In our case we used iPAP propeller flap using gluteal fold tissue for reconstruction. This has an advantage in cosmetics as the donor site suture is in the gluteal fold. Moreover, when groin dissection is planned in continuity of anogenital carcinoma resection, such in our case, femoral artery becomes unstable and reconstruction using gluteal fold tissue seems ideal as it is far away from the lymphatics that drain ano-urogenital region.

Additionally, being far away from subsequent irradiation, gluteal fold tissue can be utilized for reconstruction of radiation –induced injuries. It can reconstruct voluminous tissue defects as in obese patients, but also thinner flaps can be raised leaving smaller donor site scars. Axial rotation can be done in many directions allowing coverage of different local defects, for example ischial defects in pressure ulcers [1,9,10].

Perforators of the arteries can be indirect (myocutaneous, septocutaneous) and direct perforators such the case of iPA perforators is [11]. Here, they are branched into the fatty tissue, surrounded only with fat, without adjacent fascia, allowing a wider arc of rotation without much skeletonization. Actually, total dissection of the perforator is not even recommended because the perforator is situated only in fatty compartment allowing easy mobilization of the planned flap. [10] As described by Mardini and Wei, preoperative Doppler signal mapping is sufficient for perforator detection and with gentle dissection, freestyle perforator flap can be raised on it [12]. We applied the advices, marked the desired perforator detected with pencil Doppler, raised the flap and axially rotated for 100°, good enough to reconstruct the defect without any suture tension. It is necessary to detach any tissue resulting in pedicle kinking or twisting as these can lead to flap ischemia.

Propeller flaps can have 90 – 180° angle of axial rotation without blood supply insufficiency. They are ideal for small to moderate defects in different body parts: head and neck, upper and lower extremities, trunk. Advantages for their utilization are: freedom in their design as a freestyle perforator flaps regarding soft tissue volume needed; they are faster and simpler reconstructive method comparing to free flaps; possibility to use local tissue that is more similar to the missing one; easy and fast harvesting when adequate technique is used; the donor site morbidity is minimal. Having in mind the above and technical refinements achieved lately, indications for perforator propeller flap reconstructions are constantly increasing [4].

## **CONCLUSION**

Perineo-genital defects are challenging for reconstruction. With rich vascularization in the region, there are many reconstructive options. However, reconstruction with local similar tissue is preferred method. Reconstruction with perforator flap is novel approach for local flap usage. Reconstruction with iPAP flaps can be successful and great alternative for traditional or free flaps. When being propeller, it can cover large defect, especially when used bilaterally, gaining the possibility to reconstruct complex wound. On the other site, it is easy and fast to harvest with some specific technical considerations. This flap has many advantages over traditional flaps such as better functional, aesthetic results and low donor- site morbidity, among others.

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REVIEW ARTICLE

**ACUTE CHORIOAMNIONITIS, HISTOPATHOLOGIC CHANGES OF THE PLACENTA AND PREVALENCE OF MICROBIAL INVASION IN PATIENTS WITH PRETERM DELIVERY – REVIEW OF LITERATURE**

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**ABSTRACT**

**Aim:** To review to date published results on the topic of histopathological changes of the placenta, umbilical cord and fetal membranes in patients with preterm delivery and evaluate their association with microbial invasion of the amniotic cavity and placental disc.

**Materials and methods:** The PubMed index was searched to identify relevant articles, studies and abstracts published in English in the past 20 years (1995-2015). The following keywords were used: placenta, placental pathology, chorionamnionitis, amnionitis, funisitis, pPROM (Preterm Premature Rupture of the Membranes), intra amniotic infection and intra amniotic inflammation.

**Results:** The results of the reviewed studies show that histopathologic changes classified as chorioamnionitis, deciduitis, vilitis and funisitis, can be present even in settings that lack clinical expression of the syndrome, such as high temperature, tachycardia and leukocytosis. The presence and intensity of the inflammatory changes are inversely proportional to the gestational age. These findings are more often present in cases of extreme rather than in cases of moderate to late prematurity (94.4% in deliveries between 21-24 g.w contrary to 10.7% in deliveries at 33-36 g.w). At the same time, the detection rate of microbial invasion of the amniotic cavity varies in wide range from 7.4-74%, depending on the method used for sample collection (microbiological smear, amniotic fluid culture or PCR).

**Conclusion:** Histological verification of chorioamnionitis is far more often than the clinical manifestation of the syndrome in patients with preterm labor. Considering the discrepancy between pathohistological findings and detection rate of microorganisms ethologically associated with placental inflammatory changes, the necessity for development of more sensitive techniques for microbial detection has to be prioritized.

**Keywords:** placenta, placental pathology, chorionamnionitis, amnionitis, funisitis, PROM, intra amniotic infection and intra amniotic inflammation.

**INTRODUCTION**

Preterm delivery is one of the most challenging problems that contemporary perinatology faces. According to the World Health Organization (WHO) estimations, each year approximately 15 million babies are born prior to the 37<sup>th</sup> gestational week (g.w) [1]. Complications associated with preterm delivery are direct cause of mortality in almost 35% out of 3.1 million newborn deaths. They also represent the second largest cause of under-five mortality.

On the other hand, premature newborns are exposed to increased risk of neuro-developmental disability due to increased risk of cerebral palsy, hearing and sight impairment, behavioral and cognitive functions impairment (Attention Deficit Hyperactive Disorder) [2].

According to the globally accepted definition of WHO from 1977, preterm delivery is considered every delivery prior to 37<sup>th</sup> g.w or 259 days counting from the first day of last menstrual period. Depending on the gestational age, preterm delivery is divided into three categories: extremely preterm, less than 28 g.w; very preterm, starting from 28<sup>th</sup> until 32<sup>nd</sup> g.w, and moderate to late preterm, starting from 32<sup>nd</sup> until 37<sup>th</sup> g.w. The group of moderate to late preterm is divided into two sub groups: moderate, from 32<sup>nd</sup> to 34<sup>th</sup> g.w, and late, from 34<sup>th</sup> to 37<sup>th</sup> week [3].

Preterm delivery is considered a syndrome. There are two main groups of factors responsible. The first one are those arising from the necessity for emergency or elective termination of pregnancy due to reasons associated with the condition of the mother or the fetus, so called iatrogenic factors. These include preeclampsia, imminent eclampsia, eclampsia, HELLP Sy., intrauterine growth restriction, intrauterine fetal asphyxia or fetal distress, bleeding placenta previa or placental abruption and preexisting maternal conditions that deteriorate during pregnancy. This group of indications counts for almost 20% of all preterm deliveries. The second group are premature preterm rupture of membranes (pPROM) and preterm labor. In around 20% of cases, preterm delivery is a result of pPROM, but in almost 40% of cases preterm delivery is a result of spontaneous onset of uterine contractions. Large number of risk factors are listed in literature, which are responsible for this syndrome, starting with maternal history of preterm delivery in previous pregnancies, multiple gestation, early or advanced maternal age, short interval between pregnancies etc. [2, 4]. Among the etiological factors attracting most attention are intrauterine infections and their association with inflammatory changes of the fetoplacental unit. Nearly one quarter (26%) of premature babies die due to infection [5]. Evaluation of the histopathology of the placenta, fetal membranes and the umbilical cord in these patients shows that the prevalence of the acute inflammatory lesions varies from 22-59%, and it is inversely proportionate to the gestational age [6]. The aim of this review is to summarize data that have been published to this date, on the prevalence of histopathological changes of the placenta, fetal membranes and the umbilical cord in patients with preterm delivery and the correlation between these changes and the gestational age. We also evaluated the association between preterm delivery and microbial invasion of the amniotic space and the placenta.

## **MATERIALS AND METHODS**

Through PubMed index search, we identified articles and abstracts, written in English, published in a period of 20 years (1995-2015). We searched for the following key words: placenta, placental pathology, chorioamnionitis, vilitis, funisitis, preterm delivery, pPROM, intra amniotic infection and intra amniotic inflammation. Out of the total number of published studies, we selected those with full free access, review articles, randomized controlled trials, expert reviews, recommendations and statistical data published by relevant institutions such as WHO and NICE (National Institute for Health and Care Excellence). In the process of evaluating the quality of selected studies advantage was given to those studies dealing with the issue in question, i.e. evaluation of the degree of inflammatory changes of the placenta, umbilical cord and amniotic membranes, classified according to the criteria of the Amniotic Fluid Infection Nosology Committee of the Perinatal Section of the Society of Pediatric Pathology [7].

For the analysis of association between the inflammatory changes of the placenta and the presence of microbial invasion of the placenta and the intra amniotic compartment, we evaluated studies and articles that used microbiological smears taken from the border between the placental disc and the amniotic membranes, as well as studies and articles that used amniotic culture and PCR (Polymerase Chain Reaction) for identification of bacterial rDNA (ribosomal DNA) in the amniotic fluid, as a method for isolating and identifying microbiological agents.

## **RESULTS**

During the second half of the XX century, the scientific community started to show interest in the possible association between preterm labor and delivery, and the infections of the fetoplacental unit. During the 1960ies and the 1970ies, Blanc WA, Naeye RL and Fujikura T, started to publish data from their research which was focused on the association between ascending vaginal infections, caused by bacteria, fungi and viruses, and histopathological changes of the placenta defined as placentitis or choriamnionitis. Still, a more serious advance in this field was made in the last decade of the XX, and at the beginning of the XXI century.

In 1996, Borralho P. published the results from a study performed in 1993. In this study, he histopathologically evaluated 280 placentas from patients with preterm delivery. In as high as 38% of analyzed tissue samples, signs of chorioamnionitis were detected. Nevertheless, only 13% of the patients had clinical signs of chorioamnionitis. As the author concluded, the prevalence of the microbial invasion and the type of the microbiological agents responsible for the expression of the histopathological changes of the placenta are still unclear [8]. In 1995, Monga M. and Blanco JD, published results from a systematic review of 15 studies in which amniocentesis for amniotic culture was performed in patients with preterm delivery. They reported 12.2% success rate in isolation of microbiological agents, with variations of 0-48%, depending on the characteristics of the examined population and method used for cultivation. Histopathological analysis of the placentas in these studies showed that in 60% of the cases there were histopathological signs of chorioamnionitis. Considering that their analysis showed positive correlation between the positive amniotic cultures and histopathological changes of the placenta, it was a logical conclusion that intra uterine infection leads to inflammatory changes of the placenta. Hence, intra amniotic infection is responsible for the onset of labor and initiation of the process of preterm delivery [9].

### **Classification of the histopathological changes of the placenta**

In 2003, Redline RW and his associates, promoted and validated a complete set of patterns of placental response that can be identified in cases of infection of the amniotic compartment, hoping to establish a standard diagnostic frame that would be pragmatic for everyday use. This standard frame was established based on analysis of 20 cases from the archives of the university clinic in Cleveland, USA. Six pathologists were included in the study. Each pathologist analyzed 3 tissue samples from each of the individual cases (umbilical cord, amniotic membranes and one full-thickness section of the placental disc). After the primary evaluation of the tissue samples, the same samples were analyzed within the group. In the end, the results were revised based on modified criteria. Sensitivity of 67-100%, and specificity of 85-100% was reached, depending on the diagnostic criterion in question [10]. The whole system of classification of placental lesions is presented in Table 1.

**Table 1.** Patterns of placental response associated with amniotic infection: nomenclature and definition

Diagnostic categories	Suggested diagnostic terminology	Definition
<b>MATERNAL INFLAMMATORY RESPONSE</b>		
<b>Stage</b>		
1 – Early	Acute subchorionitis or chorionitis	PMN in sub chorionic fibrin and/or membrane trophoblast
2 – Intermediate	Acute chorioamnionitis	Diffuse-patchy PMN in fibrous chorion and/or amnion
3 – Advanced	Necrotizing chorioamnionitis	PMN karyorrhexis, amniocyte necrosis, and/or amnion basement membrane thickening/hyper eosinophilia
<b>Grade</b>		
1 – Mild – Moderate	No special terminology required	Not severe as defined below
2 – Severe	Severe acute chorioamnionitis or with sub chorionic micro abscesses	Confluent PMN ( $\geq 10 \times 20$ cells in extent) between chorion and decidua; $\geq 3$ isolated foci or continuous band
Other	Chronic (or subacute) chorioamnionitis	Subamniotic mononuclear cell infiltrate with occasional PMN (meconium and hemosiderin-loaded macrophages excluded)
<b>FETAL INFLAMMATORY RESPONSE</b>		
<b>Stage</b>		
1 – Early	With chorionic vasculitis <i>or</i> umbilical phlebitis	Intramural PMN-chorionic vessels and/or umbilical vein
2 – Intermediate	With umbilical vasculitis (one or two arteries – vein) <i>or</i> umbilical panvasculitis (all vessels)	Intramural PMN-umbilical artery or arteries ( $\pm$ umbilical vein)
3 – Advanced	With (subacute) necrotizing funisitis <i>or</i> with concentric umbilical perivasculitis	PMN $\pm$ associated debris in concentric bands-rings-halos around one or more umbilical vessels
<b>Grade</b>		
1 – Mild – moderate	No special terminology required	Not severe as defined below
2 – Severe	With a severe fetal inflammatory response <i>or</i> with intense chorionic (umbilical) vasculitis	Near confluent intramural PMN-chorionic and/or umbilical vessels with attenuation/degeneration of VSMC
Other	With associated fetal vessel thrombi	Recent thrombosis associated with intramural PMN
Other specific features	Peripheral funisitis Acute villitis Acute intervillitis with intervillous abscesses Decidual plasma cells	Focal aggregates of PMN at the umbilical cord surface PMN in villous stroma (or between trophoblast and stroma) Patchy-diffuse PMN in intervillous space Unequivocal plasma cells in decidua basalis or capsularis
PMN – polymorphonuclear leucocyte; VSMC – vascular smooth cell muscles		

The same author in 2015 published his experts review, redefining the criteria for histopathological evaluation of the placenta. These criteria were presented at the Amsterdam international consensus of criteria for diagnosis of placental lesions. According to the author, these criteria, presented in Table 2, represent an extensive basis for implementing a comprehensive system or systematized framework that includes all placental lesions [11].

Although it was not in the focus of the group, the following recommendations for tissue sampling of the placenta were introduced:

- To submit at least 4 samples
- One of the samples should include 2 cross-section blocks of the umbilical cord and a roll of extra placental membranes which includes a part of the marginal parenchyma
- Other samples should contain full-thickness sections of normal appearing placental parenchyma provided from within the central two thirds of the placental disc, including one adjacent to the umbilical cord insertion site

Using Redlines criteria, adopted by the Amniotic Fluid Infection Nosology Committee of the Perinatal Section of the Society of Pediatric Pathology, Chong JK and his collaborators analyzed 7.505 placentas from singleton pregnancies, delivered after 20<sup>th</sup> g.w. They used placentas of term pregnancies as a control. The analysis showed significantly higher presence of inflammatory changes of the placenta, defined as acute choriamnionitis, in the group delivered between the 21<sup>st</sup> and 24<sup>th</sup> g.w and the trend was descendent in groups with higher gestational age. The prevalence of the inflammatory changes of the placenta in the first group was up to 94.4%, contrary to 39.6% in the group at gestational age of 25-28 weeks, and 35.4% in the group at gestational age of 28-32 weeks. Only 10% of patients with so-called late prematurity, 33-36 weeks demonstrated inflammatory changes of the placenta. Analyzing the placentas of the term patients in the control group, they concluded that the presence of spontaneous contractions and cervical dilatation > 4 cm represent an additional poor prognostic factor, if it is to be evaluated in the function of time. The prevalence of the inflammatory changes of the placenta was 4 times higher in this group, contrary to the group of patients with no spontaneous onset of uterine contractions (11.6% vs. 4.7%,  $p < 0.01$ ). It was also presented that the frequency of acute choriamnionitis increased if the length of the delivery was higher and the cervical dilatation was more than 4 cm (30.4% vs. 11.6%) [6]. They proposed two possible scenarios as an explanation. First, microbial invasion of the amniotic space in patients with spontaneous onset of labor, when amniotic membranes are intact, is higher (17% vs. 1.5%) [12]. Second, uterine contraction, by themselves, have an inflammatory basis. The chorion and amniotic membranes, provided from the patients with evident contractions, display a high expression of chemokine receptors specific to neutrophils and monocytes, as well as for the inflammatory proteins specific to macrophages, even though there are no evident signs of chorioamnionitis. These findings correlate with the reports of increased concentration of chemokines such as IL-8, cytokines IL-1 and IL-6, chemotactic proteins, as well as growth associated oncogenes, in the amniotic fluid of term patients with spontaneous onset of contractions, contrary to those with no contractions.

**Table 2.** Placental classification (incorporating the 2014 Amsterdam Placental Workshop Group criteria)

Placental vascular processes	A. Maternal stromal-vascular lesions	Developmental	Superficial implantation/decidual arteriopathy
			Increased immature extravillous trophoblast
		Malperfusion	Global/partial Early: distal villous hypoplasia Late: accelerated villous maturation
			Segmental / complete Villous infarct(s)
		Loss of integrity	Abruptio placentae (arterial)
			Marginal abruption (venous) Acute Chronic
	B. Fetal stromal-vascular lesions	Developmental	Villus capillary lesions
			Delayed villous maturation (maturation defect)
			Dysmorphic villi
		Malperfusion	Global/Partial Obstructive lesions of the umbilical cord Recent intramural fibrin in large fetoplacental vessels Small foci of avascular or karyorhectic villi
			Segmental/complete Chorionic plate or stem villous thrombi Large foci of avascular or karyorhectic villi
			Large vessel rupture (fetal hemorrhage) Small vessel rupture (fetomaternal hemorrhage)
Loss of integrity		Villous edema	
Placental inflammatory-immune processes	A. Infectious inflammatory lesions	Acute	Maternal inflammatory response: chorioamnionitis, subchorionitis
			Fetal inflammatory response: chorionic/umbilical vasculitis
		Chronic	Villitis (CMV, others)
			Intervillousitis (malaria, others)
	B. Immune/idiopathic inflammatory lesions	Villitis of unknown etiology and related/associated lesions	Chronic villitis
			Chronic chorioamnionitis
			Lymphoplasmacytic deciduitis
		Eosinophil T-cell vasculitis	
Chronic histiocytic intervillousitis			
Other placental processes	Massive perivillous fibrin(oid) deposition (maternal floor infarction)		
	Abnormal placental shape or umbilical insertion site		
	Morbidly adherent placentas (accreta)		
	Meconium-associated changes		
	Increased circulating nucleated red blood cells		
CMV – Cytomegalovirus			

Talking about the origin of the neutrophils within the inflammatory substrate in patients with chorioamnionitis, using FISH (Fluorescent in Situ Hybridization) with probes for X and Y-chromosomes performed in cytopsin slides of placentas from male fetuses, it was determined that almost 90% of neutrophils originate from the mother. In addition, the use of FISH combined with immunohistochemistry for CD45 positive cells, for identification of leukocytes, demonstrated that CD45 positive cells in the membranes derive from the mother. On the other hand, inflammation of the umbilical cord and chorionic vessels of the chorionic plate is of fetal origin, which correlates with the anatomy of the complex. Neutrophils that migrate from the umbilical blood vessels, arteries and veins, must originate from fetal circulation in order to penetrate the wall of these blood vessels [6].

### **Microbial invasion and its role**

Usually, the intra amniotic compartment is considered to be a sterile environment. The pathway of intra amniotic infection has not been completely understood. Four basic mechanisms are suggested: ascending path of transport of the microbial agents from the lower genital tract, haemathogenous dissemination, accidental inoculation of infection during invasive procedures (amniocentesis, cordocentesis, fetoscopy etc.) and reverse or retrograde advance through the Fallopian tubes, which is supported by limited number of evidence. The ascending pathway of infection is thought to be one of the most common mechanisms for intra amniotic infection. There are two suggested sub mechanisms.

According to the first one, microorganisms pass through the cervical barrier and invade the decidua, which provides appropriate conditions for their multiplication, and from there they enter the amniotic space. The second sub mechanism suggests a direct spread of microorganisms into the amniotic space, as it is in the cases with intact membranes and no pPROM. So far, the data suggest that inflammatory response starts at the level of decidua in both previously suggested mechanisms, with the following difference. If the infectious agents advance into the decidua, maternal inflammatory cells will be concentrated in this compartment, but if there is a direct advance into the amniotic space, chemotactic gradient will attract the inflammatory cells in the chorion and further after into the amniotic space [6, 13].

The most serious evidence about the association between the intra amniotic infection and acute chorioamnionitis originates from the study of Romero R. and his collaborators. The study was performed on 92 subjects with preterm contractions and intact membranes, the material for microbiological analysis was provided by percutaneous amniocentesis, and histopathological evaluation of the placentas was performed 48 hours after the procedure. The results showed prevalence of the microbial invasion of the amniotic space in 38% of the cases. Acute chorioamnionitis was histopathologically confirmed in 71.1% of the analyzed tissue samples, while the diagnosis of acute funisitis was made in 78.7% of cases. The negative predictive value of the histopathological findings compared to the results of the microbiological analysis of the amniotic fluid was 87% for the acute chorioamnionitis and 82% for the acute funisitis respectively [14]. The prevalence of the microbial invasion of the amniotic space is similar between patients with spontaneous onset of labor at term, compared to those with premature spontaneous onset of labor and intact membranes (17 vs. 22%). Nevertheless, in preterm neonates there is more frequent early onset of neonatal sepsis, more intense inflammatory response, and more frequent presence of funisitis. There are several thesis that have been offered as an explanation to the presented differences. Microbial invasion of the amniotic space in term patients is of shorter time and it appears after the initiation of contractions.



Such microbial invasion is mainly presented with small inoculum, initiates mild inflammatory response and it is rarely followed by fetal microbial invasion. On the other hand, in patients with premature onset of contractions, with intact membranes or pPROM, microbial invasion occurs prior to initiation of contractions, the process is burdened with higher quantum of microorganisms, and probably lasts longer providing conditions for more severe inflammatory response [6].

A certain number of evidence suggest that occult intrauterine infection plays a valid role in the genesis of premature onset of contractions and preterm delivery. Majority of authors agree that the difficulties to determine the type of microbial agents are associated with the subclinical character of the infection and with inability to cultivate strains of bacteria responsible for the intrauterine infection.

Pankuch et al., in 1984 reported a prevalence of microbial invasion of the placentas in women with preterm delivery as high as 72%, but the efforts to duplicated these results in the following years, in the same category of patients, with histopathologically confirmed choriamnionitis, were unsuccessful [15]. Using PCR or amniotic fluid culture on amniotic fluid provided by amniocentesis form 166 patients, Di Giulio and his associates, reached a rate of detection of 15%, with notation that the combination of both techniques is more sensitive than the use of amniotic fluid culture as a sole method of detection (56% vs. 9.6%).

From the samples that were positive, 17 bacterial strains were isolated or detected, 6 of which by combination of both techniques (*Mycoplasma hominis*, *Ureaplasma*, *Streptococcus agalactiae*, *Lactobacillus*, *Prevotella* and *Fusobacterium nucleatum*), 6 were detected by amniotic culture only (*Staphylococcus coagulase neg.*, *Bacillus sp.*, *Peptostreptococcus*, *Gardnerella vaginalis*), while 7 were isolated with PCR (*Streptococcus mitis*, *Delfia acidovorans*, *Neiseria cinerea*, *Sneathia sanguinegens*, *Leptotrichia amnii*, uncultivated *Bacroidetes* and one unidentified phylum). Further analysis of the data revealed positive predictive value (PPV) of 100% for the combined approach, for all categories (preterm delivery prior to 25<sup>th</sup> g.w, prior to 32<sup>nd</sup> g.w, prior to 37<sup>th</sup> g.w and delivery within one day of the amniocentesis) [16]. Some of the more recent studies, such as the one from Andrew C. et al. which used material provided from 305 patients with spontaneous premature contractions and intact membranes, report even lower prevalence for the microbial invasion of the amniotic space (10.1%) with slightly lower detection rate for used molecular techniques (rDNA PCR) in comparison to amniotic culture (65% vs.16%). On the other hand, analyzing the data for the values of IL-6 in the amniotic fluid they found that the level of this inflammatory marker is high enough to suggest sever to moderate form of infection in 36.1% of the patients (20.7% with severe inflammation, 15.4% with moderate inflammation). In majority of these patients, no microbial invasion of the amniotic space was detected [17].

The question arises what initiates the inflammatory response in cases in which intra amniotic infection has not been proven? Authors theoreticize that one of the possibilities is an infection of the extra amniotic compartment (decidua, placenta, fetal membranes). Other possibility could be the inflammatory response to non-infective stimulus such as: trauma, ischemia or abruption. Interaction between the intra amniotic bacterial colonization and the inflammatory response could be summarized in 4 stages: homeostasis, initiation, evolution and resolution. Some of the microorganisms, such as *Ureaplasma* and some of the genital mycoplasmas, can be detected in small amounts in utero, in large number of uncomplicated pregnancies. If their presence does not initiate abundant inflammatory response (homeostasis), such colonization will not influence neither the mother, nor the fetus.

On the other hand, if the homeostasis is disturbed through uncontrolled proliferation or invasion with aggressive strains, the result will be intense inflammatory response (initiation). The authors considered the possibility that the inflammatory response and not microbial invasion is responsible for the liberation of prostinoids that initiate contractions and lead to cervical ripening, changes that are clinical characteristics of preterm delivery (evolution). Once the severe intra amniotic inflammation develops, it will most certainly lead, very promptly, to preterm delivery (resolution). We still don't fully understand which factors are responsible for the transition from one to the next phase, therefore it is still unclear whether clinical interventions in the early stages of the process could result in different type of resolution i.e. continuation of the pregnancy instead of unstoppable progression towards preterm delivery.

## **DISCUSSION**

Preterm delivery as a global problem along with its association with long-term risks and consequences imposes a necessity for multidisciplinary approach in the attempt to deal with the causes behind its origin. Efforts made to date have not resulted in satisfactory reduction in the rate of preterm delivery. On contraire, the rate of preterm delivery is rising. Nevertheless, if we exclude iatrogenic reasons for prematurity, what remains is a large group of patients with intrauterine inflammation, with or without confirmed infection of the fetoplacental unit, as a main etiopathogenic factor.

In the last 20 years, large number of authors tried to explain the problem of inflammation/infection of the intrauterine compartment of the gravid uterus, and to establish consequential relationship between infection and histopathological changes that develop in different parts of the fetoplacental unit: decidua, placental disc, fetal membranes, umbilical cord and amniotic fluid. Results from these studies impose consistent conclusion that the inflammatory changes of the placenta and the umbilical cord, defined as acute chorioamnionitis and funisitis, appear more often in conditions of spontaneous onset of labor. It seems that spontaneous onset of uterine contractions strongly correlates with already present inflammatory changes, regardless of the integrity of the fetal membranes, intact or not. To complicate the present situation even more, there is a confirmed correlation between the inflammatory changes of the fetoplacental unit and the gestation age. The smaller the gestational age, the higher and more severe the inflammatory changes of the placenta and the umbilical cord. From this point of view, we should concentrate on those cases with extreme and early prematurity (< 28 g.w) in which the prevalence of histopathologically proven chorioamnionitis is high (> 90%), and the rate of mortality and prevalence of late complications related to prematurity is also high.

Studies that have been carried out to date failed to provide answer to the question of correlation between the inflammatory changes of the placenta and the umbilical cord, and microbial invasion of the intrauterine compartment. Although the role of microorganisms, especially bacteria, is undoubted, in only 10-15%, maximum 38% of cases (depending on the study) bacterial presence can be detected in the samples taken from the placenta, umbilical cord and amniotic fluid. When microbiological studies are performed, advantage is given to the combination of amniotic fluid culture and molecular techniques for isolation of bacterial rDNA (PCR).

## CONCLUSION

Histopathologically verified chorioamnionitis is a more frequent finding in patients with preterm delivery if compared to clinical manifestation of the clinical syndrome presented through its pathognomic signs (fever, painful tender uterus, leukocytosis, maternal and fetal tachycardia). The presence of chorioamnionitis is associated with increased risk for perinatal mortality and morbidity (neonatal sepsis, bronchopulmonary dysplasia and cerebral palsy). Even though the association between the histopathological changes of the fetoplacental unit and premature onset of contractions is well established and proven, there is still a mist around the question, whether the inflammatory process is a trigger or a consequence of the process of the preterm delivery that has already started.

Considering the historically accepted empiric standing on the role of genital infections in etiology of preterm delivery, further analysis are needed to determine the reasons behind the relatively small percentage of isolations of microorganisms in the analyzed samples.

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REVIEW ARTICLE

**ETHICAL CONSIDERATIONS TOWARDS INDIVIDUAL RESULTS  
DELIVERY AND THE CONSEQUENCES OF INCIDENTAL FINDINGS IN THE  
WHOLE GENOME AND EXOME SEQUENCING**

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**ABSTRACT**

There are more and more genome and exome sequencing studies every day in which subjects with different demographics are included, especially age differences, from infants to adults. Precisely today's advanced techniques represent serious tools that provide not only one simple answer, but more complex plural answers. A special issue within this dilemma remains the incidental findings. It is clear that the true challenge of an investigation is solving the particular problem, disease, mutation (mystery), but it is even more challenging when an unexpected finding is discovered. In this article we review the different attitudes towards manipulation with individual results and consequently with incidental molecular findings, through presentation of some ethical dilemmas that circulate the scientific community.

**INTRODUCTION**

Ever since 1977, with the breakthrough of the technique for fast DNA sequencing by Frederick Sanger [1], through the introduction of the polymerase chain reaction- PCR, 33 years ago [2], and the beginning of one of the most prominent human undertakings – The Human Genome Project, in 1990, a new era in the field of medicine has begun [3]. Sequencing i.e. the discovery of the human genome remains to be one of the few great endeavors of the human kind and serves as a historical hallmark of science, rivalled even to the moon landing. The Human Genome Project, which sequenced the whole genome of the human species, took 13 years to complete and it cost 3 billion dollars [3].

Today, we live in the era of Next Generation Sequencing (NGS), that, in comparison to the 13 years' time of the first generation sequencing methods [4], has the ability to sequence a lot more genomes for a vastly shorter period of time – 18,000 human genomes per year or 45 genomes per day, and only for a price of less than 2000 dollars [5]. The most important methods of sequencing nowadays are genome sequencing and transcriptomes, as well as the newly popular epigenomic studies [6]. The great spectrum of challenges in the personal genomics, have unlocked the possibility for research of the individual genome testing. Hence, great opportunities of this prospect have arisen and would lead to a bright scientific future [7], but at the same time, this deeper view into oneself have provoked a number of ethical dilemmas [8]. Namely, there are more and more genome and exome sequencing studies every day in which subjects with different demographics are included, especially age differences, from infants to adults.

Precisely today's advanced techniques represent serious tools that provide not only one simple answer, but more complex plural answers. The increased activity of people on the social platforms, the curiosity to learn more about one self's genetic code, as well as the boosted benefit for the clinicians with the personalized genome results, opens the door to the delivery and importance of individual results in the genomic studies [9].

In this context, a new ethical dilemma arises, of how to operate with the said results. Specifically, is it completely regulated which particular results should be presented to the patient? Who is the one who should inform the subjects (patients) about the results with a thorough explanation? Do the results match the high criteria of good clinical practice? How is the principle of "not to know" implemented without the loss of patient autonomy?

There are an increasing number of supporters of the claim that the return of the complete individual results to the patient would have vast moral implications and would be very important in the genome/exome studies [10,11]. A special issue within this dilemma remain the incidental findings. It is clear that the true challenge of an investigation is solving the particular problem, disease, mutation (mystery), but it is even more challenging when an unexpected finding is discovered. Henceforth, the ethical dilemma of the incidental findings- should they be searched for and are they relevant or even are we obliged to individually present them to the patients? Or, should we stick to the particular analyzed gene?

Incidental findings, are defined as findings that may or may not be detected adjacent to the primary aim of the enquiry and accordingly, have potential clinical or reproductive significance [12,13]. Thus, it is interesting to point out the predicament: what if incidental findings became part of the algorithms and forms of informed consent? Will they still be considered "incidental"? Consequently, a recent study encourages a new term for these findings, naming them secondary variants, in order to bypass the flaws of the former taxonomy [14]. It is to be noted that the number of identified incidental findings in subjects is not modest in the least, on the contrary, which implicates a need of their proper regulation [15]. Studies show that there is no legal regulative for delivery of relevant individual results from the increased number of genomic studies to the subjects yet, which only intensifies the significance of the ethical questions that arise from this predicament [16]. There are more arguments in favor of giving access to the incidentalomas to the patient, since they are of great significance for the individuals [17,18, 19, 20]. Accordingly, even the studies that do not support the exposition of incident findings and go by the saying "not to transform the research setting into an extension of the clinic", are not strictly categorical, in terms of accepting the need of these results with specific relevance in particular settings and individual approach [21]. Some instances have regulated this current ethical issue by commencing directive guidelines [22, 23].

Informed consent is a convenient tool that could be used to regulate the ethical dilemmas connected to individual results. That is to say, when one signs the consent, one could have the possibility to regulate the management of incidental findings in terms of his further knowledge about the outcomes that derive from the study [17, 23, 24, 25].

### **THE DIVISION IN CLINICAL AND RESEARCH DOMAIN**

The debate around returning secondary findings in research is rather complicated by the fact that the discrepancy between clinical and research domains is becoming blurred as sequencing studies are being translated into clinical practice. Although under a research protocol, subjects' initial contact was the clinician who recruited them in a healthcare-providing facility.

Clinical standards of ethics must be therefore ensured by the clinician researchers, which becomes a dispute in pure research context where the same standards of care do not normally exist. Berkman *et al.* (2014) argue that the lack of consensus for delivery of these results comes from the nature of the field, which is perceived as a transitional one, and the collapse of this differentiation is expected and sensible [26]. Researchers, however, should be aware of their ethical duties especially in the era of advanced genomic technology implementing in various clinical trials. Therefore, higher clinical and ethical aspects of responsibilities of researchers are to be achieved in the future.

On the other hand, Hallowell *et al.* (2014) noted, that discriminating between these two undertakings is essential as those involved have different prime commitments: Researchers tend to improve scientific aspects of their research, while when it comes to the clinicians, patient duty remains their imperative [27]. Translational research is more present nowadays, where new technology advances tend to be implemented in clinical context. As Wolf (2012) argue [28], bridging researchers and clinicians through intensified multidisciplinary approach together with the pivotal role of clinician- researchers in recent research, the dilemma around secondary variants (incidental findings) should be addressed through the context of the translational research process [29]. Interestingly, a study conducted by Kaphingst *et al.* concluded that the subjects themselves do not have a preference of who will deliver the results and put less emphasis on the title of the person who delivers them, but were rather more concerned about the manner of delivery [30]. Contradicting Wolf are more than few studies which surveyed the opinions of research participants on ‘who should return incidental findings’. It was clearly instructive that the most respondents wanted such findings returned by participant’s physician or primary health care giver. In support of this point is the fact that researchers may not be adequately trained to return such findings and burdening them with this duty may distract them from achieving their primary study goal [31]. Considering the abovementioned arguments for the research clinical divide, the authors of this article lean more towards the viewpoint that suggests blurring the boundary between clinical and scientific. Thus, we will continue in the similar narrative.

## **INDIVIDUAL FINDINGS**

Reviewed in this article, are the different attitudes towards manipulation with individual results and consequently with incidental findings, through presentation of some ethical dilemmas that circulate the scientific community. One of the main doctrines of ethics is “benefacere” – doing good. It is undoubtedly a question of why this principle could not be equally projected in the research studies as well. Therefore, all scientists are bound by this norm and should always disclose the individual results to the subjects included in the research. Nevertheless, there are arguments in the literature supporting a different approach of the “benefacere” principle in doctors versus scientists. It is believed that the “doing good” norm directly depends with the role that the scientist has in the doctor-patient relationship i.e. whether the scientist has contact with the patient (is the patient’s doctor), or not.

While doctors have a clear responsibility for doing good for their patients, this obligation is still a grey area in terms of scientific work, especially in research projects where scientists have no personal contact with the subjects. Hence, this could become a burden to the researcher and an issue when it comes to future accountability [16, 32].

Furthermore, another interesting ethical question is fabricated- what is the responsibility of the researchers who are doing science on data collected in the biological banks, that is to say, scientists who are not prime collectors of material and had never had any previous contact with the subjects (referred to as secondary researchers)? In general, their duties depend on the course of the main study and how will the delivery of individual results affect its realization. From this point of view, the principle of “*benefacere*” could be satisfied by publication of the collective results and conclusions of the study only, without the responsibility of delivering individual results. On the other hand, it is reported that where individual results could have a great impact on the individual receiving them, and do not compromise the main study, their individual delivery can be a norm for the secondary researcher [33]. Therefore, new guidelines for the biological banks are constituted. Namely, it is emphasized that the clinically relevant results could be a product of the prime investigators, biobanks as well as the secondary investigators, and consequently, they should be offered to the subjects by all of the abovementioned instances [34]. Nonetheless, the views of the right of the subjects to have the availability to end results of the study is unanimous, in terms of obligating the scientists to point out the results that may have direct harmful effect of the individual’s health or may help prevent potential conditions [4, 34, 35]. Owing to all the previously mentioned ethical dilemmas, the National Heart, Lung and Blood Institute (NHLBI) has established ethical guidelines, which are regularly reviewed in accordance with the progress and growth of the genomic studies [23,24]. An interesting study from Beskow et al. (2010) shows the need of research contextualization for these guidelines. In other words, they propose that the option for delivery of individual results should be predicted and planned as early as possible in the study, or it should even be incorporated and precisely formed in the informed consent form for the selected clinical or scientific study [36]. Likewise, no less of a factor for this issue should be considered the great availability of genomic sequencing today. People are more and more interested in their codes, which is an additional reason why these ethical dilemmas should be defined most precisely.

But then what is the right way to deliver these results? Who is the one who should announce them? The rapid progress of genetic research has driven the scientists to a blind end when it comes to deciding what is the right direction in which they should advance in order to preserve the ethics in the delivery of individual results [23]. The most controversial and important dilemma is the one, whether the obtained results from a single study are as equally relevant as the results achieved in clinical trials. From the aspect of the primary goal of a study, that is generating overall results, the investigators are not compelled to deliver the results with prior verification with the standards of clinical diagnostics. Therefore, it is suggested that the results be referred to as investigatory (scientific) only, with an addendum that they be additionally evaluated, confirmed and monitored [32]. Albeit the vast number of studies, there is still no ample agreement on how these results should be delivered to the subjects. Regulations also flail in the part of who should be responsible for the delivery- does the clinician have the necessary competencies from genetics to be able to interpret and act upon the said results?

Or is the researcher trained in delivering the information appropriately to a person he has never met before? Opinions regarding this dilemma vary between clinical geneticists, genetic counselors, but also even family doctors.

What is considered, is the need for enriched education of the family doctors about genetics and a greater involvement of geneticists and clinical genetics specialists, in order to provide clinical care for patients with established relevant findings [37].



One study points out that doctors who do not have training in clinical genetics, have in general lesser knowledge about the discipline, which is seen as an additional problem for the miscommunication between doctors and patients [38]. On the other hand scientists do not have experience to communicate “bad news” of additional incidental findings. Moreover, the excitement about the unexpected findings and eagerness to publish might be a barrier to the use of these incidental findings in an ethical way. In order for the genomic research to really make a difference in the clinical practice, it is necessary to foremost educate a competent personnel between the medical workers, for proper interpretation and analysis of the results [39]. Context wise, another study concludes that the scientists working genomics do not have major experience in announcing results, but nevertheless are increasingly motivated to deliver [18]. A different study shows that the patients emphasize the importance of the nature of the results- their accuracy and possibility for prevention/treatment directly correlates to patients’ willingness for receiving them (40).

Interestingly, the offer for individual results to be delivered at the end of the study, increases the eagerness of the subjects to participate in the studies [41]. Hence, a preparation of a study with a formal study protocol, explaining the subjects the scope of the project and management with individual results, is advised [37]. Thereafter, the notion of creating a relevant and precise informed consent form presents a vital concept in regulation of this important question connected to delivery of individual results [42,43]. In this form, the subjects could individually select which, and if at all, results they would like to obtain. This approach to the matter is said to be very important for preserving the principle of autonomy [44]. In contrast, for the incidental findings, the patients do not yet have the possibility to choose whether or not they want to be presented with the data. In this context, this issue should be further discussed and regulated [45]. One study concluded that 70% of the genomic studies either did not have an individual results segment in the informed consent or even explicitly stated that there is no room for delivery of said results [46]. Given the traditional postulates of research, personal interest for benefit of the genomic studies should not be a motivation for the subjects to advance in the study [47]. Furthermore, in terms of relevance of the results with momentarily knowledge about a certain condition i.e. if the result has clinical significance, it is advised only the clinically relevant results to be included in the final medical report. But, as clinically nonsignificant results are equally relevant, it is considered to design guidelines for management and create a system of individual records with a possibility to upgrade the data for the individual clinical significance of any future investigations [37].

Although, in general, there are uniformed guidelines for managing the individual results covering their delivery in accordance to study protocols, still the scientific community is not in unison about the ethical aspects of this problem [46, 32]. It is almost unbelievable that a consensus about this question will be reached, but nonetheless future discussions about deciphering this dilemma should be strongly encouraged [48].

## **INCIDENTAL FINDINGS**

Incidental findings have lots of common traits with the individual primary results, but in the core they differ fundamentally. Namely, they demand additional investigation outside the premises of the study’s aims and inquire supplementary expert interpretation that the prime investigators involved might lack [13].

Still, it is implicated that with the advancement of the technology and implementation of such research results in clinical practice, it will be prudent to study and assess these findings.

Incidental findings should be potentially analyzed when a benefit of the patient and burden to the study balance is established, all the while bearing in mind the exclusivity of the results to the researchers (genomic researches are mostly done for scientific reasons instead of clinical practice, thus giving the investigator the sole exclusivity of the findings for certain conditions which he might choose not to publicize) [49]. Yet, with the progress of technology the access to genome sequencing is immensely increasing.

The American College of Medical Genetics and Genomics (ACMG) has established a list of minimum requirements as part of the algorithm for clinical conceptualization of genomic studies. It consists of all the clinically relevant genes (defined incidentalomes of clinical significance) which need to be routinely investigated in every genomic research [50]. Analogously, Townesend et al report similar recommendations for ethical delivery of incident findings [51]. But, it should be pondered that the patients often have unrealistic expectations of the genomic research, anticipating to receive the entire array of deviations, making the incidental findings altogether confusing [52]. Naturally, this represents a hurdle because it gets in question the autonomy of the patients and their right “not to know” a certain information. Actually, the subject who gets involved in the study for a primary indication, ends up with a whole set of results that could potentially have a negative effect [51]. Nonetheless, personal preferences of the patients are not included in the ACMG algorithm, with the justification that at this moment the principles of “doing good” and “not doing harm” outweigh the one of autonomy and individual predilections of patients in clinical trials [50]. Still, even in investigative (scientific) context, the subjects do not have the opportunity to sound their opinions whether or not they want to receive the incidental findings. One study presents scarce recommendations about upgrading the informed consent form [53], but after all, lots of questions around the obligation of the researchers to pursue incidental/secondary variants remain unanswered. Albeit all, at the present moment, there is no regulative that obligates the researchers to track and analyze incidental findings [49]. From the researcher’s standpoint, detailed and precise delivery policy of the study, balance between the scope of the study and the patients’ rights and a strong ethical principle of “primum non nocere”, is advised to be always established beforehand [54]. A study on the subjects preferences about the delivery of individual and incidental results about familial risk of cancer, concludes that the major percent of patients preferred to receive both kinds of results [55]. Likewise, another research in the field of exome sequencing, once again concludes that the patients agreed on delivery of both variants of incidental/secondary findings [56]. Further study, analyzed the attitude of parents towards the secondary variants of the clinical genome sequencing in their children [57]. Namely, with the help of these incidental findings, prevention against many conditions would be accessible and the branch of medicine that would benefit the most from these genomic researches would be, unsurprisingly, oncology [15].

It should be taken in consideration that throughout these studies a number of potentially significant results could be attained, so the scientific community should aim to overcome these dilemmas in order to provide access to the patients for the clinically significant results [58].

Furthermore, because the genome sequencing is not part of the routine practice, some results from these studies could be unique, and therefore researches should undoubtedly be burdened with the responsibility to deliver the incidental findings [57]. A recent study encloses the principles and guidelines that should be followed when delivering individual and incidental findings [59].

## **CONCLUSION**

The predicament about incidental findings represents a real ethical challenge, for which this essay is insufficient in terms of scope to process and emphasize all the aspects of ethical regulation and principles which burden the dilemma of incidental findings. New technologies have introduced new ethical dilemmas about the communication of the molecular results if incidental findings occur. Close collaboration between clinical geneticists and molecular researchers in the development of new tools such as appropriate informed consents and guidelines for information is warranted.

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REVIEW ARTICLE

**UTILITY OF EXHALED NITRIC OXIDE IN PEDIATRIC PRACTICE  
REVIEW OF LITERATURE**

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**ABSTRACT**

The field of interest for measurement of exhaled nitric oxide (NO) and nasal NO is significantly evolving over the last 25 years, with over 1000 publications published in that area. Inflammation of the airways is a central process in asthma and other lung disorders, but the monitoring of the inflammation has not been included in the current recommendations. The exhaled air contains volatile media such as nitric monoxide, carbon monoxide, ethane, pentane and non-volatile substances in the liquid phase in the exhalation, as a condensate (hydrogen peroxide). It is increasingly confirmed that the measurement of exhaled mediators in general, and especially NO, is a new way to monitor certain aspects of asthma, COPD and interstitial lung disease, which cannot be estimated with other methods, like lung function. In asthma, exhaled NO is recommended to be used as a marker for diagnosis, for monitoring the response of anti-inflammatory drugs, confirming the safety of therapy and predicting asthma exacerbation. Measurements of FeNO are easily performed, they are reproducible and technically less expensive than the analysis of induced sputum. In symptomatic patients, high FeNO levels (> 50 ppb), refer to significant eosinophilia in the airways, which will most likely respond to treatment with ICS. The current data provides support for the diagnostic use of FeNO in children with symptoms of asthma. For patients with chronic and/or severe asthma, FeNO levels are useful for determining whether eosinophilic inflammation of the airways is active or not. Both high (> 50ppb) and low (<25 ppb) levels of FeNO can be used to for predicting the outcome in patients with a definitive history of asthma who are currently in remission and who have stopped treatment with ICS.

**Key words:** asthma, children, exhaled nitric oxide, recommendations.

The field of interest for measurement of exhaled nitric oxide (NO) and nasal NO is significantly evolving over the last 25 years, with over 1000 publications published in that area. Interest is growing because the measurement of airways inflammation can contribute in the management of lung diseases and the same measurement may be included in the clinical practice [1].

**Evaluation of the airway inflammation**

Inflammation of the airways is a central process in asthma and other lung disorders, but the monitoring of the inflammation has not been included in the current recommendations [2]. Taking a direct sample of the airway cells and the mediators is invasive technique, as well as analysis of induced sputum or bronchoscopy with lavage and biopsy. The exhaled air contains volatile media such as nitric monoxide, carbon monoxide, ethane, pentane and non-volatile substances in the liquid phase in the exhalation, as a condensate (hydrogen peroxide). Non-invasive measurements of these exhaled mediators are appropriate for patient monitoring.

## **Exhaled NO**

The presence of NO in the exhaled air in both animals and humans for the first time was described in 1991. It has been shown that correlates with other indicators of moderate asthma (eosinophils in induced sputum) and bronchial reactivity in non-steroid-treated patients. Generally, the exhaled NO does not correlate with the lung function parameters in asthma. It is increasingly confirmed that the measurement of exhaled mediators in general, and especially NO, is a new way to monitor certain aspects of asthma, COPD and interstitial lung disease, which cannot be estimated with other methods, like lung function. In asthma, exhaled NO is recommended to be used as a marker for diagnosis [3], monitoring the response of anti-inflammatory drugs, to confirm the safety of therapy [4] and to predict asthma exacerbation [5-7]. It is believed that the adaptation of anti-inflammatory drugs led by the monitoring of non-invasive markers as eosinophils in the sputum and the exhaled NO can provide good control of the asthma.

## **Nasal NO**

Concentrations of nasal NO are relatively higher according to the lower respiratory tract in humans, with the highest level in the paranasal sinuses. Patients with ciliary dyskinesia and with cystic fibrosis have extremely low NO values and accordingly, the nasal NO may be a useful clinical test for early diagnosis.

## **Recommendations for a standardized procedure for the measurement of exhaled NO in adults**

### **Basic principles for clinical use of NO measurement**

In Europe, clinical testing began in the late 1990s, while American food and drug administration recommended the first NO analyzer for clinical monitoring of anti-inflammatory therapy in asthma in 2003, which was produced by Aerokrin AB from Stockholm, Sweden. Online measurements include measurement of the fraction of exhaled nitric oxide (FeNO) in real time, whereas offline measurements refer the collection of the exhale air in an appropriate time for a delayed analysis [8]. Measurement of FeNO, as a clinical agent, requires standardized measurement techniques that are obtained from previously processed data from different adult groups.

### **Standardization of terminology and measuring units for exhaled NO**

Online measurement. FeNO is expressed in parts per billion (ppb), which is equivalent to nanoliters per liter. The output of NO is the level of the exhaled NO, which is designated as volume of NO. It is obtained when the concentration of NO in nanoliters per liter is multiplied with the level of expiratory flow per minute corrected by BTPS (body temperature, pressure, saturated).

$$V_{\text{NO}} (\text{nl}/\text{min}) = \text{NO} (\text{nl}/\text{l}) \times \text{expiratory flow} (\text{l}/\text{min})$$

Offline measurements. FeNO refers to NO concentration in the exhaled air from the vital capacity. If the exhalation is carried out under a constant flow (in liters per second) and it should be written as a subscript.

### **Basic principles for the measurement of exhaled NO**

Source of exhaled NO. It is currently thought that NO is produced in the upper and lower respiratory tract and diffuses into the lumen according to the concentration gradient. Alveolar NO is probably very low because of the large hemoglobin uptake in the pulmonary capillary blood.



Although the level of gastric NO is high, it is considered that there is no effect on the level of exhaled NO due to the closure of the upper and lower esophageal sphincter.

Contamination with nasal NO. The nasal NO can be accumulated in relatively high concentrations in the low respiratory tract. But techniques that provide a lower respiratory tract NO should prevent the contamination of the sample with nasal NO.

Ambient NO Because environmental NO can reach high levels relative to those in exhaled breath, standardized techniques must prevent the contamination of biological samples with ambient NO.

Dependence on the level of expiratory flow. This flow dependence is a feature of the diffusion-based processes and relates to the transfer of NO from the wall to the lumen of the airways and can be easily explained. The fast flow minimizes the time of transit of the alveolar gas in the airways and consequently reduces the amount of transferred NO. The output level of NO as well is higher in the flow rate, analogous to the loss of respiratory heat.

Air retention. The air retention results in the accumulation of NO in the nose, the lower airways and probably in the oropharyngs, which leads to a NO peak in the NO exhalation profiles relative to the time. Accordingly, air retention is not used in standard techniques [1].

### **Other patient-related factors that affect the values of the exhaled NO**

Age/Sex: Exhaled NO levels increase with the age. As there are contradictory data for the effects of the sex, menstrual cycle and pregnancy, these characteristics should be taken in account at the time of the measurement [9-12].

Respiratory maneuvers: Spirometry reduces the level of exhaled NO and accordingly, NO analysis should be performed prior to spirometry [13, 14].

Airway caliber: It turned out that the level of FeNO may vary in relation to the degree of the airway obstruction or after bronchodilator, probably due to the mechanical effect of the NO output. Because of this, it is recommended to note the time of administration of the bronchodilator and some measurements of the caliber of the airways, like FEV1 [13].

Food and liquids: Patients should be restrained from food and drink before analyzing NO. There is an increase in FeNO after ingestion of nitrates or food containing nitrates. Drinking water and caffeine intake may lead to transient change of the levels of FeNO. It is likely that the mouthwash can also reduce the effect of foods containing nitrates. Patient should avoid to eat or drink one hour before the measurement and should be asked about the food recently consumed. The alcohol intake reduces FeNO [15].

Daily rhythm: It is not yet certain how FeNO values vary during the day and therefore it is recommended that serial measurements of NO should be done in the same time of day and the time should be recorded [16].

Smoking reduces FeNO levels. However, smokers with asthma have elevated levels of FeNO. Patients should not smoke one hour before the measurement and the history of short or long active or passive smoking should be recorded [17].

Viral infections of the upper and lower respiratory tract lead to elevated levels of FeNO in asthma. HIV infection is associated with reduction of the levels.

Other factors: The change in the blood flow in the lungs has no effect in humans, but hypoxia reduces the exhaled NO. Positive end-expiratory pressure application increases the level of FeNO in animals, while the pressure of the airways in humans does not influence the level of the exhaled NO. There are studies about the impact of exercise, with different conclusions and it is therefore considered to avoid physical effort one hour before the measurement.

**Drugs:** FeNO levels decrease after treatment with topical or oral corticosteroids in patients with asthma or after inhalation of NO synthetase inhibitors. Leukotriene antagonists also reduce FeNO. The drugs containing NO as well as oral, inhallatory and intravenous L-arginine increase the level of FeNO. Even if a medicine does not influence the production of NO can change the levels through other mechanisms, like changing the airways caliber.

### **Recommendations for online and offline measurement of exhaled NO in children**

#### **Measurements of exhaled NO in children aged 4-5 years**

**Single breath online measurement:** This method is preferred in all children who can cooperate. The child should comfortably sit down and be breath normally for about 5 minutes to acclimatize. The child inhales close to vital capacity and immediately expires under a constant flow of 50 ml/s while plateau of NO is obtained for at least 2 sec. which may be registered in an outbreak of at least 4 sec. The vaporized gas should contain low NO concentrations (<5 ppb). The expiratory pressure should be from 5 to 20 cm water column to close the velum. Repeated exhalations (2-3 which correspond to 10% or 2 to 5%) are performed at intervals of at least 30 seconds and the mean value is recorded. Audiovisual devices can ease the inhaling to the vital capacity and control the expiratory flow of 50 ml/s. The use of dynamic flow restrictors that allow the child to exhale with various pressure in the mouth, allows a constant level of expiratory flow. Dynamic flow restrictors are simple manual or mechanical devices that change their resistance depending on the blow pressure and their use is recommended in children [18, 19].

**Offline method with constant flow level:** The child blows air through a tube in the mouth that is made of a material that does not interfere with NO, whereas nasal contamination is protected by the closing of the velum in the air extraction under oral pressure of at least 5 cm water column. The gas collection can be in the Milar or Tedlar balloons. The size of these balloons is recommended to be similar or slightly higher than the vital capacity of the patient. No nasal clips or retention of air is recommended, since they may potentially lead to nasal contamination. Concentrations of NO in balloons may be stable for a few hours and the measurements can be carried out elsewhere (school or home) [20]. Great progress in offline measurements can be expected by incorporating dynamic flow restrictors into a collection system that will provide easy feasibility for children under 4 years of age. This led to a consensus and recommendations that the flow rate should be 50 ml/s for both online and offline [21].

#### **Alternative methods for pre-school children and infants**

**Online measurements of FeNO during spontaneous breathing.** This method can be applied to children from 2 to 5 years of age. FeNO can be measured in spontaneous breathing while exhaled flow can be adjusted by changing the exhaled resistance [22]. This ensures a stable and reproducible model of breath to breath. The child breathes slowly and regularly through a tube connected to a two-way valve. It is recommended to calmly breathe with normal frequency.

The level of exhaled flow is adjusted to 50 ml/s (from 40-60) with continuous adaptation of exhaled resistance manually or with automatic flow controllers.

The method still requires passive co-operation in order that the child breathes calmly and regularly through a connector, which is a limiting factor. The use of a biofeedback allows the child to visualize a model of normal respiration. Measurements in spontaneous breathing lead to variability, as there is no control over the lung volume, but the flow is measured. According to this, the measurements of NO levels during spontaneous breathing do not equate with single

breathe online measurements and it is necessary to specify this method by including a description of the normal values in healthy children [22].

Techniques of normal breathing with uncontrolled flow rates: There is no standardized method to be recommend for clinical use in infants and young children, and further investigations should resolve some methodological problems [23].

Offline measurement: Exhaled air should be collected through a tube connector or face mask connected to a valve that allows inhalation of air without NO, from a NO inert tank to avoid contamination from the ambient NO. Examples of NO are collected in NO inertial bags that are attached on the expiration side. The expiratory side provides expiratory resistance [24]. This resistance allows avoiding nasal contamination if the face mask does not cover the nose. With NO analyzers with quick response, small samples of exhaled air may be sufficient for analysis.

Online normal breathing techniques for measuring NO and the model of normal breathing in neonates and infants are described with good reproducibility [25, 26]. Infants breathe through a mask covering both the nose and mouth and the NO concentrations should be recorded during the normal-breathing stages. Reproducibility is a significant problem of the normal breathing methods and is described by many researchers [23, 27]. Since it is resistance dependent, the data for the flow rate will vary. The inconvenient side of the mixed exhaled air is that it can be contaminated with ambient NO and NO from the upper airways. As long as the contribution of NO from the upper airways is not known, it's better to collect a sample of orally exhaled air. This is facilitated by the use of a mask covering only the mouth, while the nostrils are occluded or by using a double-chamber mask.

Single breath technique during forced exhalation. Modification with rapid increase in volume, with the technique of thoraco-abdominal compression. FeNO is measured online and the NO plateau is obtained at a steady rate of expiratory flow that can vary from 10 to 50 ml/s and uses a two-chamber mask [28]. The negative side of this technique is that sedation is necessary and nowadays still it is not clear how this technique can be compared with other techniques such as single breath online or the technique of normal respiration.

### **Rational use and interpretation of NO**

There are two key issues: 1) high significant interaction of NO and eosinophilic inflammation of the airways; 2) important relationship between eosinophilic inflammation of the airways and steroid response [29]. This can be summarized as:

a) FeNO measurements are highly correlated with eosinophilic inflammation on the airways.

Many studies confirm that FeNO measurements correlate with eosinophilia in induced sputum, (30) bioptic material [31-33] and bronchoalveolar lavage as well [34].

In one study, a significant relationship of FeNO and blood eosinophilia is explained [35]. In addition, there is also a correlation between FeNO and the eosinophilic cation protein.

b) Eosinophilic inflammation of the airways is associated with a positive response to steroid treatment.

Treatment with inhaled corticosteroids (ICS) results in reduction of eosinophilia in asthmatic airways and improvement in clinical parameters. In contrast, in asthma that is not characterized by eosinophilia, the response to steroids is scarce [36]. These findings are common in patients with constant obstruction of the airways and enable distinction between asthma and COPD [37].

According to this, determination of the character of inflammation of the airways (eosinophilia) is important in the initial management of patients with chronic respiratory symptoms in order to identify those who would benefit from the steroid treatment.

c) Increased levels of FeNO predict positive steroid response in patients with non-specific respiratory symptoms. The clinical benefit of increasing steroid treatment in patients with asthma is higher in patients with increased levels of NO [38]. Some studies have shown that in determining the outcome of treatment with inhaled Fluticasone, the level of FeNO as predictor is superior than spirometry, bronchodilator test and bronchial hyperactivity. It is important that this study identifies the optimal value for the steroid response to 47 ppb. This has been proved in studies in children and adults [39, 40].

d) The use of ICS in asthma results in decreased levels of NO and according to that, the link between ICS and FeNO is dose-dependent and has a significant correlation between changes in FeNO and changes in eosinophilia in induced sputum and ICS therapy [41]. These data provide a fundamental confirmation that FeNO measurements play an important role in the evaluation and treatment of patients with airway diseases. FeNO can be used as a surrogate marker for diseases of the airways that are characterized by eosinophilia, such as atopic asthma, asthma like coughing and eosinophilic bronchitis. Because of the close relationship between the steroid response and eosinophilia in the airways, FeNO measurements play important role in predicting and monitoring the response of the treatment with ICS [29].

### **Diagnosing airway diseases**

*Asthma:* FeNO measurements are useful in distinguishing asthma from non-asthma [42]. The test should be used as diagnostic tool when chronic symptoms with a duration of 6 weeks and more are present, as the virus infection can lead to a false positive result. The predictive value is almost identical with the cell count in induced sputum. The combination of the FeNO > 33 ppb and abnormal spirometry (FEV1 <80% PV) provides great sensitivity (94%) and a specificity of 93% for diagnosing asthma. Normal values do not exclude diagnosis of asthma. This confirms the heterogeneity of asthma phenotype and FeNO measurements provide only one aspect of asthma syndrome [43, 44].

*Non-specific respiratory symptoms:* FeNO measurements have a widespread role in the examination of patients with undiagnosed chronic respiratory symptoms. Children with recurrent wheezing bronchitis, cystic fibrosis, congenital abnormalities of the airways and primary ciliary dyskinesia should also be considered. For the eosinophilic bronchitis and Cough variant asthma, which are characterized by eosinophilic inflammation of the airways and elevated FeNO levels, the positive effect of corticosteroid treatment is more likely. For other diagnoses, like vocal cord dysfunction which is presented as asthma, many clinicians often give empirical steroid treatment with very small benefit.

*Pre-school children:* Taking into account that spirometry and sputum induction cannot be easily performed in pre-school children, non-invasive measurements of the airway inflammation are potentially very useful.

The single breathing technique for measuring FeNO at this age is not suitable, so other alternatives that vary from modification of standard online techniques to offline spontaneous breathing method with flow control are developed [45-51]. Generally, these techniques are less sensitive to discriminating between asthmatic and non-asthma patients [53, 53].

In acute wheezing episode FeNO level is significantly higher in those with recurrent wheezing versus healthy controls, whereas in children with first wheezing episode FeNO levels do not differ from normal children. Also, montelukast reduces the FeNO values of small children with early onset of asthma [54, 55].

*Atopy:* There is a strong correlation between FeNO levels with total and specific IgE [56, 57]. This suggests that asymptomatic atopic children may have moderate inflammation of the airways leading to a raised FeNO level [58].

*Chronic obstructive pulmonary disease (COPD):* FeNO levels are non-consistent in patients with COPD. This may be the result of smoking or the heterogeneity of the airways inflammation [59, 60].

*Cystic fibrosis (CF):* In patients with CF, it has not been found that FeNO measurements can be clinically useful. First, there is a reduced expression of nitric oxide synthetase in patients with CF and second, elevated levels of nitrites are found in the breathing condensate of patients with cystic fibrosis [61].

*Primary Ciliary Dyskinesia (PCD):* FeNO levels are significantly lower in these patients than in healthy individuals. The nasal NO is extremely low in patients with PCD of all ages and completely separates affected from unaffected individuals. The measurement of nasal NO can probably become a screening test. The diagnostic sensitivity and specificity of nasal NO for PCD ranges from 89 to 100%, and the specificity is from 97 to 100% [62, 63]. This is due to the reduced activity of NO synthetase and the mucus that affects the diffusion of NO from sinuses in the nasal cavity or from epithelial cells to the lumen of the airways and makes the NO elimination more difficult. Even young infants with PCD, have low nasal NO [64, 65]. Probably NO is also involved in the stimulation of the ciliary motility. The nasal NO may play a role in non-specific immunity, which includes a direct toxic effect on the microorganisms [66].

*Lung transplantation:* FeNO levels are increased in post-transplant patients with an unstable lung function [67].

### **FeNO measurement for chronic asthma management**

*Prediction of exacerbations:* The prognostic value of FeNO measurements for asthma exacerbation seems to be limited. In a small study with steroidal reduction protocol, changes in sputum eosinophils were superior comparing with FeNO for prediction of exacerbation. In the second study, measurements of bronchial hyperactivity, sputum eosinophilia and FeNO measurements ranged from a sensitivity of 21% (sputum eosinophils > 4%) to 65% in FeNO greater than 10 ppb at a flow rate of 250 ml/s. Although positive predictive values ranged from 80 to 88%. Therefore, it is preferable to examine more parameters than just one. The increase in FeNO of 60% is considered to be optimal, but this has a 50% sensitivity with a positive predictive value of 83%. These studies used a protocol to exclude steroids in order to imitate clinical exacerbations and so they are not suitable [68, 69].

*Predicting the outcome of ICS reduction in stable asthma:* The main issue is when airway inflammatory markers can be used to predict a successful reduction or exclusion of ICS treatment. Studies showed that number of eosinophils in sputum was significantly more predictive than FeNO measurements in asthma control over a period of 6 months and 16 weeks.

From the results of the many studies it can be concluded that the number of eosinophils in the sputum (more than 1%) probably provides superior prognostic safety when it is necessary to determine whether a patient needs to continue treatment with ICS.

In situations where induced sputum can not be obtained (some centers and small children), the high FeNO level (> 50 ppb) probably predicts asthma relapse and a low FeNO level <20 ppb in children (<25 in adults) predicts asthma stability if the measurement is obtained at least 4 weeks after interruption or reduction in ICS in asymptomatic patient [70-73].

*Increasing ICS doses:* Correlation between airway inflammation and/or symptoms and/or pulmonary function is very poor. So, the use of these treatment should be considered as the second best [29].

### Measurement and interpretation

#### *Normal Values and Clinically Important Changes*

In the past, studies showed that the upper limit is age dependent and ranges from 15,7 ppb for the age of 4 years to 22,5 ppb for adolescents. The reason for this age-dependence is still unknown, but it can be result of an increase of the surface area in the airways which is age-related, age-dependent induction of NO synthetase as a result of recurrent immunological stimulation or progressive reduction of the constant exhalation level that is relatively high in children. So, that's why the upper limit for healthy adults is 33 ppb, while for school children is 25 ppb [74]. Measurement of FeNO level when asthma is stable may be the basic reference point for a single patient. It is considered that the mean value of FeNO change that occurs between stability and "loss of control" after stopping ICS treatment is 16,9 ppb (or mean 24,9) [41].

#### *Interpretation*

Based on the currently available data, two algorithms for interpreting the FeNO results in everyday practice had been developed. First, as a diagnostic tool and second for further management of asthma (Shown on the tables):

1) Diagnostic use is pretty clear (Table 1).

**Table 1. FeNO as a diagnostic tool**

FeNO (ppb)	Level	Eosinophil inflammation	Children
5-25	Low (< 20 in less than 12 years, <25 in more than 12 years)	Probably not	Obstructive bronchitis, GER, ENT disturbances, CF, PCD (FeNO<5 ppb check nasal NO), congenital airway anomalies, other immune deficiencies
30-45	Intermediate	Present but moderate	Interpretation based on the clinic presentation
50-65	High	Significant	Atopic asthma, if FEV1 <80% PV, diagnosis for asthma is highly probable. Eosinophilic bronchitis, a positive response to steroid treatment.

2) In order to monitor patients correctly, increased levels of FeNO in symptomatic patients refer to uncontrolled eosinophilic inflammation. This is very often result of a weak compliance with anti-inflammatory therapy or a poor inhalation technique than inadequate dosing of ICS. Low levels of FeNO implicate the absence of eosinophilic inflammation of the airways. When we assume that the result is not credible, as long as the patient has symptoms or has atopic asthma, this may be due to the use of cigarets which reduces the level of FeNO up to 60%.

It is commonly referred to nonatopic (probably neutrophilic) asthma, gastroesophageal reflux (GER), rhinosinusitis with postnasal drip or left heart failure (Table 2).

**Table 2. FeNO results for management of asthma**

FeNO (ppb)	Level	Eosinophil inflammation	Children
5-25	Low	Probably not	If there are symptoms revision of the diagnosis. Considering Obstructive bronchitis, congenital airway anomalies, CF, PCD.  If there are no symptoms and uses ICS: good compliance with treatment, reduction of the dose of ICS or, in case of a low dose, exclusion of ICS
30-45	Intermediate	Present but moderate	If there are symptoms: infection as a cause of worsening, high exposure to allergens, adding therapy other than ICS (for example long-acting $\beta$ agonist). Consider increasing ICS doses, inadequate treatment with ICS, check compliance, check the inhalation technique and consider a metered dose inhaler or spacer if the patient uses a dry powder inhaler  If there are no symptoms: no changes in the ICS dose
50-65	High	Significant	If there are symptoms of inadequate treatment with ICS: check the compliance, check the inhaler technique, inadequate ICS dose, high exposure to allergens, exacerbation or relapse, consider the metered dose inhaler or spacer if the patient uses an inhaler with a dry powder  If there are no symptoms: no changes in the ICS dose.

The data shown in the tables is for guidance only. Further studies may lead to certain changes. Patients with various clinical phenotypes may have different referent values of FeNO. Since asthma is stable, FeNO levels may remain high. Prove that "normalization" of FeNO leads to a clinical benefit is not confirmed. It may be preferable to have individual "FeNO typing" on the referent values [29].

## CONCLUSION

FeNO measurement offers a step ahead in the assessment of the airway diseases. As the "inflamometer," FeNO provides information about the nature of airway inflammation. Measurements of FeNO are easily performed, they are reproducible and technically less expensive than the analysis of induced sputum. FeNO results require careful consideration with the clinical aspect. In symptomatic patients, high FeNO levels (> 50 ppb), refer to significant eosinophilia in the airways, which will most likely respond to treatment with ICS.

The current data provides support for the diagnostic use of FeNO in children with symptoms of asthma. Whether or not it needs to be used to predict the response of steroids or to guide the need for ICS in small children with recurrent wheezing, is still not clear. For patients with chronic and/or severe asthma, FeNO levels are useful for determining whether eosinophilic inflammation of the airways is active or not. Both high (> 50ppb) and low (<25 ppb) levels of FeNO can be used to for predicting the outcome in patients with a definitive history of asthma who are currently in remission and who have stopped treatment with ICS. Depending of symptoms, at the same time, high and low levels of FeNO offer assistance to clinicians in adjustment of the dose of ICS.

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2. Mitchell JR, Horning MG, (editors). *Drug metabolism and drug toxicity*. 2nd ed. New York: Raven Press; 1984.

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3. Kutt H, Pippenberg CE et al. Plasma clearance of nor-methsuximide in a uremic patient. 223-226. In: Levy RH, Pitlick WH, Meijer J, (editors). *Metabolism of antiepileptic drugs*. New York: Raven Press; 1984. pp-1-25.

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A a	A a	N n	N n
B b	B b	W w	Nj nj
V v	V v	O o	O o
G g	G g	P p	P p
D d	D d	R r	R r
\	G g	S s	S s
E e	E e	T t	T t
@ ' .	Zh zh	] }	K k
Z z	Z z	U u	U u
Y y	Dz dz	F f	F f
I i	I I	H h	Kh kh
J j	J j	C c	Ts ts
K k	K k	^ _	Ch ch
L l	L l	X x	Dzh dzh
Q q	Lj Lj	[ {	Sh sh
M m	M m		

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