Abstract. - OBJECTIVE: When using heparin anticoagulation for continuous renal replacement therapy (CRRT), the main challenge is to tailor the dosage to patient response. This study aimed to determine if the first activated thromboplastin time (aPTT) (measured after 3 hours post heparin bolus) can be a predictor for CRRT filter survival and if the first activated clotting time-low range (ACT-LR) (10 min post heparin bolus) can be predictive for subtherapeutic or therapeutic first aPTT.

MATERIALS AND METHODS: An unfractionated heparin (UF) anticoagulation protocol was used in CRRT and heparin monitoring was performed by aPTT and ACT-LR. Extracorporeal therapy was analyzed and filter survival was assessed for general risk factors, especially coagulation tests. For statistical analysis, Logrank tests, ROC curve analysis, and the Kaplan-Meier chart for survival evaluations were utilized.

RESULTS: Using the $p_{\text{logrank}}$ test, the overall survival for the CRRT procedure was 47.8 hours ($p=0.04$), and no clotting events occurred during the first 12 hours for all examined therapies. Multivariate analysis for filter survival prediction to estimate 48 hours of CRRT revealed statistical relevance for Age (<60 years), BMI (<25.9), and INR (>1.3), with negative statistical significance for lipids, triglycerides, and fibrinogen. aPTT (180 min) values greater than 57 sec were shown to be predictive of 48-hour filter survival, and similar findings were obtained for aPTT measured at 6 hours. ACT-LR samples assessed 10 minutes after the initial heparin bolus was shown to be predictive of 48-hour filter survival, and similar findings were obtained for aPTT measured at 6 hours. ACT-LR samples assessed 10 minutes after the initial heparin bolus was shown to be predictive of 48-hour filter survival, and similar findings were obtained for aPTT measured at 6 hours. ACT-LR prediction potential for therapeutic aPTT values was evaluated. ACT-LR 10 min (cut off > 200 sec.), ACT-LR 60 min (cut off > 186 sec.), and ACT-LR 180 min (cut off > 182 sec.) were found to be predictive.

CONCLUSIONS: Based on this study and its sample size, ACT-LR can be a complimentary assessment to aPTT for monitoring anticoagulation with heparin on CRRT.

Key Words: Continuous renal replacement therapy, Heparin, aPTT predictor, ACT-LR, Filter survival.

Abbreviations
AKI - Acute kidney injury; ACT - Activated clotting time; ACT-LR - Low range Activated clotting time; APTT - Activated partial thromboplastin time; CRRT - Continuous renal replacement therapy; SHA - Systemic heparin anticoagulation; ICU - Intensive care unit; RCA - Regional citrate anticoagulation.

Introduction
Acute kidney injury (AKI) is a common complication in critically ill patients and is associated with substantial morbidity and risk of death. Approximately 5% to 10% of patients with AKI require renal replacement therapy during their Intensive Care Unit (ICU) stay and the percentage grows when sepsis is involved, with mortality rates up to 70%.

Continuous renal replacement therapy (CRRT) generally necessitates anticoagulation, and ideal anticoagulation should provide optimal antithrombotic activity, low bleeding risk, and minimal systemic adverse effects. Different anticoagulation modes are available, including systemic unfractionated heparin anticoagulation (SHA). Over the last decade, conventional systemic heparin anticoagulation has been increasingly replaced by regional citrate anticoagulation for CRRT, in approximately 50% of ICU. However, the recommendation for using Regional Citrate Anticoagulation (RCA) for CRRT in patients with Acute Kidney Injury (AKI) in the absence of contraindications is classified as Grade 2B, indicating weak evidence.

When SHA is applied during CRRT, using bolus or continuous infusion, unfractionated heparin administered in the pre-filter reaches the patient’s
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Blood reservoir by return line, generating minimal systemic anticoagulation. The problem is the anticoagulation intensity and the appropriate means for monitoring, all being risk factors for clotting or filter survival.

Heparin monitoring is performed by laboratory tests that measure the effect of given heparin on prolonging the coagulation time in seconds. Sampling site and laboratory result time are protocol settings that impact on therapeutic approach in dosing heparin. The most common tests are activated partial thromboplastin time (aPTT) and activated clotting time (ACT). The optimal therapeutic range for aPTT is 1.5-2.5 times the baseline value, which has gained widespread clinical acceptance.

According to anticoagulant protocol administration and monitoring, the first aPTT sample is taken 3-4 hours later than the initial heparin bolus. A bolus of heparin prevents clot formation but leaves the body in a delicate dynamic balance between clotting and bleeding. At this level of anticoagulation, the aPTT is no longer clinically useful as a monitoring tool. We must acknowledge that the specificity of aPTT is relevant only after at least 3 hours after a bolus.

ACT testing, in contrast to aPTT, allows for the assessment of relatively quick changes in heparin infusion, assisting in the achievement and maintenance of a consistent level of anticoagulation during surgical or medical treatment. ACT has been used successfully in cardiac surgery to evaluate the efficacy of heparin anticoagulation. The ACT is a popular point-of-care test employed to monitor UFH dosages that yield 1 to 2 units/mL plasma levels, exceeding aPTT and anti-Xa analytical range limits.

Our study sought to determine whether the first aPTT (measured 3 hours after heparin bolus, or 3 hours on CRRT) can be a predictor of CRRT filter survival, as well as whether the first ACT-LR (measured 10 minutes after heparin bolus) can be a predictor of subtherapeutic or therapeutic first aPTT, using a standard heparin administration protocol.

**Exclusion Criteria**
- Plasmapheresis therapies (n=24);
- Regional citrate therapies (n=41);
- Patients undergoing procedures with a major risk for bleeding (n=19);
- Patients presenting severe thrombocytopenia and INR >2 (n=25);
- Patients with catheter-related problems after therapy debut (n=2);
- Patients who did not consent (n=8).

**Inclusion Criteria**
- Patients receiving continuous venovenous hemodiafiltrations (CVVHDF) therapy using unfractioned heparin (UFH) anticoagulation, eligible for the study, were considered 48 therapies.

**CRRT Characteristics and Heparin Protocol**
Continuous venovenous hemodiafiltration (CVVHDF) was performed on Baxter® (Gambro®) Prismaflex TM machines equipped with the Prismaflex ST150TM set and the AN69ST membrane (Gambro Lundia, Lund, Sweden) and Fresenius Multifiltrate Machine equipped with the KIT 6 set and the Ultraflux® AV600S membrane (Fresenius, Bad Homburg v. d. Höhe, Germany). For optimal treatment, we maintained a medium blood flow of 180-230 ml/min, and we used a third of the total substitution volume in the pre-filter, using a replacement rate of 25-35 ml/kg/h.

For vascular access, 20 cm long, 13.5 French double lumen hemodialysis catheters (Joline®) were inserted into either the femoral or internal jugular vein. Catheter characteristics include a catheter tip without side holes, ensuring less clotting and low recirculation.

In our study, critical care nurses started the procedures by following ICU protocols for setting up the lines and using saline with UFH (Heparin Sodique, 5,000 UI/ml Laboratories Panpharma, Le Clairay, France) for priming solution (5,000 UI/L).

For the heparin protocol, we use a modified unfractionated weight-based heparin nomogram. The initial dose of UFH was 50 UI/kg and the maintenance rate was 10-15 UI/Kg/h. The physician takes all patients’ baseline anticoagulation status, previous therapies, and possible risks that may generate clotting or bleeding. This protocol was applied to all our ICU patients on CRRT, except for consecutive therapies (no initial bolus) and heparin usage limitations (Table I).

Anticoagulation monitoring was obtained using a point-of-care device available in the ICU. aPTT and ACT-LR were determined using a Hemochron.
Signature Elite© 2015 [Accriva Diagnostics (San Diego, CA, USA)]. This device uses clot-activator cuvettes and mechanical clotting detection. aPTT and ACT-LR are determined from whole blood, and testing is performed immediately on-site. According to producer recommendations, for aPTT and ACT-LR, a table chart for defining normal plasma equivalent range values, and specific voluntary donor groups must be defined.

aPTT normal range for plasma equivalent in volunteer donors (n=30) is 23.5-38.7 sec, and for our group of patients not receiving treatment (n=48), found baseline aPTT was 26.2-48 sec (see results) (Table II).

Other studied parameters that can be risk factors are age, BMI, complete blood count, fibrinogen, CRP, lipids, triglycerides, and platelets.

**Statistical Analysis**
All data were collected and analyzed using SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA) and Microsoft Excel.

No formal sample size calculation was performed. All eligible patients were included in the analysis. We analyzed filter lifespan using Logrank to determine the cut-off and the Kaplan-Meier method to assess the relationship of explanatory for the survival group and the non-survival group. Those tests are most likely to detect a difference between groups when the risk of an event is consistently greater for one group than another, as in our study, filter survival. The p-value was evaluated by the McNeil test.

<table>
<thead>
<tr>
<th>Plasma aPTT found</th>
<th>Continuous rate action</th>
<th>Bolus</th>
<th>Check time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma aPTT &lt;35 sec</td>
<td>+ 4 UI/Kg/h</td>
<td>bolus 50 UI/Kg</td>
<td>aPTT after (3h)</td>
</tr>
<tr>
<td>Plasma aPTT 35 - 45 sec</td>
<td>+ 2 UI/Kg/h</td>
<td>bolus 20 UI/Kg</td>
<td>aPTT after (3h)</td>
</tr>
<tr>
<td>Plasma aPTT 46 - 60 sec</td>
<td>+ 2 UI/Kg/h</td>
<td>-</td>
<td>aPTT after (3h)</td>
</tr>
<tr>
<td>Plasma aPTT 60 - 70 sec</td>
<td>-</td>
<td>-</td>
<td>aPTT after (4h)</td>
</tr>
<tr>
<td>Plasma aPTT 71 - 90 sec</td>
<td>- 2 UI/Kg/h</td>
<td>-</td>
<td>aPTT after (3h)</td>
</tr>
<tr>
<td>Plasma aPTT &gt; 90 sec</td>
<td>- 3 UI/Kg/h</td>
<td>Stop Cont. inf 60 min</td>
<td>aPTT after (2h)</td>
</tr>
</tbody>
</table>

First aPTT (3 h) after the initial bolus. * If BW > IBW x 1.3, then use ABW to estimate the initial heparin bolus. BW (Bodyweight) IBW (Ideal Body Weight) ABW (Adjusted Bodyweight).

**Table I.** Heparin protocol for bolus and dose adjustment when using UFH on CRRT.

<table>
<thead>
<tr>
<th>Target: aPTT x2</th>
<th>Initial Dose (Bolus) 50 UI/Kg</th>
<th>Maintenance 10 - 15 UI/Kg/H</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Plasma aPTT found</th>
<th>Continuous rate action</th>
<th>Bolus</th>
<th>Check time</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPTT(1) N Mean SD Range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal donors 30 34 sec. 11 sec 23.5-38.7 sec</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients 48 37 sec 9.1 sec 24.8 - 48 sec</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACT-LR(2) N Mean SD Range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal donors 20 103 sec. 12 sec 81-125 sec</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients 48 150 sec 18 sec 127-175 sec</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1The Hemochron aPTT was evaluated from normal volunteer donors (n=30) and our group of patients not receiving treatment (n=48), the results are shown as plasma equivalent in seconds. 2The Hemochron ACT-LR was evaluated using fresh whole blood from normal volunteer donors (n=20) and our group of patients not receiving treatment (n=48), the results are shown Celite equivalent Hemochron ACT values.
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Method and was considered significant under 0.05.

Receiver operating characteristic (ROC) analysis was used to identify the most statistically relevant values for filter survival between collected variables such as demographic data and different timeline sampled laboratory values. ROC curves were estimated by Bamber’s method and are used to determine the sensitivity and specificity of the relationship between two parameters. Values printed in bold have statistical relevance ($p$-value $\leq 0.05$).

**Results**

In the first 12 hours, all studied therapies had no clotting events in CRRT filters. The median filter lifespan was 38 hours. Reasons for ending the procedure were: a clogged filter (72.9%, n=35) and other reasons, including the need for surgical intervention or therapy no longer needed (27.1%, n=13). For 27 patients, the CRRT procedure ended with no clogged filter and had a median timeline of 47.2 hours. General survival for the CRRT procedure based on data analysis using the $p_{\text{Logrank}}$ test is 47.8 hours ($p=0.04$).

We agreed/accepted that the ideal timeline for filter survival is 48 hours.

Multivariate analysis shows negative results for baseline fibrinogen and lipid values, with statistical relevance for age, BMI (body mass index), INR, and aPTT (180 min) (Table III).

The baseline results had no statistical relevance for 48 hours of survival. In the general filter life span, no other parameter had predictive statistical relevance.

Analyzing data for statistical relevance in general survival, we found the patient’s age had a $p_{\text{Logrank}}$ test cutoff of 60 years ($p=0.05$). For under-60-year-old patients, for 48 hours of CRRT therapy, filter survival prediction is 49% compared to 23% of patients over that age (Figure 1).

The $p_{\text{Logrank}}$ cutoff test for BMI (body mass index) yields 25.95, with strong statistical significance ($p=0.02$). Patients with a BMI lower than 26, have a 64% chance of reaching 48 hours of CRRT therapy vs. 26% if the patient has a BMI over 26 (Figure 2).

Applying the $p_{\text{Logrank}}$ test to baseline INR, we found a 1.3 cutoff value for filter survival with strong statistical relevance ($p<0.02$). Using the Kaplan-Meier method, a 49% chance to reach 48 hours of CRRT therapy was found for patients with baseline INR values greater than 1.3, and 18% if the patient’s sampled baseline INR values were lower than 1.3 (Figure 3).

When analyzing first sampled aPTT values 180 min post bolus, the statistic cut-off ($p_{\text{Logrank}}$ test) was < 57.8 sec ($p=0.03$) (Figure 4). Using the Kaplan-Meier method, we assessed the relationship of explanatory (48 hours filter survival and > 57.8 sec aPTT cut-off) for the survival group and the non-survival group. For aPTT values greater than 57.8 sec we found a 43% predictability for filters surviving 48 hours, and for values under 57 seconds, the test showed only 24% prediction (Figure 5).

The results of the Logrank test for the sampled aPTT values at 6 hours post-bolus revealed two cutoffs: Case 1 (cutoff 50.6 sec, Logrank $p=0.04$) and Case 2 (cutoff 60.2 sec, Logrank $p=0.05$). Due to the closer proximity of 60 sec aPTT values to the applied protocol target, we used Case 2 as the threshold for the Kaplan-Meier survival test (plasma aPTT 60-70 sec). When interpreting the Kaplan-Meier test, it was shown that aPTT values

<table>
<thead>
<tr>
<th>Independent Values</th>
<th>$p$-value</th>
<th>cutoff survival prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACT 10 min</td>
<td>0.04</td>
<td>&gt; 218</td>
</tr>
<tr>
<td>ACT 60 min</td>
<td>0.35</td>
<td>&gt; 182</td>
</tr>
<tr>
<td>ACT 180 min</td>
<td>0.36</td>
<td>&gt; 175</td>
</tr>
<tr>
<td>ACT 6 Hours</td>
<td>0.09</td>
<td>&gt; 178</td>
</tr>
<tr>
<td>aPTT 180 min</td>
<td>0.04</td>
<td>&gt; 57</td>
</tr>
<tr>
<td>aPTT 6 hours</td>
<td>0.04</td>
<td>&gt; 60</td>
</tr>
<tr>
<td>Baseline INR</td>
<td>0.03</td>
<td>&gt; 1.3</td>
</tr>
<tr>
<td>Creatine kinase (CK)</td>
<td>0.02</td>
<td>&gt; 74</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.09</td>
<td>&lt; 476</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.13</td>
<td>&lt; 30.3</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>0.07</td>
<td>&lt; 9.8</td>
</tr>
<tr>
<td>Body mass index (IMC)</td>
<td>0.02</td>
<td>&lt; 25.95</td>
</tr>
<tr>
<td>Platelets</td>
<td>0.31</td>
<td>&gt; 243,000</td>
</tr>
<tr>
<td>White blood cells (WBC)</td>
<td>0.20</td>
<td>&gt; 15690</td>
</tr>
<tr>
<td>C-reactive protein (CRP)</td>
<td>0.16</td>
<td>&gt; 12.1</td>
</tr>
<tr>
<td>Total lipids</td>
<td>0.34</td>
<td>&gt; 520</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.44</td>
<td>&gt; 214</td>
</tr>
<tr>
<td>Age</td>
<td>0.05</td>
<td>&gt; 60</td>
</tr>
</tbody>
</table>

Table III. Risk factors 48 filter survival, a Multivariate Analysis.

According to producer recommendations, a table chart for defining normal range values specific to our patient group, using fresh whole blood from normal volunteer donors and our group of patients not receiving treatment.

ROC curves were estimated to determine the sensitivity and specificity of the relationship between parameters. Values printed in bold have statistical relevance ($p$-value $\leq 0.05$).
Figure 1. Filter survival based on age, cutoff of 60 years ($p=0.05$).

Figure 2. Filter survival based on Body mass index (BMI), cutoff < 25.95 ($p=0.02$). BMI > 26.
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**Figure 3.** Filter survival Based on INR, cutoff 1.3 ($p < 0.02$).

**Figure 4.** Statistic cutoff chart for aPTT 180 min Sampled values ($p_{Logrank}$ test).
acquired at 6 hours after CRRT had a 44% predictive value for the 48 hours threshold when values were larger than 60.2 sec and only 26% predictive for aPTT values below the 60.2 sec cutoff.

ACT-LR produced statistically significant findings. Significant prediction for 48 hours filter survival for ACT-LR 10 min was discovered using the Logrank test and Kaplan-Maier survival analysis, however, there was no statistical relevance for ACT LR for values obtained from samples at 60 and 180 minutes: ACT-LR values more than 218 seconds were 51% predictive for 48 hours of CRRT, while values less than 218 seconds were only 13% predictive (Figure 6).

ROC curves were computed using Bamber’s approach to establish the sensitivity and specificity of the association between the two parameters, in our study ACT-LR values and first sampled aPTT 180 min post bolus. This was done to further evaluate a potential link between ACT-LR and aPTT. We discovered that ACT-LR values have predictive power for the initial (180 min post bolus) aPTT therapeutic value (previously calculated cutoff > 57.8 Sec).

Sensitivity and specificity presented high statistical relevance for each ACT-LR sampling time, similar results were found when the area under the ROC curve (AUC) test was performed. For each measurement time, the following values were discovered: ACT-LR_10_min ($p < 0.01$; AUC=0.77; cut off > 200 sec.), ACT-LR_60_min ($p < 0.01$; AUC=0.86; cut off > 186 sec.) and ACT-LR_180_min ($p < 0.01$; AUC=0.81; cut off > 182 sec.). [Figure 7 - ROC Curve extrapolated from binary logistic regression analysis, ACT -LR prediction for APTT 180 min > 58 sec.]

Discussions

In present days, modern medicine has adopted invasive techniques that correct and compensate for various insufficiencies, including renal injury.

While once considered a treatment of last resort, continuous renal replacement therapy (CRRT) has become a routine procedure, even in the setting of marked hemodynamic instability, and is widely implemented in modern ICUs. More centers integrated anti-Xa levels alongside ACT and aPTT in their routine anti-coagulation monitoring during ECMO in recent years.

![CRRT Survival based on 180 min aPTT](image)

**Figure 5.** Filter survival predictability for found aPTT 180 min values > 57.
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Figure 6. ACT-LR 10 min predictability for filter survival.

Figure 7. Graphical representation for ROC curves when sensitivity and specificity of the relationship between ACT-LR and aPTT were analyzed. ACT-LR predictability for aPTT values > 58 sec when sampled at 180 min.
A significant restriction of any device that circulates blood through an extracorporeal circuit is the requirement for anticoagulation to prevent the blood in the circuit from clotting. Heparin is the anticoagulant used in the majority of renal replacement therapies in Romania, and exclusively in liver dialysis or extracorporeal membrane oxygenation ECMO, and is essential in maintaining homeostasis during invasive procedures, but its administration can pose a significant risk to the patient if not properly monitored. Clots in the filter and tubing might result from insufficient heparin anticoagulation. If predicted targeted aPTT levels are not rigorously specified, heparin overdose may lead to spontaneous bleeding.

Monitoring heparin treatment and its effects in inpatient settings is not only necessary but also critical to protect the patient from complications. Because CRRT is typically administered to intensive care patients with varying degrees of altered states, the likelihood of an early clogged filter can have a severe impact on the delicate metabolic balance. Randomized trials show a relationship between the dose of heparin administered and both its efficacy and its safety.

Following our anticoagulation protocol, the median filter life span of 38 (± 14.1) hours in this study was consistent with what has been reported in the literature (20-40 h). The protocol targeted aPTT values had a relevant predictive value in 48 hours of filter survival when sampled 3 and 6 hours after the initial bolus.

Using a standard heparin administration protocol, we observed that aPTT sampled 3 hours after initial bolus of heparin being higher than 57 seconds is a reliable predictor for CRRT filter survival. We discovered that the post-heparin bolus ACT (ACT-LR 10 min) value is predictive for subtherapeutic and therapeutic initial aPTT measures when used as an early assessor of heparin’s anticoagulant effect. Reiner et al discovered that ACT values greater than 225 seconds were indicative of therapeutic or supratherapeutic aPTT in 1994 research.

Predictive ACT value for subtherapeutic first aPTT can be the trigger for an extra bolus of heparin or an increase in continuous rate. Still, we need to take into consideration all factors that may influence this prediction such as nurse-dependent settings, room temperature, sampling protocol, and elapsed time until the validated result from the laboratory. Point-of-care devices are essential for quick evaluation and assessment during heparin treatment.

ACT samples obtained at 10 and 60 minutes can be used to establish if the initial aPTT post-bolus is therapeutic or subtherapeutic. The CRRT filter’s predictability to survive 48 hours may be assessed by using an aPTT cut-off of more than 57 seconds at 3 hours, and more than 60 seconds at 6 hours.

A logistic model with multivariate analysis yields negative statistical findings for filter survival statistic prediction, including lipids, triglycerides, fibrinogen, Ca, and PCR. We discovered mentions of Ph and calcium being strongly linked with circuit life in the literature.

Based on this study and its sample size, ACT-LR can be a complimentary assessment for monitoring anticoagulation with UH. Also, from our point of view, ACT-LR is a welcome predictor, especially when heparin resistance is suspected or aPTT results are delayed or have unexpected values.

The combination of improvements in staff education, cannulation techniques and CRRT giving sets and types of equipment have reduced the risk of bleeding and thrombotic events. These risks still need to be mitigated when choosing the method and dosage of anticoagulation therapy. They also do not negate the need for routine monitoring and assessment.

The study is representative of our ICU, inclusion and exclusion criteria narrowed the number of cases, this is one of the limitations of our study sure we cannot exclude certain factors in ICU patients that may influence calculation results beyond those assessed here.

**Conclusions**

The predictability for 48-hour survival of the CRRT circuit can be estimated using an aPTT threshold: for 3 hours post initial heparin bolus, a value greater than 57 sec, and for 6 hours a 60 sec aPTT. Predictability for reaching a therapeutic aPTT value of approx. 60 sec, when sampled at 3 hours of CRRT, can be estimated by ACT. We consider that ACT can be a complementary analysis in monitoring anticoagulation in CRRT, it is even a welcome predictor, especially when we suspect heparin resistance or have discordant aPTT values.

**Conflict of Interests**
The authors declare no conflict of interest.
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**Funding**
This research received no external funding.

**Informed Consent**
Before starting data collection, written, informed consent was obtained for all patients or from the next of kin.

**Ethics Approval**
The study was approved by the Ethical Committee of the University of Medicine and Pharmacy Iuliu Hatieganu (No. 102/ March 2017) and Cluj-Napoca County Hospital (No. 13911/29.05.2017).

**Data Availability**
The database is available by request to the corresponding author.

**Authors’ Contributions**
Florin Anton: Data curation, Writing- Original draft preparation, Visualization, Investigation. Paul Rus: Writing - Reviewing and Editing. Natalia Hagău:Conceptualization, Methodology Supervision and Validation.

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