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Comparative analysis of hepatic cytolysis biochemical parameters and of quantitative changes in protein fractions in patients with chronic hepatitis

Gabriela Cucu (Pîrîianu) [1], Bogdan Ionescu [1,*], Diana Ionescu [1], Mariana Carmen Chifiriuc [1], Grigore Mihaescu [1]

[1]Faculty of Biology, University of Bucharest; Research Institute of the University of Bucharest, Romania

*corresponding author e-mail address:***bogdanionescu87@yahoo.com**

ABSTRACT

Globally, chronic viral hepatitis B and C is a serious health problem, affecting one in 12 people. Out of 1825 patients with known liver disease admitted to Witing hospital between 01.10.2010 and 12.30.2012, 70 patients were selected for this study, based on the infectious etiology of the liver disease, with type B or C hepatitis viruses, being investigated for serum proteins and major immunoglobulins levels. The 21-40 years group has the largest number of patients with quantitative changes in liver enzymes levels, with a significant predominance of cases in the urban population. In case of the studied group, out of the tested parameters, the γ- globulins (G) were significantly increased, while the β proteins fraction was only slightly increased. The albumins (A) fraction and the A/G ratio were decreased. A great variation among patients with different pathologies has been observed for the γ- globulins percentage values, the highest values being registered in patients with cirrhosis, hepatic failure and evolutive chronic hepatitis. Increased titers of polyclonal γ-globulin fraction indicate a chronic immunological process associated with liver disease (chronic active hepatitis, cirrhosis). The average values obtained for IgA percentages in patients with hepatic afflictions have been slightly higher, with high individual variations, the highest values being registered for evolutive chronic hepatitis and hepatic failure, while the lowest ones in cirrhosis, demonstrating that quantitative immunological methods are essential for the diagnosis and monitoring of treatment effectiveness in case of persistent infections.

Keywords: *chronic hepatitis, immunogram, protein electrophoresis, IgA, γ- globulins, A/G ratio*.

1. INTRODUCTION

Globally, hepatitis B and C affects one in 12 people. Infections with the 2 viruses are considered 'silent killers' because of their asymptomatic nature, but the complications associated with these infections are potentially lethal being represented by cirrhosis or liver failure [1]. Viral hepatitis B is a serious health risk, given that currently, globally 350 million people are HBV positive [2,3]. Studies have estimated that about 4 million people are infected with hepatitis C annually, in addition to the already existent 180 million infected people. So more than 1.5 million people are infected with the virus B or C, which can lead to cirrhosis or liver failure [4,5]. In Romania, the prevalence of hepatitis B is 4.37% of the total population and 3.23% in the case of hepatitis C as shown in an epidemiological study [6]. Chronic viral infection with viruses B and C causes direct and indirect pathological effects mediated by persistent immune complexes [7]. The clinical analysis undertaken in laboratories effectively contributed to the development of the theoretical knowledge in the field of hepatitis pathology [8,9]. The purpose of this paper was to establish the correlation between hepatic cytolysis evidenced by biochemical analysis and quantitative changes in protein fractions.

2. EXPERIMENTAL SECTION

Out of 1825 patients with known liver disease admitted to Witing hospital between 01.10.2010 and 12.30.2012 which presented major changes in the levels of ALT and AST liver enzymes, 70 patients were selected for this study. Blood samples were collected from these patients in order to perform laboratory analyses. The patients included in the study suffered from liver diseases in different stages, consecutive to infection with type B or C hepatitis viruses. The rest of the patients were excluded from this study based on the fact that their liver afflictions were not generated by HBV or HCV. A paraclinical evaluation of each patient including biochemical and immunochemical analyses was performed. Changes in protein fractions of serum IgA, IgG and IgM antibodies were observed. Electrophoresis on agarose gel was performed for the separation of serum proteins. In this purpose, the blood samples were collected into vacuum containers without anticoagulant with / without separator gel. The serum was separated by centrifugation 15 min at 3000 rpm/min and refrigerated (at 2-8 ° C) for no more than 72 hours, because in the case of sera older than 72 hours the β, β1 and β2 fractions cannot be separated. In the two compartments of the migration chamber 45 ml of buffer were distributed. 5µl of diluted serum were applied on the gel in each slot of the device and left for 5 minutes. The excess gel wad removed using filter paper. The gel sheet wad curved downwards with the gel sheet and placed to assure the sera migration towards the cathode. The migration lasts 15 minutes at 100V. Following migration the gel was inserted into the fixing solution for 10 min, then dried in hot air.

3. RESULTS SECTION

From a total of 1825 patients with changes in liver enzyme levels a group of 70 patients was selected, in the case of which the serum protein electrophoresis and the immunoglobulins serum levels were determined. The study population was divided in groups by age, i.e. 0-20, 21-40, 41-60 and >60 years and sex criteria (Fig. 1).

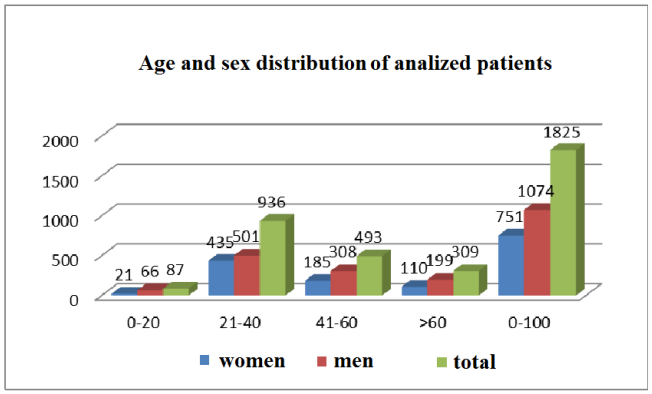

Figure 1. Distribution of studied population depending on sex and age.

The 21-40 years group has the largest number of patients with quantitative changes in liver enzymes levels, namely 936 of which 435 were women and 501 men, with a significant predominance of cases in the urban population (Fig. 1, 2). Based on disease severity 35 women and 35 men were selected for further electrophoretic and immunochemical determinations. The study group was compared with a control group of 15 patients, 9 men and 6 women, with no conditions that would cause changes in serum protein electrophoresis fractions or in the immunogram as revealed by the normal values shown in Tables 1 and 2.

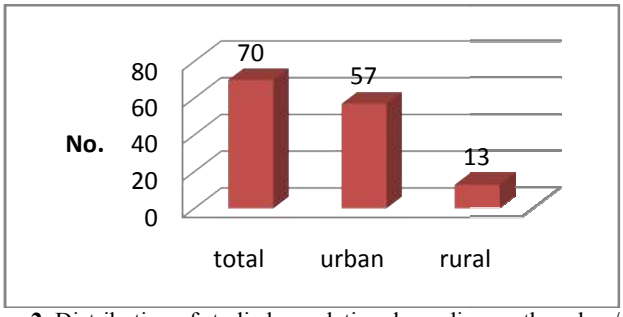

Figure 2. Distribution of studied population depending on the urban/rural provenience.

The pathology of the selected group was dominated by chronic viral hepatitis (Fig. 3).

In case of the studied group, out of the tested parameters, the γ- globulins (G) were significantly increased, while the β proteins fraction was only slightly increased. The albumin (A) fraction and the ratio A/G were decreased (Table 3).

In case of β proteins fraction, despite the average value indicated an increased level comparatively with control, the individual variation showed that the levels of this parameter were either lower or higher than the normal values, depending on the primary disease (Fig. 4). It is to be noticed the lower values registered in patients with cirrhosis, hepatic failure and evolutive chronic hepatitis.

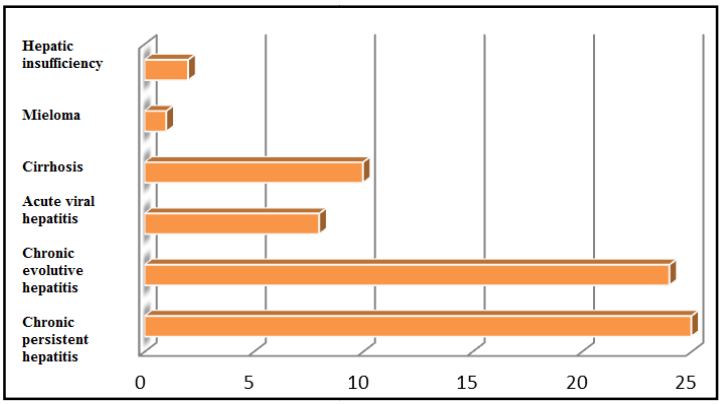

Figure 3. The distribution of cases in accordance with the primary pathology.

A great variation among patients with different pathologies has been observed for the γ- globulins percentage values, the highest values being registered in patients with cirrhosis, hepatic failure and evolutive chronic hepatitis (Fig. 5).

Concerning the A and the A/G ratio values, the lowest values have been also registered in patients with cirrhosis, hepatic failure, acute viral and evolutive chronic hepatitis (Fig. 6, 7).

The quantitative determination of IgA, IgG and IgM has great value for diagnosis of a wide range of diseases. Therefore, the IgA assay is recommended for: assessing the humoral component of the immune response and the monitoring of treatment in IgA myeloma. IgG represents 75% of total immunoglobulin levels. They are major antibodies produced in response to secondary antigenic stimulation. IgG is the only Ig transferred through the placenta and determines the state of passive immunity in the newborn. IgG neutralizes bacterial toxins and acts as an opsonin. Inherited (congenital) or acquired IgG deficiency increases the risk of bacterial infections. On the other hand, the IgG levels increase in immunocompetent individuals in response to a variety of infectious (bacteria and viruses) or inflammatory processes. Determination of specific IgM and IgG titers is indicative of a recent infection. A major cause of increased polyclonal IgG levels is the acquired immunodeficiency syndrome. Increased levels of polyclonal IgG also are registered in multiple sclerosis and in some chronic hepatitis cases. IgM antibodies are produced during primary immune responses. IgM is the first class of immunoglobulins synthesized by the fetus (after antigenic stimulation of the placental transfer path) and the newborn. The determination of specific IgM is useful for assessing the stage of infection (in acute infection IgM is synthesized in comparison to IgG which predominates in chronic infections) and the probability of congenital infections (IgM in newborns is evidence of a transplacental viral infectious process). The IgG in the serum of a newborn infant is of maternal origin. The hyper-IgM syndrome is characterized by the absence of IgG and IgA in

the serum, associated with a marked increase in IgM levels indicative of an immunodeficiency. Increased levels of polyclonal IgM can be found in various infectious or inflammatory conditions. IgM level is typically high in primary biliary cirrhosis. Decreased IgM levels are found in congenital or acquired hypo-γ-globulinemia. Determination of IgM is recommended for: assessing the humoral component of the immune response; diagnosis and treatment monitoring in patients with Waldenström macroglobulinemia; assessing the likelihood of an uterine infection. Quantification of viral specific IgM levels is very important, as it allows the diagnosis of an infection from a single serum sample taken during the acute phase of the infection. The method is applicable on an infection with a sufficient incubation period required for the synthesis of detectable IgM titers at the time of the installation of the clinical symptoms. Diagnosis of viral infections is based on seroconversion, more specifically on a significant increase of IgM titers between the acute-phase sample and the sample collected after installation of clinical symptoms and significant increase of specific IgG titers. During the primary immune response, IgG anti-viral titer remains low while after the secondary contact, the titer increases rapidly, remaining elevated for a long time (years) and may persist throughout life. In the case of HCV, IgG class antibodies have different antigenic specificities, depending on the type of infection: persistent or progressive. Serological diagnosis can only be issued by highlighting increased IgM titers after the acute phase of infection or by analyzing elevated IgG levels in serum samples taken in the acute phase and the convalescent phase.

The average values obtained for IgM and IgG percentages in patients with hepatic afflictions have been within the normal limits, i.e. 13.77% for IgG and 2.64% for IgM, while for IgA have been slightly higher, i.e. 6.76%, with high individual variations, the highest values being registered for evolutive chronic hepatitis and hepatic failure, and the lowest ones in cirrhosis (Fig. 8).

Table 1. Results regarding quantitative evaluation of serum proteins in the control group.

No.	*Sex*	*Age*	*Habitat*	*Electrophoretic panel (%)*							
				Total proteins	*A%*	*α_1*	*α_2*	*β*	*γ*	*A/G*	*G*
1.	M	56	U	77	59	4	9	11	20	1.2	41
2.	M	30	U	61	58	5	9	8	19	1.3	42
3.	M	25	U	69	60	4	9	9	16	1.2	40
4.	M	45	U	66	53	5	10	11	17	1.2	47
5.	W	48	U	80	52	4	10	11	20	1.2	40
6.	M	25	U	80	53	5	11	10	20	1.2	47
7.	M	41	U	77	54	4	11	11	20	1.2	46
8.	M	38	U	66	51	5	11	11	18	1.2	49
9.	W	66	R	64	56	5	9	13	16	1.2	44
10.	W	28	U	60	56	4	10	10	20	1.5	44
11.	W	40	U	58	58	5	12	10	20	1.5	47
12.	W	67	U	74	50	5	12	10	17	1.2	48
13.	M	55	R	60	60	5	12	10	18	1.2	47
14.	M	71	R	64	58	4	12	10	18	1.4	44
15.	W	28	U	64	59	5	9	9	17	1.3	40
Normal values				**60-80**	**52-62**	**2-5**	**6-12**	**8-11**	**11-21**	**1.2-1.5**	**38-48**

Table 2. Ig percentages (IgA, IgG, IgM) established by the turbidimetric method for the control group.

No.	*Sex*	*Age*	*Habitat*	*IgA*		*IgG*		*IgM*	
				Value	*Normal*	*Value*	*Normal*	*Value*	*Normal*
1.	M	56	U	0.71	0.7-4	10.3	7-16	1.70	0.4-2.4
2.	M	30	U	2.21	0.7-4	9.21	7-16	2.21	0.4-2.4
3.	M	25	U	2.57	0.7-4	13.4	7-16	1.67	0.4-2.4
4.	M	45	U	3.95	0.7-4	15.2	7-16	0.76	0.4-2.4
5.	W	48	U	0.80	0.7-4	10.3	7-16	1.11	0.4-2.4
6.	M	25	U	2.66	0.7-4	15.0	7-16	1.12	0.4-2.4
7.	M	41	U	2.06	0.7-4	10.6	7-16	0.94	0.4-2.4
8.	M	38	U	0.97	0.7-4	12.2	7-16	1.96	0.4-2.4
9.	W	66	R	3.18	0.7-4	14.6	7-16	2.01	0.4-2.4
10.	W	28	U	0.71	0.7-4	9.21	7-16	2.16	0.4-2.4
11.	W	40	U	1.43	0.7-4	13.0	7-16	1.20	0.4-2.4
12.	W	67	U	1.16	0.7-4	9.6	7-16	1.54	0.4-2.4
13.	M	55	R	2.40	0.7-4	10.2	7-16	1.60	0.4-2.4
14.	M	71	R	1.42	0.7-4	15.3	7-16	2.06	0.4-2.4
15.	W	28	U	1.45	0.7-4	12.2	7-16	1.03	0.4-2.4

Table 3. Procentual determination of electroforetic fractions in the case of the patients with hepatic afflictions.

Investigated parameter	**Total Proteins**	***A* %**	***α_1* %**	***α_2* %**	***B* %**	***γ* %**	***A/G* %**	***G* %**
Normal values	60-80	52-62	2-5	6-12	8-11	11-21	1.2-1.5	38-48
Average values for the studied group	69.78261	43.5942	4.676471	9.927536	13.68116	28.49275	0.780145	55.98551

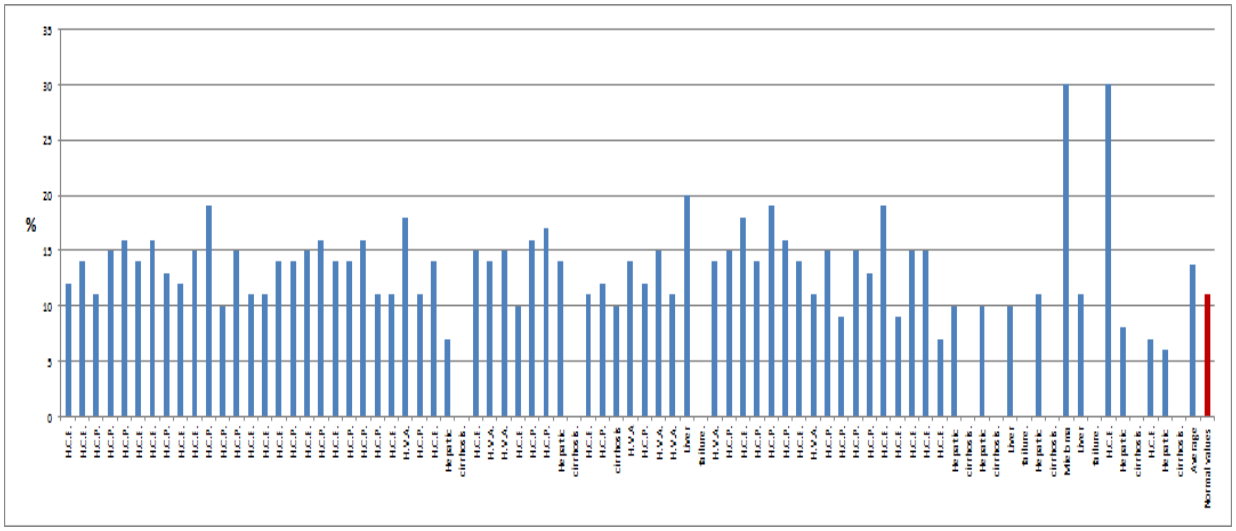

Figure 4. Distribution of ***β***- globulins percentage values within the analyzed group.

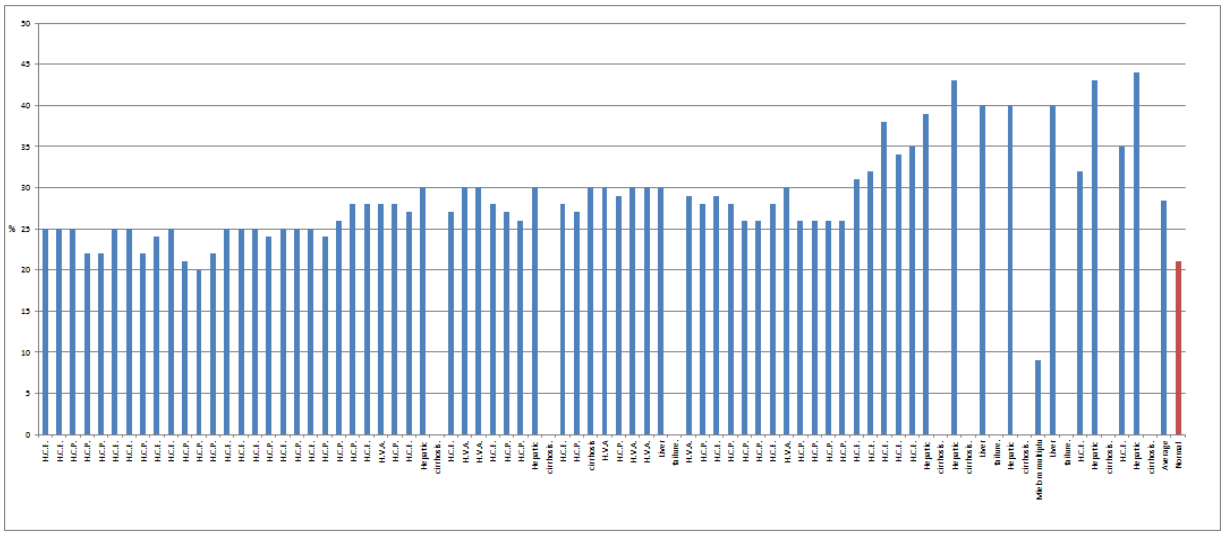

Figure 5. Distribution of γ- globulins percentage values within the analyzed group.

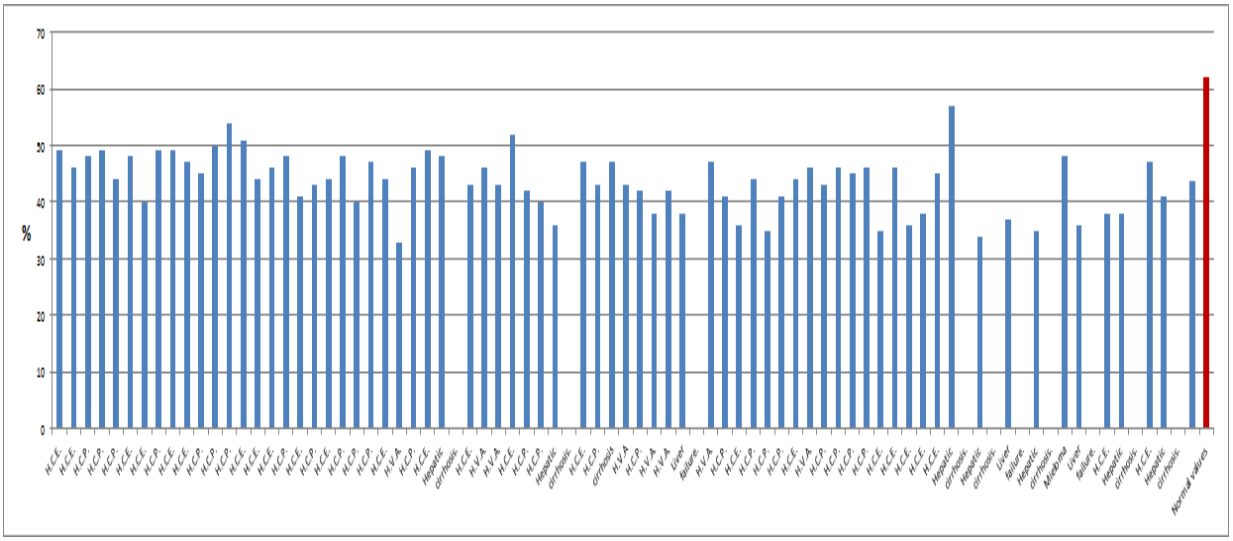

Figure 6. Distribution of *A* percentage values within the analyzed group.

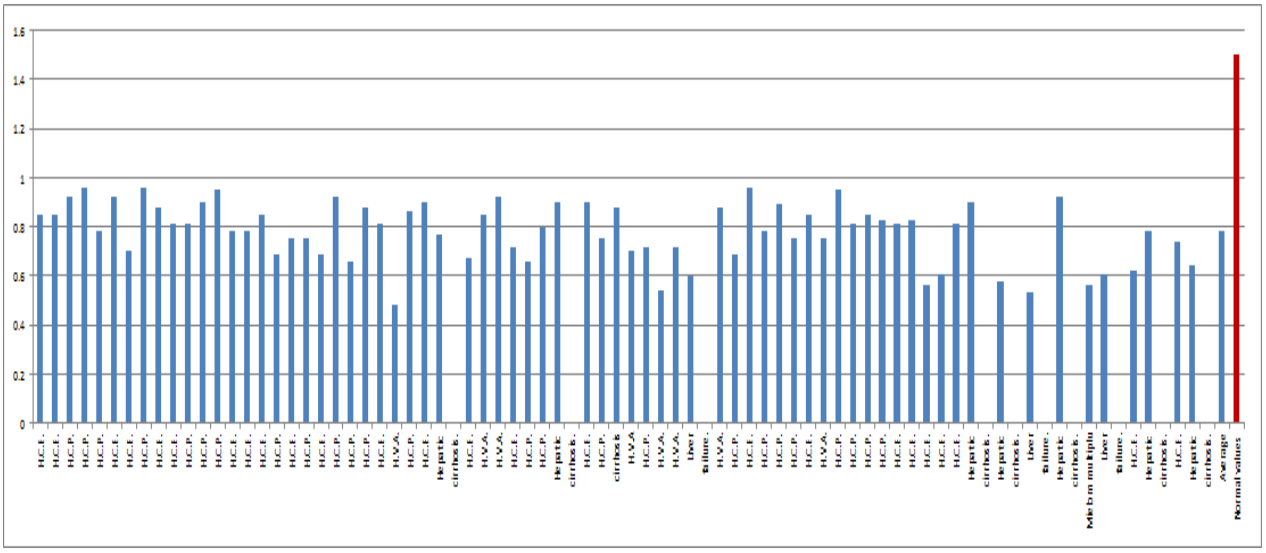

Figure 7. Distribution of *A/G* ratio values within the analyzed group.

4. CONCLUSIONS

The results of serum protein electrophoresis showed increased albumin and globulin levels, and decreased albumin/γ-globulin ratio for the majority of the tested patients. Increased titers of polyclonal γ-globulin fraction indicates a chronic immunological process associated with liver disease (chronic active hepatitis, cirrhosis). The average values obtained for IgM and IgG percentages in patients with hepatic afflictions have been within the normal limits, while for IgA have been slightly higher, but with high individual variations, demonstrating that quantitative immunological methods are essential for the diagnosis and monitoring of treatment effectiveness in case of persistent infections.

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