

# Urinary protein fractions separation by geometrical electrofocusing after spinal, Sevofluran and Desfluran anesthesia

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**Abstract.** Objective: The A compound, which results after Sevofluran degradation by CO<sub>2</sub> absorbents, induce renal toxicity in laboratory animals producing tubules necrosis which are clinically evidenced by proteinuria and glycosuria. In this study we aim to determine protein types which pass the glomerular barrier by urinary electrophoresis, after general anesthesia with Sevofluran and Desfluran and after spinal anesthesia, aiming to observe if glomeruli permeability is affected by other anesthetics then Sevofluran.

Material and method: We studied 110 patients who required anesthesia, for medium and long term surgical procedures, grouped in three batches: First batch I, comprises 61 patients anesthetized with Sevofluran; Second batch II, comprises 33 patients anesthetized with Desfluran and 3rd batch comprises 16 patients with spinal anesthesia, performed in Anesthesiology and Intensive Care Unit. The study is prospective, observational and the patients signed an informed consent for the collection of biological samples and their analysis, and consent was obtained also from the ethics committee of the Hospital and of prof. dr Alexander Schiopu to use the method for separating proteins by geometric Electrofocusing. 330 urine samples from those patients were analyzed by spectrophotometry in 600 nm, for the quantitative protein determination from urine, followed by geometrical electrofocusing. We also analyzed demographic data and anesthetic particularities. Results: After quantitative assessment and proteins separation we obtain the following statistical data. Using proteins quantitative assessment at 0, 24 and 72 hours in ANOVA GraphPad test we obtained a  $p < 0.0001$  (median: 11.46 mg% / 42.22 mg% / 22.30 mg%) statistically significant for proteins means for the batch anesthetized with Sevofluran. Urinary albumin, alpha and beta protein fractions obtained preoperatively and at 24 and 72 hours postoperatively after Sevofluran general anesthesia is statistically modified with a  $p = 0.0001$ . In Desfluran anesthesia batch we obtained a  $p = 0.01$  (mean: 29.84 mg% / 57.47 mg% / 39.45 mg%) for total proteins means. For urinary albumin the significant modification is also with a  $p = 0.03$  (mean: 0.175 g% / 0.345 g% / 0.269 g%). Alpha and beta protein fractions were also significant altered with a  $p = 0.002$  and  $p = 0.0001$  respectively. For the group with spinal anesthesia, urinary protein changes are insignificant for the 3 intervals (mean: 21.66 mg% / 23.64 mg% / 15.79 mg%). Conclusions: Changes to the glomerular filtration are significant for the group anesthetized with Sevofluran for proteins and urinary protein fractions. Long term anesthesia with Sevofluran associated to pathological conditions such as: hypertension, diabetes, obesity, the sepsis is dangerous.

**Key Words:** sevoflurane, proteinuria, creatinine, serum urea.

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## Introduction

Proteinuria, in the absence of symptomatology, is the most common laboratory sign observed being, caused by impaired glomerular permeability (Fischbach et al 2009). Physiologically, the glomerular membrane allows only small molecular mass proteins filtrations, which are lately mostly absorbed in renal tubules. Glomerular membrane alteration, low rates tubules absorption or renal tissue destruction lead to proteinuria (Fischbach et al 2009). Proteinuria after Sevofluran anesthesia is due to degradation by carbon dioxide (CO<sub>2</sub>) absorbents and to increased renal quantity in Fluor ions (Lasler et al 1994; Bito & Ikeda 1995; Keller et al 1995; Higuchi et al 1998). The A compound, which results after Sevofluran degradation by CO<sub>2</sub> absorbents, induce renal toxicity in laboratory animals producing tubules necrosis which are clinically evidenced by proteinuria and glycosuria (Edmond & Eger 2002; Stoian et al 2010). Mario et al 1998, report increased risk of renal toxicity in high levels of A

compound (CH<sub>2</sub>F-O-C=CF<sub>3</sub>). Further studies demonstrate that injury and renal lethality (cortical-medullary necrosis rate) is correlated with the compound A doses, with hour administration by concentration. Necrosis affects the cortical-medullary junction, initial in proximal tubules cells and furthermore in other medullary cells. Results from other studies suggest that compound A doses which lead to renal injuries varies from 150 to 300 drops per minute by hour (dpm/h) (25 dpm/h of compound A for 6 hours). Administering 8 hours 100 dpm/h of compound A to monkeys leads to increasing urine protein and N-acetyl b-glucose aminidase (tubules cells enzyme) following tubules cells necrosis and tubules degradation (Newton et al 1998). In this study we aim to determine protein types which pass the glomerular barrier by urinary electrophoresis, after general anesthesia with Sevofluran and Desfluran and after spinal anesthesia, aiming to observe if glomeruli permeability is affected by other anesthetics then Sevofluran.

Table 1. Perioperative and demographic data

		Sevofluran	Spinal anesthesia	Desfluran
		n = 61	n = 16	n=33
<b>Age</b>	<b>Mean</b>	57.93	65.68	57.94
	<b>Dev Std</b>	12.366	13.3	11.37
<b>BMI</b>	<b>Mean</b>	23	23.75	22.94
	<b>Dev Std</b>	3.911	1.91	11.37
<b>Sex</b>	<b>B/F</b>	32/29	13-Mar	19/14
<b>Anesthesia (min)</b>	<b>Mean</b>	212.131	241.25	192.4
	<b>Dev Std</b>	59.892	58.637	55.23
<b>MAC</b>		1.8 (1.4-2.2)	0	7.2 (5.8-8.2)
<b>Previous anesthesia</b>		52.5%	43.8%	69.70%
<b>Diabetes Mellitus</b>		14.8%	12.5%	6.10%
<b>Blood Pressure</b>		23%	62.5%	42.40%
<b>ASA</b>	<b>I</b>	1.6%	0	0%
	<b>II</b>	39.3%	31.3%	18.20%
	<b>III</b>	60.7%	68.8%	81.80%
<b>Sepsis</b>		21.3%	0	6.10%
<b>Surgery</b>				
	<b>Thoracic surgery</b>	40.98%	0	30.33%
	<b>Vascular surgery</b>	4.91%	75%	9.07%
	<b>General surgery</b>	54.09%	25%	60.60%

## Material and method

We studied 110 patients who required anesthesia, for medium and long term surgical procedures, in Anesthesiology and Intensive Care Unit. 330 urine samples from those patients were analyzed by spectrophotometry in 600 nm, for the quantitative protein determination from urine, followed by geometrical electrofocusing (method patented by Prof. Schiopu Alexandru in 1997). Samples were taken from 61 patients with general anesthesia with Sevofluran (batch 1), 33 samples from patients with general anesthesia with Desfluran (batch 2) and 16 samples from patients with spinal anesthesia (batch 3). Urine samples were obtained preoperatively, at 24 and 72 hours postoperatively and are processed without prior conservation to avoid possible mistakes. The patients admitted in study were between I to III ASA anesthesiology risk, according to the American Society of Anesthesia, without known renal impairments and normal rates for serum urea, serum creatinine preoperatively. The anesthesiological protocol included induction with Sodium Thiopental, Rocuronium and Fentanyl and maintenance with Sevofluran, Fentanyl and Rocuronium for the first batch, same protocol but replacing the volatile anesthetic with Desfluran for batch 2 and for batch 3 a lumbar spinal anesthesia with Bupivacaine 0,5%. We used a 5 l/min fresh gas flow at induction then decreased to 2 l/min on Sevofluran opening, at 1.5-2 MAC (minimal alveolar concentration) for batch 1. For batch 2 (with Desfluran) we used a same 2 l/min fresh gas flow but with a MAC between 6 and 8. Duration of anesthesia was over 100 min. The selected patients were monitored: ECG, noninvasive BP, SpO<sub>2</sub>, breath / min, Et CO<sub>2</sub>, MAC, Temperature, Diuresis, absorbents temperature of CO<sub>2</sub>. No nephrotoxic drugs were administered, or other aminoglycosides drugs that could interfere with anesthetics and affect

kidney function. Proteins electrophoresis by geometrical electrofocusing was performed in Department of Physiopathology laboratory, University of Medicine and Pharmacy Tg. Mures. For the statistical study of the data, we applied elements of descriptive and inferential statistics. For average comparison we used unpaired Student t test with two heads so one-way ANOVA, both with significance threshold of 0.05. The results obtained were statistically analyzed using Graph Pad Prisma 5 programme, which is online free. We analyzed and calculated the value of p, considered statistically significant over 0.5. We also analyzed demographic data and anesthetic particularities. The study is prospective, observational, patients signed an informed consent for the collection of biological samples and their analysis, and consent was obtained also obtained from the ethics committee of the Hospital and from prof. Dr. Alexandru Schiopu to use the method for separating proteins by geometric Electrofocusing.

## Results

In the study were included 110 who formed three batches having the demographic data represented in Table 1. After quantitative assessment and proteins separation we obtain the following statistical data. Using proteins quantitative assessment at 0, 24 and 72 hours in ANOVA GraphPad test we obtained a p<0.0001(20.01 mg%/ 55.82 mg%/ 30.73 mg%) mean protein statistically significant for proteins means (Fig. 1). Urinary albumin at 0h/ 24h/ 72h obtained mean 0.13 g%/ 2.36 g%/ 2.25 g%. Alfa proteins means 0h/ 24h/ 72h obtained: 0.03 g%/ 0.8 g%/ 0.05 g% (Fig. 2) and beta protein fractions obtained preoperatively and at 24 and 72 hours postoperatively

after Sevofluran general anesthesia is statistically modified with a  $p=0.0001$ , mean: 0.016 g%/ 0.07 g%/ 0.04 g% (Fig. 3). In Desfluran anesthesia batch we obtained for total proteins means, for 0h/ 24h/ 72h (29.84 mg%/ 57.47 mg%/ 39.45 mg% Fig. 4). For urinary albumin the significant modification is also in 0h/ 24 h with a  $p=0.03$  (mean: 0.175 g%/ 0.345 g% Fig. 5). Alpha protein fractions were significant altered for 0/24 h with a  $p=0.002$  (mean 0.02 g%/ 0.08 g%) and beta proteins were also modification  $p=0.0001$ (0.01 g%/ 0.06 g%/ 0.03 g%) respectively (Fig. 6). In spinal anesthesia beach we obtained for 0h/ 24h/ 72h,  $p=0.176$  (21.66 mg%/ 23 mg%/ 15.79mg%) total proteins means.

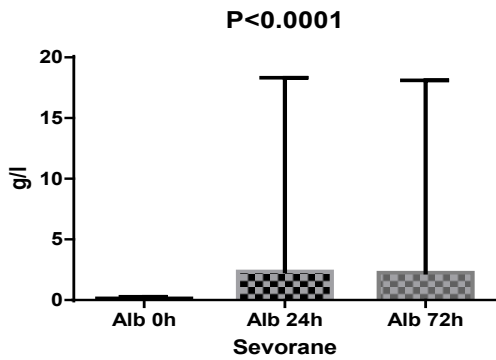


Fig. 1. Preoperatively and at 24 and 72 h postoperatively urinary albumin in batch 1 (Sevofluran)

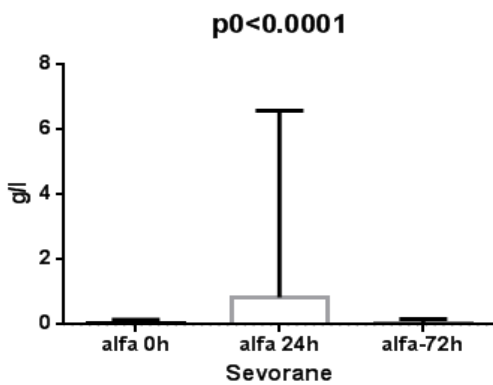


Fig. 2. Alfa-globulins after Sevofluran (batch 1)

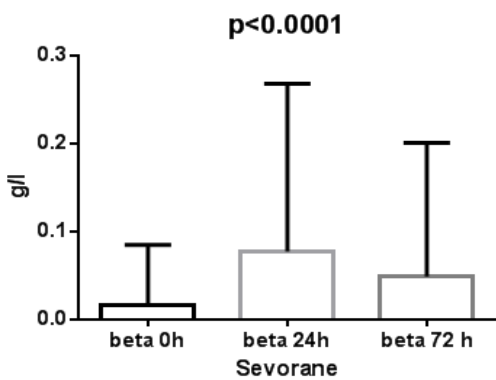


Fig. 3. Beta-globulins preoperatively and at 24 and 72 h postoperatively batch 1 (Sevofluran)

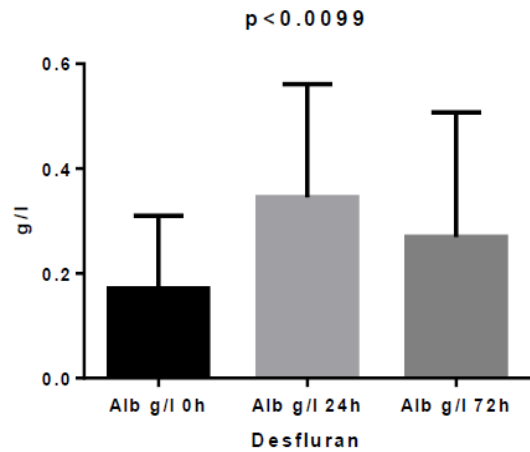


Fig. 4. Preoperatively and at 24 and 72 h postoperatively urinary albumin in batch 2 (Desfluran)

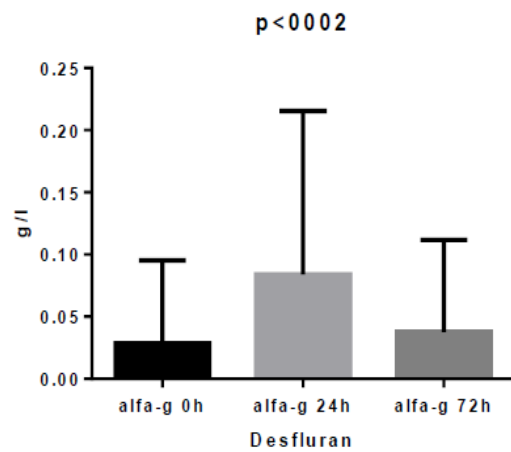


Fig. 5. Alfa-globulins after Desfluran (batch 2)

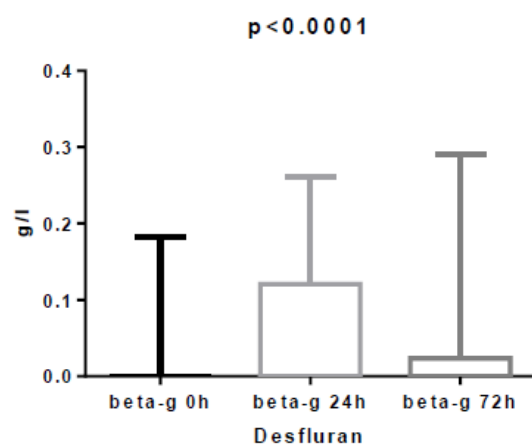


Fig. 6. Beta-globulins preoperatively and at 24 and 72 h postoperatively batch 2 (Desfluran)

### Discussions

Testing urinary functions in laboratory animals, volunteers and patients revealed minimal and transitory alterations after

profound and prolonged inhalator anesthesia. Actual evidences suggest that inorganic Fluor resulting from metabolic degradation of Sevofluran did not induce renal injury. It is to be discussed interaction between Sevofluran degradation compounds in particular of the A compound with CO<sub>2</sub> absorbents, which induces renal injury. Compound A production is produced approximately 150 ppm/h while renal injury appears at 660 ppm/h and is transitory. Inhalators anesthetics have minimal effects on the renal blood flow. Yearly, millions of patients underwent Sevofluran anesthesia but reports of renal injuries after this anesthetic are very rare in studies including patients with pre-existing renal pathology (Higuchi et al 2001), cardiac pathology, prolonged and repeated anesthesia (Kobayashi et al 1992). Has the inorganic Fluor a nephrotoxic effect? Metoxifluran anesthesia experience suggested that a serum concentration of inorganic Fluor ions above 50-100 micro mol/l induces nephrotoxic effects such as renal insufficiency, urine concentrating defects and decrease in response to Vasopresin. Also, Fluor ions induce tubules cells injury in vitro (Cittanova et al 1996). Results from other studies suggest that Fluor ions production after Sevofluran exposure could induce transitory renal injury without clinical expression similar with alterations produced by other anesthetics like Isofluran (Nishyama et al 1998). In our study we observed that glomerular permeability alterations is also for other types of proteins than albumin (alpha- and beta-proteins), changes that occur in all three batches with statistically significance. Considering lack of data involving post anesthesia proteinuria, we wish to emphasize about its real existence, fact that cannot be ignored taking into consideration public health affections that imply persistent proteinuria (Ninomiya ED et al 1997; Ninomiya et al 2009; Hillege et al 2001; Hiddo et al 2010). That particular type protein separation by electrophoresis, patented by Prof. Schiopu Alexandru, helped us to separate protein fractions without their preliminary concentrations (Shiopu & Schiopu Jr 2002).

## Conclusions

Changes to the glomerular filtration are significant for the group anesthetized with Sevofluran for proteins and urinary protein fractions. Lengthy anesthesia with Sevofluran associated with pathological conditions such as: Hypertension, Diabetes, Obesity, the Sepsis is dangerous. We shall not ignore post anesthesia proteinuria, since it rises public health affections given that it is not a routinely post anesthesia monitoring criteria, due to its costs. The admittance of these changes by the anesthesiologists and nephrologists could be a step ahead for limiting pre- and post-anesthesia renal injury. Using cautiously anesthetic low gas flows and assuring adequate, patient adapted, renal blood flow could diminish glomerular effects. The increased number of patients with pre- and post-anesthesia glomerular injuries becomes of great concern and must be kept in mind before any therapeutic or anesthetic intervention.

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