Evaluation of Plasmatic Levels of Negative and Positive Acute Hepatic Phase Proteins in Patients who Underwent Major Surgery

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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 Jamovi 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,

The acute hepatic phase response occurs as a result of tissue trauma, infection or a systemic inflammation. It is triggered in the liver by interleukin 1(IL1) and interleukin 6(IL6). This reaction has been proved to be the main mechanism of decreasing the plasmatic level of albumin in patients undergoing major surgery [1,48].These interleukins are particularly produced by the macrophages in the damaged tissue [2,3]. This acute phase reaction belongs to a systemic response, coordinated by interleukins [4].

An acute phase protein is considered a protein which changes its plasmatic concentration with at least 25% of its initial value [24]. The role of positive acute phase proteins is mainly to stimulate the innate immune system by activating the complement system as well as antibody opsonization [51].

These positive acute phase proteins are divided into two groups. The first one is represented by the serum amyloid, C reactive protein(CRP), Complement 3, Complement 4 and alpha glycoproteins. Their synthesis in the liver is initiated by the IL1-like cytokines, which comprises different types of IL1-cytokines and tumor necrosis factors[25,26]. The second group is represented by fibrinogen, alpha 1 antichymotripsyn, alpha 1 trypsin, and alpha 2 macroglobulin. These proteins are also synthesized by the liver in response to IL6 as well as lL6-like cytokines (leukemia inhibitor factor, oncostatin, M,cardiotrophin-1) [27-29].

The action mechanism of both interleukins is similar. It consists in binding to a specific receptor. The receptor for IL1 has a molecular mass of 80 kda[5-7] and the receptor for IL6 has a molecular mass of 68 kda[9-11]. Structurally, both receptors belong to the immunoglobulin superfamily. Their concentration, low in normal conditions, increases in the liver as a response to systemic inflammation [8].

The genetic transcription factor plays an essential role in the synthesis of these proteins and is produced by two separate mechanisms [18-20]. First, in case of IL1, the enzymatic system is stimulated by the synthesis of ceramide proteins which activate protein kinase [16,17]. Second, in case of IL6, the enzymatic system is stimulated by the phosphorylation of tyrosine [12-15].

This genetic transcription factor is represented by messenger Ribonucleic Acid (mRNA) and has a role in transporting the genetic information from the nucleus to ribosomes. Genetic information codes amino acid sequences into protein structures [21-23].Finally, protein synthesis takes place in this ribosomes by binding mRNA with transport Ribonucleic Acid (tRNA).

In order for these proteins to be synthesized in the ribosomes, an amino acid substrate and an energetic substrate need to exist beside the genetic transcription factor [30,31].

Former authors indicate that acute positive phase proteins increase as the negative proteins decrease. These authors consider that the available amino acid substrate is limited in the setting of systemic inflammation, due to the catabolism of proteins[32].

Negative acute phase proteins are mainly represented by albumin(its main role being to determine the colloid-osmotic pressure) and transferrin(a plasmatic globulin which has the role in transporting iron from the blood to its storages found in the liver, spleen and bone marrow) [49].

The latest study done on this topic is from 2005 where Gruys et.al. studied the acute phase reaction in animals [48]. To our knowledge, our study is the first one on humans, which determines fibrinogen, CRP, albumin and transferrin and compares them statistically.

Experimental part

Material and methods

Sixty two patients were included in this study. Thirty patients were male and thirty two were female, the average age was 55, 3 years old(ranging from 39 years old to 91

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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. Hemolyzed 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:

Albumin + Brooches purple (BCP) → Ph. Acid BCP - Albumin Complex

Total proteins were determined on Samsung LABGEO. The method used was based of 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.

Descriptives

	ALBUMIN	FIB	CRP	TRANSFERRIN
N	62	62	62	62
Missing	0	0	0	0
Mean	4.13	270	17.3	2.74
Median	4.10	260	12.0	2.70
Standard deviation	0.460	60.9	17.1	0.321
Variance	0.211	3715	293	0.103
Minimum	3.20	120	1.00	2.10
Maximum	4.80	440	85.0	3.40
25th percentile	3.80	230	6.11	2.50
50th percentile	4.10	260	12.0	2.70
75th percentile	4.47	298	24.8	2.98

Table 1
VALUES OF ALBUMIN, FIBRINOGEN, CRP AND TRANSFERRIN PRIOR TO THE MAJOR SURGERY

Descriptives

	ALB /24	FIB /24	CRP /24	TRANSFERRIN /24
N	62	62	62	62
Missing	0	0	0	0
Mean	3.24	444	71.9	1.96
Median	3.20	440	74.0	2.00
Standard deviation	0.262	105	41.1	0.263
Variance	0.0687	11085	1692	0.0690
Minimum	2.70	270	5	1.50
Maximum	3.60	870	160	2.40
25th percentile	3.02	364	35.0	1.72
50th percentile	3.20	440	74.0	2.00
75th percentile	3.40	498	90.0	2.20

Table 2
VALUES OF ALBUMIN, FIBRINOGEN, CRP AND TRANSFERRIN - 24 hours AFTER THE MAJOR SURGERY

Descriptives				
	ALB /48	FIB /48	CRP /48	TRANSFERRIN /48
N	62	62	62	62
Missing	126	126	126	126
Mean	2.99	572	146	1.60
Median	3.00	565	145	1.60
Standard deviation	0.275	143	65.4	0.229
Variance	0.0759	20518	4272	0.0526
Minimum	2.50	340	31	1.30
Maximum	3.50	980	350	2.10
25th percentile	2.73	450	95.3	1.40
50th percentile	3.00	565	145	1.60
75th percentile	3.20	678	180	1.80

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

Tukey Post-Hoc Test – FIB 0 -24 -48				
		1	2	3
1	Mean difference	—	-174	-302
	p-value	—	< .001	< .001
2	Mean difference		—	-127
	p-value		—	< .001
3	Mean difference			—
	p-value			—

From the inferential statistics, our results demonstrate that ANOVA test is validated in case of all determined proteins.

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

Tukey Post-Hoc Test – ALB 0 -24-48				
		1	2	3
1	Mean difference	—	0.887	1.140
	p-value	—	< .001	< .001
2	Mean difference		—	0.253
	p-value		—	< .001
3	Mean difference			—
	p-value			—

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

Tukey Post-Hoc Test – TRANSFERRIN 0 -24 -48				
		1	2	3
1	Mean difference	—	0.777	1.132
	p-value	—	< .001	< .001
2	Mean difference		—	0.355
	p-value		—	< .001
3	Mean difference			—
	p-value			—

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

Tukey Post-Hoc Test – CRP 0 -24 -48				
		1	2	3
1	Mean difference	—	-54.6	-129.2
	p-value	—	< .001	< .001
2	Mean difference		—	-74.6
	p-value		—	< .001
3	Mean difference			—
	p-value			—

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).

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

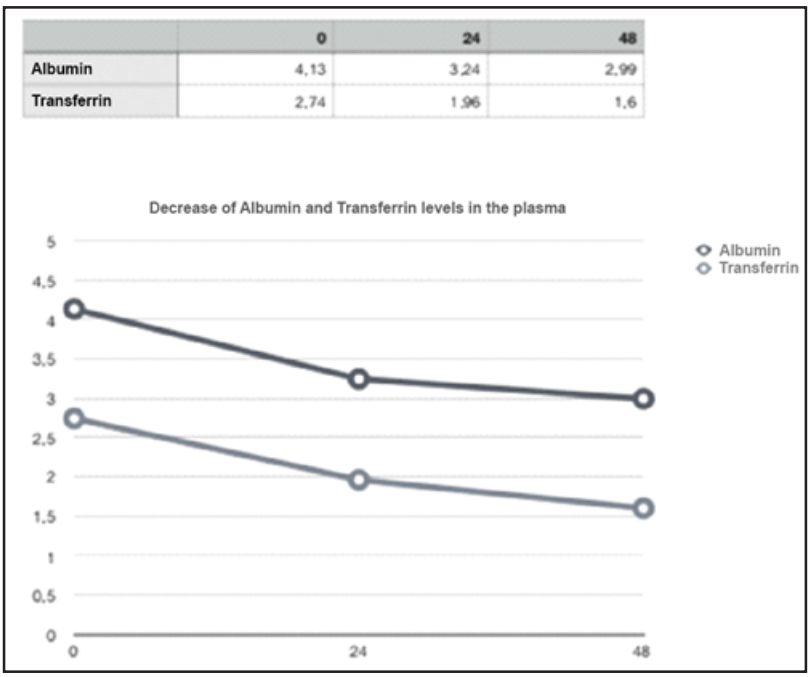
The descriptive statistics that the standard deviations and variations are low in case of negative acute phase proteins and different in case of positive acute phase proteins.

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].

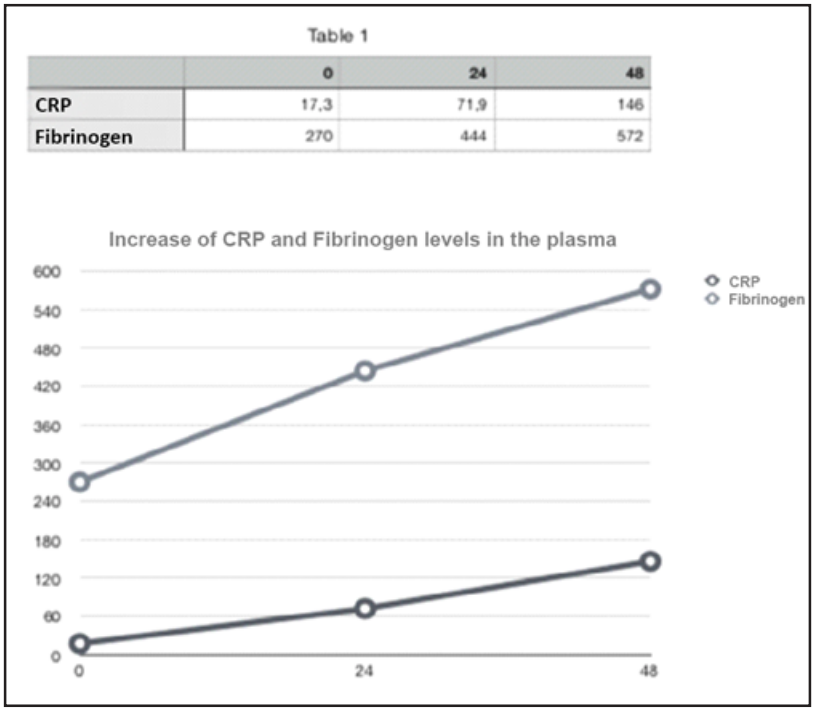
	0	24	48
Albumin	4,13	3,24	2,99
Transferrin	2,74	1,96	1,6



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

Table 1

	0	24	48
CRP	17,3	71,9	146
Fibrinogen	270	444	572



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

Correlation Matrix

		ALBUMIN	FIB	CRP	TRANSFERRIN
ALBUMIN	Pearson's r	—			
	p-value	—			
FIB	Pearson's r	0.056	—		
	p-value	0.667	—		
CRP	Pearson's r	-0.009	-0.074	—	
	p-value	0.948	0.568	—	
TRANSFERRIN	Pearson's r	-0.010	-0.051	-0.012	—
	p-value	0.936	0.696	0.924	—

Table 8
CORRELATIONS BETWEEN THE VALUES OF ALBUMIN, TRANSFERRIN, CRP AND FIBRINOGEN TEST RESULTS BEFORE THE MAJOR SURGERY

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.

Correlation Matrix

		ALB /24	FIB /24	CRP / 24	TRANSFERRIN / 24
ALB /24	Pearson's r	—			
	p-value	—			
FIB /24	Pearson's r	0.364	—		
	p-value	0.004	—		
CRP / 24	Pearson's r	-0.108	0.032	—	
	p-value	0.403	0.807	—	
TRANSFERRIN / 24	Pearson's r	0.162	0.280	-0.225	—
	p-value	0.208	0.028	0.079	—

Table 9
CORRELATIONS BETWEEN THE VALUES OF ALBUMIN, TRANSFERRIN, CRP AND FIBRINOGEN TEST RESULTS 24 hours AFTER THE MAJOR SURGERY

		ALB /48	FIB /48	CRP /48	TRANSFERRIN /48
ALB /48	Pearson's r	—			
	p-value	—			
FIB /48	Pearson's r	-0.017	—		
	p-value	0.899	—		
CRP /48	Pearson's r	-0.203	0.298	—	
	p-value	0.113	0.019	—	
TRANSFERRIN /48	Pearson's r	-0.046	0.199	-0.105	—
	p-value	0.724	0.121	0.416	—

Table 10
CORRELATIONS BETWEEN THE VALUES OF ALBUMIN, TRANSFERRIN, CRP AND FIBRINOGEN TEST RESULTS 48 hours AFTER THE MAJOR SURGERY

Correlation Matrix

		ALBUMIN	TP
ALBUMIN	Pearson's r	—	
	p-value	—	
TP	Pearson's r	0.633	—
	p-value	< .001	—

Table 11
CORRELATIONS BETWEEN ALBUMIN AND TOTAL PROTEIN TEST RESULTS

	Sum of Squares	df	Mean Square	F	p
Albumin	33.35	2	16.6733	414	< .001
Residual	4.91	122	0.0402		
Transferrin	31.19	2	15.5953	641	< .001
Residual	2.97	122	0.0243		
Albumin ✻ Transferrin	1.93e-28	4	4.82e-29	1.59e-10	1.000
Residual	7.40e-17	244	3.03e-19		

Table 12
MULTIPLE ANOVA TEST COMPARING ALL THE VALUES OF ALBUMIN AND TRANSFERRIN AT THE THREE DIFFERENT TIME INTERVALS (BEFORE THE MAJOR SURGERY, 24 AND 48 hours AFTER THE INTERVENTION)

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
CRP	391376	2	195688	219	< .001
Residual	108845	122	892		
FIBRINOGEN	2.13e +6	2	1.07e +6	225	< .001
Residual	578554	122	4742		
CRP ✻ FIBRINOGEN	2.67e-25	4	6.68e-26	-1.40e-13	1.000
Residual	-1.16e-10	244	-4.77e-13		

Note. Type 3 Sums of Squares

Table 13
MULTIPLE ANOVA TEST COMPARING ALL THE VALUES OF CRP AND FIBRINOGEN AT THE THREE DIFFERENT TIME INTERVALS (BEFORE THE MAJOR SURGERY, 24 AND 48 hours AFTER THE INTERVENTION)

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.

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 synthetized 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.

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