EUROPEAN ACADEMIC RESEARCH
Vol. III, Issue 6/ September 2015

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Value of Interleukin-7 and Interleukin-15 as Predictors of Graft Versus Host Disease in Recipients of Allogeneic Bone Marrow Transplantation

ABDEL GHANY S. MOHAMMED
HODA A. GAD ALLAH
MONA A.WAHBA
NERMEEN A. NABIH
RASHA I. MUSTAFA
ALIA M. AHMED
Department of Internal Medicine
Clinical Hematology, Ain Shams University
Cairo, Egypt

Abstract:

Background: *Allogeneic hematopoietic stem cell transplantation is a curative therapy for a wide spectrum of hematological and non-hematological disorders. The development of post-transplant complications- notably GVHD, has limited the broader application of the technique for so long. Researchers have been looking for a biomarker that can help them in early detection of patients at risk of GVHD for early intervention or implementation of aggressive management. Many molecules were studied in this prospective including interleukin-7(IL-7) and interleukin-15(IL-15).*

Objectives: *To evaluate plasma levels of both IL-7 and IL-15 pre-transplant as a baseline, and all through transplant procedure at defined intervals. To correlate these levels with GVHD process either acute or chronic. To interpret the findings to have a reply on the question of whether a correlation means that level of interleukins are a cause of the complication or a result.*

Patients and Methods: *We study evaluated the association of both plasma IL-7 and IL-15 levels with GVHD either acute or chronic, in 30 patients who were eligible for allo-HSCT hematopoietic stem cell transplantation with different hematological benign and malignant*

disorders. They received their allo-transplant from fully matched sibling donors except for two (having single locus mismatch). Plasma IL-7 and IL-15 levels were determined pre-transplant as a baseline and at certain intervals post-transplant.

Results: *It was found that plasma level of IL-7 at day+14 was positively correlated to hepatic and gut a GVHD with P-values of 0.003 and (p<0.001) respectively. It was also correlated to GVHD free survival and mortality with a P-value of 0.012 and 0.002. But rate of change of plasma values of IL-7 over transplant procedure was only correlated with gut a GVHD with high statistical significance (P-value<0.001). On the other hand, plasma level of IL-15 at day +14 correlated with cutaneous c GVHD with a P-value of <0.001, but it showed no association with any type of aGVHD. It showed no association with GVHD free survival or mortality. It was generally observed that rate of change of plasma level of IL-15 correlated with hepatic aGVHD.*

Conclusion: *Plasma levels of IL-7 at day+14 correlated well with gut and hepatic aGVHD. Also, plasma level of IL-15 at day +14 correlated strongly with cutaneous cGVHD. Rate of uprise of plasma levels of IL-15 over transplant procedure period correlated well with hepatic aGVHD.*

Key words: Allo-HSCT, IL-7, IL-15, aGVHD, cGVHD.

Introduction

The distinctive characteristics of allogeneic HSCT are that the stem cell graft is free of contamination by malignant cells and contains T-cells that are capable of mediating an immunologic reaction against foreign antigens [1]

Graft versus host disease is a complex disease resulting from donor T-cell recognition of a genetically disparate recipient that is unable to reject donor cells after allogeneic HSCT [2]

It is thought to be mediated by donor T-cell recognition of disparate major and minor histocompatibility antigens, as well as tumor-associated antigens [3]

Multiple cytokines contribute to the initiation, severity, and persistence of acute GVHD. Prior studies have mainly focused on inflammatory cytokines; however, murine models indicate that IL-7 is necessary for the initiation of GVHD. Data demonstrated that higher serum IL-7 levels in the early post-transplantation period were strongly associated with both the subsequent development and the severity of acute GVHD [4]

Interleukin 15 (IL-15) is a cytokine critical for the survival and homeostasis of memory CD8+ T cells, which are known to actively promote acute GVHD. Exogenously administered IL-15 can increase both autoimmune disease and memory CD8+T-cell function against autologous tumor targets. It has been shown that deregulation of endogenous IL-15 expression or administration of exogenous IL-15 can increase acute GVHD lethality in the presence of donor-derived allogeneic T cells [5]

Patients and Methods

This study had been conducted on 30 patients eligible for allo-HSCT in period of 2 years from January 2011till January 2013 They received their allo-transplant from fully human leucocyte antigens matched sibling donor except for two having single locus mismatch. All patients gave institutional ethics approval before undergoing transplantation procedure. This study was reviewed and approved by Ain Shams University Ethical Committee, and all patients provided written informed consent according to declaration of Helsinki which allows collection, storage and analysis of blood samples for research purposes. The patients age ranged from16 to 45 years. Their diagnoses varied between benign and malignant hematological disorders

with majority of cases being acute myeloid leukemia (AML) (n=14), followed by acute lymphoblastic leukemia (ALL) (n=8), very severe aplastic anemia (AA)(n=3),chronic myeloid leukemia (CML) (n=2), high risk myelodysplastic syndrome (MDS) (n=2) and biphenotypic acute leukemia (BAL) (n=1).

Patient selection

Patients with haematological disorders indicated for allogeneic HSCT with complete remission (CR) in cases of malignant disorders. We used for defining complete remission criteria supplied by ***de Greef et al., 2004*** [61] for AML cases and by ***Gökbuget et al., 2012*** [7] for ALL patients. With exclusion of any patient in relapse or partial remission, and those suffering from viral infections causing lymphopenia namely: HIV, HBV, HCV, CMV and EBV, or rheumatological disorders.

Transplant procedure

Conditioning regimens

Patients received their conditioning protocol in accordance with their diagnoses from one side, and in relation to degree of anticipation of occurrence of aGVHD from the other side. Conditioning regimens given pre-transplant included **fludarabine/busulphan protocol** that was given to 16 cases, AML (12cases), CML (2cases) and high risk MDS (2cases). It was constituted of fludarabine 30mg/m^2/day IV (day-6 to -3), busulphan 1mg/kg/6hours orally (day-6 to -3).

Fludarabine/busulphan /ATG/ LD TBI protocol was given for two AML cases, that were having single locus mismatch on high resolution HLA typing. It comprises the same fore-mentioned doses of fludarabine and busulphan in addition to rabbit anti-thymocyte globulin offered at a dose of 0.5 mg/kg intravenously over 4-6 hours as a test dose at day -2, followed by 2mg/kg intravenously over 4-6 hours (at day -1 and

0) with precautions against anaphylaxis done. Low dose TBI was given at a dose of 200centiGray (cGy) (on day -1 and 0) keeping in mind that it should be completed before stem cell infusion. BAL case received **fludarabine/busulphan/cytosine arabinoside /vepeside protocol**. This protocol is composed of five days of intravenous fludarabine at a dose of 30 mg/m^2/day (day -6 to -2), cytosine arabinoside given at a dose of 1200 mg/m^2/day intravenously (at day -6 and -5), busulphan at a dose of 4.5 mg/kg/day (at day -4 and -2) orally, and etoposide at a dose of 6 mg/kg/day intravenously (from day -6 to day -2). ALL patients(8) received **TBI/cyclophosphamide protocol**, constituted of fractionated 12 Gray TBI over 3 days followed by cyclophosphamide 120 mg/kg over 2 days; Three cases administered **fludarabine/cyclophosphamide/ATG** protocol, They administered fludarabine at a dose of 30 mg/kg/day (day -6 to -3), cyclophosphamide at a dose of 300mg/m2/day (at day -6 and day -3), and ATG at a dose of 3.75 mg/kg/day (day -6 to -3) after giving the test dose. They were the cases of aplasia.

Donor peripheral stem cell mobilization

Donor peripheral-blood hematopoietic progenitor cells were mobilized with filgrastim given at a dose ranging from 5 to 10 μg/kg from day -4 to 0 with evaluation of CD34 cells in peripheral blood at day-1. If their number is satisfactory, donor will be attached to apheresis machine at day 0 using COBE Spectra apheresis system. None of the grafts were ex-vivo T-cell depleted.

GVHD prophylaxis

First line GVHD prophylaxis were a combination of calcineurin inhibitors namely cyclosporine A (given at a dose of 3-5mg/kg/day, it was started at day -1, given initially intravenously till patient condition permitted oral intake, its dose was adjusted so as to maintain a serum level of 200-

400ng/ml with twice weekly follow up which was increased in frequency on demand), and methotrexate at a dose of 10mg/kg intravenously at day +1, +3,+6, and +11. This GVHD prophylaxis protocol was given to 25 cases, it was maintained for 6-9 months post-transplant. This period shortened when delayed completed donor chimerism took place, or lengthened in case of evolvement of acute GVHD. Remaining five cases had ATG as a part of their conditioning in addition to CNI/MTX that added to the power of GVHD prophylaxis. These were the three cases of aplasia along with the two cases of single locus mismatch.

Granulocyte colony stimulating factor (G-CSF) began to be given to patients at day +6 at a dose of10 μg/kg/day. It was continued till myeloid engraftment which was considered the first of three consecutive days with an absolute neutrophil count (ANC) of $0.5*10^9$/L.

The post-transplant evaluation:

The follow up was for 6 months post-transplant for each patient. It included:

1- **CBC, PT, INR, PTT along with full chemistry** including liver and renal profiles. They were done regularly with special tests ordered on demand.
2- **Donor-Recipient Hematopoietic Chimerism:** Chimerism analysis was performed at day+28, day +90 , and + 180 months post-transplantation on total peripheral-blood mononuclear cells, using the variable number tandem repeats–polymerase chain reaction method in a Clinical Laboratory Improvement Amendments– certified laboratory.
3- **Early detection of the complications** was achieved by history, clinical examination, biochemical and radiological investigations according to each disease state.

4- **Monitoring of CMV infection** was done on weekly basis using PCR for CMV DNA assay.
5- **Multiple bone marrow aspirate**, trephine biopsy, flowcytometry, cytogenetics, immunohistochemistry according to each disease state.
6- **Measurement of IL-7 and IL-15 level:** EDTA-anti-coagulated blood samples for IL-7 and IL-15 determination were obtained at enrollment, before conditioning at day-7, on the day of transplantation before infusion of the allograft, then at day +14, +28 post transplantation. Additional samples were collected at day +90 when patient developed signs and symptoms of acute GVHD. Plasma was aliquoted shortly after collection and stored at -60^{0}C until analyzed. Samples were analyzed using a high-sensitivity colorimetric enzyme-linked immunosorbent assay for IL-7 (ELISA; Avibion human IL-7 ELISA kit, Orgenium, Finland) and IL-15 (ELISA; ID ELISA human IL-15 ELISA kit, Biotechnology labs, Canada).
7- **Statistics:** Statistical presentation and analysis of the present study was conducted, using the mean, standard error, student t- test, Paired t-test, Chi-square, Mann-Whitney and Analysis of variance [ANOVA] tests by SPSS version 17. Putting in mind that a P-value > 0.05 non significant, $P \leq 0.05$ significant, $P \leq 0.01$ highly significant.

Results

We recruited a total number of 30 patients, 21 were males (70%) and 9 were females (30%). They were admitted at Ain Shams University Hospital-BMT unit from January 2011till January 2013. Their ages ranged from 16 to 45 years (with a

mean of 28 ±8.182 years). They received their allo-transplant from fully matched donors except for two having single locus mismatch. Donors were 14 males (46.66%) and 16 females (53.33%). Twenty cases were ABO compatible with their donors (66.66%), eight had bidirectional ABO incompatibility (26.66%) while two had major ABO incompatibility (6.66%). At day 0, infusion of stem cells was achieved with a mean of 6* 10^6 CD34 cells/kg BW of recipients (minimum of 3.8 and maximum of 7.09* 10^6). Engraftment occurred in most cases around day +13 post-transplant±2.586, with minimum time to engraftment of 9 days and maximum of 17 days. Two cases failed to achieve initial engraftment and were labeled as primary graft failure. Hospital stay showed great variability among transplant recipients, minimal hospital stay in the study patients was 25 days and maximal of 114 days. With a mean value of 36 days ±23.657. Patients with longest hospital stay were those having grade IV aGVHD.

Acute and chronic GVHD

Table (1) Percentage of occurrence of post-transplant complications to which the study population was subjected.

Complication			No.	Percent (%)
Acute GVHD	**Cutaneous**	Positive	1	3.33
	Hepatobiliary	Positive	7	23.33
	Gastro-intestinal	Positive	6	20.00
	Bronchopulmonary	Positive	0	0.00
	Grade of aGVHD	II	2	6.66
		IV	6	20.00
Chronic GVHD	**Cutaneous**	Positive	3	10.00
	Hepatobiliary	Positive	15	50.00
	Bronchopulmonary	Positive	3	10.00
	Gastrointestinal	Positive	4	13.33
	Mucous	Positive	4	13.33
	Ophthalmic	Positive	7	23.33

Regarding **aGVHD**, its incidence was 26.66% in our study, meaning that it had affected eight patients. Two of them (6.66%) had mild grade II aGVHD, whilst 6 of them (20%) had severe grade IV aGVHD. In contrast to what is well-known, cutaneous aGVHD had the least incidence affecting only 1 patient (3.33%). Seven patients had hepatic acute GVHD (23.33%), two of them had grade II hepatic affection (6.66%) while five (16.66%) were having grade IV acute GVHD with both liver and gut affection. Only 1 case (3.33%) had grade IV gut GVHD without associated liver involvement, it was the case of cutaneous aGVHD. . All patients with grade IV aGVHD succumbed their illness, four of them died before day 90 whilst two survived beyond day 90.

Chronic GVHD emerged as an important complications in our study and its incidence was significantly higher than that of aGVHD. It affected 18 patients out of 30 ones enrolled in the study (60%). Hepatobiliary cGVHD had the highest incidence occurring in 15 patients (50% of study population). It was followed by ophthalmic cGVHD occurring in 7 cases (i.e: 23.33%). Gut and mucus cGVHD shared the same incidence occurring in 4 cases (13.33%), and bronchopulmonary and cutaneous cGVHD came at the end of the list with an incidence of 3 cases for each (10%).

Comparison of plasma levels of both interleukins at different days with levels obtained pre-transplant showed that there is a trend towards a higher plasma values of IL-7 at day 0 and day +14 with median values of 17.37±8.60 and 16.20±9.20 pg/ml respectively. When compared to levels at day-7 which had a mean value of 12.20±7.26 pg/ml, there is a statistical significance at day+ 14 with a P-value of 0.04 and a very high statistical significance at day 0 with P-value of < 0.001.

The issue regarding plasma levels of IL-15 also showed the same, it was found that there is high statistical significance between levels at day 0 and day +14 and baseline with P-

value< 0.001. Mean values of IL-15 at day0 and day +14 were 20.62±9.77 and 16.25±7.33 pg/ml respectively in relation to mean baseline of 8.45±4.28 pg/ml.

Correlation between plasma levels of IL-7 and IL-15 at day +14 with GVHD encountered in the study population was done. Plasma level of IL-7 at day+14 was positively correlated to both GIT and hepatic aGVHD with high statistical significance. P-value was <0.001 and 0.003 respectively. In the setting of chronic GVHD, plasma level of IL-7 at day +14 was negatively correlated to mucus cGVHD with a P-value of 0.034. Also, day+14 plasma level of IL-7 was correlated to both GVHD free survival being higher in the group that suffered from GVHD either acute or chronic with a P-value of 0.012. It was also higher in the group that succumbed their illness (transplant related mortality) with statistical significance (P-value of 0.002).

Table (2): Relation between plasma level of IL-7 at day+14 and post-transplant complications

		IL7 at D+14 (pictogram per millilitre) (pg/ml)			T-test	
		N	Mean	SD	t	P-value
Cutaneous aGVHD	**Negative**	29	15.931	9.242	-0.858	0.398
	Positive	1	24.000	.		
GIT aGVHD	**Negative**	24	13.083	6.698	-5.032	0.000
	Positive	6	28.667	7.174		
Hepatic aGVHD	**Negative**	23	13.565	7.216	-3.290	0.003
	Positive	7	24.857	10.205		
Pulmonary aGVHD	**Negative**	30	16.200	9.200		
Cutaneous cGVHD	**Negative**	27	15.741	9.538	-0.815	0.422
	Positive	3	20.333	4.041		
Hepatobiliary cGVHD	**Negative**	15	17.933	12.056	1.033	0.310
	Positive	15	14.467	4.853		
Bronchopulmonary cGVHD	**Negative**	27	16.000	9.695	-0.352	0.728
	Positive	3	18.000	0.000		
GIT cGVHD	**Negative**	26	17.269	8.812	1.672	0.106
	Positive	4	9.250	9.845		
Mucous cGVHD	**Negative**	26	17.577	8.905	2.228	0.034
	Positive	4	7.250	5.852		
Ophthalmic cGVHD	**Negative**	23	17.130	9.353	1.004	0.324
	Positive	7	13.143	8.611		

Plasma level of IL-15 at day+14 did not correlate well with occurrence of aGVHD, but it correlated to cutaneous cGVHD whose patient demonstrated higher mean values than their partners who had not the complication with a P-value <0.001 (i.e: very high statistical significance). Plasma level of IL-15 at day+14 did not correlate well with GVHD free survival or mortality with P-values>0.05.

Table (3): Relation between plasma level of IL-15 at day+14 and post-transplant complications.

Items	data	IL15 at D14 (pg/ml)			T-test	
		N	Mean	SD	t	P-value
Cutaneous aGVHD	Negative	29	16.46552	7.364164	.	.
	Positive	1	10	.		
GIT aGVHD	Negative	24	16.3125	7.219859	0.083	0.936
	Positive	6	16	8.479387		
Hepatic aGVHD	Negative	23	14.84783	6.085442	-1.567	0.158
	Positive	7	20.85714	9.577379		
Pulmonary aGVHD	Negative	30	16.25	7.331733		
Cutaneous cGVHD	Negative	27	15.38889	7.21288	-5.033	0.000
	Positive	3	24	1.732051		
Hepatobiliary cGVHD	Negative	15	14.56667	6.041129	-1.271	0.215
	Positive	15	17.93333	8.293428		
Bronchopulmonary cGVHD	Negative	27	15.7963	7.495203	-1.496	0.223
	Positive	3	20.33333	4.618802		
GIT cGVHD	Negative	26	16.51923	7.729788	0.789	0.456
	Positive	4	14.5	4.123106		
Mucous cGVHD	Negative	26	16.51923	7.729788	0.789	0.456
	Positive	4	14.5	4.123106		
Ophthalmic cGVHD	Negative	23	15.32609	7.767369	-1.599	0.130
	Positive	7	19.28571	4.956958		

Rate of change of interleukins levels over the transplant procedure also was correlated to GVHD. We see that there is a high statistical significance between percentage of change of plasma levels of IL-7 and gut aGVHD (P-value<0.001). It was noticeable that there was a statistical significance between

percentage of change of plasma levels of IL-15 on one side and hepatic aGVHD on the other side, with P-value=0.01. Also, the percentage of change of plasma levels of IL-15 affected the incidence of bronchopulmonary cGVHD with being statistically significant (P-value=0.04). But none of them (percent of change of IL-7 or IL-15) was well correlated to mortality or GVHD free survival.

Table (4): Relation between percentage of change of plasma IL-7 level and post-transplant complications.

		% of change IL7								Mann-Whitney Test	
				Range			Median	Interquartile Range	Mean Rank	Z	P-value
GIT aGVHD	Negative	24	80.00	-68.00	:	1450.00	-35.00	122.50	13.17	-2.91	0.00
	Positive	6	20.00	105.88	:	257.14	106.67	44.12	24.83		
Hepatic aGVHD	Negative	23	76.67	-68.00	:	1450.00	-33.33	155.00	14.04	-1.64	0.10
	Positive	7	23.33	-46.43	:	257.14	105.88	153.10	20.29		
Ophthalmic cGVHD	Negative	23	76.67	-68.00	:	1450.00	-35.00	160.88	14.26	-1.40	0.16
	Positive	7	23.33	-33.33	:	212.50	50.00	245.83	19.57		
Cutaneous cGVHD	Negative	27	90.00	-68.00	:	1450.00	-33.33	155.88	14.48	-1.90	0.06
	Positive	3	10.00	75.00	:	212.50	212.50	.	24.67		
Hepatobiliary cGVHD	Negative	15	50.00	-68.00	:	1450.00	100.00	156.67	17.80	-1.43	0.15
	Positive	15	50.00	-67.74	:	212.50	-35.00	105.00	13.20		
Broncho-pulmonary cGVHD	Negative	27	90.00	-68.00	:	1450.00	-33.33	155.88	14.59	-1.70	0.09
	Positive	3	10.00	-29.41	:	212.50	212.50	.	23.67		
GIT cGVHD	Negative	26	86.67	-68.00	:	1450.00	-32.21	157.33	15.08	-0.67	0.50
	Positive	4	13.33	-33.33	:	114.29	8.33	131.55	18.25		
Mucous cGVHD	Negative	26	86.67	-68.00	:	1450.00	-32.21	157.92	15.42	-0.12	0.90
	Positive	4	13.33	-33.33	:	50.00	-4.17	77.08	16.00		

Table (5): Relation between percentage of change of plasma IL-15 level and post-transplant complications.

		% of change IL7								Mann-Whitney Test	
				Range			Median	Interquartile Range	Mean Rank	Z	P-value
GIT aGVHD	Negative	24	80.00	-68.00	:	1450.00	-35.00	122.50	13.17	-2.91	0.00
	Positive	6	20.00	105.88	:	257.14	106.67	44.12	24.83		
Hepatic aGVHD	Negative	23	76.67	-68.00	:	1450.00	-33.33	155.00	14.04	-1.64	0.10
	Positive	7	23.33	-46.43	:	257.14	105.88	153.10	20.29		
Ophthalmic cGVHD	Negative	23	76.67	-68.00	:	1450.00	-35.00	160.88	14.26	-1.40	0.16
	Positive	7	23.33	-33.33	:	212.50	50.00	245.83	19.57		
Cutaneous cGVHD	Negative	27	90.00	-68.00	:	1450.00	-33.33	155.88	14.48	-1.90	0.06
	Positive	3	10.00	75.00	:	212.50	212.50	.	24.67		
Hepatobiliary cGVHD	Negative	15	50.00	-68.00	:	1450.00	100.00	156.67	17.80	-1.43	0.15
	Positive	15	50.00	-67.74	:	212.50	-35.00	105.00	13.20		
Broncho-pulmonary cGVHD	Negative	27	90.00	-68.00	:	1450.00	-33.33	155.88	14.59	-1.70	0.09
	Positive	3	10.00	-29.41	:	212.50	212.50	.	23.67		
GIT cGVHD	Negative	26	86.67	-68.00	:	1450.00	-32.21	157.33	15.08	-0.67	0.50
	Positive	4	13.33	-33.33	:	114.29	8.33	131.55	18.25		
Mucous cGVHD	Negative	26	86.67	-68.00	:	1450.00	-32.21	157.92	15.42	-0.12	0.90
	Positive	4	13.33	-33.33	:	50.00	-4.17	77.08	16.00		

Figure (1): (a) Relation between plasma level of IL-7 at day+14 and post-transplant complications. (b) Relation between D+14 plasma level of IL-15 and post-transplant complications.

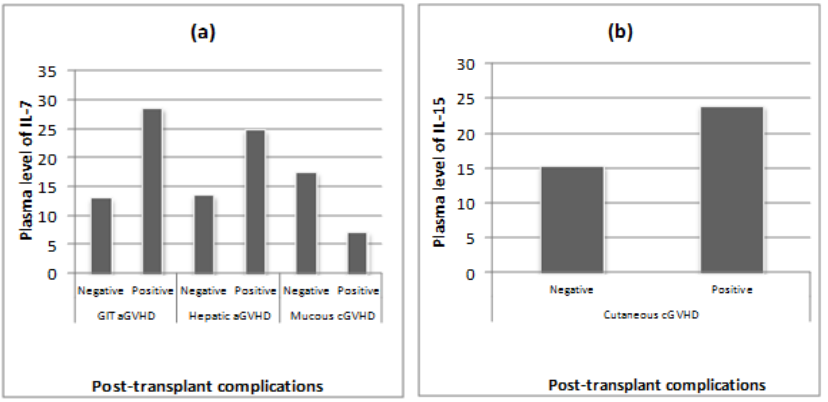
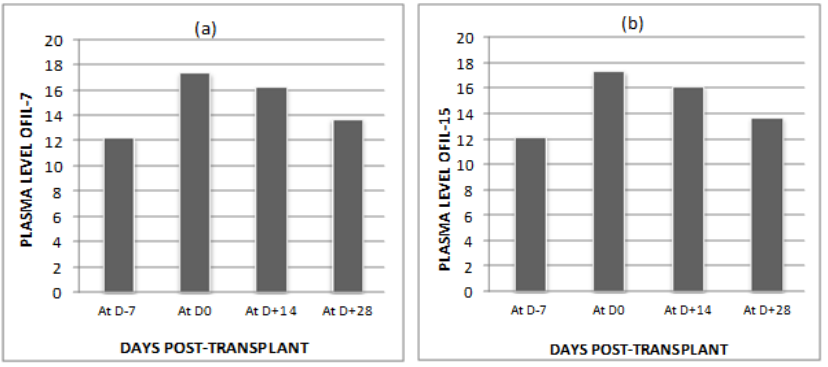


Figure (2): (a) Relation between different plasma levels of IL-7 post-transplant. (b) Relation between different plasma levels of IL-15 post-transplant



Discussion:

Allogeneic stem cell transplantation (HSCT) is currently the standard therapy for many hematologic disorders and its use has markedly increased over the past two decades [8]

Acute graft-versus-host disease (GVHD) remains a significant complication of allogeneic HSCT and limits the broader application of this therapy [9]

In our study, incidence of acute GVHD was 26.66% affecting eight recipients. This incidence was much less than what was described by **Lugt et al., 2013** who stated that acute GVHD remains a major complication of allogeneic transplantation, occurring in approximately half the transplant recipients [10]

We failed to prove an association between transplantation from a female donor to a male recipient and aGVHD, for only two out of eight patients with aGVHD were males receiving their allograft from a female donor. All two patients having single locus mismatch had grade IV aGVHD which supported what **Park and Seo, 2008** said about the degree of HLA disparity as one of the major determinants of aGVHD [11]

Also, TBI was associated with aGVHD in our study population. All cases receiving TBI containing regimen (either standard or low dose) had aGVHD except for two of them (i.e: 6 cases=13.33%). This supports a finding of **Flowers et al., 2011** that found TBI as a great risk factor for aGVHD [12]

In contrast to what was stated by **Jacobsohn and Vogelsang; 2007,** cutaneous aGVHD had the least incidence affecting only 1 patient in our series (3.33%). While it was considered to be the commonest form of aGVHD by **Jacobsohn and Vogelsang** [13]

All patients who had grade IV aGVHD (i.e: 20%) received methyl-prednisolone at a dose of 1-2 mg/kg/day for 14 days as a front line agent, but unfortunately they did not show any response dictating choosing second line agents. All of them did not respond to these agents and died as a consequence. This supports data from **Lugt et al., 2013** that patients who do not have a response to GVHD therapy are at high risk for death without relapse of the primary disease for which the transplantation was performed within 6 months after therapy initiation [10]

Chronic GVHD was a major problem in the study population affecting 18 cases (i.e: 60%). This seems to be concordant with **Ratanatharathorn et al., 2001** that said that cGVHD occurs in approximately 60–80% of long-term survivors of allo-HSCT [14]

Correlation between plasma values of both interleukins at day +14 and post-transplant complications was done. Plasma level of IL-7 at day +14 correlated strongly with hepatic and gut aGVHD with P-values 0f 0.003 and <0.001 respectively. Cutaneous aGVHD did not occur except in one case. This scarcity of cases of skin GVHD might explain the lack of association with IL-7. Our findings are concordant with **Dean et al., 2008** that considered day+14 plasma level of IL-7 as the strongest single parameter associated with acute GVHD [4].These data were affirmed by **Thiant et al., 2011** that considered IL-7 as a strong predictor of aGVHD but this was applied to day+30 levels and not day +14 [15]. But these findings stand in contrast to experimental study held by **Alpodgan et al.,2001** on mice models, that showed that administration of exogenous IL-7 to the animal improved immune reconstitution without aggravating aGVHD, regardless timing of administration or degree of genetic disparity [16]. This might be related to differences between human and animal models.

Finally, it was generally observed that there is a positive correlation between day +14 plasma level of IL-7 and GVHD free survival as well as mortality, with a P-value of 0.012 and 0.002 respectively. This might be an indirect consequence of its direct association to aGVHD, given especially the fact that 75% of patients developing aGVHD were grade IV aGVHD and resistant to treatment, so they died as a result.

We failed to confirm any association between day +14 plasma level of IL-15 and acute GVHD with P-values of 0.936 and 0.158 for gut and hepatic aGVHD respectively. This is compatible with findings of **Thiant et al., 2011**, **Thiant et al.**

only confirmed the direct relation between C-reactive protein (CRP) levels and IL-15 levels. So, you could rely on IL-15 as a marker of systemic inflammation but could not conclude an association between it and aGVHD [15]. Despite the fact that pro-inflammatory milieu available after conditioning is a fuel for ignition of the process of aGVHD.

But this viewpoint was counteracted by findings of **Chik et al., 2003**, that showed significant elevation of plasma level of IL-15 in the early post-transplant period (2 months post-transplant) in GVHD group in contrast to those without GVHD [17].

A strong association between plasma level of IL-15 at day+14 and cutaneous cGVHD was present with a P-value of <0.001. This is the contrast to what was stated by **Pratt et al., 2013** that considered low values of plasma level of IL-15 as a strong predictor for development of extensive cGVHD and considered this finding as an important clue that can be used for guidance of pre-emptive therapy [18].

In conclusion it is recommended to assess plasma level of IL-7 at day +14 post-transplant to anticipate the occurrence of aGVHD.but Plasma level of IL-15 is not a preferable biomarker to predict aGVHD. But, it can give you a better expectation of cGVHD especially cutaneous type.

We need to evaluate benefit of implementation of more aggressive immunosuppression in patients with high day+14 values of IL-7 and whether this may be a preventive strategy for aGVHD especially given data that prevention of GVHD is much easier than treatment.

REFERENCES

[1] Giralt SR and Bishop MR: Principles and overview of allogeneic hematopoietic stem cell transplantation. In:

Hematopoietic stem cell transplantation, Bishop MR; chapter 1, ISBN: 978-0-387-78579-0, Springer 2009: 1-20.

[2] Socie´ G and Blazar BR: Acute graft-versus-host disease: from the bench to the bedside. Blood 2009; 114: 4327-36.

[3] Pidala J and Anasetti C: Can antigen-specific regulatory T cells protect against graft versus host disease and spare anti-malignancy alloresponse? Haematologica. 2010; 95: 660–65.

[4] Dean RM, Fry T, Mackall C, Steinberg SM, Hakim F, Fowler D, Odom J, Foley J, Gress R, and Bishop MR**:** Association of Serum Interleukin-7 Levels With the Development of Acute Graft-Versus-Host Disease**.** Journal of clinical oncology 2008; 28: 5735-41.

[5] Blaser BW, Schwind NR, Karol S, Chang D, Shin S, Roychowdhury S, Becknell B, Ferketich AK, Kusewitt DF, Blazar BR, and Caligiuri MA: Trans-presentation of donor-derived interleukin 15 is necessary for the rapid onset of acute graft-versus-host disease but not for graft-versus-tumor activity. Blood. 2006; 108: 2463–69.

[6] de Greef GE, van Putten WL, Boogaerts M, Huijgens PC, Verdonck LF, Vellenga E, Theobald M, Jacky E, Löwenberg B; Dutch-Belgian Hemato-Oncology Co-operative Group HOVON; Swiss Group for Clinical Cancer Research SAKK: Criteria for defining a complete remission in acute myeloid leukaemia revisited. An analysis of patients treated in HOVON-SAKK co-operative group studies. Br J Haematol. 2005;128: 184-91.

[7] Gökbuget N, Kneba M, Raff T, Trautmann H, Bartram CR, Arnold R, Fietkau R, Freund M, Ganser A, Ludwig WD, Maschmeyer G,Rieder H, Schwartz S, Serve H, Thiel E, Brüggemann M, Hoelzer D; German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia: Adult patients with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell

transplantation and targeted therapies. Blood. 2012; 30;120:1868-76.

[8] Labrador J, Lopez-Anglada L, Perez-Lopez E, Lozano FS, Lopez-Corral L, Sanchez-Guijo FM, Vazquez L, Rivera JAP, Martin-Herrero F, Sanchez-Barba M, Guerrero C, del Cañizo MC, Caballero MD, Miguel JFS, Alberca I, and Gonzalez-Porras JR: Analysis of incidence, risk factors and clinical outcome of thrombo-embolic and bleeding events in 431 allogeneic hematopoietic stem cell transplantation recipients. haematologica 2013; 98:437-43.

[9] Choi SW, Kitko CL, Braun T, Paczesny S, Yanik G, Mineishi S, Krijanovski O, Jones D, Whitfield J, Cooke K, Hutchinson RJ, Ferrara JLM, and Levine JE: Change in plasma tumor necrosis factor receptor 1 levels in the first week after myeloablative allogeneic transplantation correlates with severity and incidence of GVHD and survival. 2008 112: 1539-42.

[10] Lugt MTV, Braun TM, Hanash S, Ritz J, Ho VT, Antin JH, Zhang Q, Wong CH, Wang H, Chin A, Gomez A, Harris AC, Levine JE, Choi SW, Couriel D, Reddy P, Ferrara JLM and Paczesny S: ST2 as a Marker for Risk of Therapy-Resistant Graft-versus-Host Disease and Death. NEJM 2013; 369: 529-39.

[11] Park M and Seo JJ: Role of HLA in Hematopoietic Stem Cell Transplantation. Bone Marrow Research 2012; 2012, Article ID 680841, 7 pages**.**

[12]Flowers MED, Inamoto Y, Carpenter PA, Lee SJ, Kiem HP, Petersdorf EW, Pereira SE, Nash RA, Mielcarek M, Fero ML, Warren EH,Sanders JE, Storb RF, Appelbaum FR, Storer BE, Martin PJ: Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. Blood. 2011;117: 3214-19.

[13] Jacobsohn DA and Vogelsang GB: Acute graft versus host disease. Orphanet Journal of Rare Diseases 2007, 2:35.

[14] Ratanatharathorn V, Ayash L, Lazarus HM, Fu J, and Uberti JP: Chronic graft-versus-host disease: clinical manifestation and therapy. Bone Marrow Transplantation 2001; 28: 121–29.

[15] Thiant S, Labalette M, Trauet J, Coiteux V, de Berranger E, Dessaint JP and Yakoub-Agha I: Plasma levels of IL-7 and IL-15 after reduced intensity conditioned allo-SCT and relationship to acute GVHD. Bone Marrow Transplantation 2011;46:1374–81.

[16] Alpdogan O, Schmaltz C, Muriglan SJ, Kappel BJ, Perales MA, Rotolo JA, Halm JA, Rich BE, van den Brink MR: Administration of interleukin-7 after allogeneic bone marrow transplantation improves immune reconstitution without aggravating graft-versus-host disease. Blood. 2001;98: 2256-65.

[17] Chik KW, Li K, Pong H, Shing MM, Li CK, Yuen PM: Elevated serum interleukin-15 level in acute graft-versus-host disease after hematopoietic cell transplantation. J Pediatr Hematol Oncol. 2003 ;25: 960-64.

[18] Pratt LM, Liu Y, Ugarte-Torres A, Hoegh-Petersen M, Podgorny P J, Lyon A W, Williamson S, Khan M, Chaudhry A, Daly A, Stewart A, Russell A, Grigg A, Ritchie D, Storek J: IL15 levels on day 7 after hematopoietic cell transplantation predict chronic GVHD. Bone Marrow Transplant. 2013;48: 722-28.