

# High sensitive troponin concentration stability in dialysate of anuric patients on hemodialysis

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## ABSTRACT

**Background.** High sensitive troponin I (hsTnI) and high sensitive troponin T (hsTnT) are markers of cardiac damage. Cardiomyocyte necrosis increases its blood levels. It is known that dialysis is cardiotoxic and that results in lack of contractility of certain myocardial segments. This mechanism is primarily due to hypo perfusion of the myocardium during dialysis. The dialysis itself increases cardiovascular (CV) risk in patients by many different mechanisms. It has been proven that the incidence of heart failure is much more frequent in patients on hemodialysis than in healthy population.

The aim of this pilot study was to investigate the presence of troponin T molecules and troponin I in dialysate and compare their concentrations.

**Materials and Methods.** The study included 5 anuric patients (4M) on hemodialysis. The dialysate samples were sampled for each patient three times during a dialysis cycle. The first sample was taken after thirty minutes, the second sample was taken in the middle of dialysis (120 minutes) and the third sample was taken thirty minutes before the end of dialysis. The value of hsTnI was measured using a high-sensitivity test on the Immuno-enzymatic analyzer Abbott Architect i1000SR. According to CLSI EP15-A2 protocol verification of hsTnT chemiluminescent micro-particle immunoassay on the analytical platform Roche cobas e411 was performed.

**Results.** Altogether 15 samples (three for each patient) were processed. hsTnT was detected in all 15 samples ( $13.42 \pm 1.18$

ng / L), while hsTnI was detected in only 8 samples ( $0.14 \pm 0.16$  ng / L). To test the difference in detectability between hsTnT and hsTnI, chi square test was used and the difference was statistically significant (Yates chi-square 6.708,  $p = 0.009$ ).

**Conclusion.** The presence of troponin molecules in dialysate was determined for the first time in scientific literature. This study has confirmed that TnT is present in all dialysate samples and that its concentration is stable in dialysate. TnI concentrations were detectable in significantly lower concentrations.

**Key words:** hemodialysis, hs troponin T, hs troponin I, dialysate

## INTRODUCTION

Cardiac - specific troponins are troponin T (TnT) and troponin I (TnI), as different genetic variants of troponins exist. Since a morphologically different form of troponin is expressed in skeletal muscle cells, these two types of troponin are used as a specific marker of cardiac damage. In situations where cardiac necrosis occurs, they increase their blood concentrations. The most common conditions that lead to elevation of the troponin level in the blood are myocardial infarction, pulmonary embolism and toxic heart damage. Not only conditions that cause heart cell damage cause elevated troponin levels in the blood. Recent studies have shown that the kidneys are the major organs that eliminate troponin from the blood<sup>1</sup>. This statement is also confirmed by the fact that in end stage

renal disease (ESRD) patients, especially in patients on hemodialysis, had elevated concentration of troponin in blood.<sup>2</sup> High sensitive troponin T (hsTnT) and high sensitive troponin I (hsTnI) are used today as specific cardiac damage markers. The increase of their concentration above cut-off level indicates that cardiac events are most commonly reported. hs-TnT test has a detection limit of 5 ng / L (pg / mL), while the quantification limit (LoQ) is 13 ng / L on the analytical system applied 3,4. hsTnI can be detected by Chemiluminescent Microparticle Immuno Assay (CMIA) method<sup>5</sup>. TnT blood levels in anuric patients on hemodialysis was increased in more than 90% of patients, and the levels of TnI in 2/3 of them<sup>6</sup>. Therefore, the diagnosis of acute cardiac event in these patients should include dominantly clinical symptoms and oscillations of troponin in time. Recent studies have shown that TnT and TnI concentrations in blood have prognostic value. Studies have shown that overall dialysis duration is not the main risk factor for mortality and that Tn has shown good correlation with mortality risk. TnT is a good indicator of all-cause mortality, but TnI is proven to be in strong prognostic correlation only with cardiac incidents<sup>7-9</sup>.

Hemodialysis (HD) is one of the three replacement renal therapies. HD is usually performed three times a week for four hours. In case of instability of the patient, other replacement renal therapies are performed with the aim of preventing the development of complications and improving survival rate and quality of life.<sup>10</sup> According to the study, during HD, myocardial perfusion decreases up to 44%.<sup>11</sup>

The decrease in perfusion causes changes in contractility of certain myocardial segments, and in about 30% of patients on HD, the ultrasound imaging of the heart can detect such changes.<sup>12</sup> These patients are at increased risk of development of systolic or diastolic heart failure. In addition, HD stimulates the inflammatory response of the body, and the inflammation enhances atherosclerotic changes in the blood vessels. Accelerated atherosclerosis leads to premature atherosclerosis of coronary blood vessels inducing hypoperfusion of the myocardium. In addition to the above-mentioned, the frequency of systolic heart failure in the first year in patients on HD is about 40%, while the overall incidence of diastolic heart failure in patients on HD is 25-87%, depending on the literature.<sup>13-16</sup> Since hsTn concentrations in blood are prognostic factors in patients on hemodialysis, we wanted to examine how the hemodialysis affects the elimination of TnT and TnI, and are TnT i TnI even excreted in dialysate. TnT is a molecule of about 40 kDa in size and is predominantly degraded in blood on a 20 kDa immunoreactive segments. TnI is about 20 kDa in size and is also degraded in blood on smaller immunoreactive segments, dominantly 5-10 kDa in size.<sup>17</sup> As the HD dominantly removes molecules of up to 10 kDa<sup>18</sup> we have assumed that TnI will be dominantly removed from the blood in dialysate and that its dialysate concentrations will be greater than TnT concentrations.

The primary objective of this study was to determine the possibility of detection of TnT and TnI in dialysate using high-sensitive tests (hsTnT, hsTnI). The secondary goal was quantification and comparison of TnT and TnI in dialysate samples of anuric patients. We also wanted to examine whether the value of Tn concentration in dialysate measured 30 minutes before end of HD would be representative of the Tn concentration measured throughout dialysis.

## PATIENTS AND METHODS

The study included 5 anuric patients attending HD procedure at KB Merkur on a regular basis. Patients were median age 70 years old (MIN 58y, MAX 74y), without heart failure and acute coronary disease. Three patients involved in the study had diabetes. The HD procedure was performed in all patients three times a week for 4h, the ultrafiltration volume of dialysate was  $2.2 \pm 0.9$  liters, depending on the required volume for removal.

The first sample of dialysate was taken after thirty minutes, the second sample was taken in the middle of dialysis (120th minute) and the third sample was taken thirty minutes before the end of dialysis. We performed dialysate sampling in the amount of up to 5 ml in each patient three times during one HD procedure to establish if there were differences between the measured concentrations in the different parts of the HD procedure.

The samples were stored at -80C until analysis. Dialysate used in HD procedure contains sodium (139 mmol / L), chloride (108 mmol / L), potassium (1.5 mmol / L), magnesium (0.5 mmol / L), calcium (1.5 mmol / L), acetate mmol / L), bicarbonate (34 mmol / L) and glucose (1.0 g / L). Dialysate flow rates were 500 ml / min in all patients, respectively. Two patients included in the study were connected to the Rexed dialysis membrane while the other three were using the Frenius FX10.

hsTnI in dialysate was determined by Chemiluminescent Microparticle Immuno Assay (CMIA) method, on an immunochemical analyzer Abbott Architect i1000SR using 3P25 ARCHITECT STAT High Sensitive Troponin-I Reagent Kit, 3P25 ARCHITECT STAT High Sensitive Troponin-I Calibrators and 3P25 ARCHITECT STAT High Sensitive Troponin-I Controls<sup>19</sup>. The calibrators were prepared gravimetrically using recombinant human cardiac troponin IC complex and calibrator B was traced to NIST SRM 2921 at a concentration of 20 ng / L 5. The value of hsTnT in dialysate was determined by immunochemical method with electrochemiluminescent detection on Roche cobas® e411 using Roche Diagnostics Troponin T high sensitive (TnT-hs) test, Roche Diagnostics PreciControl Troponin T as control, and Roche Diagnostics Troponin T hs CalSet calibrators<sup>3</sup>. The detection limit (LoD) by the reagent manufacturer (Roche Diagnostics) is 5 ng / L (pg / mL) and the limit of quantification (LoQ) 13 ng / L on the applied analytical system 4. Verification of the manufacturer's LoQ value hsTnT is determined by CLSI EP17-A protocol. The same protocol was also applied to determine the limit of quantification of hsTnT in dialysate. Verification of hsTnT chemiluminescent micro-particle immunoassay on the analytical platform Roche cobas e411 was performed according to CLSI EP15-A2 protocol<sup>20</sup>. Limit of quantitation (LoQ) was determined according to CLSI EP17-A protocol.

The statistical analysis was made using statistical program Statistics (version 13.1). Descriptive data analysis was performed for all attributes.

## RESULTS

There were altogether 15 samples (three for each patient). The hsTnT level was detectable in all 15 samples ( $13.42 \pm 1.18$  ng / L), while hsTnI was only detectable in 8 samples ( $0.14 \pm 0.16$  ng / L). The results are shown in Table 1. To test the difference in detectability between hsTnT and hsTnI, chi square test was used and the difference was statistically significant (Yates chi-square 6.708,  $p = 0.009$ ). To test the difference between total TnT and TnI concentrations in dialysate, Student's T test for dependent samples was used, which showed that the difference was statistically significant ( $t = 44.9028$ ,  $p < 0.001$ ).

The mean difference of TnT level in dialysate 30 minutes before the end of dialysis ( $13.27 \pm 0.74$  ng/L) did not show statistically significant compared to the average TnT concentration measured in dialysate of all samples ( $13.42 \pm 1.1$  ng / L) (for the analysis we used Wilcoxon W test,  $p$ -value: 0.9530). The difference between TnI in dialysate 30 minutes before dialysis endpoint ( $0.16 \pm 0.23$  ng / L) was not statistically significant compared to the mean TnI concentration in dialysate of all samples ( $0.14 \pm 0.16$  ng / L) (for the analysis we used Wilcoxon W test,  $p$ -value: 1.0000).

## DISCUSSION

This research has for the first time shown that TnT and TnI are removed by dialysis in anuric patients on HD. Dialysate is an isotonic fluid with normal blood osmolarity and serves to allow the removal of excess metabolic waste substances from a patient's body. Dialysate flow, used in HD, is usually 500 ml/min, which was the case in this study, but may range from 350 to 600 ml / min<sup>10</sup>. Its flow determines the total amount of dialysate to be used in dialysis, so the most commonly used dialysate volume is around 120 L.

In accordance with our previous research, we have proved that it is possible to measure hsTn in urine, and this research confirmed the possibility of measuring hsTn in dialysate<sup>1</sup>. We have confirmed that a measurement finding is not random because we measured the hsTn concentration on several occasions during HD. Another reason why we measured Tn concentrations three times during dialysis was that we were unable to store the entire dialysate (around 120L) in laboratory conditions to determine the troponin values in the overall dialysate. Dialysate samples were taken from each patient three times during one

Table 1. Values of hsTroponin I and hsTroponin T in dialysate of anuric patients

Pt (N)	TNT1	TNT2	TNT3	TnI1	TnI2	TnI3
1.	16,65	13,55	14,12	0,1	0,2	0
2.	13,81	11,6	12,66	0,2	0	0
3.	12,14	12,51	12,35	0	0	0
4.	14,02	13,21	13,5	0,2	0,4	0,5
5.	13,93	13,64	13,7	0,2	0	0,3

TnI=hsTroponin I, TNT=hsTroponin T (ng/l). TNT1 and TnI2=the first sample of dialysate, taken after thirty minutes. TNT2 and TnI2=the second sample, taken in the middle of dialysis (120th minute). TNT3 and TnI3=the third sample, taken thirty minutes before the end of dialysis.

HD procedure, which have proven the differences between the measured concentrations of TnT and TnI in dialysate. hsTnT was present in all dialysate samples and its concentration was stable in dialysate. hsTnI concentrations were detectable in significantly lower concentrations. The literature states that TnI binds to the dialysis membrane<sup>21</sup>. This claim has so far been confirmed only in *in vitro* conditions. Our research confirmed this claim, but in *in vivo* conditions.

One of the potential limitations of this study is that hsTnT and hsTnI assays are not standardized for dialysate. Since the manufacturers claim that it is possible to use the mentioned tests in all body fluids, and that dialysate is a fluid similar to plasma, there is no reason not to apply these tests for dialysate<sup>19-20</sup>. So, there is

no reason, for the Tn concentrations measured in dialysate, to be invalid. The results obtained confirm that the results could be valid because, as expected, the TnI values were significantly lower than the TnT concentration due to the TnI binding to the dialysis membrane<sup>22</sup>.

### CONCLUSION

The presence of Tn molecules in dialysate was determined for the first time. TnT values in dialysate were much higher than the TnI values that were barely measurable. These TnI concentrations are consistent with the expected values since TnI has been shown to bind to the dialysis membrane.

Since differences in Tn concentration be-

tween the measured values were not statistically significant, we can safely claim that one-time measurement of troponin (either TnT or TnI) in dialysate during dialysis represents the representative Tn value in dialysate.

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