Transplant Immunology 41 (2017) 22–26

Contents lists available at ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

Free light chain and intact immunoglobulin abnormalities in heart transplant recipients: Two year follow-up timelines and clinical correlations

Peter Sečník Jr [a,d], Petra Lavríková [a,d], Lenka Hošková [b], Pavlína Malíková [c], Antonín Jabor [a,d], Janka Franeková [a,d,*]

[a] Department of Clinical Biochemistry, Institute for Clinical and Experimental Medicine, Vídeňská 1958/9, 14021 Prague, Czech Republic
[b] Cardiology Clinic, Institute for Clinical and Experimental Medicine, Vídeňská 1958/9, 14021 Prague, Czech Republic
[c] Department of Clinical Hematology, Institute for Clinical and Experimental Medicine, Vídeňská 1958/9, 14021 Prague, Czech Republic
[d] Third Faculty of Medicine, Charles University, Ruská 87, 10000 Prague, Czech Republic

ARTICLE INFO

Article history:
Received 19 September 2016
Received in revised form 1 November 2016
Accepted 6 January 2017
Available online 4 February 2017

Keywords:
Posttransplant lymphoproliferation
Heart transplantation
Free light chains
Electrophoresis
Immunofixation

ABSTRACT

Objectives: To assess the timelines of serum free light chain (sFLC) concentrations and the kappa/lambda light chain (K/L) ratio in heart transplant (HTX) recipients. To analyze the performance of serum protein electrophoresis (SPE), serum immunofixation (sIFE) and sFLC measurements for gammopathy detection following a HTX.
Methods: A total of 96 patients who underwent a HTX were analyzed during a two-year follow-up period. The relevant clinical data were obtained from patient medical records. SPE, sIFE and sFLC methods were used for the detection of free light chain and intact immunoglobulin gammopathies at 4 time points after HTX.
Results: A statistically significant decrease in sFLC K and L (a decrease of 39.1% and 27.6%, respectively, when compared to pretransplant values) was found 9 months after the HTX ($p < 0.001$, Friedman test). We detected SPE or sIFE abnormalities in 23 (8.4%) samples, and sFLC K/L ratio abnormalities in 34 (12.4%) samples. All of the K/L ratio abnormalities had normal SPE/sIFE values, and 19% of the findings were persistent.
Conclusions: A significant and consistent dynamics in the sFLC concentration was found in the HTX patients during a 2-year follow-up period, which reflected changes in the immunosuppressant dosage. A remarkable number of monoclonal and polyclonal gammopathies was identified with some persistent abnormalities, using the SPE/sIFE and sFLC methods. Some of the detected abnormalities, which might possess a higher risk for PTLD if interpreted according to common practice in nonTX patients can only be detected by sFLC methods.

1. Introduction

Solid organ transplant recipients are at an increased risk of lymphoproliferative disorders with anti-rejection treatment, and infection with oncogenic *Epstein-Barr virus* (EBV) and *Cytomegalovirus* (CMV) being important predisposing factors [1,2,3]. EBV viral load is associated with immunosuppression type and dosage, however data on association with PTLD risk are unequivocal [4,5]. The reported prevalence of posttransplant lymphoproliferative disease (PTLD) in heart transplant patients (HTX) varies from 1.7–6.3% [4,6]. The prevalence of gammopathy in HTX varies from 25 to 50%, depending on the immunosuppression protocol used [7,8,9]. It is assumed that the hyperplastic proliferation of the lymphoid tissue (the early stage of PTLD) might be linked to polyclonal and subsequent monoclonal gammopathy. However, some researchers have expressed their doubts as to whether the detection of monoclonal gammopathy is significant in PTLD diagnostics at all [10].

The serum free light chain (sFLC) concentration and light chain kappa to lambda (K/L) ratio have become internationally recommended biomarkers for monoclonal gammopathy and plasmocellular dyscrasia diagnostics and follow-up in non-transplant patients [11]. There are also published data exploring the use of sFLC in Hodgkin, nonHodgkin and diffuse large B-cell lymphomas for prognostication and/or therapy monitoring [12,13,14,15]. They are, therefore, logical candidate biomarkers for the early detection of PTLD and were already marginally tested in this setting [16,17,18,19,20]. However, no common opinion on their practical implementation was reached. There is also limited data on the dynamics and changes of sFLC during the posttransplant period and practically no data specific for HTX patients in the literature [16,17].

* Corresponding author at: Department of Laboratory Methods, Institute for Clinical and Experimental Medicine, Videnska 1958/9, Prague 14021, Czech Republic.
E-mail address: jafa@ikem.cz (J. Franeková).
URL: http://www.ikem.cz (J. Franeková).

http://dx.doi.org/10.1016/j.trim.2017.01.003
0966-3274/

2. Objectives

We aimed to analyze the dynamics of the sFLC concentration and the K/L ratio during a two-year follow-up period after a HTX and to assess the performance of serum protein electrophoresis (SPE) or serum immunofixation (sIFE) and the sFLC measurement for monoclonal gammopathy detection. Moreover, being potentially the most relevant biological factor, we assessed the influence of renal function on sFLC values in this cohort.

3. Patients and methods

We analyzed a total of 96 patients (81 men and 15 women) between the age of 21–74 years old (median 54 years) who underwent a heart transplantation during the years 2010–2012. The patient characteristics, including potential PTLD risk factors, are presented in Table 1. The number of patients with a sFLC K/L ratio pathology and an SPE or sIFE pathology (monoclonal, biclonal and oligoclonal gammopathy combined) is shown as well. The length of the follow-up was 24 months. A high-dosage multiple immunosuppressant regimen (tacrolimus, mycophenolate mophetil, and corticosteroids and/or sirolimus/everolimus) is used after HTX in our center.

Table 1
Characteristics of the analyzed cohort of 96 heart transplant recipients.

Characteristics			No. of patients		
			Total	With sFLC ratio pathology	With SPE/sIFE pathology
Sex	Men		82	21	12
	Women		14	3	4
Age (years)	<40		19	3	2
	40–50		17	4	6
	50–60		33	11	4
	>60		27	6	4
Diagnosis leading to HTX	Ischemic heart disease		33	8	4
	Cardiomyopathy		61	16	11
	Other		2	0	1
CMV serostatus before HTX	CMV infection in history (IgG reactive, IgM nonreactive)		68	19	7
	Seronegative (IgM + IgG negative)		19	4	8
	Active infection (IgM reactive, PCR positive)		0	0	0
	False IgM reactivity (PCR negative)		3	0	0
	Not tested		6	1	1
CMV infection after HTX (confirmed using PCR)	Yes		16	5	10
	No		80	19	6
EBV serostatus before HTX	Acute infection reactivation		2	0	2
	EBV infection in history		89	22	14
	Seronegative		1	0	0
	Not tested		4	2	0
Tacrolimus posttransplant concentration (average, μg/l)	0–9th month	<5	0	0	0
		5–10	1	0	0
		10–15	85	22	13
		>15	1	1	0
		Not tested[a]	9	1	3
	9th–18th month	<5	0	0	0
		5–10	51	13	7
		10–15	33	10	6
		>15	3	0	0
		Not tested[a]	9	3	3
	18th–24th month	<5	3	2	0
		5–10	72	18	10
		10–15	8	2	1
		>15	2	0	0
		Not tested[a]	11	4	5

[a] Patients with an alternative immunosuppressant regimen and/or not attending follow-up examinations regularly were not included.

Four sequential serum samples were obtained from each patient at defined time points (before the HTX and the 9th, 18th and 24th month after the HTX). The samples were separated from the blood cells and were stored at −70 °C, in accordance with the kit manufacturer's recommendation. All sFLC, SPE and sIFE analyses were performed in batches to minimize analytical variability.

The SPE and sIFE were performed as a gold standard method for the detection of monoclonal immunoglobulin using a Sebia Hydrasys 2 analyzer and Sebia gels and antisera (Sebia, Parc Technologique Léonard de Vinci-Rue Léonard de Vinci, CP 8010 Lisses, 91008 EVRY CEDEX, France). The SPE and sIFE findings were divided into 4 categories (normal, monoclonal, biclonal and oligoclonal patterns).

The sFLC concentrations were measured using Binding Site kits on an SPA Plus analyzer (Binding Site Group Ltd., 8 Calthorpe Road, Edgbaston, Birmingham, UK), and the K/L ratios were calculated. Renal reference ranges for the K/L ratio (0.37–3.1) were used if the estimated glomerular filtration rate (eGFR) was below 60 mL/min/1.73 m^2. The eGFR was calculated from the serum creatinine using CKD-EPI 2009 [21].

The sFLC findings were divided into 3 categories - normal sFLC concentration and K/L ratio, K and/or L concentration pathology with a normal K/L ratio and K/L ratio pathology, and the latter was further subdivided into the ratio only pathology without a pathologic sFLC concentration, the ratio pathology due to an elevated sFLC concentration (also referred to as the monoclonal pattern) and the ratio pathology due to a suppressed sFLC concentration. In the case of a modification of the type of pathology over time (detected by the same method and in the same patient), the finding was classified according to the last pathology detected.

The plasma tacrolimus and serum creatinine analyses were measured using Abbott kits on an Abbott Architect ci16200 (Abbott Laboratories, Abbott Park, Illinois, USA).

Relevant clinical data were obtained from the patient medical records. All of the patients gave informed consent to all of the diagnostic and therapeutic procedures used. The study was approved by Ethics Committee of the Institute of Clinical and experimental medicine under G14-08-26. The laboratory is accredited according to ISO 15189.

A Friedman test with post-hoc Conover analysis was used to assess the dynamics of the sFLC.

4. Results

4.1. Timelines of the sFLC K and L concentrations

First, we evaluated the timelines of the sFLC K and L concentrations using data from 96 patients in 4 time points before and after their HTX. The concentration dynamics in all of the time points are given in Figs. 1 and 2. A statistically significant decrease in sFLC K and L (a decrease of 39.1% and 27.6%, respectively, when compared to the pretransplant values) was found 9 months after the HTX ($p < 0.001$, Friedman test). The concentrations of all of the markers showed a steady rise during the following 15 months; however, they did not reach the pretransplantation levels at the 24th month (Figs. 1 and 2).

4.2. A simultaneous sFLC and SPE/sIFE finding comparison

Second, we compared the sFLC and SPE/sIFE findings simultaneously in a total of 274 samples after HTX (Table 2). All patients had negative SPE/sIFE before heart transplantation. After HTX, 168 (61.3%) of the findings were negative using both of the methods, 23 (8.4%) were