The acute hepatic phase response is defined as a reaction that includes hepatic synthesis of proteins, consisting in the increase of some proteins called positive acute phase proteins and the simultaneous decrease of others called negative acute phase proteins. This study describes this hepatic reaction, based on a series of consecutive determinations, at three different time intervals (right before a major surgery event, 24 and 48 hours after the intervention), of the plasmatic levels of transferrin, albumin, fibrinogen and C reactive protein (CRP). Subsequently, the data was analyzed using anovia 2019, version 0.9. The inferential statistics consisted in calculating an ANOVA test that compared the values at 24 hours and 48 hours versus the values right before the major surgery. The results were validated by calculating the p value (p<0.05) as well as conducting correlation tests by determining the Pearson coefficient which shows the values of CRP, fibrinogen, transferrin and albumin are independent of each other and do not interact. The multiple ANOVA comparative test reveals the lack of interaction between the values of determined proteins, regardless of the moment of determination.

Key words: albumin, transferrin, C reactive protein, fibrinogen, acute phase protein,
years old). Patients presenting associated hepatic or renal pathology that can lead to a decrease of albumin and transferrin levels in plasma, malnutrition and/or diarrheal disease were excluded from the study. Hemolized or lipemic serums were not investigated.

The determinations done for these patients were: total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin, CRP, fibrinogen, transferrin and albumin.

Determination of albuminemia was done using a dry chemistry method on Samsung LABGEO. The method is based on binding albumin with a pigment substance in an acidic environment. This binding produces a change in color from yellow to purple. This change is quantified using spectrophotometry. Normal values of serum albumin range between 3.5 - 5.0 g/dL. The reaction at the basis of determining the albuminemia level is:

\[
\text{Albumin} + \text{Brooches purple (BCP)} \rightarrow \text{Ph. Acid BCP} - \text{Albumin Complex}
\]

Total proteins were determined on Samsung LABGEO. The method used was based on the reaction of these proteins with Cu\(^{+2}\) and measuring the resulting complex using spectrophotometry. Normal values of total proteins range between 6 - 8 g/dL.

Total protein + Cu\(^{+2}\) → OH - Protein-Cu complex

The determination of liver transaminase levels (ALT and AST) was based on the colorimetric method done on Samsung LABGEO. Bilirubin levels were measured using the enzyme oxidation method. The precision of the measurements were evaluated according to the standard guidelines issued by the Institution of Clinical Studies, USA. The precision was evaluated using BIO-RAD level 1-3 and the result showed that the variation coefficient was less than 5% in each determination.

To determine the plasmatic transferrin levels, the method was imunenephelometry, using BN ProSpec system by Siemens. The principle used by this method is based on forming complexes with specific antibodies. This complex diffuses a light beam which runs through the test. The intensity of the diffused light beam is proportional with the relevant protein concentrate in the test. The result is assessed by comparing it with a standard concentration. Normal transferrin levels in the plasma are between 2.00-3.60 g/L in subjects older than 19 years.

To determine the fibrinogen level in the plasma, we used Diagnostica Stago STA fully automated Coagulation Analyzer by Artisan Technology Group. This analyzer determines the fibrinogen concentration in the plasma quantitatively using the Clauss clotting method. This testing method involves the measuring the rate of fibrinogen to fibrin conversion in a diluted sample under the influence of excess thrombin. Since under these conditions the fibrinogen content is rate limiting, the clotting time can be used as a measure of the concentration of the fibrinogen and in fact the clotting time is inversely proportional to the level of fibrinogen in the plasma. Normal values are between 150-400 mg/dL.

CRP was determined quantitatively in vitro using Immunoturbidimetric assay on Roche/Hitachi Cobas c501 by Siemens. CRP in human plasma agglutinates with latex particles coated with monoclonal-CRP-antibodies. The aggregates are determined turbidimetrically. Normal values range between 0.00 and 0.50 mg/dL.

This study was approved by the Ethical Committee of Arad Clinical County Hospital. Written consent was obtained from all participants.

The statistical part of this study is based on descriptive statistics and inferential statistics. The inferential statistics are comprised of ANOVA tests as well as correlation tests. In both situations we consider a statistically significant \(p<0.05\). (\(p\) representing the error probability)

### Results and discussions

Results are presented in the tables below, our results indicate that the variances and standard deviations are low in case of negative acute phase proteins and high in case of positive acute phase proteins.

<table>
<thead>
<tr>
<th>Descriptives</th>
<th>ALBUMIN</th>
<th>FIBRINOGEN</th>
<th>CRP</th>
<th>TRANSFERRIN</th>
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<td>250</td>
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<td>2.70</td>
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<tr>
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<td>0.460</td>
<td>60.9</td>
<td>17.1</td>
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<tr>
<td>75th percentile</td>
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<tr>
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<tr>
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<td>5</td>
<td>1.50</td>
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<tr>
<td>Maximum</td>
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<td>870</td>
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<td>3.02</td>
<td>364</td>
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<td>50th percentile</td>
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<tr>
<td>75th percentile</td>
<td>3.40</td>
<td>408</td>
<td>90.0</td>
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From the inferential statistics, our results demonstrate that ANOVA test is validated in case of all determined proteins.

We also conducted correlation tests between the values of albumin, transferrin, CRP and fibrinogen test results before the major surgery, 24 hours and 48 hours after the intervention.

We also evaluated the correlation between of albumin and total protein test results in order to show the tests performed above are accurate (Table 11).

Multiple comparison ANOVA tests were also performed (Table 12 and 13)

The descriptive statistics that the standard deviations and variations are low in case of negative acute phase proteins and different in case of positive acute phase proteins.

All the differences between the mean values of each protein (before the major surgery, at 24 and 48 hours after) were statistically significant (p<0.001).

We observed the same trend change in case of albumin and transferrin but a different trend change in case of fibrinogen and CRP. This may be explained by the molecular structure of these proteins and the common amino acid substrate needed for synthesis [34]. Albumin and transferrin have a relatively similar composition. Albumin has a molecular mass of 68 kda and 585 amino acids in its structure, while transferrin has a molecular mass of 79 kda and 670 amino acids in its structure [43, 44]. The molecular structure of these proteins was entirely described using the cyanogens cleavage method. The fragments obtained were analyzed and the results showed the abundance of tyrosine, lysine and particularly glutamic acid, in case of both proteins [46,47].

---

Table 3
VALUES OF ALBUMIN, FIBRINOGEN, CRP AND TRANSFERRIN 48 hours AFTER THE MAJOR SURGERY

Table 4
DIFFERENCE IN FIBRINOGEN AT THREE DIFFERENT TIME INTERVALS: BEFORE THE MAJOR SURGERY, 24 AND 48 hours AFTER THE INTERVENTION AS WELL AS BETWEEN 24 and 48 hours

Table 5
DIFFERENCE IN PLASMA ALBUMIN AT THREE DIFFERENT TIME INTERVALS: BEFORE THE MAJOR SURGERY, 24 AND 48 HOURS AFTER INTERVENTION AS WELL AS BETWEEN 24 AND 48 hours

Table 6
DIFFERENCE IN PLASMA TRANSFERRIN AT THREE DIFFERENT TIME INTERVALS: BEFORE THE MAJOR SURGERY, 24 AND 48 hours AFTER THE INTERVENTION AS WELL AS BETWEEN 24 and 48 hours

Table 7
DIFFERENCE IN PLASMA CRP AT THREE DIFFERENT TIME INTERVALS: PRE-SURGICAL TRAUMA, 24 AND 48 hours AFTER THE INTERVENTION AS WELL AS BETWEEN 24 AND 48 hours

After obtaining these values (p<0.001), we proceeded in illustrating the graphics in order to compare the changes between albumin and transferrin and also, between fibrinogen and CRP.

We also conducted correlation tests between the values of albumin, transferrin, CRP and fibrinogen test results before the major surgery, 24 hours and 48 hours after the intervention.

We also evaluated the correlation between of albumin and total protein test results in order to show the tests performed above are accurate (Table 11).

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CRP has a molecular mass of 115 kda and 1200 amino acids in its structure while fibrinogen (being one of the largest hepatic protein) has a mass of 340 kda and 2400 amino acids in its structure [33-37]. If we consider the same amino acid substrate needed for protein synthesis [35], we can explain the same trend change in case of negative acute phase proteins and the differences found in case of positive acute phase proteins (graph 2 shows the slight increase after 24 hours in case of fibrinogen compared to CRP) [41]. Fibrinogen, being one of the largest acute phase proteins, requires for its synthesis a higher number of amino acids [42]. This is how we could explain the slight increase of fibrinogen in the plasma particularly in the second day after the intervention when it is compared with the increase of CRP in the plasma.

**Graph 1**
Changes of plasma values of transferrin and albumin test results at 24 hours and 48 hours

**Graph 2**
Changes of plasma values of CRP and fibrinogen test results at 24 hours and 48 hours

### Table 1

<table>
<thead>
<tr>
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<tr>
<td>CRP</td>
<td>17.3</td>
<td>71.9</td>
<td>146</td>
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<tr>
<td>Fibrinogen</td>
<td>720</td>
<td>444</td>
<td>572</td>
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### Table 8

<table>
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<tr>
<th></th>
<th>ALBUMIN</th>
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<td></td>
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<tr>
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<tr>
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<tr>
<td></td>
<td>p-value</td>
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<td></td>
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<tr>
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<td>Pearson's r</td>
<td></td>
<td>-0.009</td>
<td>-0.012</td>
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<tr>
<td></td>
<td>p-value</td>
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<tr>
<td>TRANSFERRIN</td>
<td>Pearson's r</td>
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<tr>
<td></td>
<td>p-value</td>
<td>0.925</td>
<td>0.656</td>
<td>0.924</td>
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Correlation tests do not show any interaction between these four proteins at all three moments of determination. The only validated correlation (p < 0.001) is between albumin and total protein [38]. This strong correlation is explained by the fact that the albumin concentration determines around 60% of total protein concentration.

The statistical tests show that there are no interactions between the values of transferrin and albumin on one hand and fibrinogen and CRP on the other. Furthermore, we conducted the multiple ANOVA correlation test which showed no interaction between albumin, transferrin, fibrinogen and CRP in the plasma at all three moments of determination.

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A major limitation of this study is that the interleukins were not determined (IL1 and IL6). As a result, we are not able to clearly evaluate the relation between these interleukins and acute phase proteins. Further studies should be performed to investigate this aspect as well. So far, it has been demonstrated that it is very hard to highlight a correlation between the values of interleukins and the values of these plasma proteins [52].

There should also be mentioned that in case of systemic inflammation, acute phase proteins are synthesized also in the vascular endothelium and macrophages besides the liver. This secondary synthesis does not seem to contribute significantly to either the normal values in the plasma or to
increase the concentration in case of the acute phase response [45].

Conclusions
From this study, it can be concluded that positive acute phase proteins have different trend changes when compared to negative acute phase proteins. So far, the main explanation accepted by the majority of authors is the common amino acid substrate needed for protein synthesis. This substrate is very limited due to protein catabolism which occurs in the setting of systemic inflammation. According to this study, the protein synthesis depends on the molecular mass of each protein and even the types of amino acids found in their structure.

In conclusion, protein synthesis in the acute phase response is a highly complex mechanism which involves multiple factors and this study has demonstrated that these processes are independent of each other and further research is needed to clearly understand the phenomenon.

References
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