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USEFULNESS OF CD44 LEVEL AS A BIOMARKER IN DIAGNOSIS OF ACUTE LIVER REJECTION

By
ESAM M. BAYOMY[1], TAREK M. YOUSEF[1], KHALID M. MANSOUR[1], DOAA Z. ZAKY[2], AHMED M. EL GHANDOUR[1] AND MOHAMED B. AHMED[3]
Department of Internal Medicine and Gastroentrology[1], Department of Tropical Medicine[2] and Department of General Surgery and Liver Transplantation, Faculty of Medicine, Ain Shams University, Cairo 11566, Egypt
(*Correspondence: drdoaazakaria@gmail.com)

Abstract

The incidence of acute and chronic rejection has declined with improvement of immunosuppression regimens in liver transplant recipients. The CD44 protein belongs to a large family of type I Trans membrane glycoproteins and expressed on the surface of most vertebrate cells and is an important receptor for the components of extracellular matrix.

The study evaluated if the serum level of CD44 had a value in diagnosis of acute rejection depending on proved rejection by liver biopsy. The patients were recruited from ASCOT from May 2017 to December 2018. They were 20 adults with Post LDLT with elevated liver function tests and were reviewed.

The results showed that serum level of CD44 was significantly lower in the rejection group in our study in patient with post liver transplantation**.** There was highly significant difference between controls & patients \as regards serum level of CD44. Negative correlation between serum levels of CD44, WBCs & AST. There was highly significant difference between patients with different pathological findings as regards serum level of CD44. The best cut off point for serum level of CD44 as a marker of rejection was found ≤ 44ng/ml with sensitivity of 85% and specificity of 95% in diagnosis of acute cellular rejection.

Key words: Egyptian patients, Liver transplantation, CD44, Transplant immunology.

Introduction

Liver transplantation (LT) is considered as an established therapeutic option for patients with the acute and chronic liver failure and hepatocellular carcinoma (Graziadei *et al*, 2016). It evolved as a highly effective approach to treat many end-stage liver disease (ESLD) cases that had had no treatment before LT (Sagmeister *et al*, 2002). Patients with liver cirrhosis were more susceptible to infections, due to the alterations in the gut microbiota, intestinal barrier dysfunction, genetic predisposition, and immunodysfunction (Jalan *et al*, 2012). These immunocompromised patients are predisposed to develop bacterial infections and sepsis, due to endothelial alterations, leukocyte dysfunction, bacterial translocation, and iatrogenic factors (Acevedo and Fernandez, 2014). Moreover, the acute-on-chronic liver failure may develop as sequela of a superimposed bacterial infection or sepsis (Moreau *et al*, 2013).

Generally, many Egyptian authors declared that the Egyptian patients suffered from the increasing number of chronic LDs and LDs, due to the high prevalence of hepatitis C virus among the population, with an increasing need for LT (Sharaf-Eldin *et al*, 2016; Ahmad *et al*, 2017; 2018; Abd-Elsalam *et al*, 2018).

The first human orthotropic liver transplantation (LT) in Europe was performed by Sir Roy Calne in Cambridge in 1968 (Calne *et al*, 1968). One year after the first successful human liver transplantation reported by Thomas Starzl in the United States (Starzl *et al*, 1967). The LT has been evolved rapidly, as standard therapy for acute and chronic liver failure of all etiologies, with more than 80,000 procedures performed to date. Survival rates were improved significant in the last two decades, achieved rates of 96% & 71% at 1 & 10 years post-LT respectively (Adam *et al*, 2012**).** This great success was mostly attributable to several advances such

as the introduction of new immunosuppressive agents and preservation solutions, to the improvements in surgical techniques and to early diagnosis and management of complications after LT (Dutkowski *et al*, 2010). Despite the continuous optimization of immunosuppression protocols, the acute cellular rejection (ACR) occurred in about 15%-60% of liver allograft recipients in the first postoperative months (Rostaing *et al*, 2012).

In contrast to many aspects of clinical transplantation, the algorithm for diagnosing ACR has not changed since the advent of clinical transplantation in the 1960s. The diagnosis of ACR requires evidence of graft dysfunction (e.g., elevated aminotransferases), which was typically followed by confirmed allograft biopsy. Liver biopsy was the best organ for ACR diagnosis (Demetris *et al*, 2010).

The postoperative monitoring of liver transplant recipients in regard to ACR was based on measurements of transaminase levels and markers of bile synthesis and accumulation. In cases of parameters increase, a biopsy was indicated for histopathological validation and differentiation from other reasons for graft dysfunction, such as ischemia/reperfusion injury or recurrent hepatitis (Rook and Rand, 2011).

Biomarkers for noninvasive diagnosis and, ideally, for the prediction of ACR, would be a valuable tool for the postoperative care of liver transplant recipients. Such biomarkers could facilitate a considerably earlier diagnosis and enable individual risk stratification and the adjustment of immunosuppressive regimens to prevent rejection (Roedder *et al*, 2011).

When a foreign graft is inserted into a recipient, recipient leukocytes respond to the foreign antigens by producing soluble factors that induce both lymphocyte and antigen proliferation. Thus, cytokines as interleukins (IL) IL-1, IL-2, IL-5, IL-6, tumor necrosis factor (TNF), and g-interferon (g-IFN) may be expected to rise in response to an episode of acute rejection. Substances such as b2-microglobulin (b2-M), intercellular adhesion molecule- 1 (ICAM-1) and neopterin, which were induced by cytokines, may also be expected to be of potential use. However, secretion of these substances is a reflection of leukocyte activation and is not specific for rejection. The IL-1, TNF, & g-IFN measurement proved to be disappointing, with increases in all agents in rejection, infection, and other complications (Tilg *et al*, 1990).

The CD44 can be used as a biomarker for the blood test-based diagnosis of rejection in liver transplant recipients. These results may help us identify patients who are at risk for ACR in the first months after transplantation to improve the clinical care of liver transplant recipients (Raschzok *et al*, 2015).

CD44 is a family of type I transmembrane glycoproteins with a wide tissue distribution that is involved in various physiological and pathological processes, such as cell–cell and cell–matrix interaction, leukocyte extravasation, cell migration, and lymphocyte activation (Yan *et al*, 2015). Serum soluble CD44 can be generated through the proteolytic cleavage of membrane- or receptor-bound CD44 or by cell activation, for example, in autoimmune disorders (Katoh *et al*, 1994). Also, the involvement of CD44 in inflammatory T helper 1 immune responses, its role in regulatory T cell function (Sharma, 2017).

The study aimed to assess value of serum level of CD44 as a marker of rejection in patients with post liver transplantation

Materials and Methods

This study included two groups of Egyptian patients selected from Ain Shams Center of Organ Transplantation (ASCOT). Patients were recruited from May 2017 to December 2018. They were divided into two groups: G1 (patients): Twenty post Living Donor Liver Transplantation (LDLT) patients with elevated liver enzymes within first three months post-operative. G2 (control): Twenty persons with normal liver profile and free from any diseases (Donors preoperative were included). The study was explained, and written consents were taken from all patients. Patients with the following

criteria were excluded: 1- Patients with Infection excluded with CRP, Procalcitonin, culture and sensitivity of body fluids and secretions, 2- Patients with Vascular complication excluded with Hepatic Venous and Arterial Duplex, and 3- Patients with surgical biliary tract complications.

Patients assessments: History taking with special emphasis on: All patients were subjected to: age, sex, fever, date of surgery (LDLT), drug history, drug history of immunosuppression, complications and symptoms of hepatic decompensating pre-operative e.g. hematemesis, ascites, hepatic encephalopath….etc. Itching, abdominal pain, vomiting. Full physical examination included abdominal examination with special emphasis on jaundice, drains and wound.

Biochemical analysis: The following tests were done: Complete blood count (CBC), liver profile tests, alanine transaminase (ALT), aspartate transaminase (AST), alkaline Phosphatase, GGT, serum Bilirubin, serum Albumin and prothrombin time (PT). CRP, Procalcitonin, virology tests e.g. HCV RNA PCR, CMV PCR, EBV IgM, HBV DNA PCR, Culture and sensitivity of body fluids, secretions, serum level of immunosuppression drug and serum CD44 level.

Imaging: Ultrasound, hepatic venous and arterial duplex were done.

The liver biopsy and histopathological examination: ACR changes were divided into 3 pathological pictures; mixed (predominantly mononuclear activated lymphocytes, neutrophils & eosinophils) portal inflammation, bile duct inflammation/damage and subendothelial inflammation of portal veins or terminal hepatic venules (Choudhary *et al*, 2017).

Each of these parameters was scored as 1 to 3 and sum was called rejection activity index, thus a maximum score of 9 was possible. The various possible rejection grades were: a score of 0-2 without rejection, 3 borderlines (with consistent), 4-5 mild, 6-7 moderate and 8-9 as severe ACR. Higher rejection activity index did not translate into less response to steroid (Höroldt *et al*, 2006). CD44 serum level assay2.2.5: After collection of blood, blood was clot by leaving it undisturbed at room temperature, 10-20 minutes. Remove clot by centrifuging at 2,000-3,000rpm for 20 minutes. If precipitates appeared during reservation, the sample must be centrifuged again.

Statistical analysis: Data were analyzed by statistical package (SPSS) software version 13.0. Data were expressed as number and percentage and continuous data as in laboratory data as mean and SD. If data was normally distrusted Chi-square test (x^2) (test of significance in categorical data). Paired T test was used for comparing between 2 dependent means. If data was not normally disturbed Mann Whitney U test for two-group comparisons was used. P value above 0.05 was significant.

Ethics and consent: The participants provided written informed consent, and the study was approved by the Ain Shams University Faculty of Medicine Research Ethical Committee.

Results

The results were shown in tables (1, 2, 3, 4, 5, 6, 7, 8 & 9)

Table 1: Comparison between Age, Sex, Hx of HCC, Co-morbidity and Fever among controls

Variants		Control (n=20)	Patients (n=20)	P-value	Significant
Age	M±SD	29.80±4.25	42.60±12.73		
	Range	26- 41	16- 59	0.000	HS
sex	Male	14 (70.0%)	14 (70.0%)		
	Female	6 (30.0%)	6 (30.0%)	1.000	NS
Co-morbidities	No	20 (100.0%)	12 (60.0%)		
	DM	0 (0.0%)	2 (10.0%)	0.007	HS
	DM & Hypothyroidism	0 (0.0%)	6 (30.0%)		
Fever	No	20 (100.0%)	4 (20.0%)		
	Yes	0 (0.0%)	16 (80.0%)	0.000	HS

*:Chi-square test; •: Independent t-test

Table 2: Comparison between different labs (CBC, CRP, INR, PTT, Bili, AST, ALT) among controls & patients.

Variables		Control (n=20)	Patients (n=20)	Test value	P-value	Significant
Hb (gm/d)	M±SD Range	13.36±1.27 11.1-15.2	8.73±0.99 7.1- 10.6	12.877*	0.000	HS
Plt (× 10^3/ml)	M±SD Range	225.55±88.47 188- 325	127.40±92.61 32- 308	3.427*	0.001	HS
WBCs(× 10^3/ml)	M±SD Range	6.20±1.18 4.5- 8.5	7.62±5.22 1.8- 17.9	-1.186*	0.243	NS
CRP (mg/L)	M±SD Range	0.26±0.16 0- 0.5	2.86±3.80 0.07- 12.2	-3.058*	0.004	HS
INR	M±SD Range	1.05±0.07 0.9-1.1	1.27±0.29 1- 2	-3.278*	0.002	HS
PTT (sec)	M±SD Range	33.66±5.36 21.9- 43	37.85±5.77 31- 50.3	-2.380*	0.022	S
T. Bili (mg/dl)	M±SD Range	0.52±0.15 0.3- 0.7	11.86±9.80 1.2- 25.9	-5.171*	0.000	HS
D. Bili (mg/dl)	M±SD Range	0.17±0.08 0.1- 0.3	6.85±5.31 0.3- 13.7	-5.626*	0.000	HS
AST (U/L)	Median (IQR) Range	17 (16-20) 14- 28	59.5 (51- 94) 36- 244	-5.423ǂ	0.000	HS
ALT (U/L)	Median(IQR) Range	15 (1 -16) 11- 18	82 (71-136) 49-638	-5.421ǂ	0.000	HS

*= Independent t-test, ‡=Mann Whitney test

Table 3: Comparison between ALK, GGT, T. Prot, S.Alb, BUN, S.Creat, Na, K, P.Cal in controls & patients.

Variables		Control (n=20)	Patients (n=20)	Test value	P-value	Significant
ALK P (U/L)	M±SD Range	80.00±21.72 42-120	400.80±280.56 126-909	-5.098*	0.000	HS
GGT (U/L)	M±SD Range	30.00±12.19 12-48	378.80 ± 256.35 44-866	-6.078*	0.000	HS
T. Prot (g/dl)	Median(IQR) Range	7.2 (6.9-7.8) 5.9-71	5.8 (5.5 - 6.6) 3.8-7.6	-3.957ǂ	0.000	HS
S. Alb (g/dl)	M±SD Range	4.32±0.56 3.5-5.2	2.75±0.67 2-4.4	8.065*	0.000	HS
BUN (mg/dl)	M±SD Range	14.60±2.98 10-19	38.50±26.58 11-104	-3.996*	0.000	HS
S. Creat (mg/d)	M±SD Range	0.76±0.20 0.5-1.2	1.12 ± 0.41 0.6-1.9	-3.527*	0.001	HS
Na (m Eq/L)	M±SD Range	141.20±2.42 136-145	132.0±4.83 122-139	7.611*	0.000	HS
K (m Eq/L)	M±SD Range	4.34±0.38 3.8-5	3.95±0.74 2.3-4.9	2.094*	0.043	S
P. Cal (ng/ml)	M±SD Range	0.18±0.12 0-0.4	0.31 ± 0.13 0.1-0.5	-3.547*	0.001	HS

*=Independent t-test; ‡= Mann Whitney test

No significant difference between controls & patients as regards WBC but, significant difference as regards PTT with (P =0.022 and highly significant difference as regard Hb (P = 0.000), Plt (P = 0.001), CRP (P = 0.004), INR (P = 0.002), T. Bili (P = 0.000), D. Bili (P = 0.000) AST (P = 0.000), ALT (P = 0.000). There was highly significant difference between controls & patients \as regards ALK (P = 0.000), GGT (P = 0.000), T. protein (P = 0.000), S.Alb (P = 0.000), BUN (P = 0.000), S. ceat. (P = 0.001), Na (P = 0.000), PCal (P = 0.001) but significant difference as regards K (P = 0.043).

Table 4: Comparison between serum level of CD44 among controls & patients.

CD44	Control (n=20)	Patients (n=20)	Test value	P-value	Significant
Median(IQR) Range (ng/ml)	61.25 (51.25± 90) 35-120	14.5 (13±40.25) 6.5- 75	-4.460	0.000	HS

‡=Mann Whitney test

There was highly significant difference between controls & patients as regards serum level of CD44 with (P = 0.000) high with controls and low with patients.

Table 5: Comparison between PA U/S parameters and biopsy among controls & patients.

Variables		Control (n=20)	Patients (n=20)	Test value	P-value	Significant
P.V PSV	M±SD Range	35.00±1.72 32-37	56.05±12.79 38.5 – 86	-7.294•	0.000	HS
H.A RI	M±SD Range	0.57±0.18 0.07-0.69	0.56±0.18 0.06 – 0.71	0.292•	0.772	NS
Hepatic vein	Triphasic	20 (100.0%)	20 (100.0%)	0.000*	1.000	NS
Collection	No Yes	20 (100.0%) 0 (0.0%)	18 (90.0%) 2 (10.0%)	2.105*	0.147	NS
Liver Biopsy	Rejection	0 (0.0%)	14 (70.0%)			
	+vascular insult Cholangitis +	0 (0.0%)	2 (10.0%)	40.000*	0.000	HS
	Obs	0 (0.0%)	4 (20.0%)			
	Normal	20 (100.0%)	0 (0.0%)			

*:Chi-square test; •: Independent t-test

Non-significant difference was between controls & patients as regards H.A R.I, Hepatic vein and collection, But, a highly significant difference between PSV & different pathological findings in liver biopsy among controls & patients.

Table 6: Correlation between variable parameters and serum level of CD44 in patients

Variables	r	P-value
Age	0.294	0.209
Hb	-0.110	0.643
Plt	-0.628**	0.003
WBCs	-0.572**	0.008
CRP	-0.109	0.648
INR	-0.073	0.760
PTT	-0.104	0.663
T.Bili	-0.048	0.842
D.Bili	-0.097	0.686
AST	-0.483*	0.031
ALT	-0.222	0.346
ALK P	-0.239	0.310
GGT	-0.322	0.166
T.Prot	0.316	0.175
s.Alb	-0.057	0.812
BUN	0.035	0.882
S.Creat	-0.196	0.408
Na	0.722**	0.000
K	-0.215	0.362
P.Cal	0.011	0.963
Level of FK	-0.351	0.263
Level of Neoral	0.395	0.333
P.V PSV	0.172	0.469
H.A RI	-0.173	0.466

Table 7: Comparison between CD44 levels among patients with different pathological findings in liver biopsy.

CD44	Rejection (=14)	Rejection+ vascular insult (n=2)	Cholangitis + Obs (n=4)	Controls (n=20)	Test value	P-value	Significant
Median(IQR)	13.00 (13.0 – 14.5)	72.50 (70.0 – 75.0)	41.00 (36.5 – 48.5)	61.25 (51.25 - 90)			
Range (ng/ml)	6.5 – 42.5	70 – 75	35 – 53	35 – 120	28.841	0.000	HS
	Post Hoc analysis by LSD						
	P1	P2	P3	P4	P5	P6	
CD44	0.022	0.005	0.064	0.000	0.042	0.018	

‡=Kruskal Wallis test, P1: Rejection Vs Rejection+ vascular insult P2: Rejection Vs Cholangitis + Obs,P3: Rejection + vascular insult vs cholangitis + obs , p4: control vs rejection ,p5: control vs rejection+ vascular insult and P6: control vs cholangitis + Obs

Table 8: Comparison between sex, comorbidities, fever, immunosuppression drug, collection in PA U/S, liver biopsy, and serum level of CD44.

Variables		CD44 Median(IQR)	Range	Test value	P-value	Significant
Sex	Male	14.25 (13.00 – 32.50)	13.00 – 53.00			
	Female	41.00 (6.50 – 70.00)	6.50 – 75.00	-0.921ǂ	0.357	NS
Co. Morbidities	No	14.50 (13.00 – 37.50)	13.00 – 75.00			
	Dm	44.00 (35.00 – 53.00)	35.00 – 53.00	3.289ǂǂ	0.193	NS
	Hypothyroidism	13.00 (6.50 – 38.00)	6.50 – 44.00			
Fever	No	13.00 (13.00 – 13.75)	13.00 – 14.50			
	Yes	23.50 (13.00 – 43.25)	6.50 – 75.00	-1.391ǂ	0.164	NS
Drug	F.K	24.75 (13.00 – 48.50)	13.00 – 75.00			
	Neoral	13.50 (9.75 – 23.50)	6.50 – 42.50	-1.645ǂ	0.100	NS
Collection	No	14.25 (13.00 – 35.00)	6.50 – 75.00			
	Yes	41.00 (38.00 – 44.00)	38.00 – 44.00	-1.407ǂ	0.159	NS
Liver Biopsy	Rejection	13.00 (13.00 – 14.50)	6.50 – 42.50			
	Rejection+ vascular insult	72.50 (70.00 – 75.00)	70.00 – 75.00	11.703ǂǂ	0.003	S
	Cholangitis + Obs	41.00 (36.50 – 48.50)	35.00 – 53.00			

ǂ= Mann Whitney test; ǂǂ= Kruskal Wallis test.

Table 9: Sensitivity & specificity of CD44 serum level as a marker for acute cellular rejection in post-liver transplantation

Parameter	AUC	Cut of Point	Sensitivity	Specificity	PPV	NPV
CD44	0.911	≤44	85.00	95.00	94.4	86.4

AUC: Area under Curve

Best cut off point for of CD44 serum level as a marker of rejection was ≤ 44ng/ml with sensitivity of 85% and specificity of 95% in diagnosis of acute cellular rejection.

Discussion

The identification and clinical establishment of easily obtainable and reliable non-invasive biomarkers for ACR would mean substantial progress for postoperative management of the liver transplant recipients (Raschzok *et al*, 2015)**.**

Biomarkers for the noninvasive diagnosis of ACR may prevent delays in therapy because of sampling or interpretation error, and biopsy-associated complications such as bleeding, infection, or pain (Lee, 2014).

Biomarkers for the stratification of the risk of ACR could help to prevent rejection and individualize the immunosuppression therapy. Noninvasive biomarkers could enable personalized and optimized therapy for liver transplant recipients in an attempt to prevent morbidity and improve allograft survival, thereby reducing hospital stay and limiting the expense of the treatment (Morris and Anderson, 2013)**.**

The aim of this study was to evaluate the significance of serum level of CD44 as a Marker of acute cellular rejection in patients with post liver transplantation.

The strength of the current study was that the subjects included in our study were with abnormal liver profile all of them underwent liver biopsy to investigate underlying pathology and all of them were examined with one single histopathologist and all laboratory investigations were done in the same laboratory. In our study we depended on diagnosis of acute cellular rejection on histopathological findings in liver biopsy. All patients group were selected from ASCOT after monitoring elevation of the liver functions tests. All controls were selected completely clinically and lab normal persons. Our reference range could be calculated by using the mean of serum level of CD44 of control group (being = 61.25 (51.25 - 90).

Chen *et al.* (2010) described CD31, CD44, and chemokine (C-X-C motif) ligand (CXCL) 9, biomarkers for cross-organ allograft rejection. Rouschop *et al.* (2010) observed high CD44 protein levels during renal allograft rejection in a study with 23 patients with biopsy-proven acute renal allograft rejection compared with 9 transplant recipients without ACR. Moreover, they reported increased CD44 serum protein levels

in 24-hour pretransplant blood analyses of renal transplant recipients who later developed acute allograft rejection (Raschzok *et al*, 2015)**.**

In the present study, serum level of CD44 was significantly lower in the rejection group of patient with post liver transplantation, whereas it was reported to be increased during allograft rejection in the post cardiac and kidney transplantation. The interestingly, through the differentiation of CD4- and CD8-positive lymphocytes in the pretransplant analysis, there is lower CD44 expression for CD8-positive cells and a similar trend for CD4-positive cells in the rejection group. Raschzok *et al*. (2015) speculated on the reasons for the discrepancy between our findings of low CD44 serum protein levels in liver transplant recipients prior and during rejection and the previously reported high CD44 levels during cardiac and kidney allograft rejection.

In the present study, there was highly significant difference between control group & patients group as regards serum level of CD44. Negative correlation between serum level of CD44, WBCs and AST .There was highly significant difference between patients with different pathological findings as regards serum level of CD44. The best cut off point for Serum level of CD44 as a marker of rejection was found ≤ 44ng/ml with sensitivity of 85% and specificity of 95% in diagnosis of acute cellular rejection.

In a cohort study, serum samples were collected in standard serum tubes immediately before transplantation, at POD 1, 3, 7, & 14, and when a biopsy was performed all patients underwent follow up serial serum levels of CD44 & CXCL9 then the comparison between serum levels of CD44 & CXCL9 at different times and pathological findings in liver biopsy (Raschzok *et al*, 2015)**.**

In the present study, on the basis of a ROC curve analysis, cutoffs were calculated to define the levels of CD44 that differentiate with the highest specificity and sensitivity between patients with and without risk for ACR. At POD 1, the cutoff values of CD44 for the risk of ACR were identified to be <200.5ng/ mL (sensitivity, 88%; specificity, 61%), whereas the cutoff values of CXCL9 were >2.7ng/mL (sensitivity, 60%; specificity, 79%). A combination of both biomarker cutoffs at POD 1 (<CD44 & >CXCL9 cutoff) enabled the best prediction of patients at risk for ACR with a positive predictive value (PPV) of 91% and a negative predictive value (NPV) of 67%, (Asaoka *et al*, 2014).
In the study of Raschzok *et al.* (2015) the reference range was different from the present study due to different kits. Massoud *et al.* (2010) postulated that C4 With a cutoff value of ≤0.31 g/L, C4 had a sensitivity of 97%, a specificity of 62%, a positive predictive value of 74%, and a negative predictive value of 94% as a noninvasive marker of acute cellular rejection in post liver transplant. Feussner *et al.* (1994) postulated that the mean postoperative SAA plasma concentration in liver allograft recipients was about ten times higher (9.76±6.60mg/dl) as compared to mean value in healthy controls (0.98±0.42 mg/dl).

Brouard and Soulillou (2010) evaluated the intracellular IL-2 quantification in CD3+CD8+ cells in 21 liver transplant recipients for 6 months after liver transplantation, showing that intracellular IL-2 expression in CD8+ T cells before transplantation was closely related to the development of ACR. These results were confirmed by Germani *et al.* (2009) reported that patients experiencing ACR showed a significantly higher intracellular percentage of IL-2+ in CD8+ T cells compared to stable liver transplant recipients. Graft eosinophilia was identified as an independently associated feature of ACR in liver transplantation. The absence of peripheral eosinophilia predicted the absence of moderate/severe ACR , however it could not be used to predict or to assess the response to corticosteroids for the treatment of acute rejection. In a more recent study, based on 690 consecutive first liver transplant patients and using protocol liver biop-

sies, peripheral eosinophil count was strongly associated with moderate-severe ACR. These investigators also found that the delta in eosinophil count between the biopsies performed before and after ACR treatment was the only independent predictor of histological improvement (Germani *et al*, 2015).

The role of IL-9, IL-23 and IL-17 in liver transplantation remains to be clarified. As far as IL-9 is concerned, when serum levels were determined in 50 liver transplanted patients (15 patients with ACR episodes, and 35 patients without ACR) on day 1 & 7 after liver transplantation and on the day of liver biopsy, with neither difference between patients nor ACR. The serum concentrations of IL-23 and IL-17 were not different early in the post-trans-plantation period. However, a significant increase in serum IL-23 levels in the ACR group was seen at the time of liver biopsy (Fábrega *et al.*, 2009)**.** The data were confirmed by a latter prospective study (Fan *et al.*, 2012) showing that the levels of circulating CD4+IL-17+ T cells were higher in ACR patients than those without. The frequency of CD4+IL-17+ cells in peripheral blood correlated with ACR histological severity (Flores *et al*, 2016). Raschzok *et al.* (2015) postulated that CD44 can be used as a biomarker for the blood test–based diagnosis of rejection in liver transplant recipients. These results may help to identify patients at risk for ACR in first months after transplantation to improve clinical care the recipients**.**

Conclusion

No doubt, the liver transplantation (LT) has emerged as an established therapeutic option for patients with chronic liver disease worldwide.

There was correlation between different pathological findings in liver biopsy and serum level of CD44, lower levels being associated with rejection. CD44 may be used as a marker to detect acute liver rejection post transplantation. Prospective multicenter studies with longer follow up period and more patients will confirm and prove the update findings.

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