Sapovirus – An emerging pathogen in renal transplant recipients?

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Abstract

Purpose

After including sapovirus to the viral GI-screening routine of our institution's laboratory, we noticed an increasing number of sapovirus diagnosis among kidney transplant recipients. Therefore, we assumed former GI-tract infections with unidentified pathogens could have been caused by sapovirus as well. In order to better understand the characteristics of a sapovirus infection in a high-risk group we initiated this study.

Methods

13 renal transplant recipients with GI-tract symptoms and later identified viral/unknown pathogens were included. Four patients suffered from a sapovirus infection. Kidney function, levels of immunosuppressants, CRP-levels and acid-base balance at admission and dismission, as well as onset of symptoms and time of hospitalization were analyzed.

Results

Even though statistically not significant, creatinine levels at admission tended to be higher in sapovirus patients (p = 0,710, sapovirus: 3,3 mg/dl (1,3; 5,0), non-sapovirus: 2,5 mg/dl (1,1; 4,9)). Also, Tacrolimus levels at admission showed the same trend (sapovirus: 13,6 ng/ml (12,9; 13,6), non-sapovirus: 7 ng/ml (2,6; 22,6), p = 0,279). At dismission creatinine levels improved equally in both groups (sapovirus: 1,7 mg/dl (1,4; 3,2), non-sapovirus: 2 mg/dl (1,0; 3,6), p = 0,825).

Conclusion

Especially in high-risk patients early symptomatic treatment remains crucial to protect the transplant's function. In our cohort all patients recovered equally well from the sapovirus infection as well as from other viral GI-tract pathogens. Larger cohorts and long-time follow-ups are needed in order to detect the long-term consequences and a potential need for further research regarding specific treatment.

Trial registration number:

DRKS00033311 date: December 28th 2023

Introduction

Diarrhea is generally known as one of the most common diseases especially among children but also in adults. In 3rd world countries it is a leading cause of death due to bad general health and lack of adequate medical treatment (1). However, also in Europe and Northern America, acute gastroenteritis (AGE) defined as three or more looser unformed stools per day often presenting as watery diarrhea in combination with vomiting, is a common reason for emergency department presentation (2). Usually, hospitalization is not necessary for immunocompetent healthy adults, whereas in immunocompromised hosts such as kidney transplant recipients diarrhea of any cause usually leads to an in-patient hospital stay requiring intravenous anti-infective therapy and search for pathogen or non-infectious causes(3, 4). In Germany, on average 350.879 patients are hospitalized due to AGE every year (4) (5), and on average 2792 death per year are caused by AGE (4). Among transplant recipients AGE is also one of the most common infections when presenting at an emergency department (5). In a case series study about reasons for admission to emergency department in renal transplant recipients Uysal et al. identified AGE with a share of 11% as the number one leading cause, followed by upper respiratory tract (9%) and urinary tract infections (4%) (5).

Especially in immunocompromised hosts there are two main reasons for GI-related symptoms problems, i.e., infectious and not-infectious causes.
Common pathogens of diarrhea in general are either bacterial (shigella, salmonella, E. coli, campylobacter, clostridium), viral (norovirus, enteric adenovirus, sapovirus, rotavirus, astrovirus, SARS-CoV-2) or, especially in travelers, parasites (cryptosporidia, giardia, yersinia, listeria) (3). Also in transplant recipients norovirus, adenovirus, rotavirus, and cytomegalovirus (CMV) are the leading viral infectious causes for diarrhea (6). Since more sensitive techniques are available, detection of previously not identified pathogens – such as the sapovirus – became possible (7), leading to an increasing frequency of its diagnosis (6). Sapovirus for example has been primarily discovered in 1976 and is since then known to be an important pathogen of AGE causing 2.2–12.7% of all AGE infections in the general population worldwide (8).

Nonetheless, in transplant recipients norovirus is still the leading cause for AGE related to 34.8% of infections (9, 10). For sapovirus-infections in transplant-recipients no valid data is yet available (6). norovirus as well as sapovirus belong to the calicivirus family (11). Both occur commonly during the cold season and are transmitted by fecal-oral transmission from human-to-human or contaminated foods (8, 12).

In transplant recipients immunosuppressive therapy can also cause diarrhea. Unfortunately, standard medication such as Calcineurin Inhibitors (CNI) or Mycophenolate-Mofetil (MMF) frequently lead to GI-complications by damaging the intestinal mucosa, inhibiting the proliferation of GI-epithelial cells or causing a release of cytokines leading to chronic inflammation (13, 14). Especially Tacrolimus and MMF are associated with GI-complications, whereas for example Azathioprine seems to have a rather protective effect (14).

Regardless of the underlying cause AGE patients often present with severe symptoms like diarrhea, stomach cramps, fever or weight loss (3, 8). Renal transplant recipients are at a high risk to present further complications like dehydration (15) acute renal failure (10) and Tacrolimus lapse (16).

Diagnostic of viral pathogens is usually performed by stool sampling with subsequent multiplex PCR including the most common regional pathogens (17). Regular monitoring of clinical parameters including vital signs as well as kidney function, infectious parameters and immunosuppressant serum levels is essential (18, 19).

Management of viral infections such as noro- or sapovirus consists of rehydration, electrolyte replacement and monitoring of the patient (8). In renal transplant recipients a reduction of immunosuppressants might be considered (20). To date, there is no approved specific treatments for most viral pathogens associated with AGE including noro- and sapovirus.

After sapovirus detection has been included in our institution’s routine gastroenteritis screening in the end of 2022 we noticed an increase of diagnoses at our nephrological ward. Therefore, we presumed that earlier gastroenteritis infections which had been declared unclear might had been caused by sapoviruses as well. In order to evaluate characteristics of sapovirus-infections compared to other viral gastroenteritis pathogens in a high-risk group such as renal transplant recipients this study was initiated.

**Methods**

**Study design and participants:**

In a retrospective analysis a cohort of 13 renal transplant recipients, admitted to our institution’s nephrology ward due to diarrhea between 01/01/2023 and 30/06/2023, were included. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Medical Faculty of LMU University (December 19th 2023, Number: 23–0926).

**Techniques:**
Stool samples were tested for norovirus and sapovirus using Multiplex Real-time PCR (Multiplex Real-time PCR by Seegene, year 2019 and 2021, including norovirus 1 and 2, rotavirus, adenovirus, astrovirus and sapovirus). Also, for CMV-detection an in-house-PCR methods was used. IBD was diagnose via Colonoscopy and histological analyzation. Serum creatinine was detected by kinetic color test based on Jaffé-method. For the measurement of CRP the particle enhanced immunological turbidity test and for Tacrolimus levels HPLC-MS/S-method were used.

**Statistics:**

Statistic analyzation was performed using IBM SPSS® Statistics Version 29. Baseline characteristics and other values were reported by median and 25th – 75th quartile. For detection of group differences in non-parametric data Man-Whitney-U-Test was used. For detection of differences within one group we used Kruskal-Wallis-Test.

**Results**

**Study participants:**

Starting in December 2022, Sapovirus has been included in the LMU Hospital laboratory standard viral stool screening for GI-diseases. From 01/01/2023 and 30/06/2023, four renal transplant recipients were hospitalized due to ongoing diarrhea at the renal division at LMU Hospital, in which sapovirus infection was detected in a stool sample. In the same period nine other renal transplant recipients were also hospitalized because of diarrhea. Of these patients, three suffered from a norovirus Type II, one from CMV-Colitis and one was primarily diagnosed with inflammatory bowel disease (IBD). In five patients neither bacterial nor viral pathogens could be detected (see Fig. 1).

**Baseline Characteristics:**

Mean age of all included patients was 51 years showing no significant differences (p = 0,710) between the sapovirus- and non-sapovirus groups. Onset of symptoms was reported in a median of 7 days (min. 1, max. 7)) before hospitalization in the sapovirus-group, whereas first symptoms were noticed in a median of 6 days (min. 1, max. 90 d; p = 0,825) in the non-sapovirus group. Even though not statistically significant (p = 0,710) creatinine levels at admission tended to be higher (3,3 [mg/dl] (1,3; 5,0)) in the sapovirus-group than in the non-sapovirus group (2,5 [mg/dl] (1,1; 4,9). Also, the Tacrolimus levels at admission appeared to be higher in the sapovirus-group (13,6 ng/ml (12,9; 13,6) than in the non-sapovirus group (7 ng/ml (2,6; 22,6), p = 0,279). Mean CRP levels at admission were low in both groups (sapovirus: 0,15 mg/dl (0,1; 2,6), non-sapovirus: 0,2 mg/dl (0,1; 21,3)) and showed no significant difference between the groups (p = 0,260). Metabolic acidosis was present in 2/4 sapovirus-patients and in 3/9 non-sapovirus patients. In both groups duration of hospitalization was similar (sapovirus: 8,5 days (8; 12), non-sapovirus: 9 days (5; 20)). Creatinine at dismissal did not differ significantly in both groups (sapovirus: 1,7 mg/dl (1,4; 3,2), non-sapovirus: 2 mg/dl (1,0; 3,6), p = 0,825).
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sapovirus</th>
<th>Non-Sapovirus</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Median (min; max))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects</td>
<td>4</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Age [years]</td>
<td>52 (37; 84)</td>
<td>48 (19; 78)</td>
<td>0.710</td>
</tr>
<tr>
<td>Gender female [%]</td>
<td>50</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Number of NTx</td>
<td>1 (1; 2)</td>
<td>1 (1; 3)</td>
<td>0.825</td>
</tr>
<tr>
<td>Onset of symptoms [d]</td>
<td>7 (1; 7)</td>
<td>6 (1; 90)</td>
<td>0.727</td>
</tr>
<tr>
<td>Creatinine at admission [mg/dl]</td>
<td>3.3 (1.3; 5.0)</td>
<td>2.5 (1.1; 4.9)</td>
<td>0.710</td>
</tr>
<tr>
<td>Tacrolimus levels at admission [ng/ml]</td>
<td>13.6 (12.9; 13.6)</td>
<td>7 (2.6; 22.6)*</td>
<td>0.279</td>
</tr>
<tr>
<td>CRP at admission [mg/dl]</td>
<td>0.15 (0.1; 2.6)</td>
<td>0.2 (0.1; 21.3)</td>
<td>0.260</td>
</tr>
<tr>
<td>Metabolic Acidosis at admission [%]</td>
<td>50</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Duration of stay [d]</td>
<td>8.5 (8; 12)</td>
<td>9 (5; 20)</td>
<td>1.000</td>
</tr>
<tr>
<td>Creatinine at dismissal [mg/dl]</td>
<td>1.7 (1.4; 3.2)</td>
<td>2 (1.0; 3.6)</td>
<td>0.825</td>
</tr>
</tbody>
</table>

p values ≤ 0.05 were considered statistically significant, +: n = 3, *: n = 7

**Overall symptoms:**

All patients regardless of the final diagnosis presented with ongoing diarrhea. Two patients of the sapovirus-group and three of the non-sapovirus group also reported nausea and vomiting. Also, gastric cramps, fever, reduced overall health were repeatedly reported symptoms, especially in the non-sapovirus group. Some patients were additionally suffering from oliguria leading to huge concerns regarding the return of their transplant’s function.

**Treatment:**

No specific treatment for patients with sapo- or norovirus infections was used. All sapovirus patients received intravenous fluids and sodium hydrogen carbonate to buffer a metabolic acidosis when necessary. Also, symptomatic treatment with loperamide and dimenhydrinate were applied if necessary.

In the non-sapovirus group treatment depended on the underlying pathogen. Because of relatively high CRP levels four patients received antibiotic treatment, assuming a bacterial infection, which was stopped after the viral pathogen diagnosis. The patient suffering from ulcerating colitis received immunomodulatory treatment with Glucocorticoids, Budesonide and Azathioprine. The patient suffering from CMV-Colitis received anti-viral treatment with Valganciclovir. Also, one patient calculatedly received Fluconazole having had a mycotic infection shortly before.

**Complications:**

Acute renal failure or at least an acute decline in renal function was observed in all patients regardless of their cause for diarrhea. Diarrhea and loss of renal function also led to acute metabolic acidosis in 5 patients (2 sapovirus, 1 norovirus, 1 IBD, 1 none). Also, a lapse of immunosuppressants, especially Tacrolimus, was detected in 2 sapovirus- and 3 non-sapovirus patients.

**Discussion**
Even though sapovirus has been known as a GI pathogen for a long time, there seems to be an increase in its detection recently. Reasons might be improved technical standards as well as improvement in technical devices themselves as they can increasingly perform more tests at one time (e.g., through Multiplex-PCR-testing) (17). As the detection of sapovirus-infections will further increase over the next years, a profound understanding of the disease as well as treatment algorithms especially in a high-risk cohort such as immunosuppressed patients are of high clinical relevance. In order to understand and highlight the characteristics of a sapovirus infection compared to other causes of diarrhea, all kidney transplant recipients presenting with diarrhea at our nephrological ward from January to June 2023 and a positive viral GI pathogen diagnosis were included in our analysis (Fig. 1).

Even though not statistically significant, one of the major findings in our study cohort was the tacrolimus overexposure detected in the group of patients infected by sapovirus (13,6 mg/dl (12,9; 18,3) (Median and Range) in comparison to the non-sapovirus group (7,1 (2,6; 22,6), p = 0,279) (Fig. 2). Small sample size might be the reason for lack of statistically significant difference. Tacrolimus overexposure is a common complication of a diarrheal disease in medically immunosuppressed patients and is associated with altered pharmacokinetic interactions of the immunosuppressant (16) leading to limited renal function/acute renal failure (21). Therefore immunosuppressants` levels, especially when elevated, should be monitored closely.

Also, acute renal failure or at least a decline in renal function was detected among the sapovirus patients primarily caused by the huge fluid losses due to the diarrhea leading to a reduced perfusion of the transplant-kidney (creatinine at admission sapovirus: 3,3 mg/dl (1,3; 5,0), non-sapovirus: 2,5 mg/dl (1,1; 4,9), p = 0,710.

Most importantly, when studying a cohort such as renal transplant recipients relevant alternative diagnoses for diarrhea have to be taken into account in order to delineate infectious patients, find the right treatment option and recover renal function as quickly as possible. The major causative classes can be divided up into infectious reasons and non-infectious reasons e.g., side effects from immunosuppressive treatment.

As seen in our cohort other viral pathogens like the most common AGE virus the norovirus, but also other viral pathogens especially popular among transplant recipients such as CMV and BK-virus have to be taken into account (22). Furthermore, bacterial infections (23) or especially in immunosuppressed patients mycotic infections should be considered (22). For initial differentiation between bacterial and other pathogens levels of infectious parameters such as CRP are commonly used as an indicator for bacterial infections when strongly elevated (24). In our cohort two patients presented with strongly elevated CRP levels (21,3 mg/dl and 17,7 mg/dl) at admission but later were diagnosed with a viral (norovirus) or non-identified pathogen. Procalcitonin and IL-6 might be useful to better predict different classes of pathogens (24). In three patients no underlying pathogen could be detected. These patients might have been infected by another virus that as the sapovirus earlier is not part of the routine-Gastroenteritis screening. Overall, also in immunocompromised patients, an advanced approach to identify the underlying pathogen (e.g., via gastro-/colonoscopy including biopsies) should only be considered if symptoms cannot be cured by symptomatic treatment and when a causal treatment option after the pathogen`s identification is expected (19, 25, 26).

Another very important differential diagnosis which has to be considered in medically immunosuppressed patients is the immunosuppressant-induced diarrhea which is most popular in MMF treatment (13). It is caused by blockade of essential enzymes for replication and growth of GI epithelial cells leading to a reduced fluid absorption and therefore diarrhea (13, 27). When MMF-induced diarrhea is suspected a diurnal profile of MMF levels is necessary as a single value can be misleading (28).

Regarding the treatment of diarrheal diseases symptomatic treatment usually is the first option leading to a recovery of symptoms in approximately 50% of all cases in transplant recipients (13). This includes an intravenous fluid substitution as well as a hold of a diuretic therapy and anti-motility or anti-emetic drugs if necessary. Also, intravenous buffer solutions can be administered if a metabolic acidosis is present. Especially the use of anti-motility drugs is highly controversial as
they only mitigate symptoms but therefore inhibit the pathogen clearance (19). Their use should therefore be well considered.

Secondly, if available, after identification of the underlying pathogen a specific treatment would be eligible. For sapovirus infections no specific treatment is yet available. Even in norovirus infections which are very common and therefore well-known pathogens only trial phase specific treatment under study conditions is available. Given our cohort’s good recovery from sapovirus infections the need for specific treatment options can be questioned. To identify the real necessity for specific treatment options in sapovirus infections larger cohorts and long-time follow-ups are necessary. If a real need becomes apparent, given the affiliation to the same viral family (calciviridae) of sapo- and norovirus, medical studies on Nitazoxanide (29–32), Immunoglobulins (33, 34) or other anti-viral drugs (Polymerase Inhibitors, Protease Inhibitors, Immunomodulators (35) which have shown positive effects on norovirus infections should be tested.

The patients represented in our study cohort only received symptomatic treatment including the above-mentioned treatment options. Fortunately, all patients and their transplant-kidney function recovered well from sapovirus infection with no need of permanent or intermittent hemodialysis. Results of larger cohorts including more patients and maybe more difficult progressions would be interesting at this point. Apart from their severe symptoms such as nausea, vomiting, gastric cramps and fever, patients complained about tremendous worries regarding the regain of their transplant’s function which we noticed as a considerable burden for our patients. Also, in this regard an available specific treatment might have relieved most patients.

Similar to other GI-tract infections in immunocompromised hosts, the development of chronicity is of concern (36). Alike norovirus-infections, also for sapovirus there are reports on chronicity and prolonged symptoms especially in immunocompromised patients (10, 37).

Also, regarding the transplant-kidney function another dreaded complication is the transplant rejection which for example Servais et al. found to be elevated in sapovirus-infections in intestinal transplant recipients (38). In our study cohort no rejection was observed and also given the small sample size of sapovirus-infected patients at Servais et al (n = 4, thereof 2 rejections) (38) no reliable evidence can be drawn from these results. Longer follow up times and bigger sample sizes are needed to answer this question.

In our sapovirus patients age span (37–84 years) and time after transplantation (10 month – 25 years) ranged widely but interestingly no difference in the time of hospitalization (p = 0,392) and improvement of kidney function (p = 0,392) could be detected in this group. One patient aged 61 years and only 10 months after transplantation was admitted with a serum-creatinine of 2,6 mg/dl and was dismissed 8 days later with a serum-creatinine of 1,4 mg/dl. The oldest patient (84 years) with the longest time after transplantation (25 years) was admitted with a serum-creatinine of 3,9 mg/dl and was also dismissed after 8 days with a markedly improved serum-creatinine of 1,9 mg/dl. Of course, given the small sample size of this study cohort larger study cohorts are needed to verify the results.

In many of our patients tacrolimus levels were markedly elevated. Due to a shorter retention time during diarrheal diseases Tacrolimus uptake is shifted to the lower GI tract where uptake mechanisms are worse (21), causing higher Tacrolimus blood levels during diarrheal diseases. Given the elevated Tacrolimus levels of our sapovirus-Patients one could therefore hypothesize that the retention time might be even shorter in sapovirus-infections than in other viral pathogens explaining the higher Tacrolimus blood levels in this cohort. A bigger study cohort and clinical studies would be necessary to test this hypothesis. While adjustment of Tacrolimus dose during the diarrheal episode is warranted, the utility of any long-term reduction of immunosuppressive therapy remains to be determined. Hence, recovery from viral infection translates into normalized Tacrolimus absorption and requirement of dose adjustment, unlike diarrheal episodes secondary to immunosuppressants (13, 27)
Due to a lack of specific treatment options and the potential of rare but severe complications like chronicity and transplant-rejection prevention should be improved (39). Trials on norovirus vaccinations already exist but the development appears to be difficult (40). For sapovirus there is no vaccination yet available (7). So given the fecal-oral transmission path basic hygiene measures, especially hand hygiene, are essential (39).

Conclusion

In a high-risk cohort such as renal-transplant-recipients early symptomatic treatment of any type of diarrheal disease is crucial to prevent patients from severe complications such as acute renal failure and overdose of immunosuppressants.

Search for an underlying pathogen should be initiated as early as possible to offer the optimal treatment and to reduce disease transmission.

Regardless of age and time after transplantation, all patients in our study cohort recovered well from sapovirus infection by symptomatic treatment. To date, there is no available specific treatment for sapovirus infections but given its affinity to the norovirus for which at least specific treatment is tested under study-conditions further studies including testing of specific treatment for sapovirus should be performed.

A clear limitation of our study is the small sample size including a heterogeneous group of kidney transplant patients admitted to our ward. As patients were included in our study at least 10 months after transplant, conclusions cannot be drawn regarding short-time posttransplant patients.

Declarations

Author contributions:

MR and ABM collected data and prepared data. MR performed analyzation and designed the methodology. ABM reviewed the methodology. US and MF reviewed the analysis. MF supervised the project. MR wrote the first draft of the manuscript. All authors revised and approved the final version of the manuscript.

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Availability of data and materials

The clinical dataset will be made available upon reasonable request to the corresponding authors.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This study was approved by the ethics committee of the Medical Faculty of the LMU Munich (December 19th 2023, Number: 23-0926)

Clinical trial registration

The study has been registered on DRKS (trialsearch.who.int), Reg. Nr. DRKS00033311 (December 28th 2023)

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References


Figures
Figure 1

Count of identified pathogens among included patients (n = 13)
Figure 2

a: Comparison of Tacrolimus-levels between patients with sapovirus infection (n = 4) vs. other cause for AGE (n = 9) at admission to nephrologic ward

b: Comparison of Tacrolimus-levels at admission by pathogens (upper limit > 6 month after Transplantation: 6 ng/ml; proband 3, 9 and 10 received different immunosuppressants).