Journal of Mass Spectrometry and Advances in the Clinical Lab 28 (2023) 9–12

Contents lists available at ScienceDirect

Journal of Mass Spectrometry and Advances in the Clinical Lab

journal homepage: www.sciencedirect.com/journal/journal-of-mass-spectrometry-and-advances-in-the-clinical-lab

Case Report

A persistently febrile patient post-bone marrow transplant

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ARTICLE INFO

Keywords:
Laboratory-developed test
Immunosuppressant
Drug-drug interaction
Therapeutic drug monitoring
Anti-infective
Mass spectrometry

Case description

A cis male patient in his mid-30s received a non-myeloablative haploidentical bone marrow transplant (NM-BMT) following repeated hospitalizations for vaso-occlusive crises (VOC) as a complication of sickle cell disease (Day 0). Following NM-BMT, the patient was treated with mycophenolic acid (1,000 mg 3x a day), sirolimus (3 mg daily), and valacyclovir (500 mg 2x a day) prophylactically to prevent host rejection and viral infection. Additionally, the patient was prescribed a 3-month course of posaconazole (300 mg daily) for bilateral pulmonary nodules, which were unrelated to sickle cell disease or NM-BMT. His initial clinical course was complicated by multiple infections and stage IV graft-versus-host disease of the stomach. At day 27 post-transplant, the patient was discharged and continued with outpatient prescriptions for posaconazole, valacyclovir, and sirolimus. Of note, there was also a spike in sirolimus concentrations around this time, due to an increase in the dosing regimen (from 2 mg to 3 mg daily).

On day 45 following NM-BMT, the patient presented for a routine outpatient visit with a fever of 101 °F. Blood cultures were collected to assess infection status; over the course of the intervening days the patient's fever was continuous and persistent. On Day 49, the blood culture result was positive for *candida parapsilosis* fungemia and the patient was admitted. The patient began a course of micafungin (100 mg daily), which is the primary therapy for *candida* fungal infections. At this time posaconazole was held with plans to resume following a completed course of micafungin. On Day 50, the patient tested positive for human herpesvirus 6 (HHV-6) and cytomegalovirus (CMV), for which he was prescribed Valcyte® (valganciclovir hydrochloride, 900 mg 2x a day). Due to emesis, the oral Valcyte® treatment was paused and the patient was switched for two days to intravenous ganciclovir (5 mg/kg every 12 h). Following completion of his course of Valcyte®, he was treated with letermovir (480 mg daily) for maintenance suppression of CMV. A timeline of the patient's admission and medications is shown in Fig. 1.

From Day 45 to 52 the patient remained continuously febrile (100.0–101.8 °F) despite line removal, antifungal and antiviral treatment, and decreasing HHV-6 and CMV viral loads. On Day 52 the care team raised suspicion of sirolimus-induced fever. At the time, the patient's sirolimus concentrations were borderline low, but three weeks prior had been above the trough target (5–15 ng/mL), peaking at 23.1 ng/mL on Day 28 (Fig. 2). Within 24 h of sirolimus cessation, the fever resolved, and the patient was afebrile for the remainder of his admission. Concurrently, the care team noticed the patient's hepatic injury markers (alanine aminotransferase and aspartate aminotransferase) were trending upwards, raising concern for micafungin-induced hepatotoxicity, so the patient was then transitioned from micafungin to fluconazole (400 mg daily).

To maintain immunosuppression, the patient was started on tacrolimus (starting dose of 2 mg 2x a day, total daily dose of 4 mg) and blood concentrations were diligently monitored and adjusted to ensure the patient achieved a trough concentration within the target range of

Abbreviations: NM-BMT, non-myeloablative haploidentical bone marrow transplant; VOC, vaso-occlusive crises; LC-MS/MS, liquid chromatography-tandem mass spectrometry; CMV, cytomegalovirus; HHV-6, human herpesvirus 6.

Peer review under responsibility of "MSACL".

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https://doi.org/10.1016/j.jmsacl.2023.01.005
Received 11 November 2022; Received in revised form 5 January 2023; Accepted 11 January 2023
Available online 28 January 2023

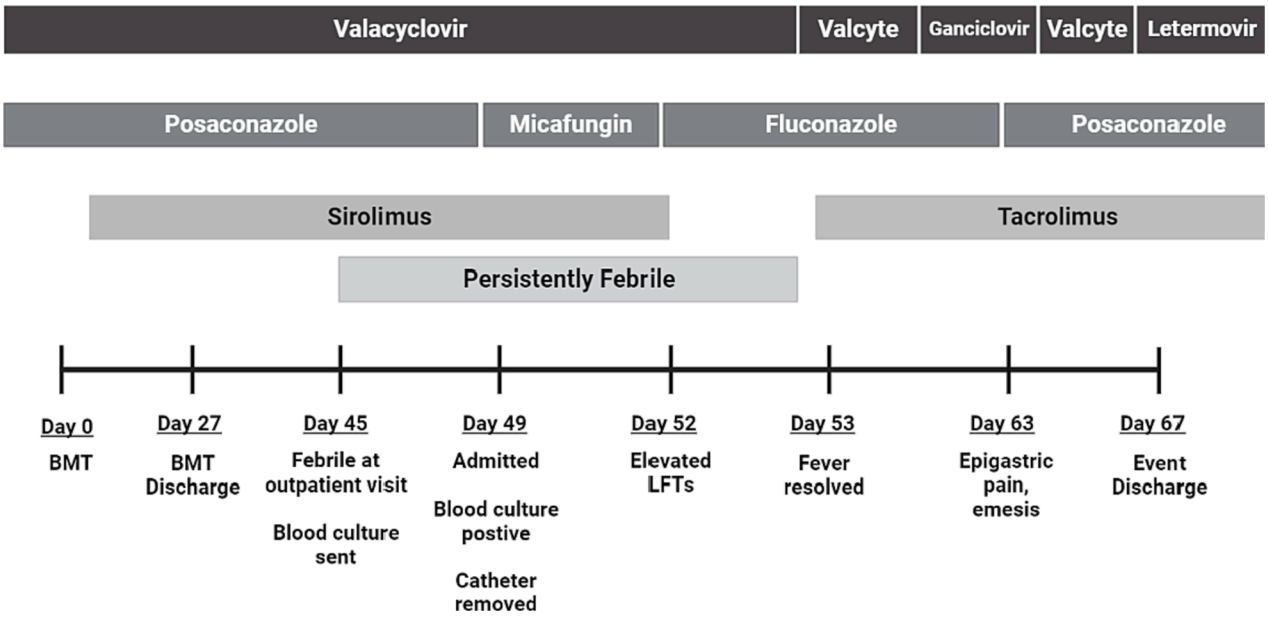

Fig. 1. Patient admission and medication timeline.

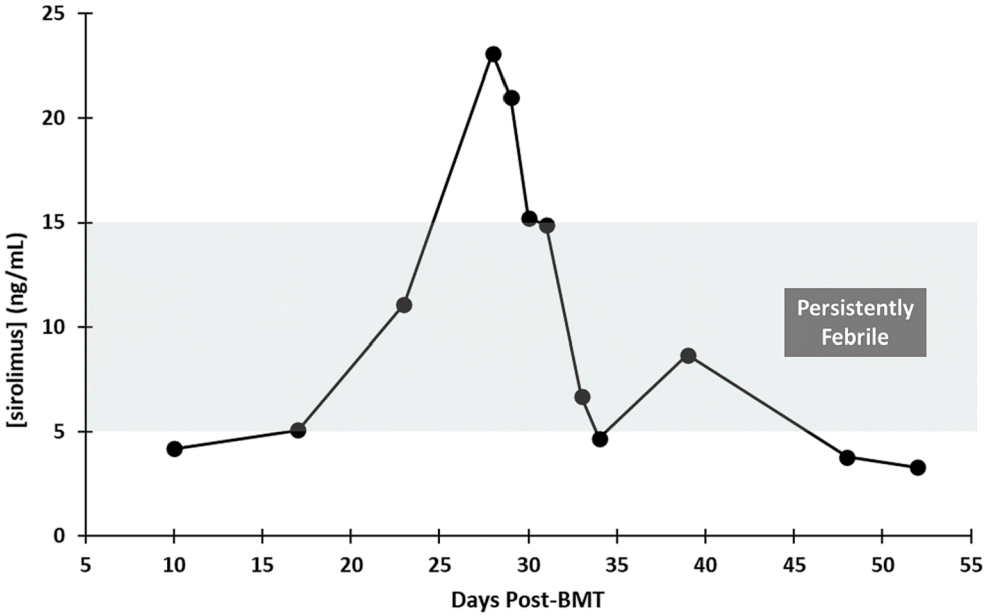

Fig. 2. Timeline of sirolimus concentrations with febrile status indicated. Shaded area indicates target trough concentration.

10–15 ng/mL (Fig. 3). The patient completed his course of fluconazole and on Day 63 resumed his course of posaconazole (400 mg/day). As posaconazole is a strong CYP3A4 inhibitor and tacrolimus is metabolized via CYP3A4, the tacrolimus concentration increased significantly following initiation of posaconazole (Fig. 3). Guided by therapeutic drug monitoring results, tacrolimus was held for one day and the dose was subsequently lowered to achieve concentrations within the target range. Following dose reduction, tacrolimus concentrations fell below the target range and the care team increased the dose two more times to reach the therapeutic range.

By Day 67 the patient's abdominal pain was manageable, his infection had resolved, and he was successfully discharged. Regular therapeutic drug monitoring was continued in the outpatient setting, and additional tacrolimus dose adjustments were made following posaconazole completion.

Case discussion

Inadequate immunosuppression is well correlated with acute immune activation resulting in graft rejection. However, immunosuppressant concentrations above the therapeutic range increase the risk of serious adverse events including nephrotoxicity, neurotoxicity, hypertension, leukopenia, thrombocytopenia, anemia, and long-term risk of malignancy. Supratherapeutic dosing may cause symptoms that confound treatment course, obfuscate the differential diagnosis, and negatively impact patient outcomes. In addition, many immunosuppressants, including sirolimus and tacrolimus, are metabolized via the highly polymorphic enzyme CYP3A4. The narrow therapeutic window, significant risk for toxicity, and genetic diversity in metabolic status underscore the importance of therapeutic drug monitoring for immunosuppressants.

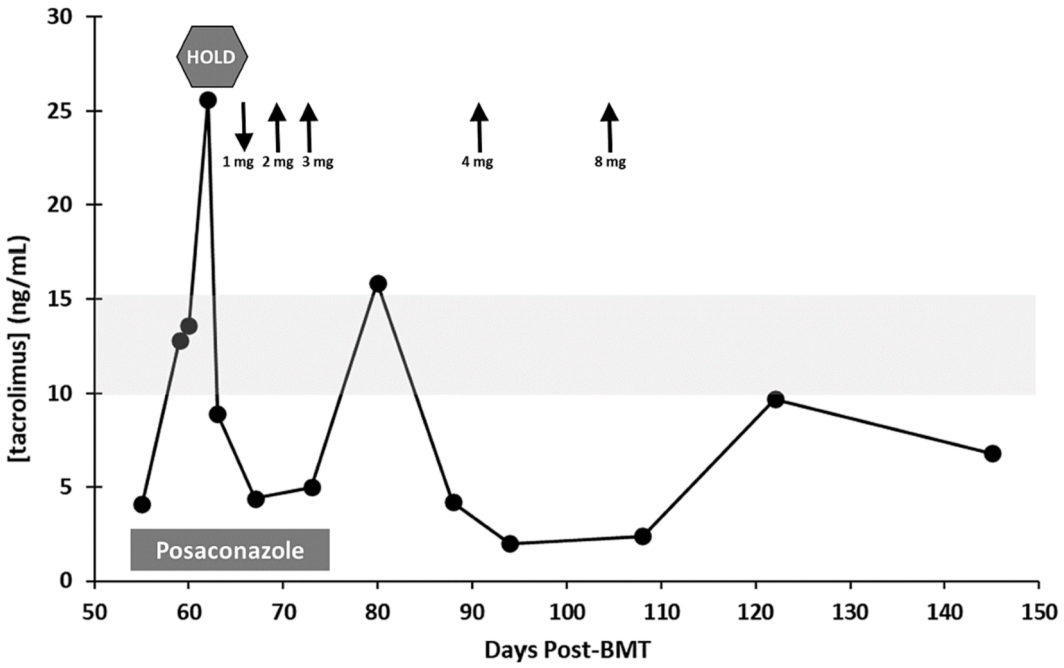

Fig. 3. Timeline of tacrolimus concentrations and dose adjustments (tacrolimus doses shown as total daily dose), along with posaconazole administration. Vertical arrows indicate tacrolimus dose adjustments and shaded area indicates target trough concentrations.

Close monitoring of immunosuppressant levels allows for optimization of dosing to account for differential medication response due to differences in metabolism, drug-drug interactions, and adverse effects. The main adverse effects associated with sirolimus include hyperlipidemia, impaired glucose tolerance in diabetic patients, renal toxicity, and proteinuria [1]. In addition to these adverse effects, treatment with sirolimus is associated with pneumonitis, often with accompanying fever [2]. Fevers of unknown origin are difficult to identify, and drug induced fevers are only considered following rule out of other febrile sources including infectious, inflammatory and malignant etiologies [3]. The diagnosis of a drug-induced fever is confirmed if the fever resolves within 72 h of medication cessation. Several case reports describe immunosuppressive medication-induced fevers in transplant patients [3–5] and although the precise mechanism of action remains unknown, it is possible that pulmonary inflammation may precipitate a febrile state [2]. By process of elimination, the patient's persistent fever was attributed to sirolimus and resolved upon ceasing this medication. Tacrolimus, which acts through inhibition of calcineurin, was initiated to maintain immunosuppression, and did not induce fever.

Sirolimus and tacrolimus may be measured via immunoassay or utilizing laboratory developed tests, primarily performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Although some immunoassays correlate well with LC-MS/MS analysis [6], other assays show significant variability and poor sensitivity [7]. Immunosuppressant monitoring via LC-MS/MS avoids interferences of parent drug metabolites, which can be significant, particularly with impaired metabolism and increased metabolite concentrations due to genetics or drug-drug interactions, as seen in this case [8,9]. Currently, LC-MS/MS is the gold standard for therapeutic drug monitoring [10]. Additionally, laboratory-developed mass spectrometric methods are readily multiplexed and have increased sensitivity to allow for low blood volume sampling [11,12]. Clinically, target ranges for immunosuppressants depend on the analysis methodology and thus, cannot be used interchangeably. For this patient, all whole blood measurements of tacrolimus and sirolimus were performed by LC-MS/MS using a methodology developed and validated by the hospital laboratory for the simultaneous quantification of sirolimus, tacrolimus, everolimus, and cyclosporine [13–15].

Transplant patients are often treated with complex pharmaceutical regimens that include immunosuppressants, antivirals, and antifungals, all of which have the potential for toxicity and drug interactions. Many anti-infectives are CYP3A4 inhibitors, which alter the pharmacokinetics and pharmacodynamics of both anti-infective and immunosuppressive therapy [16]. For example, azole antifungals inhibit CYP3A4, which decreases the rate of metabolism of drugs like tacrolimus and sirolimus, which are metabolized via CYP3A4. This phenomenon is demonstrated in Fig. 3, where there is a significant elevation in tacrolimus concentration above the therapeutic range upon administration of posaconazole. In an even more striking example, antivirals, such as ritonavir and cobicistat, used mainly for the treatment of human immunodeficiency virus, but also for immunocompromised patients, may require tacrolimus dose reductions in excess of 100-fold due to their potent CYP3A4 inhibitory capacity [17].

This case highlights the complexity of transplant medicine while emphasizing the need for rapid adaptability in response to evolving patient needs. Although rare, sirolimus is reported to induce fevers [3,4]. Despite relatively low sirolimus levels during the febrile period, evaluation of the full clinical context allowed the care team to efficiently identify and eliminate the fever-inducing agent. In addition to medication-related adverse events, awareness of drug-drug interactions is key in complex care populations [18].

Therapeutic drug monitoring of immunosuppressants empowers physicians to make evidence-based clinical decisions centered on the individual patient and clinical context, optimizing therapeutic benefit while minimizing the risk of adverse events. Laboratory developed tests, as seen in this case, are instrumental for patient care, allowing clinical laboratorians to tailor their test menu to the needs of their patient population. In return, this allows physicians to rapidly gain information about a patient's status to make informed decisions that guide complex care strategies, improve patient outcomes and advance the field of precision medicine.

Case resolution

Approximately-three months after discharge the patient was doing well, he had discontinued tacrolimus and made a full recovery. At the

time of transplant the patient had a dangerously low body weight of 115 lbs, but following recovery his body weight increased to 144 lbs, putting him back in a healthy range. Impressively, the patient remained VOC- and infection-free, achieving his longest admission-free period in several years. The vigilance of the care team and excellent use of therapeutic drug monitoring with a laboratory-developed test was essential to guide care and treatment strategies for this complex patient case.

Points of Interest

1. Drug induced fevers should be considered in the list of differential diagnoses following exclusion of infectious, inflammatory and malignant etiologies in the transplant population
2. Many anti-infective medications inhibit CYP3A4 activity and can cause significant increases in immunosuppressant blood concentrations
3. Consistent, locally-available therapeutic drug monitoring allows providers to optimize therapy in the presence of drug-drug interactions and complex treatment regimens
4. Laboratory-developed tests can be designed to meet the clinical needs of specific patient populations, such as transplant recipients

IRB statement

This case study was reviewed by the Johns Hopkins Medicine IRB (IRB00354513) and determined as not constituting human subjects research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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