Bone marrow suppression and associated consequences in patients after heart transplantation: A 6-year retrospective review

Michal Pazdernik, Ivan Malek, Eva Koudelkova, Jan Sochman, Josef Kautzner

Aims. To evaluate the incidence of bone marrow suppression and consequences of MMF dose adjustment in patients within the first year after heart transplantation.

Methods. Group I (n=47) was treated with a regimen currently used in patients after heart transplantation (mycophenolate mofetil - MMF, valganciclovir - VGC and trimethoprim/sulfamethoxazole - TMP-SMX). Group II (n=47) received only MMF of potentially myelotoxic medications. The myelotoxic effect and need for dose modification were assessed. The incidence of rejections and infectious episodes associated with MMF adjustment were analyzed during the first 12 months in Group I.

Results. There was a significantly greater proportion of patients with leukopenia (leukocyte count < 4 x 10^9/L) at 3 months after orthotopic heart transplantation in Group I compared with Group II (19.1% vs 2.1%; \( P = 0.02 \)). The difference in lymphopenia (lymphocyte count < 0.8 x 10^9/L) at 3 months follow-up was highly significant (38.3% vs 6.4%; \( P = 0.0002 \)). MMF was modified due to bone marrow suppression or severe infection in 63.8% patients in Group I and in only 8.5% of patients in Group II (\( P < 0.001 \)). Reducing or stopping MMF was not associated with increased rejections. In Group I, at least 1 episode of higher degree cellular or humoral rejection occurred in 35% of patients with the standard MMF dosage compared with only 26% in patients with modified MMF (\( P = 0.0534 \)).

Conclusions. Addition of VGC+TMP-SMX to current immunosuppressive medication regimen in patients after heart transplantation is associated with significant lymphocytopenia and leukopenia. Importantly, modification of immunosuppressive prophylaxis (reducing or stopping MMF) leads to normalization of blood count without increased incidence of rejections.

Key words: heart transplant, medication regimen, myelotoxicity, rejections

Received: December 2, 2014; Accepted with revision: April 22, 2015; Available online: May 14, 2015
http://dx.doi.org/10.5507/bp.2015.022

Department of Cardiology, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic
Corresponding author: Michal Pazdernik, e-mail: Michal.Pazdernik@email.cz

INTRODUCTION

In the early period after orthotopic heart transplantation (HTx), patients are particularly vulnerable to rejection\(^1\) and infectious complications\(^2,3\). Immunosuppressive and anti-infective drug prophylaxis consists of drugs with potentially myelotoxic effects.

Current maintenance immunosuppression is achieved with a combination of different immunosuppressive agents. This generally comprises corticosteroids in combination with calcineurin inhibitors, tacrolimus or cyclosporin, and the antiproliferative agent mycophenolate mofetil (MMF). In addition, current guidelines\(^4\) recommend dual anti-infective prophylaxis composed of valganciclovir (VGC) and trimethoprim/sulfamethoxazole (TMP-SMX). One of most serious adverse reactions associated with immunosuppressive and anti-infective drug prophylaxis is bone marrow suppression. In this respect, leukopenia\(^5\) and anemia\(^6\) are the most frequent consequences.

On the one hand MMF significantly reduces acute rejection rates after heart transplantation\(^7,8\). But the benefits are often offset by drug intolerance or myelotoxicity\(^9,10\) that necessitate dose reduction or complete withdrawal. Besides shock and multi-organ dysfunction following the intense inflammatory reaction to sepsis, complications arising from sepsis-related immunoparalysis contribute to morbidity and mortality\(^12,13\). Hence, adjustment of the MMF dose is also required in patients with severe infection. Data on the clinical consequences of MMF dose reduction are scarce. The goal of this study was to assess the potential myelotoxic effects of MMF, VGC and TMP-SMX after heart transplantation. Second, we evaluated whether MMF dose reduction due to excessive bone marrow suppression or severe infection is associated with subsequent risk of acute rejection.

MATERIALS AND METHODS

Potential myelotoxic effects of MMF, VGC and TMP-SMX after heart transplantation was analyzed retrospectively in a study population from a period spanning 6 years (2004-2009). All patients were in a terminal state of heart failure and underwent orthotopic heart transplant at the Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic. Prophylaxis comprising the above potentially myelotoxic agents was initiated in all patients within one month after the transplantation.
An internal institutional database was used to identify all patients. The medical records of all 94 adult patients (aged >18 years) were subsequently analyzed.

The patient population was divided into two subgroups. Group I, called a “current regimen group” (period between 2008 - 2009), included 47 patients in whom the immunosuppressive regimen during the first month after the heart transplant consisted of MMF, VGC and TMP-SMX. Group II, entitled the “previous regimen group” (period between 2004 - 2007), also included 47 patients treated with only one potentially myelotoxic agent, MMF (as regular VGC and TMP-SMX administration started in our center in 2008). Patients treated with VGC for cytomegalovirus infection and patients treated with TMP-SMX, respectively, were not included in this group. The dosage of potentially myelotoxic drugs according to our institutional recommendations was as follows: MMF was started at 500 mg i.v. peroperatively, continued by 500 mg i.v. BID on the first day after heart transplant, followed by 1g p.o. BID. MMF blood levels were not monitored during the follow-up. VGC as a general prophylaxis was administered in a dose of 900 mg per day and TMP-SMX in a dose of 480 mg per day (TMP 80mg/SMX 400mg) and the duration of treatment was 100 and 90 days, respectively. Anti-thymocyte globulin (ATG-Fresenius S.) 1.25 mg/kg i.v. was used as induction therapy for all patients in the first two days. In the days following, ATG was carefully administered depending on lymphocyte count. In case when optimal lymphocyte count was reached, ATG was administered not any more. Corticosteroids were administered according to inner institutional protocol; all patients in the cohort used at least 5 mg of Prednisone in 12th month after HTx.

The data collected in both patient groups included basic demographics, information on the etiology of heart failure and blood element counts (erythrocytes, leukocytes, neutrophils, lymphocytes, thrombocytes) in selected periods (before heart transplant; 3, 6 and 12 months after heart transplant). The need for dose adjustment or complete withdrawal of MMF due to bone marrow suppression or severe infection was also recorded.

The incidence of graft rejections during the first 12 months after heart transplant in both groups was analyzed. Endomyocardial biopsies were planned and performed according to the institutional protocol. Patients underwent biopsy every week until 30 days post-transplant, and then every 2 weeks for 3 months, followed by once a month for 6 months. The last regular biopsies were scheduled on the 9th and 12th month after the heart transplant.

The MMF dose was modified or treatment was terminated depending on all available information such as leukopenia <4000 leukocytes/mm³ and/or severe lymphopenia and in certain cases, of severe infection. In case of blood count normalization, the dose of MMF was either individually enhanced or MMF was reinitiated in lower doses under careful monitoring.

The data are expressed in medians. Both absolute and relative frequencies were used for description. For testing discrete variables, the chi-squared test was applied and for continuous variables the t-test or Mann-Whitney test was used. Bonferroni correction for significance levels was applied in case of multiple testing.

Definitions

According to the revised (R) ISHLT classification we distinguished three grades of acute rejection: Grade 0 R-no rejection (no change from 1990 classification); Grade 1 R-mild rejection (corresponding to 1990 Grades 1A, 1B and 2); Grade 2 R-moderate rejection (replacing 1990 Grade 3A); and Grade 3 R-severe rejection (Grades 3B and 4 in 1990 classification). Antibody-mediated rejection after heart transplantation was based on evidence of endothelial injury and antibody and complement deposition.

RESULTS

A total of 94 patients were identified. There were 47 patients in Group I - Tacrolimus (44 patients) / Cyclosporin (n=3 patients), Prednison, MMF, VGC, TMP-SMX and 47 patients in Group II - Tacrolimus (10 patients) / Cyclosporin (37 patients), Prednison, MMF. No significant differences in patient demographic data and predisposing conditions of heart failure were found between the two groups. Group I consisted of 40 men, median age 53.1 years, proportion of ischemic vs dilated cardiomyopathy 30/47%. Group II comprised 36 men, median age 50.5 years, proportion of ischemic vs dilated cardiomyopathy 43/43% (Table 1).

A significantly higher proportion of patients with leukopenia (leukocyte count < 4 x10⁹/l) at three months after orthotopic heart transplantation was found in Group I compared to Group II (19.1% vs 2.1%; \( P = 0.02 \)). Similarly, lymphopenia (lymphocyte count < 0.8 x10⁹/l) at 3 months of follow up was significantly more frequent in Group I (38.3% vs 6.4%; \( P = 0.0002 \)). This finding was valid at one year after transplantation (19.1% vs 4.3% Group II; \( P = 0.05 \)) (Table 2, Fig. 1). There were no major differences in number of other blood elements (erythrocytes, neutrophils) between the two groups in the reference periods (1 month, 3 months, 6 months and 12 months after heart transplantation). Thrombocyte count showed a statistically significant difference between groups 3 and 12 months after heart transplant, but this finding was clinically insignificant. 3 patients from Group I developed symptomatic CMV infection after VGC withdrawal during follow-up.

Severe infection was found in 25.5% (12/47) of patients in Group I – 3 cases of symptomatic CMV infection, 7 cases of pneumonia (5x Aspergillus fumigatus, 1x Pseudomonas pseudoalcaligenes, 1x Candida albicans) and 2 cases of sepsis (Staphylococcus aureus, Klebsiella pneumoniae). Compared to Group II where severe infections were noted in 12.8% (6/47) - Klebsiella oxytoca sepsis (2 cases), Burkholderia cepacia pneumonia (1 case), Toxoplasma gondii myocarditis (1 case), Staphylococcus coagulase negative+Corynebacterium species mediasti-
Table 1. Patient characteristics data.

<table>
<thead>
<tr>
<th></th>
<th>Anti-Infective prophylaxis</th>
<th>Number of men/women</th>
<th>Median age</th>
<th>Ischemic/Dilated cardiomyopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (n=47)</td>
<td>VGC + TMP-SMX</td>
<td>40/7</td>
<td>53.1 years</td>
<td>30/47%</td>
</tr>
<tr>
<td>Group II (n=47)</td>
<td>-</td>
<td>36/11</td>
<td>50.5 years</td>
<td>43/43%</td>
</tr>
</tbody>
</table>

VGC – valganciclovir, TMP-SMX – trimethoprim/sulfamethoxazole

Table 2. Percentage of patients who developed significant bone marrow suppression during follow up.

<table>
<thead>
<tr>
<th></th>
<th>“Current” regimen</th>
<th>“Previous” regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0 (%)</td>
<td>M1 (%)</td>
<td>M3 (%)</td>
</tr>
<tr>
<td>Erythrocyte &lt; 4 (\times 10^{12}/L)</td>
<td>31.9</td>
<td>51.1</td>
</tr>
<tr>
<td>Thrombocyte &lt; 130 (\times 10^{9}/L)</td>
<td>0</td>
<td>2.1</td>
</tr>
<tr>
<td>Neutrophil &lt; 2 (\times 10^{9}/L)</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Lymphocyte &lt; 0.8 (\times 10^{9}/L)</td>
<td>6.4</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Table 3. Association of severe infections with MMF dose adjustment.

<table>
<thead>
<tr>
<th></th>
<th>Current regimen (n=47)</th>
<th>Previous regimen (n=47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of severe infections</td>
<td>12/47 (25.5%)</td>
<td>6/47 (12.8%)</td>
</tr>
<tr>
<td>Number of patients with MMF dose adjustment independent on severe infection</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Number of patients with MMF dose adjustment due to severe infection</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

MMF- mycophenolate mofetil

Table 4. Distribution of rejections in both regimens.

<table>
<thead>
<tr>
<th></th>
<th>No rejections</th>
<th>1R/1A rejections</th>
<th>1R/2 and higher rejections</th>
<th>Humoral rejections</th>
<th>Humoral+cellular rejections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current regimen (n=47)</td>
<td>8</td>
<td>25</td>
<td>11</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Previous regimen (n=47)</td>
<td>4</td>
<td>17</td>
<td>16</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

nitis (1 case) and Pseudomonas aeruginosa pneumonia (1 case). MMF was reduced or discontinued in 63.8% (30/47) of patients in Group I, while only 8.5% (4/47) of patients in Group II required the same adjustment (\(P < 0.001\)). The proportion of patients with withdrawal vs reduced dose of MMF was 22/8 in Group I, compared to 2/2 in Group II. The main reason for MMF dose adjustment was bone marrow suppression - in 44.7% (21/47) patients in Group I and in 6.4% (3/47) patients in Group II. MMF dose was adjusted during severe infection in 7 patients in Group I and in 1 patient in Group II (Table 3). In addition to regulation of MMF dosage, VGC had to be temporarily withdrawn in 14.9% (7/47) of patients in Group I due to inadequate compensation of blood count. Agranulocytosis requiring administration of G-CSF (Filgrastim) was reported in 6.3% (3/47) patients in Group I. Administration of MMF had to be modified due to gastrointestinal toxicity in addition to 4.3% (2/47) of patients in Group I. No gastrointestinal intolerance in relation to MMF administration was found in Group II. The number of patients with at least one episode of graft rejection (n=94) within 12 months after index procedure in Group I versus Group II had the following values: without rejection (8/4), 1R/1A rejection (25/17), 1R/2
and higher rejection (11/22), humoral rejection (2/2), humoral + cellular rejection (1/2) (Table 4).

Interestingly, modification of immunosuppressive therapy was not associated with increased incidence of rejections. At least 1 episode of higher degree cellular or humoral rejection occurred in Group I in 35% (6/17) of patients with the standard MMF dosage compared with only 26% (8/30) occurrence rate in patients with dose reduction or complete withdrawal of MMF ($P = 0.0534$).

**DISCUSSION**

Data are scarce on the clinical impact of different immunosuppressive regimens used in cardiac transplant care on bone marrow suppression. Clinicians are compelled to choose between interim ignoring low WBC count, adjusting the MMF/VGC dose or using G-CSF. Moreover severe infections require adjustment of the patient’s immunosuppressive regimen after heart transplantation. This therapy modification increases the risk of graft rejection.

Infection remains a major cause of morbidity and mortality following transplantation, but antimicrobial prophylaxis regimens continue to improve. The most recommended prophylactic antimicrobial therapy consists of VCG and TMP-SMX. Although these drugs decrease the risk of infectious complications, they may increase the myelotoxic effects of immunosuppressives. Since 2008, both VGC and TMP-SMX have been added to immunosuppressive regimen of all patients undergoing heart transplant at our institution. In this study, we found that “the current medication regimen” (including VGC+ TMP-SMX) causes more pronounced bone marrow suppression than the “previous medication regimen”. A significant proportion of patients on current medication had lymphopenia and leukopenia. No other blood elements were

---

**Fig. 1.** Comparison of both regimens in terms of myelotoxicity (number of blood elements in reference periods).
MMF in patients with lowered dose of the drug. Only both drugs. VGC could potentially increase the level of 
ability on potential pharmacokinetic interaction between 
nated by renal excretion, no convincing data are avail 
crease the incidence of CMV infections that have been 
findings are consistent with the results of the TICTAC 
acute graft rejection. In contrast, Khosroshahi et al. showed that MMF dose reduction was not associated with 
mmunal side effects related to 
forcement in the “current regimen” group. The reason for 
this significant difference was pronounced bone marrow 
and graft loss. However, studies investigating this relation 
are mainly related to renal transplants, while data from cardiac transplant registries are scarce. Knoll et al. found that the cumulative number of days on reduced MMF dose increased significantly the risk of acute graft rejection. In contrast, Khosroshahi et al. showed that MMF dose reduction was not associated with an increased risk of acute allograft rejection or impaired 
s, Withdrawal due to intolerance or toxicity is nec 
complete withdrawal have not been clearly established. Some evidence suggests that MMF dose modification, either reduction or discontinuation, may increase rejection rate and graft loss. However, studies investigating this relation 
importantly, modification of immunosuppressive therapy in Group I was not associated with increased incidence of rejections ($P = 0.0534$). These findings are consistent with the results of the TICTAC trial, which showed that addition of MMF to single-agent immunosuppression does not provide any advantage over single-agent immunosuppression in terms of rejections. Similar results were also found by Teuteberg who reported a regimen consisting of a dose reduced MMF in patient after HTx.

In our study population, we modified or completely withdrew MMF from therapy in 63.8% of patients in the “current regimen” group and only in 8.5% patients in the “previous regimen” group. Importantly, modification of immunosuppressive therapy in Group I was not associated with increased incidence of rejections ($P = 0.0534$). These findings are consistent with the results of the TICTAC trial, which showed that addition of MMF to single-agent immunosuppression does not provide any advantage over single-agent immunosuppression in terms of rejections. Similar results were also found by Teuteberg who reported a regimen consisting of a dose reduced MMF in patient after HTx.

In our study population, we modified or completely withdrew MMF from therapy in 63.8% of patients in the “current regimen” group and only in 8.5% patients in the “previous regimen” group. Importantly, modification of immunosuppressive therapy in Group I was not associated with increased incidence of rejections ($P = 0.0534$). These findings are consistent with the results of the TICTAC trial, which showed that addition of MMF to single-agent immunosuppression does not provide any advantage over single-agent immunosuppression in terms of rejections. Similar results were also found by Teuteberg who reported a regimen consisting of a dose reduced MMF in patient after HTx.

CONCLUSIONS

Contemporary immunosuppressive and anti-infective drug prophylaxis is associated with significant lympho-
cytopenia and leukopenia. In case of a profound drug-
induced myelotoxicity, reducing or stopping MMF leads 
to normalization of blood count and does not inflict in-
creased incidence of rejection episodes.

ACKNOWLEDGEMENT

We would like to thank Vera Lanska for help with statistical analysis.

Authorship contributions: MP, IM: research, manu-
script writing; EK, JS, JK: manuscript writing.

Conflict of interest statement: The authors state that 
there are no conflicts of interest regarding the publication 
of this article.

REFERENCES


