THERAPEUTIC DRUG MONITORING OF MYCOPHENOLIC ACID
A retrospective statistical study
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<td>Autoimmune diseases</td>
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<tr>
<td>aGvHD</td>
<td>acute graft versus host disease</td>
</tr>
<tr>
<td>AMP/ATP</td>
<td>Adenosinmonophosphate/Adenosinetriphosphate</td>
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<tr>
<td>AUC&lt;sub&gt;0-12h&lt;/sub&gt;</td>
<td>Area under curve – 12 hours after MPA administration</td>
</tr>
<tr>
<td>CoV</td>
<td>Coefficient of variation</td>
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<tr>
<td>CsA</td>
<td>Cyclosporine A</td>
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<td>EMIT</td>
<td>Enzyme multiplied immunoassay technique</td>
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<td>fMPA</td>
<td>unbound/free fraction of MPA</td>
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<tr>
<td>fMPAG</td>
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<td>GMP/GTP</td>
<td>Guanosinemonophosphate/Guanosinetriphosphate</td>
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<td>HLPC</td>
<td>high performance liquid chromatography</td>
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<td>HTX</td>
<td>Heart transplantation</td>
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<td>IMPDH</td>
<td>inosine monophosphate dehydrogenase</td>
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<td>LC-MS</td>
<td>Liquid chromatography–mass spectrometry</td>
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<td>LTX</td>
<td>Liver transplantation</td>
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<td>MMF</td>
<td>mycophenolate mofetil</td>
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<tr>
<td>MPA</td>
<td>mycophenolic acid</td>
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<tr>
<td>MPAG</td>
<td>mycophenolic acid glucuronide</td>
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<tr>
<td>MRAP-2</td>
<td>multidrug resistance-associated protein 2</td>
</tr>
<tr>
<td>RTX</td>
<td>kidney transplantation</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SLE</td>
<td>systemic lupus erythematosus</td>
</tr>
<tr>
<td>tMPA</td>
<td>total (bound + unbound) MPA</td>
</tr>
<tr>
<td>tMPAG</td>
<td>total (bound + unbound) MPAG</td>
</tr>
<tr>
<td>TRL</td>
<td>Tacrolimus</td>
</tr>
<tr>
<td>UDPGT</td>
<td>uridine diphospahte-glycuronosyl transferase</td>
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1 Introduction

The discovery of immunosuppressants has always been in progress. The first used immunosuppressant was the classical Azathioprin, which was then replaced by Cyclosporin A (CsA) and later by Mycophenolate mofetil. Advancement in the invention of new immunosuppressive drugs offers us an opportunity to combine them in order to block different pathways of the immune system simultaneously. By combining multiple drugs low dose treatment is allowed, to lessen the side effects [26].

In 1896, Gosio succeeded in deriving Mycophenolic acid (MPA) from cultures of Penicillum spp.. And later, it was purified by Alsberg and Black in 1913. It is said to have antibacterial, antifungal and antitumor activity [26]. MPA, an active metabolite of the prodrug mycophenolate moefitil (MMF), is now counted among the best immunosuppressants in the prevention of transplant rejections, acute graft versus host disease (aGvHD) and also in the treatment of autoimmune diseases, such as Lupus nephritis, IgA nephritis, rheumatoid arthritis, antineutrophil cytoplasmic antibody-associated vasculitis and autoimmune hemolytic anemia [18,27].

As we all know every medication has risks, as well as benefits. Main side effects of MMF are gastrointestinal discomforts and hematological toxicity. With the help of therapeutic drug monitoring by measuring MPA blood concentrations one can control the MPA level, to which the body is exposed. On the one hand it helps us achieve enough serum MPA level to prevent graft rejection or deterioration of kidney function and on the other hand helps us prevent the occurrence of adverse effects.

In this work, our main objective was to find out which actions were taken, regarding the MPA serum levels and how did it influence the clinical outcome.
1.1. Mycophenolic acid

1.1.1. Pharmacodynamics

MPA is a selective, non-competitive and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), an enzyme, which is important for the biosynthesis of guanine nucleotids in the de novo pathway of DNA synthesis.

As lymphocytes are totally dependent on the de novo pathway, the inhibition through MPA is specific. From the two isoforms of the IMPDH, IMPDH1 is expressed in most cell types while IMPDH2 is only expressed in activated lymphocytes. MPA inhibits IMPDH2 up to 4-5 fold more than the other isoform [2].

![Diagram of DeNovo pathway]

Figure 1: The diagram shows where MPA interacts in the de novo pathway and depletes the production of GMP. Illustration by KajalEichner, based on Immunopharmacology 47 (2000) 85 - 118

Due to a decrease in the number of guanosine nucleotides the glycolysation of lymphocyte and monocyte glycoproteins is also suppressed. The expression of adhesion molecules, which are important for recruiting lymphocytes to the site of inflammation and graft rejection is also suppressed [2,18,25,26]. This inhibition is said to be dose dependent [25]. As not only proliferation of T-cells but also of B-cells is inhibited, we can observe a reduction in antibody production [26,63]. A non-randomized study, which included 65 stable renal transplant recipients showed that interleukin-2 production was significantly more reduced in the combined therapy group (CsA+MMF and Tacrolimus+MMF) than in
CsA or TRL alone. This outcome shows the additional inhibitory effect of MPA on interleukin-2, which could be explained as follows: MPA inhibits the clonal expansion of activated lymphocytes; decreases the population of activated lymphocytes and thereby indirectly contributes to the inhibition of calcineurin activity and interleukin-2 production. According to this study, IL-2 is a good predictor “for monitoring combined therapies comprising calcineurin inhibitors (CNIs) and MMF” [47].

MPA has not only build up its place in transplant recipients but also in the treatment of autoimmune diseases. Many randomized controlled studies could show its effectiveness in the treatment of systemic lupus erythematoses (SLE) and of glomerulonephritis, both in induction and maintenance therapy [12].

SLE is a systemic autoimmune disease, which is often characterized by frequent renal involvement and T- and B-cell dysfunction. According to historical data and small pilot studies MPA is said to be beneficial in the treatment of other autoimmune diseases like IgA nephritis, rheumatoid arthritis, antineutrophil cytoplasmic antibody-associated vasculitis, severe psoriasis and autoimmune hemolytic anemia. It could be shown that MPA had efficacy in controlling major renal manifestations caused by lupus nephritis [18, 34]. Child-onset systemic lupus erythematosus (cSLE) is treated with off-label use of MPA (weight-adjusted). This study consisted of patients with cSLE. It shows that peak MPA concentrations coincide with the greatest suppression of IMPDH activity. Even weight-adjusted dosing of MMF, however, was a bad predictor of MPA exposure [61].
In case of rapidly progressive glomerulonephritis, maintenance therapy with MPA showed a reduction in disease activity, which was measured with the help of Birmingham vasculitis activity score and proteinuria. With regard to adverse effects “the overall frequency of malignancies in the transplant studies in which MMF was combined with other immunosuppressive drugs was as low as 1%”[52].

A. N. Abd Rahman showed that a target MPA AUC$_{12}>35$ mg.h/l is likely to have better efficacy outcomes in patients with autoimmune diseases. A. N. Abd Rahman also observed that serum albumin was responsible for 70% of between subject variability in the unbound fraction of MPA and that serum albumin together with age and co-medication accounted for 60% of the between-subject variability in the unbound fraction of MPAG [1]. It was found that high albumin levels (>25 g/L) led to a reduction in free fraction of MPA. These low levels of free MPA fraction correlated with low immunosuppression [63].

Not only in renal but also in heart transplantation, MPA showed lower rejection rates and overall better survival in comparison to Azathioprin treatment [4].
In a preliminary study conducted, it could be demonstrated that MPA can be successfully used to prevent acute graft versus host disease (aGvHD). No nephrotoxicity, liver toxicity and overall low rate of adverse events were associated with the MPA treatment. Even steroid sparing effect of MPA became apparent as MPA administration allowed a significant dose reduction of prednisolone during the treatment, which was not the case when treated with Cyclosporin [4].

1.1.2. Pharmacokinetics

MMF is a pro-drug of the 2-morpholinoethylester of MPA. After oral administration it is completely hydrolyzed in the upper gastrointestinal tract [64] into its active metabolite, MPA and hydroxyethyl morpholine by plasma esterases [26]. Therefore, it is not possible to measure MMF concentration levels in plasma after oral administration [33, 67]. The oral bioavailability of MPA after MMF administration is about 94% [40].

Hydroxyethyl morpholine is an inactive metabolite that is rapidly eliminated via urinary tract. MMF is less soluble in water. MPA in serum is 97-99% bound to albumin and the binding merely correlates with MPA concentration. Lowering in the serum albumin (caused by liver and renal abnormalities) causes rise in the free fraction but not the absolute free amount of MPA concentrations (fMPA) [67,77]. The absolute unbound concentration (fMPA) however, is responsible for the immunosuppressive effect [16].

![Chemical structures of MMF, MPA and MPAG and their metabolization. Illustration by Kajal Eichner](image-url)
MPA is metabolized to MPAG (mycophenolic acid glucuronide) and AcMPAG (minor acylglucuronide) by UDPGT (uridine diphospho-glucuronosyl transferase) mainly during its passage through the liver, but also in the GI tract and kidney [27, 64]. MPA plasma concentration reaches its peak one hour after the oral administration, later comes a second plateau between 3-4 hours [64]. In a study performed by van Hest RM et al the mean metabolic half-time of MPA came out to be about 11 h in kidney transplant recipients [74]. Thus, supporting the fact of at least 2 doses per day are needed to maintain the MPA levels.

MPAG itself is pharmacologically inactive and is bound in serum approximately 82% to protein [33]. It underlies the enterohepatic recirculation (EHC) where it’s transported into the bile with the help of Multidrug Resistance-Associated Protein 2 (MRAP-2) and later deconjugated via glucuronidases shed by GI tract bacteria in the small intestine in MPA, which can be absorbed again and is excreted via the kidney. This procedure of the EHC is responsible for the second peak appearing under the area under curve 6-8 hours after been orally taken [27, 33, 64]. “EHC contributes approximately 40% to MPA exposure” [1]. Therefore, any disturbances in the transportation or in the glucuronization would increase or decrease the exposure to MPA and MPAG [27]. MPAG also seems to hold an immunosuppressive effect but doesn’t inhibit IMPDH [63].
fMPA has an higher affinity to protein binding sites in comparison to fMPAG. In normal conditions MPAG cannot displace MPA from albumin as its accumulation leads to increased enterohepatic recirculation. It isn’t the case while co-treated with CsA as it inhibits MRAP-2 and thus the recirculation of MPA. CsA causes displacement of MPA by MPAG [16].

MPA is mainly excreted by active tubular secretion of MPAG into urine (up to 87%) [1], therefore making the role of kidney function very important [27]. Transporters that play a role in the secretion of MPAG are organic anion transporters and multidrug resistance protein 2 [1].

While administration of EC-MPS (enteric-coated mycophenolate sodium), an enteric coated form of MPA, maximum levels after dose administration are achieved later than MMF [64]. This new formulation has been developed to avoid gastrointestinal adverse events and to prevent consistent dose interruptions and dose reductions in order to prevent gastrointestinal discomforts [36]. As we know that MMF is needed to be hydrolized to
form the active metabolite, MPA, this isn’t the case with EC-MPS. In addition, EC-MPS isn’t affected by the concomitant use of proton pump inhibitors (PPIs) [1,38]. Patients with autoimmune diseases showed a higher MPA exposure after administration of EC-MPS compared to MMF but the difference in exposure didn’t seem to effect the inhibition of IMPDH activity [1]. Many patients get pantoprazole as concomitant drug. Pantoprazole increases the pH in the stomach, which causes a slow release of MMF, as it has a typical pH-dependent solubility profile. The solubility of MMF decreases with rising pH-values [42]. A single and blind, randomized, multicenter trial showed that EC-MPS and MMF are therapeutically similar and have a comparable safety profile [36].

1.1.3. Adverse effects
Gastrointestinal side effects (diarrhea, nausea, vomiting), hematologic effects (leucopenia and anemia), cardiovascular risk factors (hypercholesterinemia, hypertension), infections (cytomegalovirus, polyomavirus, bacterial infections like pyelonephritis, pneumonia), osteoporotic/osteonecrosis fractures and neurologic effects (headache, tinnitus), genitourinary symptoms (urgency, dysuria, burning, sterile pyuria) are said to be associated with MPA [34,57,62]. As it’s also a teratogenic agent, contraception is required in ladies of childbearing age [13]. A prospective, open-label study performed by Kuypers et al demonstrated that anemia and leucopenia yet show correlation with high MPA trough levels [39].

As the unbound concentration is responsible for the immunosuppressive effect, an increase causes infections and haematological side effects. Decreased unbound concentrations correlate with a higher risk for acute rejection [16]. Acute rejection is the most feared outcome in the early phase after transplantation.

In 2003, a prospective study including kidney transplant recipients, reported that 35% of the patients with persistent diarrhoea developed Crohn’s-like diseases [45]. Later, Dalle IJ et al reported same changes [15]. But this development seems to be reversible after discontinuation of MMF [19]. The development of this inflammatory bowel disease is explained as follows: AcMPAG, one of the metabolite of MPA, binds to proteins which are then chemically modified and act as antigens for the immune system. In the gut this leads to the activation of the immune system, thus causing autoimmune reaction [69].
Figure 5: Duodenal biopsy: demonstrates the dilated duodenal crypt with necrotic debris [30]. Courtesy and © ACG Case Rep J 2016;3(2):101-103. doi:10.14309/crj.2016.13. Published online: January 20, 2016, reprinted with permission.

In a large, randomized trial MMF, CsA and steroids, given together in a combination, have emerged as a standard therapy [62]. MPA-AUC correlates with the MPA exposure highly. While many studies could show the relationship between low MPA exposure and acute rejections, no convincing relationship could be found between high MPA exposure and toxicity.

Psoriasis patients treated with MMF rarely developed leukopenia in comparison to transplant recipients [26]. Leukopenia is explained as follows: free MPA can more easily access to bone marrow from the circulation than protein-bounded MPA where precursor cells are inhibited and leucopenia is caused [78]. Trough concentrations >3mg/L were associated with the higher incidence of side effects. But no association could be found between MPA and CMV infection within small bowel, liver and pancreas transplantation [40].
1.1.4. Drug interactions

Between-subject variability has many reasons, which include differences in hepatic and renal function, serum albumin, bilirubin and haemoglobin concentrations, concomitant administration of interacting drugs, co-morbidities, body weight, gender, race, time after transplantation, genetic polymorphism [1].

*Table 1: Primary factory that can alter MPA exposure. Adapted from the Clinical Journal of the American Society of Nephrology 2: 1062-1072, 2007*

<table>
<thead>
<tr>
<th>Drugs, Disease</th>
<th>Effect and Site of Interaction</th>
<th>Effect on MPA AUC</th>
</tr>
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<tbody>
<tr>
<td>Cyclosporin A</td>
<td>Suppression of export of MPAG and EHC of MPA into bile by inhibition of MPAG transport via Mrp2 transporter</td>
<td>Decreased MPA AUC, increased MPAG AUC</td>
</tr>
<tr>
<td>Antacids, PPIs</td>
<td>Decreased absorption in the gastrointestinal tract</td>
<td>Decreased MPA AUC</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Suppression of EHC of MPA by loss of shed glucuronidase as a result of inhibition of anaerobic bacteria</td>
<td>Decreased MPA AUC</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Stimulation of biosynthesis of UGT and consequent increased MPA clearance</td>
<td>Decreased MPA AUC</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Impaired absorption of MPA</td>
<td>Decreased MPA AUC</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>Increased fMPA, possibly reduced intrinsic clearance</td>
<td>Increased free MPA AUC</td>
</tr>
<tr>
<td>Promotor variants within the UGT1A9 gene</td>
<td>Genetic variability of expression of UGT1A9</td>
<td>Decreased MPA AUC or trough</td>
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</table>

MPA interacts with many drugs. Co-administration with CsA and cholestyramin leads to lower exposure by inhibiting the enterohepatic circulation [40]. It’s proved that CsA inhibits MRAP-2, therefore, stopping the transportation of MPAG into the bile, resulting in
high MPAG exposure in plasma. The function of MRAP-2 is not only the excretion of MPAG into the bile but also other endogenous (e.g. Bilirubin) and exogenous (e.g. diclofenac and valproic acid) conjugates. CsA is clinically relevant because it can reduce the MPA exposure up to 30-40% and it is nephrotoxic [27, 60]. In a 2-year, randomized study, L Frimat et al showed an improvement in the kidney function without increasing the risk for graft rejections on 50% CsA dose reduction in combination with MMF [24]. There also exists a recommendation to measure MPA plasma levels dependent on co-medication, such as CyA or Tacrolimus. According to Shaw LM et al and if MPA is prescribed together with CyA, the trough MPA levels should range between 1.0-3.5 μg/mL. The AUC target is between 30-60 μg*h/mL [67] after renal or heart transplantation. And if prescribed together with Tacrolimus then trough levels differ a lit bit (≥ 1.9 μg/mL) [71].

Corticosteroids and rifampicin also decrease MPA exposure via stimulating the biosynthesis of UGT (uridine diphosphat-glycuronosyl transferase). Antibiotics reduce exposure by affecting MPA re-absorption, as glucuronidases are shed by the GI tract bacteria for the formation of MPA from MPAG via reducing its hydrolization. Genetic variability in the expression of UGT1A9 gene can also cause decrease MPA trough levels [65].

There are also other drugs that seem to influence the binding of MPA to human serum albumin (HSA). Sodium salicylate and furosemide decrease the binding of MPA to HAS, whereas CyclosporinA, prednisone, warfarin, digoxin, phenytoin, ranitidine, cimetidine, heparin, ibuprofen do not effect its binding to HAS [68].

It could be shown in different studies that MPA exposure is subjected to inter-patient variability. One study showed that impaired renal function, low albumin levels and CsA co-administration tend to increase the clearance of MPA. Whereas these changes influenced the total fraction of MPA (tMPA), the unbound fraction was hardly affected. An impaired renal function and co-treatment with CsA correlated with high concentrations of both fMPAG and tMPAG and low concentration of tMPA, whereas opposite is the case in co-treatment with tacrolimus [16].
1.1.5. Therapeutic drug monitoring

The need of MPA TDM is still been questioned although many studies have been done on this topic. TDM seems to be very helpful in cases where exposure of a particular drug can’t be predicted dependent on the dose of that drug. Vast interpatient-variability in the MPA concentration found in patients on standard dose and in the pharmacokinetics of MMF is evidence enough for many institutes to do TDM. Interpatient-variability is mainly controlled by renal function, serum albumin and co-therapy with CNIs [73]. With the help of TDM the following structural change in the clearance of MPA could be shown: The clearance of MPA decreases in the first year after transplantation whereas the exposure gradually increases [29,75].

MPA AUC 0-12 hours shows a stronger correlation with acute rejection than the pre-dose plasma concentration but a weak correlation with drug-related toxicity [40]. Co-administration with CsA causes reduction in MPA concentration, therefore, the tMPA AUC 0-12 should amount to 30-60mg h/l in renal transplant recipients [16]. No correlation was found in MPA AUC 12 and side effects of MPA.

Table 2: Indications for TDM [40]; CNI = calcineurin inhibitors, such as Cyclosporin A. Adapted and modified from Clinical Journal of the American Society of Nephrology 5: 341 – 358, 2010

<table>
<thead>
<tr>
<th>Indications for a MPA-Therapeutic drug monitoring are as follows:</th>
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<tr>
<td>Co-therapy with CNI</td>
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<td>Drug interactions, such as pantoprazole</td>
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<td>Recipients with high immunologic risk</td>
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<tr>
<td>Delayed graft function</td>
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<tr>
<td>Altered gastrointestinal/hepatic/renal function</td>
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<tr>
<td>Haematologic toxicity</td>
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<tr>
<td>Non-compliance</td>
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Leslie M Shaw et al also defined main characteristics for immunosuppressive drugs such as cyclosporine, tacrolimus, MPA and sirolimus: ‘a narrow therapeutic window; variable absorption, distribution and elimination; and evidence for correlation between drug concentration and clinical outcome, efficacy and toxicity’ [66]. These factors favor the establishment of TDM. In some patients MPA-AUC exceeds higher values than needed and according to T van Gelder et al it would be of advantage to identify those patients with the help of therapeutic drug monitoring in order to reduce not only drug dose but also medication cost and improve safety [73].

The Adaption de Posologie du MMF en Greffe Renale (APOMYGRE) trial is about a randomized study performed in France on kidney transplant recipients. Patients were either assigned to the fixed-dose therapy regime group or to the concentration-controlled group. MPA-AUC target was set to 40 mg*h/L and MMF dose adjustments were calculated with the help of a computer program. After 12 months there was a significant difference between the two groups, which included less treatment failures and less biopsy-proven acute rejections [41]. A second larger study, known as FDCC (Fixed-dose versus concentration-controlled) included 901 renal transplant patients, both adults and children. This study couldn’t find any difference between the both groups as the MMF dose adjustments were made by the investigators and not with the help of a computer program as in APOMYGRE trial. This could also be the reason for different results as the adherence maybe lower in the FDCC study [72]. Because of an indefinite outcome, these studies could not enforce the establishment of TDM for MMF.

MPA plasma levels can be measured with the help of HLPC (high performance liquid chromatography) and EMIT (enzyme-multiplied immunoassay technique). EMIT is less specific than HPLC because it cannot differentiate AcMPAG, a metabolite of MPA, from the main drug, therefore MPA levels occur to be higher if measured with this tool [31]. Schutz E et al also showed that plasma levels of MPA when measured with EMIT were up to 24-35% higher [63].
1.2. Hypothesis and objectives

MPA has gained a lot of popularity in the recent years due to its tolerable side effects, a comparable high efficacy and safety profile. It offers a most favorable substitute for other immunosuppressant therapies till date. But it’s still in debate, whether it can completely displace the classical immunosuppressants like azathioprin or CyA. Therefore, more research has to be done to answer these questions.

The clinical parameters offer us a good way to watch the efficacy of MPA. Haematological parameters (hemoglobin, leukocytes and platelets) and kidney function (serum creatinine level and proteinuria) are the main factors this study lays pressure on. And also adverse events will be analyzed. Most common complications, which lead to the discontinuation of MMF are gastrointestinal discomforts (up to 40%) [44].

The objectives of this retrospective study are not generalizable, but explorative and hypothesizing and this research also ensures quality assurance. The main objectives of this study were as follows:

1. How high were the trough levels?
2. Which actions were taken, regarding the trough levels of MPA?
3. How was the clinical course of the patients regarding serum creatinine levels and proteinuria? Were trough levels of the patients with successful outcomes higher or lower?
4. Were there any complications, such as infection or gastrointestinal toxicity or hematological toxicity? How was the correlation between complications and the trough levels?

Since this study was retrospective, data extraction and final outcome assessment from medical records might not have been optimal, because the recorded information varied among patients.
2 Material and Methods

2.1. Patient data

This retrospective study takes a look at patients, who were treated with MPA in the division of nephrology and hematology, at the University of Ulm. In most cases therapy isn’t completed yet. It specifically applies for patients with autoimmune diseases, such as IgA nephritis and lupus nephritis. The indication for the treatment with MPA was evidence-based; however the evidence varied from degree 1a to 2b. Mycophenolate drug monitoring was performed in the institution for clinical chemistry, Ulm University. Approval has been obtained from the ethics committee of the Ulm University under the proposal number 17/14.

Measured MPA trough level concentrations were provided by the clinical chemistry with patients’ code, name and age. The patient data was collected with the help of SAP database (University hospital of Ulm, clinical record and discharge reports). To access the SAP username and password was required. Confidentiality and data protection regulation was observed strictly and patients’ data was only accessible from the university computers with SAP software installed. The data was captured and evaluated anonymously.

The study involved a total of 50 consecutive patients (23 males and 27 females) to restrict the volume of work and due to the limited observation period. The age of the patients at the beginning of the therapy ranged between 18 to 84 years (mean age 51 year).
Figure 6: x-axis shows the age of the patients and y-axis the gender and total number of patients treated with MMF at the University of Ulm. n=50

2.2. Protocol

The main aim of this study was to check which actions were taken regarding the MPA trough level concentration. The trough levels were measured with a validated HPLC (High-performance liquid chromatography) and LC-MS (Liquid chromatography–mass spectrometry) procedure. LC-MS is an HPLC system with a mass spec detector.
Before the beginning of the therapy with MPA following data was obtained: weight, blood pressure, haemoglobin, white blood cell count, platelets, serum creatinine, proteinuria, co-medication: antihypertensive, antidiabetic, other immunosuppressant drugs, (such as cyclosporin, tacrolimus, prednisolone) and pantoprazole. On follow-up, haemoglobin, white blood cell count, platelets, serum creatinine and proteinuria were measured after the first week, one month, three months, 6 months and one year after the therapy. Attention was also paid to the occurrence of infections (such as pneumonia), leucopenia and gastrointestinal side-effects, transplant rejection and respectively acute graft versus host disease (aGvHD). The therapy was modified when trough levels were lower or higher than the 2,5-4,5 mg/l or terminated, if no improvement was seen in laboratory parameters, in case of pregnancy, side effects or death. The dose of MPA varied from 360 mg/d to 3000 mg/d. MMF or MPA were given in two different formulations: Cellcept and Myfortic. Dependent on the development of gastrointestinal side effects the medication was switched from Cellcept to Myfortic. The duration of MPA therapy varied from three months to more than ten years. MPA was given either as induction therapy or maintenance therapy in case of autoimmune diseases and kidney, heart or liver transplantation. And patients with stem
cell transplantation were given MPA as prophylaxis to prevent acute graft versus host disease.

2.3. Statistical analysis

Microsoft Excel (Office 2010) was used for statistics, calculations and graphic interfaces. The data was analyzed with descriptive statistics. Our main objective was to investigate the development of the MPA trough levels over time. Furthermore, other variables such as creatinine, proteinuria, leukocytes, haemoglobin and platelets were examined.

Coherences and correlations between the variables mentioned above and MPA trough levels were shown in bivariate scatter diagrams. Statistical parameters used, are median, mean, maximum and minimum, standard deviation (SD), coefficient of variation (CoV) as continuous parameters and absolute and relative frequency as categorical parameters. The influences of outliers and wrong data were also examined and discussed. To identify potential outliers, scatter diagrams and calculative results of collected data and parameters (mainly minimum, maximum) were compared. The influences of outliers on results were examined by cross analyzing data calculated with and without outliers. In addition subgroups were created to capture disturbance values during data analysis and compare the behaviour of mycophenolate trough levels in different cohorts oriented on age, gender, diagnosis and co-medication. In case of a disturbance the model and database could be further adjusted.

Since it is a retrospective study, results are interpreted for explorative purposes. A p-value of less than 0.05 was considered statistically significant. The results were compared and evaluated with other respective studies.
3 Results

In our retrospective study we gathered data of 50 patients (23 males and 27 females). Mean age at the beginning of the therapy with MPA was 51 years (ranged from 18 to 84 years). MPA was administered to patients suffering from one or more of the following medical entities:

- autoimmune diseases (lupus nephritis, IgA nephritis, chronic glomerular disease, rapid progressive glomerulonephritis)
- transplant recipients (kidney, heart, liver)
- prevention of acute graft versus host disease

The observation time varied from less than a year to more than 10 years at the time of data collection. At the time of data assessment patients cohorts with autoimmune diseases varied in their MPA treatment period from less than a year to more than 10 years, whereas transplant recipients (8 patients) received MPA for at least more than 2 years. The average administration period for MPA to prevent aGvHD was around a year.

The MPA dose given was based on the diagnosis with an average dose of 1456 mg per day. The dose varied from 360 mg per day to 3000 mg per day. Patients not only received MPA as immunosuppressant but also others, such as CyA, tacrolimus and prednisolone. Other medications included pantoprazole, oral anti-diabetics and anti-hypertensives.

3.1. How high were the trough levels?

The trough levels of mycphenolate mofetil were analysed dependent on the gender, age of the patients, dosis per day, diagnosis and therapy type. For every individual case the mean value of measured MPA concentration was calculated. The mean MPA level of all patients was evaluated from mean individual values:
Figure 8: Median: 2mg/L; Minimum: 0.1 mg/L; Maximum: 11 mg/L. The mean value of trough level measurements per patient was 3 mg/L (range: 1 to 14 mg/L) and the median of all trough levels is 2 mg/L (range: 0.1 to 6.4 mg/L), which is lower than the target range of 2.5-4.5 mg/L, n=50.

Figure 9: Males showed an average trough level of 1.865 mg/l (SD: +/-1.620, CoV: 0.868) whereas females were in the target range of 3.088 mg/l (SD: +/-1.620, CoV: 0.525), male: n=23, female: n=27.
Figure 10: Influence of age on the mean trough levels of MPA. With the aging the average trough levels seem to rise but the result is not significant, $r^2=0.003$, $n=50$

![Figure 10](image)

Figure 11: This diagram depicts that increased dosage leads to increased trough levels of MPA, $r^2=0.10$, $n=50$

![Figure 11](image)
Figure 12: This diagram shows that even though in all three groups the dose per day did not differ a lot (diamond), there was difference in the mean trough levels (square). Whereas transplant recipients and patients with a GvHD prophylaxis had almost the same mean trough levels (which was under the target range), the patients with autoimmune diseases showed mean trough levels of above 3 mg/l. (AD = autoimmune diseases; KTX/HTX/LTX = Kidney, heart, liver transplant recipients; aGvHD = acute graft versus host disease), n=50
Figure 13: MPA trough levels were in the reference range in case of induction therapy (2.99 mg/L; SD: +/- 1.789; Coefficient of Variation 0.59) and maintenance therapy (2.83 mg/L; SD: +/- 1.561; CoV 0.55) and below the reference range in case of aGvHD (1.49 mg/L; SD: +/- 0.816; CoV 0.54), n=50.

Figure 14: This figure shows us the influence of Cyclosporin on the trough levels of MPA. The mean levels of MPA were in the range if they did not receive Cyclosporin as co-medication. Whereas Tacrolimus did not seem to influence the trough levels of MPA as compared to Cyclosporin. Cyclosporin (SD: +/- 0.914 vs 1.734; CoV: 0.71 vs 0.61, n=11); Tacrolimus (SD: +/- 0.697 vs 1.883; CoV: 0.33 vs 0.77, n=10).
Figure 15: This diagram shows how concomitant treatment with pantoprazole effects the mean MPA trough level concentration. Group YES: SD: +/- 1.487, CoV: 0.58; Group NO: SD: +/- 2.256, CoV: 0.8, n=50

Figure 16: This diagram reflects the influence of oral antidiabetic medication on MPA trough levels. The trough levels of MPA were higher by 1.0 mg/l if the mentioned medication was not taken. Group YES: SD: +/- 1.671, CoV: 0.686; Group NO: SD: +/- 1.731, CoV: 0.506, n=50
Figure 17: This diagram shows that if blood pressure (BP) medication is given mean MPA trough level is higher and in the therapeutical range. BP medication YES: SD +/- 1.547; CoV: 0.55; BP medication NO: SD +/- 1.997; CoV: 0.96, n=50
3.2. Which actions were taken, regarding the trough levels of MPA?

As shown below in Figure 18 and Figure 19 different actions were taken regarding the trough levels: 243 times MPA trough levels were measured; 183 times no changes were made; 7 times no changes were made in MPA dose even if it was under the target level of 2,5 mg/L because it was administrated in combination with at least one other immunosuppressant (such as CyA, tacrolimus or steroids); 9 times no changes were made even though the trough levels were lower but they were reconsidered and justified; 17 times dose was reduced due to high trough levels. When they were in target range than the dose was reduced due to occurrence of side effects such as infections, leucopenia or gastrointestinal discomfort. 15 times dose of MPA was increased because of low target level; 7 times it was aborted due to pregnancy, death, no improvement or unnecessity.

Figure 18: Response to MPA drug level measurements. n=50
The medication continuation, change of dose, dose adjustment or discontinuation was obviously not influenced by the MPA trough levels measured. Thus, an influence of TDM on MPA dosing could not be demonstrated.
3.3. How was the clinical course of the patients regarding serum creatinine levels and proteinuria?

This diagram shows the changes in the serum creatinine concentration over the time period of one year. 0= at the beginning of the therapy. The standard deviation varies from 71.27 to 183.01 (coefficient of variation from 0.46 to 0.91) because though there were 50 patients, labs were not checked always.

**Figure 20:** There was a trend to an improvement of kidney function during 12 months. n=34

The course of creatinine and thus of kidney function was stable over the time of 12 months.
This diagram shows the changes in the proteinuria over the time period of one year. 0= at the beginning of the therapy. The standard deviation varies from 980.67 to 2078.82 (coefficient of variation from 1.08 to 1.61) because over the period of time checkups were not done properly and regularly due to lack of compliance from patient’s side.

![Proteinuria over the course of time](image)

**Figure 21:** Proteinuria improved under MPA treatment. n=11

The urinary protein excretion decreased from 1700 mg to 700 mg after 12 months. Maximum proteinuria was assured on week 1 in agreement with the presumed indication for MPA treatment.
This diagram illustrates that patients with autoimmune diseases and transplant recipients (n=38) had higher creatinine levels (standard range: 59-104μmol/l) before the beginning of the therapy and after three month of therapy their creatinine levels began to decline. But patients who received MMF to prevent aGvHD (n=9) creatinine levels increased over three months period.

Figure 22: In patients with kidney disease, serum creatinine improved. Patients with graft versus host disease had low and later normal creatinine levels. n=38, 12 subjects not specified
This diagram only reflects the result of 11 patients where a follow up of proteinuria took place. Proteinuria 0 = at the beginning of the therapy (mean: 1469 mg/l; SD: +/- 2118.565 mg/l; CoV: 0.873); Proteinuria 1= three months after the beginning of the therapy (mean 1189 mg/l; SD: +/- 1285.565 mg/l; CoV: 1.165).

**Figure 23:** The results show that over the period of three months proteinuria decreased. n=11, 39 subjects not specified
There was a trend that autoimmune and kidney transplant patients with low MPA levels had higher creatinine but patients with high MPA troughs had better creatinine levels.

**Figure 24**: This graph demonstrates the correlation between mean MPA trough level concentration and serum creatinine after three months of the initiation of the therapy. *n=21, 29 subjects not specified*
As stronger correlation was observed between proteinuria and MPA troughs. Low MPA levels were associated with high proteinuria.

**Figure 25:** This graph demonstrates the correlation between mean MPA trough level concentration and proteinuria after three months of the initiation of the therapy. n=14, 36 subjects not specified
3.4. Complications

Were there any complications, such as the influence of other immunosuppressant, gastrointestinal toxicity or hematological toxicity? How was the correlation between complications and the trough levels?

This diagram states that if ecMPA (Myfortic) is given to the patients instead of MMF (Cellcept), not less cases of GI-discomfort occur. Both ways we have a probability of around 33% that patients will have to deal with gastrointestinal discomfort.

**Figure 26**: No difference between MMF and ecMPA with regard to gastrointestinal adverse events. Cellcept n=33; Myfortic n=17
The three main side effects of MPA such as infections, leukopenia and gastrointestinal discomfort (diarrhoe) are set in relation to the diagnosis. The frequency of adverse events was not different between the three groups.

**Figure 27:** AD: Autoimmune diseases; RTX/HTX/LTX: Kidney/Heart/Liver transplantation; aGvHD: acute graft versus host disease in case of stem cell transplantation. MPA was given to prevent aGvHD. n=50

**Table 3:** Information that can be derived from the table is the percentage of side effects, which occurred dependent on the diagnosis. According to the table infections were the most common side effects. n=50

<table>
<thead>
<tr>
<th></th>
<th>Infections</th>
<th>Leukopenia</th>
<th>GI-discomfort</th>
<th>No side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>42%</td>
<td>6%</td>
<td>15%</td>
<td>33%</td>
</tr>
<tr>
<td>RTX/HTX/LTX</td>
<td>70%</td>
<td>20%</td>
<td>5%</td>
<td>0</td>
</tr>
<tr>
<td>Prophylaxis:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aGvHD</td>
<td>66%</td>
<td>33%</td>
<td>55%</td>
<td>0</td>
</tr>
</tbody>
</table>
In this figure we only set the trough levels of the patients in correlation with the side effects who really were associated with symptoms. Our study shows that the side effects did not really correlate with the MPA trough levels.

*Figure 28*: Contrary to our expectations, the side effects were not explained by increased MPA troughs. 
n\(n=50\)
4 Discussion

The main aspect of our study was to evaluate whether it is meaningful to implement the drug monitoring of MPA. Based on the studies till date, there are different opinions represented among the researchers. According to Imagard Neumann et al individual variability in MPA kinetics, drug interactions and changes in the parameters of MPA over the period of time make it impossible to predict the systemic exposure of this medication if given at a fixed-dose regime [51]. To answer our hypothesis we analyzed MPA trough levels with the help of clinical data and set them in correlation to outcome.

4.1. MPA trough levels

First of all, we measured the trough levels and set them in correlation to factors, such as gender, age, co-medication, diagnosis, form of therapy and co-medication.

Gender

In our study, females not only had higher MPA trough levels than men but also their trough levels were in the target range. Coefficient of variation was 52.5% in female and 86.8% in male. This might indicate that adherence to drug medication was better in females than in males.

The other reason behind this is that MPA metabolism is reduced in women. According to Morissette P et al these findings could be a result of inhibition of uridine 5’-diphosphate glucuronosyl transferase (UGT) enzymes by estrogen [49]. This enzyme converts MPA to MPAG [65]. Joy et al found out that MPAG Clearance$_{0-12}$ is also by 6-fold higher in females than males (p=0.047) and also creatinine clearance was higher by 21% in females [32].
Age

With the progression of age MPA trough levels seemed to increase but because $r^2 = 0.0030$ which means that the mean MPA trough level concentration cannot be explained by the factor of age in our case. The reason for the increase in MPA trough levels may be the renal impairment, which could be due to ageing itself but also due to the progression of the disease.

Dose

Figure 11 illustrates that higher MMF dose result in higher mean MPA trough level concentration but the result is not clinically relevant ($r^2=0.104$). Only 10% of the total variation in MPA exposure can be explained by MPA dose/day. Our finding totally support the data from other studies that dose of MMF did not specifically correlate with MPA exposure or mean MPA trough level due to high inter-patient variability.

Children and adolescents need higher doses because they are said to have higher drug-drug interactions and other reason maybe the different enzyme activity. Few studies have been performed considering children and adolescents, that’s why they might benefit from TDM immensely contrasting to adults [23].

Diagnosis

Cellcept was normally given at the dose of 1g twice a day and Myfortic 720 mg twice a day. Depending on why MMF was given, whether it was to prevent the progression of autoimmune diseases (AD) or to prevent graft rejection or acute graft versus host disease, mean trough levels came out to be different. The mean trough levels were 1.44 mg/L in the transplantation and aGvHD group and below the target range, whereas in the AD group trough levels were 3.04 mg/L.

Similarly, Neumann et al also found that mean MPA trough level concentrations (12 h after last dose) were higher in the AD group in comparison to kidney transplant recipients (4.1 mg/L versus 1.8 mg/L, $p=0.018$ s). The reason behind this outcome could be the triple immunosuppression given in case of transplantation [51].
Form of therapy

MMF can be given as induction or maintenance therapy or as prophylaxis to prevent aGvHD. In case of induction and maintenance therapy MPA trough levels were almost the same (2.99 mg/l; 2.83 mg/l) and in the reference area. But in aGvHD group it was only 1.49 mg/l. The reason for this finding could be because in case of aGvHD patients had received stem cell transplantation and therefore, they received more than one immunosuppressants and it was not important to keep the MPA level in the range.

The treatment of lupus glomerulonephritis with MMF is effective in the induction or as the maintenance therapy [28]. Another study compared MMF with intravenous cyclophosphamide (IVC) for the treatment of lupus nephritis over a period of 24-week induction therapy and found no significant difference between both groups [3]. Bomback et al found out that treatment with MMF not only maintained remission in patients with lupus nephritis but also had lower rates of long-term toxicity [7].

Co-medication

Immunosuppressants

Most commonly the immunosuppressants such as CyA and Tacrolimus influence the plasma levels of MPA differently. In our case as shown in Figure 14 CsA leads to significantly lower MPA trough levels. Tacrolimus did not seem to influence MPA trough levels that much. The same findings were also supported in other studies, such as that of Dennis A Hesselink et al [27]. As Tacrolimus also inhibits uridine 5’-diphosphate glucuronosyl transferase (UGT), it may also cause increase in MPA trough levels but this effect was not clinically significant [81].

Mikako Kobayashi et al found out that diarrhea, one of the side effects associated with the intake of MMF, was caused by mycophenolic acid glucuronide (MPAG). As CyA inhibits the multidrug resistance-associated protein 2, less MPAG is excreted with the bile and therefore resulting in less incidents of diarrhea. The opposite is applicable for tacrolimus [37]. Diarrhea can lead to a rise in tacrolimus trough concentrations – thus making diarrhea even worse.

Ferjani Hanen et al showed that MMF can restore creatinine levels and also has a reno- and hepatoprotective effect if given in combination with tacrolimus [22].
Pantoprazole

Allegedly pantoprazole affected the mean MPA trough level concentration (Figure 15). Even though mean MPA trough level seem almost same in our study, the coefficient of variation (CoV) shows the difference. CoV is 58% if pantoprazole is given and 88% if not. Rupprecht et al demonstrated that mycophenolic acid exposure is significantly lowered by pantoprazole (p<0.001). Pantoprazole only affects mycophenolate mofetil, but not enteric-coated mycophenolate sodium. The reason behind this is thought to be the poor solubility of MMF in higher pH and as we know that pantoprazole increases the pH in stomach [38].

Anti-diabetics

In our study oral anti-diabetic medications also seemed to have influence on the MPA trough levels (Figure 16). Trough levels were almost 1 mg/l higher if this medication was not prescribed. It’s not clear whether it’s the medication or the diabetes itself. Some studies like that of Pescovitz found no difference in MPA exposure between diabetics and non-diabetics [56]. Whereas van Hest et al found out that MPA T_max was increased in patients with diabetes, the reason could be the gastroparesis, a complication of diabetes that slows absorption of the medication [74].

Blood pressure medication

Antihypertensives seemed to influence the mean MPA trough level concentration positively (Figure 17). The reason might be that the normalization of blood pressure prevents further destruction or helps recovering of kidney, which results in normal renal function and therefore normal MPA clearance. On the other side, patients who need antihypertensives might be more inclined to fake MPA regularly.
4.2. Which actions were taken, regarding the trough levels of MPA?

Dependent on the mean MPA trough levels either the therapy was continued or modified. If patients did not show any complications and their trough levels were in the target range, no changes were made. Dose adaptation took place at mean MPA trough level of 2.4 mg/l. The reasons behind it were among others the progression of the disease, estimated with the help of renal retention parameters (creatinine plasma level, proteinuria), side effects, low trough levels or combination with other immunosuppressants.

The reasons for medication shifts were symptoms and not drug levels. Medication shift means that the Myfortic was started instead of Cellcept due to GI-discomfort, mostly diarrhea. GI side effects are one of the most common side effects of MMF with the occurrence rate of 45% [5]. Many studies revealed that GI side effects were the main reason for the MMF dose reduction. Vogt et al showed in a 12-month, open-label, multicenter, prospective study that if patients were given Myfortic less interventions regarding dose adaptations and less interruptions had to be made [77]. Another study observed that if GI side effects took place, less dose adjustments had to be made in case of EC-MPA (15%) versus Mycophenolate Mofetil (19.5%) [11].

Medication was aborted in case of serious infections, pregnancy, no improvement or death. MPA during pregnancy can lead to first-trimester pregnancy loss and malformations, such as that of external ear, cleft palate and lip [58].

The need of MPA TDM is still being questioned. With the help of TDM we can not only investigate MPA exposure (over- and underexposure) but also get a better understanding of the pharmacokinetics of the respective drug [57,66]. As dose is the poor predictor of exposure for MMF due to high within-patient variability, therapeutic drug monitoring (TDM) is an option. Furthermore, many studies stated that underexposure to MPA in the first week after transplantation lead to rejection. This also shows the significance of TDM as the pharmacokinetics of this drug are complex and unpredictable [57,73]. According to Leslie M Shaw et al MPA monitoring can be scheduled at the end of first week, one month
and two months after the transplantation. Furthermore, monitoring is to be done if rejection, side effects, changes in immunosuppression or non-compliance is suspected [65]. Borrows et al found out that the median value for MPA trough levels correlated significantly with the risk of rejection during the first 30 days after transplantation [9]. On the other hand Kuypers et al found no correlation between MPA trough level and clinical efficacy or toxicity [39]. Van Gelder T et al recommended a formula to calculate the dose of MMF based on MPA trough level concentration ($C_0$) [71].

\[
\text{New Dose} = \text{old dose} \times \frac{\text{target } C_0}{\text{measured } C_0}
\]

To ensure the establishment of TDM, also the assay methods have to be cost-effective, and easy to perform. In case of MMF the HPLC and Emit assay are performed. Lutz T Weber et al found out that HLPC measured a MPA trough level concentration of 1.2 mg/l, but Emit assay measured a higher concentration of 1.4 mg/l, respectively [79]. In our study only HPLC and LC- MS were used.

4.3. How was the clinical course of the patients regarding serum creatinine levels and proteinuria? Were trough levels of the patients with successful outcomes higher or lower?

The effectiveness of the therapy was measured based on the improvement of renal parameters. Therefore, successful outcomes meant normalization of creatinine levels and decrease in proteinuria. In our case, Figure 22 and 23, show the decrease in proteinuria and plasma creatinine levels after the initiation of the therapy. This evidence is also supported by other studies like that of Daniela Corna et al and William A Briggs [14,10]. Many reasons regarding the function of MPA are to be mentioned for this outcome: On the one side MPA suppresses the formation of antibodies and thereby prevents its deposition in the
kidney. Secondly, it also leads to decreased binding of leukocytes to the endothelium by inhibiting the glycosilation of proteins involved in this process [14].

In our study creatinine levels and proteinuria showed a fluctuation over the period of one year after the initiation of the therapy. Overall the creatinine levels and proteinuria sunk. In Figure 22 we could show that MMF is nephroprotective: Creatinine levels sank within the period of three months in the therapy of autoimmune diseases and prevention of transplant rejection. Patients with haematological disease had low normal creatinine levels at the beginning of the therapy. But during the therapy their creatinine levels increased. One explanation could be that it’s because MMF was never given alone but in combination with other immunosuppressants such as CyA or Tacrolimus, which are said to be nephrotoxic [59].

A four year, prospective, multicenter study was conducted among heart transplant recipients, where they found that if MMF was given in combination with CyA not only dose of CyA could be reduced but also MMF improved renal parameters: The CrCl at month 6 and at 4 years were 51.0 ± 15.6 and 54.1 ± 15.6 mL/min versus 41.9 ± 11.1 mL/min at baseline (P < .0001) [47]. A 12-month, open-label, multicenter, prospective study also observed renal function over the period of one year. Mean creatinine clearance increased from 58.6 mL/min at month 1 to 63.2 mL/min at month 12 [77].

Figure 24 demonstrates the trend for a negative correlation between MPA trough levels and serum creatinine level ($r^2=0.087$). Even though our results are not significant, it could be interpreted as if patients are underexposed to MMF than no improvement in renal parameters takes place, i.e. serum creatinine levels are high (creatinine clearance is low) and vice versa. Borrows R et al also found out that high levels of serum creatinine and decreased glomerular filtration rate was associated with lower MPA predose levels [8]. In other words, MPA clearance is dependent on the creatinine clearance. This is one other factor that accounts for the intra-patient variability and therefore, supporting the impact of therapeutic drug monitoring [75,76].

Proteinuria also decreased over the same period of time as serum creatinine levels. Proteinuria was not measured in patients with haematological diseases where MPA was given to prevent acute graft versus host disease. Briggs et al found that treatment with
MMF over 12 months lead to substantial decrease in proteinuria. Not only this but the suppression was at least as good as that under CyA therapy or better [10]. Similarly, Dooley et al demonstrated significant decrease in proteinuria and serum creatinine with MPA. Mean urinary protein-to-creatinine ratio (uP/C) also decreased from 5.45 to 2.92 (p=0.039) [18]. In the maintenance therapy of granulomatosis polyangiitis and microscopic polyangiitis with MMF at 2 g/d proteinuria declined from a median of 0.5 g/d to 0.2 g per day [53]. Another study that showed decline in proteinuria (<0.5 g/L) and increase in protein plasma levels is that of Dimitrakov et al [17].

Figure 25 demonstrates a negative correlation between MPA trough concentration and proteinuria (r²=0.37), which is significantly stronger than in case of serum creatinine (Figure 24). A prospective, randomized, controlled trial consisting of two groups with IgA nephritis (MMF with ongoing treatment and without MMF) compared the decline of proteinuria. In the MMF group a decline in proteinuria was upto more than 50% in 80% of the patients, whereas in the control group only 30% of the patients showed the effect [70]. In a recent meta-analysis, Liu at al compared different immunosuppressants with steroid therapy in treatment of IgA nephropathy. MMF showed a significant increase in complete response/partial response (CR/PR) proteinuria remission rates (p=0.006) [43]. But a prospective placebo-controlled randomized study could not demonstrate any beneficiary effect of MMF regarding the renal function or proteinuria [46]. A long term follow-up found out that progressive IgA nephropathy patients significantly benefit from sequential therapy with cyclophosphmaid pulse therapy followed by MMF maintenance therapy. It resulted in improved mean renal survival time with better glomerular filtration rate, decrease in proteinuria [21].
4.4. Were there any complications, such as gastrointestinal or hematological toxicity? How was the correlation between complications and the trough levels?

We focused in our study on three main side effects: infections, GI-discomfort and leukopenia. All these side effects were set in correlation to the diagnosis. Side effects seem to occur when the maximum concentration of MPA is above 10 mg/l and trough concentrations >3 mg/L [6,40]. In our case the side effects occurred at the following trough level: Infections at 2.4 mg/l, GI-discomfort at 2.18 mg/l and leukopenia at 1.45 mg/l. Information that can be derived from the table 3 is that if MPA is given due to autoimmune diseases then lesser side effects occur in comparison to the other diagnosis (transplantation or prophylaxis of aGvHD).

Infections occurred mostly after organ transplantation or if MPA was given as prophylaxis against aGvHD. The reason for this occurrence might be the treatment of patients with more than one immunosuppressants, which is almost the case after such diagnosis. Leukopenia occurred mostly after stem cell transplantation, which can be explained as follows: Leukopenia is generated in patients with chemotherapy on purpose if stem cell transplantation is foreseen and it takes months till the normal cell count is recovered. GI discomfort is also one of the most often side effects in transplant recipients, resulting in the prescription of PPIs [76]. Behrend M et al showed that if the MPA medication is given in 3 doses per day, GI symptoms could be minimized as in this way the maximum concentration of MPA (C_{max}) can be reduced [6].

MPA is available in two formulations: Cellcept and Myfortic. Enteric-coated mycophenolic acid (EC-MPA = Myfortic) is highly soluble in the small intestine where the pH is neutral than in stomach (acidic pH). The bioavailability of Myfortic is only 72% in comparison to Cellcept (94%) [52]. This could be the reason for our finding that under Cellcept 37,5% of the patients had their trough levels in reference area and under Myfortic only 20%.
When we compared both groups, we found no relevant differences concerning the side effects. As well as in Cellcept and in Myfortic group around 33% of the patients had to deal with gastrointestinal problems. From that 33% who developed diarrhoe under Cellcept only 9% of the patients were then given Myfortic, which resulted in reduced gastrointestinal problems. The reason why not all patients with this side effect were given Myfortic was maybe less compliance from doctors or patients’ side, or the fact that Myfortic itself did not completely prevent diarrhoe.

Many other studies also showed that from 59% to 70% of the transplant recipients, who required dose reduction, 20% was due to GI-discomfort [35,54]. In vitro studies such as that of Wieland et al showed that AcMPAG induce the release of cytokines, such as tumor necrosis factor α and interleukin 6. These cytokines are responsible for inflammatory processes, among others like those seen in Crohn’s disease [80]. A single and blind, 12-month multicenter trial showed that 42.1% of the patients under Cellcept required two or more dose reductions due to adverse side effects (such as GI-discomfort, leukopenia) in comparison to Myfortic where only 26.9% required dose reductions (p=0.048) [36].
5 Conclusion

In this retrospective study we compared mean MPA trough level concentrations and set them in correlation with clinical factors, such as gender, age, diagnosis, renal parameters, co-medication and side effects. Many studies support the results we achieved as discussed before.

Considering our results, TDM of MPA is not only cost and time inefficient, nor proves to be relevant. Therefore, decision to perform TDM should be considered depending upon clinical issues, such as in case of dual immunotherapy with CsA, or in patients at high risk of rejection after transplantation. More discipline is needed for significant results, as it cannot be assured whether the pre-dose levels were measured exactly after 12 hours of MPA intake or not. It’s more important to monitor renal parameters, such as creatinine clearance or CsA concentration, as they can help predict changes in MPA exposure.

Every new medication poses a new challenge. With it comes new hope, possibilities to finally prevail the disease. MPA is on its way to overtake other immunosuppressants but it needs more investigation and studies to finally obtain definitive data.
6 Summary

Mycophenolate mofetil (MMF) is a prodrug of the 2-morpholinoethylester of mycophenolic acid (MPA). MPA inhibits the key enzyme of the de novo pathway, inosine monophosphate dehydrogenase (IMPDH). This factor is responsible for the immunosuppression. MPA is not only used to prevent transplant rejection, but also to treat autoimmune diseases. Therapeutic drug monitoring (TDM) for MPA has always been questioned, because studies had not offered a definitive outcome whether it is useful or not to implement TDM.

In this retrospective study we analysed MPA trough levels of 50 patients. These patients had either autoimmune disease (lupus nephritis, IgA nephritis, chronic glomerular disease or rapid progressive glomerulonephritis), or were transplant recipients (kidney, liver, heart) or it was given to prevent acute graft versus host disease. Our main objective was to determine the usefulness and clinical relevance of TDM for MPA trough level concentrations. To verify this, we compared mean MPA trough level concentrations and set them in correlation with clinical factors, such as gender, age, diagnosis, renal parameters, co-medication and side effects.

MPA trough levels were higher in patients with autoimmune diseases than in transplant recipients. Gender, diagnosis, concomitant therapy with CyclosporinA, pantoprazole and blood pressure effects MPA trough levels and MPA trough levels in return effects the outcome, which correlates with renal retention parameters. Furthermore, few actions were taken regarding the MPA trough level concentrations. We do not know whether it was lack of compliance, discipline or some other reasons. No correlation was found between side effects (gastrointestinal discomfort, infections or leukopenia) and MPA trough level concentrations.

The results of our retrospective study do not support the implementation of TDM. Renal retention parameters, CyclosporinA dose should be considered to predict MPA exposure. The vast intra- and inter-patient variability in the pharmacodynamics and pharmacokinetics of MPA mentioned in many studies needs to be studied in more detail to deliver a clear answer for the TDM for MPA. Based on current findings, TDM for MPA is still a big question mark; more studies are eagerly awaited.
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