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THE DIVISION OF INFECTIOUS DISEASES
Karolinska Institutet, Stockholm, Sweden

VIRAL GASTROINTESTINAL INFECTIONS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT PATIENTS

Lisa Swartling

Stockholm 2020

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Printed by US-AB 2020
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ISBN **978-91-8016-010-0**

Viral gastrointestinal infections in allogeneic hematopoietic stem cell transplant patients
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Lisa Swartling

Principal Supervisor:
PhD Elda Sparrelid
Karolinska Institutet
Department of Medicine, Huddinge
Division of Infectious Diseases

Co-supervisor(s):
Professor Per Ljungman
Karolinska Institutet
Department of Medicine, Huddinge
Division of Hematology

Professor Jonas Mattsson
Karolinska Institutet
Department of Onchology and pathology,

Opponent:
Professor Thomas Lion
Children's Cancer Research Institute,
Medical University of Vienna,
Department of Pediatrics
Vienna, Austria

Examination Board:
Professor Niklas Arnberg
Umeå University
Department of Clinical Microbiology, section of Virology

Professor Håkan Hanberger
Linköping University
Department of Clinical and Experimental Medicine

PhD Jacek Toporski
Lund University
Department of Clinical Sciences

ABSTRACT

Gastrointestinal symptoms, and elevated liver enzymes, are common after HSCT, often due to drug toxicity, graft-versus-host disease (GVHD) or infections. It is essential to distinguish between GVHD and infection, since both conditions may progress to lethal disease, but require opposite strategies for the immunosuppressive treatment. Several of the viral gastrointestinal infections are easily transmitted and can cause outbreaks in health care facilities.

In this thesis I studied viral gastrointestinal infections in HSCT patients, with focus on human adenovirus (HAdV), norovirus and hepatitis E virus (HEV), addressing transmission within health care, clinical importance, risk factors for severe/prolonged disease and the importance of secretor-status.

In paper I we analyzed an outbreak of HAdV at the Center for Allogeneic Hematopoietic Stem Cell transplantation (CAST), Karolinska University Hospital. We identified nine patients with HAdV A31. Hygiene measures were implemented, but the outbreak continued for a prolonged time. High strain on the staff during the early part of the outbreak, possible contamination of the facilities of the ward, and unidentified cases with sparse symptoms, may have contributed to the prolonged outbreak. The clinical consequences were significant, although no patient developed severe HAdV disease.

Paper II was a retrospective study of the clinical importance, and risk factors for long-term symptoms, in 63 HSCT patients with norovirus infection. In paper III, we analyzed if secretor-status influenced the clinical course of norovirus infection in 89 HSCT patients with norovirus infection, of whom 63 also had been included in paper II.

we found chronic symptoms of norovirus (>30 days) in 18/89 (20%) of the patients. Severe combined immunodeficiency (SCID) diagnosis was associated with chronic norovirus symptoms in both paper II and III, which may be due to the delayed immune reconstitution in many of these patients. The number of secretor-negative patients was low compared to the general population, indicating that secretor-negative genotype may protect against norovirus even when the patient is severely immunocompromised.

Paper IV was a retrospective study of the frequency and clinical importance of HEV infection in a cohort of 236 HSCT recipients. HEV RNA was detected in 8/236 (3.4%) patients 6 months after HSCT. We found that elevated alanine aminotransferase (ALT) at six months after HSCT was associated with HEV infection. Spontaneous clearance was common, but one patient died of multiorgan failure where HEV infection may have contributed.

In conclusion, we found that an outbreak of HAdV can be difficult to control and may have

serious consequences. Norovirus causes chronic symptoms (> 30 days) in 20% of HSCT patients, and SCID as indication for HSCT is associated with a chronic course of norovirus infection. We found that problems discriminating symptoms of HAdV, or norovirus, from symptoms of gastrointestinal GVHD, are a significant clinical challenge. HEV infection is an infrequent, but potentially severe, differential diagnosis in patients with elevated ALT six months after HSCT.

LIST OF SCIENTIFIC PAPERS

I. Prolonged outbreak of adenovirus A31 in allogeneic stem cell transplant recipients.
Swartling L, Allard A, Törlen J, Ljungman P, Mattsson J, Sparrelid E.
Transpl Infect Dis. 2015 Dec;17(6):785-94

II. Norovirus causing severe gastrointestinal disease following allogeneic hematopoietic stem cell transplantation: A retrospective analysis.
Swartling L, Ljungman P, Remberger M, Sundin M, Tiveljung A, Mattsson J, Sparrelid E.
Transpl Infect Dis. 2018 Apr;20(2)

III. The Importance of Secretor-status in Norovirus Infection Following Allogeneic Hematopoietic Stem Cell Transplantation
Swartling L, Sparrelid E, Ljungman P, Boriskina K, Valentini D, Sharma S, Svensson L, Nordgren J.
Manuscript

IV. Hepatitis E virus is an infrequent but potentially serious infection in allogeneic hematopoietic stem cell transplant recipients.
Swartling L, Nordén R, Samuelsson E, Boriskina K, Valentini D, Westin J, Norder H, Sparrelid E, Ljungman P.
Bone Marrow Transplant. 2020 Jul;55(7):1255-1263

INNEHÅLLSFÖRTECKNING

LIST OF ABBREVIATIONS

ALC	Absolute lymphocyte count
ALT	Alanine amino transferase
ANC	Absolute neutrophil count
APC	Antigen presenting cell
CAST	The Center for Allogeneic Hematopoietic Stem Cell Transplantation
CB	Cord blood
CD	Cluster of differentiation
CMV	Cytomegalovirus
Ct	Cycle threshold
ddPCR	Digital droplet PCR
DNA	Deoxyribonucleic acid
FUT2	α1,2-fucosyltransferase 2
GVL	Graft-versus-leukemia effect
HAdV	Human adenovirus
HBGA	Histo-blood group antigen
HEV	Hepatitis E virus
HLA	Human leukocyte antigen
HSCT	Allogeneic hematopoietic stem cell transplantation
Ig	Immunoglobulin
L	Litre
MAC	Myeloablative conditioning
mL	Millilitre
NK cell	Natural killer cell
non-MAC	Non-myeloablative conditioning
OR	Odds ratio
PCR	Polymerase chain reaction
PJP	Pneumocystis jirovecii
qPCR	Quantitative PCR
RIC	Reduced intensity conditioning

RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase PCR
SCID	Severe combined immunodeficiency
ULN	Upper limit of normal
URD	Unrelated donor

1 BACKGROUND

1.1 ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

Allogeneic hematopoietic stem cell transplantation (HSCT) is a treatment where an abnormal hematopoiesis is replaced by hematopoetic cells from a healthy individual. As survival after HSCT has improved over the years, the indications for HSCT have broadened. In adults the majority of HSCTs are performed for treatment of hematological malignancies (mainly acute leukemia, but also chronic leukemia, myelodysplastic syndrome, lymphomas and multiple myeloma), whereas in children, non-malignant diagnoses dominate. HSCT can eliminate or control residual malignant cells, since the new immune system will attack these cells. This is termed graft-versus-leukemia (GVL) effect and is considered to be the main requirement for long-term disease control in patients with hematological malignancy[1,2]. It has been shown that graft-versus-host disease (GVHD) is associated with a GVL effect[2]. In addition to this effect, HSCT enables a more intensive chemotherapy, and total body irradiation, since bone marrow toxicity is a main limiting factor.

HSCT is also used as replacement therapy for non-malignant disorders. These are congenital or acquired deficiencies of marrow function, the immune system, or enzyme deficiencies, including a wide range of diseases, such as thalassemia, aplastic anemia, inborn errors of metabolism, and severe combined immunodeficiency (SCID). In patients with non-malignant disorders, GVHD should be avoided since there is no beneficial anti-tumor effect and GVHD prophylaxis should therefore be optimized.

1.1.1 The donor and source of stem cells

The question of which donor to choose has become more complicated in recent years with the introduction of haploidentical donors. Traditionally, the donor of stem cells should be as well matched for human leukocyte antigen (HLA) as possible, to avoid severe complications of HSCT. However, several studies have shown that comparable or even better results can be obtained by using a haploidentical donor as with an unrelated donor with "moderate degree" of mismatch (1/8)[3]. Other factors that can be considered for the choice of a suitable donor, are cytomegalovirus (CMV) match, sex, age of the donor and blood group.

The preferred choice is still an HLA-matched sibling, which is available in approximately 25% of the patients. The second choice is usually a well-matched (8/8) unrelated donor (URD) obtained from the worldwide registries of stem cells donors. Haplo-identical family donors (usually child or parent, with half of the HLA-antigens identical) have been

increasingly used lately with the improving outcome. For children, umbilical cord blood (CB) donor is an alternative, but are rarely used for adults, due to the low cell dose. The source of hematopoietic stem cells is today mostly peripheral blood, after mobilization of stem cells with colony stimulating factor, but hematopoietic stem cells can also be harvested from bone marrow, or from umbilical cord blood. The preferred stem cell source may however depend on the diagnosis and can vary between different HSCT centers.

1.1.2 Conditioning regimen

Prior to infusion of harvested hematopoietic stem cells, the patient receives a cytotoxic conditioning treatment. The purpose of the conditioning treatment is to minimize the number of malignant cells in the host, if the reason for HSCT is malignant disease, and to prevent graft rejection. The conditioning therapy has historically been myeloablative (MAC), where the hematopoiesis may not recover without infusion of hematopoietic stem cells. Reduced intensity conditioning (RIC) is associated with less toxicity and shorter neutropenia and has enabled HSCT in older patients, and in patients with co-morbidities. Since it has been acknowledged that cure of malignant disease after HSCT, to a large extent depends on the GVL effect, RIC regimens have become more commonly used in recent years. There are many different conditioning protocols and they are now divided into MAC, RIC and non-myeloablative (non-MAC) regimens, with non-MAC being the least intense. The choice of conditioning depends on the indication for HSCT, the age of the patient and occurrence of co-morbidities.

1.1.3 GVHD

GVHD appears when the donor immune cells react to the recipient cells and is one of the most severe complications after HSCT. Acute GVHD usually occurs during the first 3 months after HSCT, and chronic GVHD later than 3months. However, the timing has become more diverse and is overlapping with the introduction of RIC, donor lymphocyte infusion (DLI) and late tapering of immunosuppressive drugs[4,5].

Acute GVHD most commonly affects the skin, the gastrointestinal tract and the liver. Pathophysiology involves three steps. (1) Tissue damage caused by the conditioning, the underlying disease and/or its treatment, causes release of inflammatory cytokines and chemokines, which will activate recipient APCs. Disrupted barriers with translocation of microbial products, such as lipopolysaccharides, will further enhance this activation. (2) Recipient APCs interact with donor T-cells, causing activation, proliferation and migration of allo-reactive T-cells, and also of NK-cells. (3) Tissue damage by cytotoxic T-cells and NK-

cells, with release of proinflammatory mediators, further contributing to the cytokine storm. The incidence of acute GVHD varies between 30-70%, depending on the degree of HLA matching, type of donor, conditioning regimen and age of the patient. A commonly used prophylaxis against GVHD is cyclosporine and short term methotrexate, with addition of in vivo depletion of T-cells in patients with an increased risk of GVHD, such as those with an unrelated donor.

Acute GVHD is graded I-IV according to the severity of organ involvement[6], with GVHD grade III-IV carrying a high mortality[7]. The diagnosis is mostly clinical, although histopathological evaluation can be essential to differ from other diseases, such as CMV colitis[8]. Standard first line treatment for acute GVHD is corticosteroids and continued prophylaxis with cyclosporine (or another calcineurin inhibitor). The response rate on corticosteroid treatment decreases with the grade of GVHD but is generally approximately 40-50%. Steroid refractory acute GVHD is a severe complication with significant mortality. Several different therapies have been used over the years with varying efficacy but none of these have been shown to be clearly superior. Ruxolitinib has recently been shown in randomized controlled trials to be more effective than "best available therapy" and is now seen as the primary choice for treatment of steroid refractory acute GVHD[9]. Other promising options are mesenchymal stem cells, especially in children, and photopheresis. Several other agents have been tried for second-line therapy but data for these therapies are insufficient for a general recommendation[10]

Chronic GVHD affects 25-65% of long-term survivors and is one of the leading causes of late morbidity and mortality after HSCT. A major risk factor for chronic GVHD is preceding acute GVHD. The main target is connective tissue in various organs, resulting in dermatitis, keratoconjunctivitis, oral mucositis, enteritis or liver affection. A particularly difficult form of chronic GHVD is obliterative bronchiolitis that can cause respiratory failure. The diagnosis depends primarily on clinical signs (with exclusion of other differential diagnoses) and is graded mild, moderate or severe[4]. The first line treatment for chronic GVHD is corticosteroids, usually in combination with a calcineurin inhibitor to reduce the dose and duration of corticosteroids. Approximately 20% of adults and 50% of children respond to corticosteroids. There is no consensus concerning second-line therapy for chronic GVHD, but a large variety of treatments are used, including extracorporeal photopheresis, mycophenolate mofetil, rituximab, calcineurin inhibitors, mammalian target of rapamycin (mTOR) inhibitors, tyrosine kinase inhibitors, and ruxolitinib[10]

Both acute and chronic GVHD impair the immune reconstitution, due to GVHD itself and the immunosuppressive treatment[11,12.]

1.1.4 Immune reconstitution

Reconstitution of the immune system is affected by various factors, such as age, conditioning regimen, type of donor, stem cell source, graft manipulation (T-cell depletion), the development of GHVD or recurrence of the underlying disease, illustrated in Figure 1. The cells of the innate immune system, including granulocytes, monocytes and NK cells, normally recover in number and function during the first 1-2 months after HSCT, with neutrophils being the first leucocyte to appear in peripheral blood. However, important functions of innate immune cells, such as chemotaxis and phagocytosis, can remain impaired over a longer period, especially if the patient develops GHVD. Macrophages are more resistant to chemotherapy and early on tissue macrophages of host origin remain, but gradually they are replaced by donor macrophages.

The reconstitution of the adaptive immune system takes longer. The reconstitution of T-cells (CD3+ cells) occurs by two main pathways. Initially there is an early thymus-independent peripheral expansion of mature donor T-cells from the graft, providing a limited repertoire of T-cells during the first year after HSCT. Secondly, there is generation of naïve donor T-cells, that undergo thymus-dependent maturation. This process takes six months up to two years. The thymus function, and thereby the thymus-dependent maturation of T-cells, is dependent on several factors, especially older age and the occurrence of GVHD[13]. The CD4+ helper T-cells reconstitute later than CD8+ cytotoxic T-cells, resulting in an inversed CD4/CD8 ratio seen early after HSCT.

B-cells usually reach normal levels after 1-2 years, but recovery of memory B-cells takes longer, leading to a prolonged defect humoral immune response, especially in patients with GVHD. The impaired function of CD4+ helper T-cells further hampers an efficient antibody response during the first couple of years after HSCT.

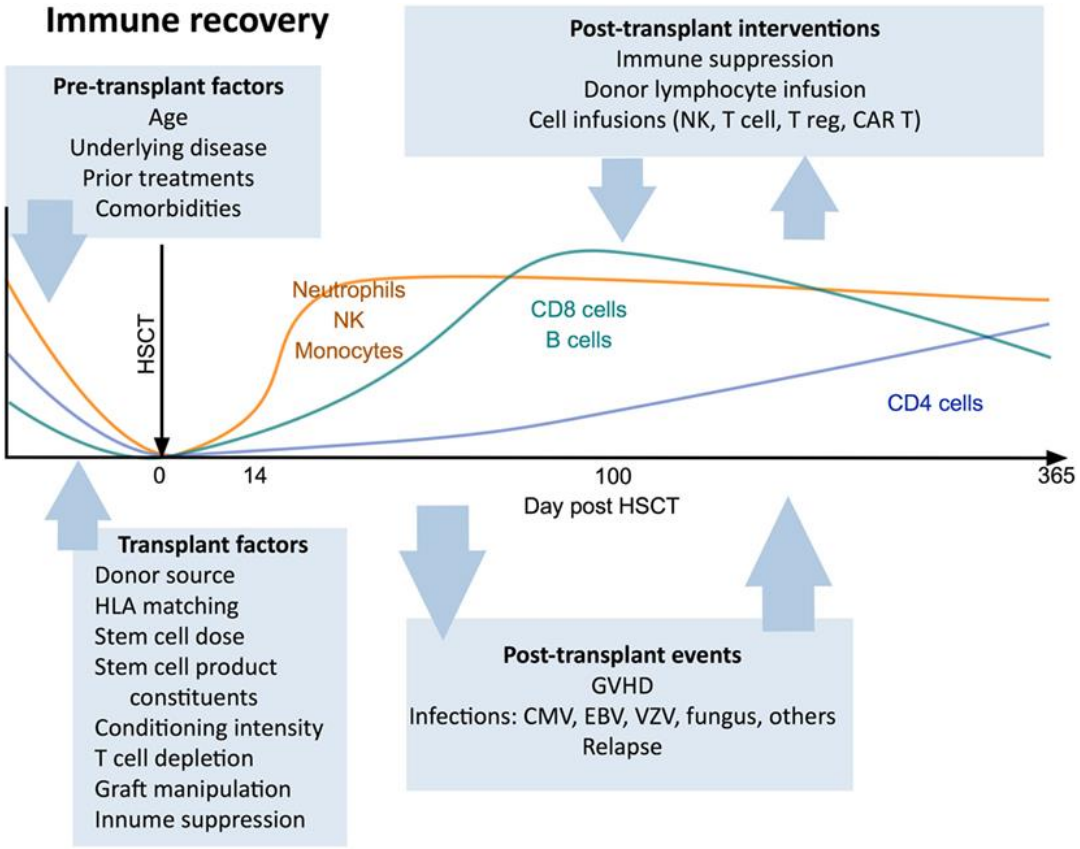

Figure 1. Illustration of the recovery of the number of different immune cells after HSCT, and factors that can influence the immune reconstitution.

Image from Stern L, McGuire H, Avdic S, Rizzetto S, Fazekas de St Groth B, Luciani F, Slobedman B and Blyth E (2018) Mass Cytometry for the Assessment of Immune Reconstitution After Hematopoietic Stem Cell Transplantation. Frontiers in Immunology[14]

1.1.5 Infections after HSCT

Infections are a major cause of morbidity and mortality following HSCT. The spectrum and risk for infections varies depending on the time that has passed since HSCT, and if complications affecting the immune recovery, especially GVHD or relapse, have evolved.

The process of immune reconstitution, and the corresponding risk for infections, has traditionally been divided into three phases: the pre-engraftment phase (0-30 days after HSCT), the early post-engraftment phase (30-100) days after HSCT), and the late post-engraftment phase (>100 days after HSCT). However, in recent years, the infectious panorama of the early post-engraftment period can be found for longer, until approximately 180 days post HSCT, depending on the type of conditioning, type of donor, T-cell depletion, and stem cell source, all possibly impacting immune reconstitution. GVHD is the most

important risk factor for infections in the late post-engraftment phase, and if GVHD does not occur, the risk is generally lower for most infections after day 100 (180).

The pre-engraftment phase (day 0-30)

During the first 2-4 weeks after HSCT, bacterial infections are dominating, usually as blood stream infections, due to neutropenia, disruption of the mucosal barriers, and indwelling catheters. The dominating etiologies are enteric gram-negative rods and gram-positive cocci, translocated from the gastrointestinal tract, and coagulase-negative staphylococci, originating from the skin and central venous catheters[15,16]. Bacteremia in a neutropenic patient carries a high mortality, if not treated adequately, and empirical broad-spectrum antibiotic treatment should always be administered to neutropenic patients with fever[17,18]. Bacteremia with gram-negative rods are associated with the highest mortality, and many centers provide the patients with quinolone prophylaxis during the neutropenia. Quinolone prophylaxis reduces the number of episodes with fever and bacteremia, respectively, but there is no confirmed effect on mortality[19]. During pre-engraftment there is also a risk for candida mucositis and invasive candida infection unless prophylaxis is given. The risk for invasive mold infections is generally moderate but increases especially in patients with previous invasive mold infection, cord blood transplantation, or patients with active leukemia at the time of HSCT[20]. Prophylaxis with antifungal agents is generally recommended: fluconazole if there is a low incidence of mold infections at the center, or a mold-active agent such as posaconazole if the incidence is high, or in patients with an increased risk[21].

Herpes simplex 1 and 2 reactivations are common during this phase motivating prophylaxis with acyclovir. On the contrary, the risk of reactivating other latent or persistent viruses is low during this phase, although children with pre-transplant shedding of human adenovirus (HAdV) in stool, can develop invasive HAdV infection early after HSCT[22-24]. Community acquired respiratory viral infections, especially influenza, respiratory syncytial virus (RSV), and parainfluenza, can cause severe pneumonia with high mortality during the pre-engraftment phase. The new coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS CoV2), may cause lethal pneumonia in 20% of patients who become infected within the first year after HSCT[25].

If the mentioned respiratory viral infections (including HAdV in respiratory secretions), or HAdV in stool, are identified prior to HSCT, postponing the transplantation is recommended[24,26].

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The early post-engraftment phase (day 30-100 (180))

Following recovery of neutrophils, the risk of infections with enteric bacteria is greatly diminished. The risk of bacteremia with coagulase-negative staphylococci is also reduced, but the risk remains as long as the patient has a central venous catheter. During the early post-engraftment phase the infections mainly reflect the profound defect of cellular immunity in the patients. Reactivations of cytomegalovirus (CMV) are common, and since there is a high risk of disease, monitoring and pre-emptive therapy with ganciclovir have been standard[22,27]. Recently, prophylaxis with a new antiviral drug, letermovir, was shown to reduce the number of clinically significant CMV infections and reduce all-cause mortality by week 24 after HSCT[28]. Prophylaxis with letermovir has therefore been implemented at many centers. Reactivation of Epstein-Barr virus (EBV) with the development of post-transplant lymphoproliferative disease is a serious complication with high mortality[23]. High risk patients should be monitored for EBV in blood [29]. HAdV can be reactivated or acquired with the risk of lethal disseminated disease, which is described in more detail in the HAdV section. Children should be monitored for HAdV in blood, and some centers also monitor the level of HAdV in stool[30]. There is a substantial risk for reactivation of varicella zoster virus and seropositive patients should receive prophylaxis with acyclovir/valaciclovir during the first year post HSCT[31]. Community acquired respiratory virus infections, especially influenza and respiratory syncytial virus, continue to be important pathogens during this period.

The risk for invasive mold infection is high in patients with acute GVHD III-IV, acute GVHD $\geq$ II in patients > 40 years or with an alternative donor, acute GVHD followed by chronic GVHD, or GVHD unresponsive to steroids[21,32,33]. Pneumocystis jirovecii pneumonia (PJP) is common without prophylaxis, which is routinely given during this period[34]. Other fungal infections are uncommon in the absence of GVHD.

The late post-engraftment phase (> day 100 (180))

The risk of infections during this phase is highly associated with the occurrence of chronic GVHD. In patients without GVHD, there is a moderate risk for infections with encapsulated bacteria (*Streptococcus pneumoniae and Haemophilus influenzae*). Vaccination against these bacteria with conjugated vaccines is recommended from 3 months after HSCT, and during season patients should be vaccinated against influenza[35,36]. The risk for PJP continues, and

prophylaxis should be prescribed at least until the GVHD prophylaxis has been terminated. Reactivation of VZV can also occur, motivating continued prophylaxis for at least a year.

In patients with GVHD, the risk of infections is related to the severity of GVHD, and the treatment required, including the dose and duration of corticosteroids. The infections seen are related to the delayed reconstitution of both the cellular and humoral immune system in patients with GVHD. Hence, there can be reactivations of CMV, which may require continued monitoring[27,37]. There is an increased risk for infections with *Streptococcus pneumoniae and Haemophilus influenzae,* and antibiotic prophylaxis can be considered in patients with severe chronic GVHD. Influenza continues to be important and yearly vaccination is recommended as long as the patient is assessed to be immunocompromised. Prophylaxis for PJP needs to be continued, and patients with severe GVHD are at risk of invasive mold infections.

Hepatitis B can cause severe complications including fibrosing cholestatic hepatitis due to reactivation of the infection after HSCT[38]. All patients with chronic hepatitis B infection (who are not on antivirals) should receive antiviral treatment, life-long. Patients with previous hepatitis B also have a high risk of reactivating the infection and antiviral prophylaxis is recommended from the time of HSCT and until at least a year after the immunosuppression has been terminated[38]. HSCT patients with previous or chronic hepatitis B should be monitored for reactivation. For patients without hepatitis A or hepatitis B antibodies, vaccination can be considered, before and after the HSCT. Patients with chronic hepatitis C are at risk for accelerated development of cirrhosis after HSCT and should receive treatment as soon as regarded possible[38]. In some countries, although not in Sweden, screening for hepatitis E has been implemented, since hepatitis E has emerged as a cause of liver injury following HSCT[39].

1.2 HUMAN ADENOVIRUS (HADV)

Infections with HAdV are common, especially in young children. There are currently more than 100 HAdV types, which are grouped into seven species (A-G)[40]. HAdVs are genetically heterogenous viruses that can infect several different cell types and organs. The tissue tropism varies between HAdV types, rendering a broad variety of symptoms, including upper or lower respiratory tract infection, gastroenteritis and conjunctivitis[41]. In immunocompetent individuals HAdV infections are usually mild and self-limiting, although severe manifestations with lethal pneumonia, encephalitis or myocarditis also may occur[42,43].

The circulating types of HAdV varies over time and between geographical areas. However, the knowledge of circulating HAdV types, both in the society and in health care facilities, is limited, since HAdV typing is uncommon in routine laboratory testing. The most common HAdV types reported in the general population are types C1, C2, C5, B3, B7, B14, B21, E4, and F41 [44,45]. The types of HAdV reported in HSCT patients are similar to those found in the general population, with C1, C2, C5, B3 and B7 dominating, apart from HAdV A31 having been found more frequently in HSCT patients[41,46-49].

1.2.1 HAdV persistence and reactivation

Healthy children can excrete virus in stool for months or even years after the primary infection[50,51], and HAdV has been shown to persist in different cell types and tissues[52]. Garnett et al reported of HAdV DNA in lymphocytes in tonsils and adenoids. The prevalence was highest in young children and declined with increasing age. Infectious HAdV was rarely found, but HAdV DNA replication and production of infectious virus appeared at stimulation of the lymphocytes, indicating a latent form of HAdV[52,53]. Since clearance of HAdV DNA seems to occur with increasing age, unlike what is seen in other viruses capable of true latency (herpes viruses and human immunodeficiency virus)[54], we here use the term "persistence" for HAdV.

Kosulin et al. studied gastrointestinal biopsies from immunocompetent and HSCT children. Using *in situ* hybridization, HAdV DNA was identified in gastrointestinal lymphocytes from the immunocompetent children, whereas in HSCT children HAdV DNA was located mainly in the gastrointestinal epithelial cells. This could indicate that lymphocytes are the primary site of HAdV persistence, but when reactivated, leaking viruses from lymphocytes replicate more readily in epithelial cells[55]. HAdV DNA has also been detected in lymphocytes infiltrating tumors, but it has not been shown whether HAdV has a potential oncogenic role in humans[56]. Persistent HAdV may reactivate in severely immunocompromised patients, especially in the HSCT setting[41]. Detection of HAdV antibodies before HSCT has predicted reactivation of the same HAdV type after HSCT[57], and a HAdV strain identified prior to HSCT was with few exceptions identical to the HAdV strain found after HSCT[57,58]. Although HAdV may persist in several tissues, many studies indicate that the gastrointestinal tract is the primary site of HAdV reactivation in children[59-61].

1.2.2 HAdV, transmission

HAdV is transmitted by droplets, direct contact, fecal-orally, or indirectly by contaminated objects[41]. HAdV particles are highly resistant physically. HAdV has been shown to remain infectious on dry surfaces for up to four weeks[62,63] and HAdV DNA can remain detectable on surfaces for three months[64]. Since HAdV are non-enveloped viruses, they are resistant to many hand disinfectants, although the sensitivity can vary between HAdV types[65]. For inactivation of HAdV, a solution with 85% ethanol for at least 2 minutes is effective[65]. Surfaces and fomites can also be disinfected with an oxidative agent or sodium hypochlorite for 10 minutes[66,67].

HAdV can be easily transmitted and cause outbreaks in closed or crowded environments, such as among military recruits[68]. HAdV has also been responsible for many outbreaks within health care facilities, including neonatal wards[69], ophtalmological clinics[70] and pediatric long-term care facilities[71]. There are several reports of HAdV transmission in HSCT wards, of which three (including our own) were type A31, one type F41 and one type A12[49,72-75]. In a couple of the reports of HAdV A31 transmission continued during a long time, up to several years[49,75].

1.2.3 HAdV in the HSCT population

1.2.3.1 Epidemiology

The reported incidence of HAdV infection in HSCT patients varies depending on age of the patient, "transplant procedures", the extent of testing and the material tested. Recent studies, from the last 10 years, have reported HAdV viremia in 10.5-26% of pediatric[60,76-79] and 1.5-12% of adult HSCT recipients[76,80-83]. HAdV disease has been diagnosed in 4-11% of pediatric[47,59,84,85] and in 2-4% of adult patients after HSCT[80-82].

1.2.3.2 Definitions

There are no universally used definitions for manifestations of HAdV in the HSCT population. The following definitions were recommended by the 4th European Conference of Infections in Leukemia (ECIL) 2011[86]:

- Systemic infection/viremia: Positive HAdV PCR, virus isolation, or antigen detection in peripheral blood.

- Local infection: Positive HAdV PCR, virus isolation, or antigen detection in biopsy material or in body fluids other than peripheral blood.

- Probable disease: HAdV infection plus corresponding symptoms and signs without histological confirmation.

- Proven disease: HAdV infection plus corresponding symptoms related to the infection and histological confirmation of HAdV in the appropriate location.

Histological confirmation is not always possible, whereby proven disease may be less common. In this thesis, HAdV infection refers to the detection of HAdV in any sample, and disease refers to HAdV infection with corresponding signs and symptoms (with or without histological confirmation).

1.2.3.3 Clinical picture

Subclinical HAdV infections are common in patients having undergone HSCT[58,83,84]. Screening studies have shown that gastrointestinal symptoms are absent in 54-70% of patients with HAdV detected in feces, and 50-82% of patients with viremia remain asymptomatic, with or without pre-emptive treatment[86]. HAdV can cause a wide range of symptoms in HSCT patients: mild disease including enteritis or upper respiratory symptoms, severe localized disease, such as colitis, hepatitis, hemorrhagic cystitis or pneumonia, or severe disseminated disease with multiple organ involvement and high mortality[30]. Young children have the highest risk of both HAdV infection and disease[41]. However, in cases of dissiminated disease with multiple organ involvement, the mortality is high in both children and adults[40,80,87]. Other risk factors for HAdV infection and disease include *in vivo* or *ex vivo* T-cell depletion, grafts from unrelated or haploidentical donors, cord blood grafts, and

lymphopenia with CD3+ T-cell counts < 0.3 x 10^9/L [30]. Absence of HAdV-specific T-cells is also linked to the development of HAdV disease[88].

1.2.4 Diagnosis and monitoring of HAdV

Diagnosis of HAdV infection is today usually based on polymerase chain reaction (PCR) of peripheral blood or samples from feces, urine or respiratory secretions. Quantitative (q) PCR is a general routine for analyses of blood samples. Some centers also use qPCR for analyses of fecal samples, but this is not done at the Karolinska University Hospital.

Rational for monitoring HAdV in peripheral blood: Rising levels of HAdV in peripheral blood is associated with HAdV related death and has been shown to precede clinical symptoms of HAdV by up to several weeks[82,84,86]. Due to the limited effect of HAdV therapy, especially in patients with disseminated disease, monitoring high risk patients for HAdV with qPCR in peripheral blood, and pre-emptive treatment is recommended in current guidelines[30,86]. However, there are no data showing beneficial effects on mortality with this strategy[30].

Rational for monitoring HAdV in feces: Several authors have reported of increasing HAdV levels in feces preceding viremia in pediatric HSCT patients[24,47,59-61,79,89]. In some reports a critical threshold of HAdV > 10^5-10^6 copies/gram feces preceded viremia by in median 6-11 days[59,60,89]. Others have reported of a high amount of HAdV in feces being associated with viremia, although without a specific HAdV thresold in feces clearly preceding viremia[47,61,79]. In addition, Kosulin et al found that children with HAdV shedding in stool prior to HSCT had a significantly increased risk of viremia post HSCT[24]. These data have prompted recommendations of screening children for HAdV in feces prior to HSCT, and monitoring feces with qPCR during the first 100 days post HSCT[30,40]. In contrast, there are no data supporting monitoring adults for HAdV in fecal samples[30]. In a recent European survey, most HSCT centers monitored the patients for HAdV in blood (all patients 66%, and only high-risk patients 19% of centers). Monitoring of fecal samples was less common (26% of centers)[90].

1.2.5 HAdV antiviral treatment

Cidofovir has *in vitro* virostatic effect against HAdV and has been reported to have some positive effect as pre-emptive treatment of HAdV infection and disease, although the efficacy can be limited when there is no concomitant immune reconstitution or in cases of multiorgan disease[48,84,86,87,91]. Renal toxicity is a common and serious side-effect[40]. The most frequently

used dose is 5mg/kg once a week, but for pre-emptive treatment a dosing regimen of 1 mg/kg three times per week is also used[40,90,92].

Brincidofovir is a lipid-conjugated derivative of cidofovir that has been associated with a significantly better viral response, even in patients with profound lymphopenia [93-95], but no prospective controlled study has been performed. Oral brincidofovir is not associated with nephrotoxicity, but gastrointestinal toxicity is common and therapy limiting[96]. The substance seems to be a promising treatment option for HAdV but is presently not available[40]. An intravenous formulation of brincidofovir is under investigation[97].

1.2.6 HAdV adoptive cellular therapy

Adoptive transfer of donor derived HAdV-specific T cells have been studied with promising results[98-101]. The isolation and expansion of donor-derived HAdV-specific T-cells is time consuming and may not always be possible. HAdV-specific or multipathogen-specific T-cells from third party donors are being evaluated, which may provide a more universal and faster access to cell therapy[102].

1.2.7 Management of HAdV infection and disease

In guidelines from the European Society of Blood and Marrow Transplantation (EBMT) 2018, screening of feces for HAdV twice prior to HSCT, and weekly monitoring of HAdV in both blood and feces with qPCR after HSCT, until recovery of CD3+ cells above 300/µL, is recommended for pediatric patients [30]. For adults, there is no strong recommendation on HAdV monitoring[30]. At our center, monitoring of high-risk children for HAdV in blood was introduced in recent years. In addition, screening children for HAdV in stool, and screening all patients for respiratory viruses (including HAdV), prior to HSCT, has been implemented at the center lately. However, the method for quantification of HAdV in feces is currently not available at Karolinska University Hospital. There is no established threshold of HAdV DNA in blood or feces for initiating pre-emptive HAdV treatment. Tapering of immunosuppression should be done whenever possible, especially in patients with HAdV disease[86]. In a recent European survey, HAdV DNA levels of > 100 to > 10.000 copies/mL blood had been used as threshold for initiating pre-emptive treatment in the included HSCT centers[90]. Hiwarkar et al recommend pre-emptive treatment with cidofovir in the presence of viremia > 1000 copies/mL and/or HAdV DNA in feces with rapidly rising levels above the suggested critical threshold[30]. In patients with HAdV disease treatment with cidofovir is recommended[40,86].

1.3 NOROVIRUS

Norovirus is the leading cause of viral gastroenteritis worldwide. Norovirus gastroenteritis affects all age groups and is estimated to be responsible for 21 million cases and 500-800 deaths yearly in the United States[103,104]. Typical symptoms are sudden onset of vomiting and diarrhea usually resolving within 2-3 days. However, symptoms lasting for a median of 4-6 days have been reported during hospital outbreaks and in immunocompetent young children, and in elderly people with co-morbidities norovirus has been associated with excess mortality [105,106]. The diagnosis of norovirus is mostly based on PCR of fecal or vomit sample[107]. There exists no specific treatment or vaccine against norovirus infection [108].

Noroviruses are small non-enveloped RNA-viruses belonging to the family caliciviridae. The norovirus genus is divided into 6 genogroups (G), of which GI, GII and (to a very little extent) GIV cause infection humans[109]. The human genogroups are further divided into at least 34 genotypes[110]. Norovirus GII.4 is the dominating genotype globally since many years, and still is the most prevalent genotype in Sweden, although other genotypes have emerged as predominant in some other countries[104,111]. Globally circulating GII.4 strains are every few years replaced by new antigenically divergent GII.4 strains, which coincides with recurrent increased activity of norovirus outbreaks[112]. In immunocompetent individuals, norovirus GII.4 has been associated with a more severe clinical course than other genotypes, and more frequently causes vomiting[113,114].

1.3.1 Transmission

Noroviruses are transmitted primarily by the fecal-oral route, but air-borne transmission (of aerosolized infectious vomit) also occurs[115]. Noroviruses are transmitted very efficiently due to several characteristics: the infectious dose is low (estimated to 18-1000 virus particles), viral shedding precedes the onset of symptoms and may peak after symptoms have resolved, and noroviruses are stable in the environment, persisting on surfaces, in food and in water[114,116,117]. Common hand disinfectants are not effective for norovirus, in parity with what is seen with HAdV[107]. Outbreaks are common, both in the community and, particularly, in health care facilities, and there are several reports of norovirus outbreaks in HSCT units[118-121]. Most nosocomial outbreaks of norovirus are caused by GII.4, possibly because norovirus GII.4 is associated with an increased risk for vomiting, in addition to GII.4 being the dominating strain in the communities[114].

Norovirus RNA can be detected in stool for up to 100 days in immunocompetent people, and for even longer periods in immunocompromised individuals, after resolution of symptoms[122,123]. However, it is not known if detection of norovirus RNA a long time after symptoms have resolved represents infectious viruses with the potential of transmission.

1.3.2 Secretor-status

Host genetic factors play an important role for the susceptibility to norovirus infection. The ability of several norovirus strains to infect humans strongly depends on the presence of different histo-blood group antigens (HBGAs) on gut mucosal surfaces. At least 20% (21.5-28%) of the Swedish population do not express certain HBGAs on their epithelial cells due to homozygous inactivating mutations in the α1,2-fucosyltransferase 2 (FUT2) gene[124,125]. These individuals are termed secretor-negative and are with few exceptions protected against the dominating genotype of norovirus (GII.4)[110]. It is not known if secretor-status is associated with the susceptibility to norovirus infection also in immunocompromised individuals, or if secretor-status can influence the clinical course of norovirus infection in these patients.

1.3.3 The immune response to norovirus

There is very little knowledge of how the human immune system interacts with norovirus, both in terms of protective immunity and clearance of the infection. Humoral response may have an important role, since increased levels of antibodies blocking the binding between virus like particles (VPL, a surrogate for virus) and HBGA is associated with a lower risk of becoming ill from norovirus[107] Further, in a case report of a patient with chronic norovirus infection, the infection resolved at the development of strain-specific HBGA-blocking antibodies[126]. Thus, B-cells might be important both for preventing and clearance of norovirus infection[107]. T-cells are also activated when exposed to norovirus, in line with what is seen in other viral infections, although their role in controlling norovirus has been less clarified[127].

1.3.4 Norovirus in the immunocompromised patient

In immunocompromised patients, norovirus is increasingly acknowledged as a cause of chronic and/or severe gastroenteritis. This has been described in patients after solid organ

transplantation[128-131], and in patients with hematological malignancy [120] and primary immunodeficiency disorders [132-134]. There is limited knowledge of norovirus in the HSCT setting. Some authors have reported of norovirus infection causing chronic diarrhea and malnutrition, requiring TPN for several months up to a year, occasionally with lethal outcome [135-137]. In other reports the duration of symptoms was considerably shorter (median 7-12,5 days), although the clinical picture was severe in some of the patients, requiring admission to the intensive care unit, and with several deaths that may have been complications of norovirus infection [120,138]. Further, in two reported outbreaks of norovirus in HSCT units, there were no severe cases [118,119]. There is little knowledge of risk factors associated with a severe or prolonged course of norovirus infection in patients undergoing HSCT.

1.4 HEPATITIS E VIRUS

There are four genotypes of HEV causing disease in humans. HEV genotype 1 (HEV1), and to a lesser extent HEV 2, are responsible for large waterborne outbreaks as well as sporadic cases of acute hepatitis in low-income countries [139]. HEV 3 and HEV 4 are endemic in industrialized countries, causing zoonotic infections, with HEV3 being the predominant genotype, and HEV4 mainly found in high-income areas in Asia[139]. Recently an additional four HEV genotypes (HEV5-8) have been discovered in wild animals. Infection with these HEV genotypes have been described in a couple of case reports, although the clinical relevance for humans remains to be clarified[140].

The clinical picture varies depending on the HEV genotype. HEV1 and HEV2 cause acute icteric hepatitis in 5-30% of the cases, and fulminant hepatitis with high mortality can occasionally occur, especially in pregnant women for whom mortality can be as high as 15-25%[139]. HEV1 and HEV2 have not been associated with chronic infection. The diagnosis of HEV should be based on a combination of serology and HEV RNA, and for immunocompromised individuals only HEV RNA is recommended[141].

1.4.1 HEV3, epidemiology

The prevalence of anti-HEV IgG in Europe varies greatly depending on the serological method used, geographical area and type of population. The seroprevalence increases with age and is higher among individuals exposed to swine and/or wild animals[142]. In Sweden the

prevalence of anti-HEV IgG is 9-16%[143,144], whereas in France and Germany the reported seroprevalence is higher, up to 52% in southwest of France[142].

1.4.2 HEV3, transmission

Transmission of HEV3 occurs through the fecal-oral route, mainly by ingestion of insufficiently cooked meat from infected animals[145]. The primary reservoir for HEV3 are pigs, but other domestic and wild animals, such as wild boar, deer, rabbits, cattle, sheep and horses, can also be infected. In addition, shell-fish and fruits or vegetables watered with contaminated water may transmit HEV[140]. There are also increasing data supporting transmission by blood products[146], and a few cases of transmission from donors of stem cells or solid organs have been reported[147,148]. HEV ribonucleic acid (RNA) has been detected in 0.01 % of Swedish blood donors [149]. The prevalence of HEV RNA in blood donors varies between different European regions and has been up to 0.12–0.13% in certain regions of France and Germany[150]. Several European countries have implemented HEV RNA screening of blood products and donors of stem cells, but such screening is currently not performed in Sweden[150].

1.4.3 HEV3, Clinical picture

HEV3 causes acute self-limiting hepatitis in healthy people, but the majority of HEV3 infections are subclinical[151]. Individuals with pre-existing liver disease carry an increased risk for acute liver failure upon infection with HEV3[140], and extrahepatic manifestations including thrombocytopenia, glomerulonephritis and neurological symptoms have been linked HEV infection[139,152].

1.4.4 HEV3 in immunocompromised patients and in the HSCT setting

HEV3 can cause chronic infection and rapid advancement to liver cirrhosis in immunocompromised patients. This has mostly been described in SOT recipients[153], but also in patients with HIV [154], rheumatological disorders[155] and hematological malignancy [156]. The reported frequency of patients tested positive for HEV RNA after HSCT has been 0% in two small cohorts[157,158], and 0.4- 2.4% in larger cohorts with 111-328 patients[39,159,160]. Moreover, Willemse et al detected HEV RNA in 5/123 (4%) HSCT patients with elevated ALT[161]. In

HSCT patients infected with HEV, up to 63% may develop chronic infection, and the clinical course can be severe[39,161]. Carre et al have reported of a case of fatal fulminant HEV infection early after HSCT[162], and in a retrospective European multicenter study, HEV contributed to liver related death in two patients[163]. In contrast in another multicenter study, no HEV related deaths were found among 25 HSCT patients with HEV infection[164].

1.4.5 HEV3, treatment

The experience of treating HEV infections is limited, and mostly involves solid organ transplant recipients[165]. Reduction in immunosuppression can lead to clearance of HEV infection in approximately 30% of solid organ transplant patients with persistent infection[166]. Also in HSCT patients, chronic HEV infection has resolved during tapering of immunosuppressive drugs[39,164]. Ribavirin has been associated with clearance of HEV infection in several studies of solid organ transplant patients[167-169]. Although data on treatment of HSCT patients with HEV is limited, case reports and series indicate that ribavirin may be effective and should be considered for these patients[38,161,163,164].

2 AIMS

2.1 GENERAL AIMS

To study the clinical importance of viral gastrointestinal infections in HSCT recipients, with focus on adenovirus, norovirus and hepatitis E.

2.2 SPECIFIC AIMS

- To investigate transmission within a health care facility

- To determine the clinical picture and outcome

- To determine risk factors for severe and/or long-term infection

- To determine the importance of genetic factors (secretor-status)

3 STUDY POPULATION

The patients studied had all undergone HSCT 2005-2016 at CAST, Karolinska University Hospital Huddinge. The conditioning regimens, GVHD prophylaxis and supportive care procedures at the center were previously described[170,171]. Haploidentical transplants were performed using post-transplantation cyclophosphamide according to Luznik et al or Raiola et al[172,173] and since 2013 alpha/beta T-cell depletion was used for some patients[174].

All patients were monitored for cytomegalovirus (CMV) on a weekly basis by a quantitative PCR and preemptive antiviral treatment was given as previously described. [175], Patients considered at high risk for PTLD (serological mismatch, unrelated or haplo-identical donor, cord blood stem cell source, aGVHD $\geq$ II) were also monitored weekly for Epstein–Barr virus (EBV) during the first 3 months after HSCT[176]. No general surveillance for HAdV was performed at our unit during the time of our studies, but testing was promptly done on suspicion of infection. In patients with gastrointestinal symptoms, microbiological testing of fecal samples was conducted, including PCR for noro-, sapo-, rota-, and adenovirus and detection of *Clostridium difficile* toxin, and in cases of protracted diarrhea also culture for *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* and microscopy for parasites. Upper and lower endoscopies were performed at suspicion of gastrointestinal GVHD.

Other methods, including microbiological and statistical analyses, are described in the section of results and discussion.

The studies were approved by the Regional Ethical Board of Stockholm:

Study I and II: DNR 2012/1506-31/4

Study III: DNR 2017/1508-31/2

Study IV: DNR 2016/381-31/4 and 2017/1909-32

4 RESULTS AND DISCUSSION

4.1 PAPER I

December 2011 to March 2012 there was an outbreak of HAdV A31 at our unit. We analyzed the outbreak, the possible routes of transmission, and the medical consequences.

We studied all patients who were admitted at CAST during the outbreak (defined as the period from the first to the last verified case of HAdV A31 infection: December 12, 2011 to March 22, 2012). We also studied all patients diagnosed with HAdV infection from November 2011 to July 2012. Clinical data, and information of hospitalizations, visits to the out-patient clinic and the patient housing (mainly housing families with children), were retrieved from the medical records. We also collected information of hygiene and cleaning routines, and measures during the outbreak, respectively, from the staff at the ward.

4.1.1 Detection and phylogenetic analyses of HAdV

For detection of HAdV, a Taqman real-time PCR targeting the hexon gene was used, as previously described[177,178]. All HAdV strains identified during the outbreak, and the following 4 months, were typed. The HAdV type was determined as described by Allard et al[179]. Phylogenetic analysis of the hexon gene was conducted using primers and PCR programs according to Leruez-Ville et al[73], with slight modifications presented in paper I.

For phylogenetic analysis of the E3 gene, a 2-step PCR amplification of almost the entire HAdV E3 gene was performed using 8 sets of different pairs of primers designed from the known sequence of the HAdV A31 reference strain (AM749299.1). Further details are outlined in paper I. Sequencing and phylogenetic analyses were performed at the Department of Virology, Umeå Universtity, Umeå.

4.1.2 Description of the outbreak

Nine patients with HAdV A31 were identified. The outbreak is illustrated in Figure 2. Several measures were implemented:

1. HAdV screening of all admitted patients on two occasions, and regular testing of those with symptoms,

2. closing and disinfecting the common kitchen (day 38 of the outbreak),

3. transfer of all HAdV positive patients from the ward (day 46 of the outbreak).

After these steps were taken, there were no new cases identified in the ward for a period of four weeks, although three patients in the out-patient clinic were found to be HAdV A31 positive. These patients had all been previously admitted at the ward, at the same time as HAdV A31 positive patients. Transmission in the ward was thought to be over, and the kitchen reopened (on day 59 of the outbreak). However, two more cases of HAdV A31 were diagnosed on day 78 and 101 respectively (Figure 2)

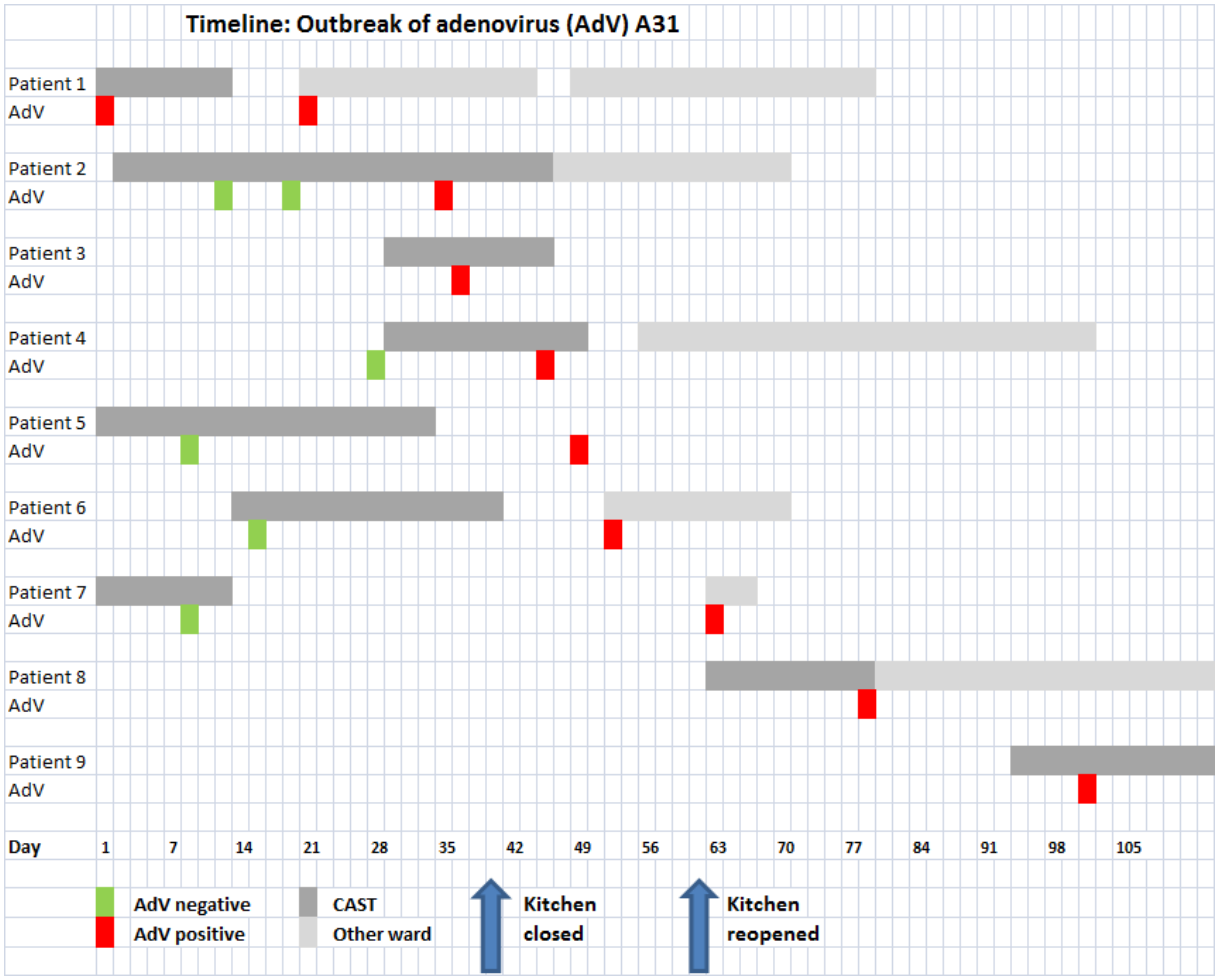


Figure. 2. Timeline of the human adenovirus (HAdV) outbreak. All samples were fecal samples except in Patient 5 (plasma sample). Day refers to the day after the first identified HAdV A31 case. The bars indicate admission at CAST or "other ward" (intensive care unit or department of infectious diseases).

4.1.3 Did transmission between the patients occur?

Phylogenetic typing was conducted in all nine cases of HAdV A31 infection. Sequencing the hexon gene showed 100% homology between the patient strains, but also to reference strains of HAdV A31, whereby transmission among the patients could neither be concluded or ruled out. We therefore sequenced the more diverse E3 gene, revealing that the patient strains contained identical mutations not present in the reference strains, hence suggesting a common source of transmission. The hexon gene has a relatively low genetic variability[49]. In another reported HAdV outbreak among HSCT recipients, sequencing revealed similar results, with homology between the patient strains as well as the reference strains[74].

4.1.4 Possible routes of transmission

All patients with HAdV A31 had been admitted to the ward. The two last cases (patients 8 and 9) had no connection in time to the other identified HAdV positive patients in the ward, but no other possible interactions with HAdV infected patients (out-patient clinic or patient housing), were identified for those patients.

The following factors may have contributed to transmission of HAdV:

1. Although HAdV positive patients were isolated, transmission may have occurred indirectly through contaminated surfaces or fomites in the ward (HAdV contamination may have occurred before hygiene measures were implemented, and after reopening the kitchen).

HAdV has been shown to remain infectious on surfaces for up to four weeks, and HAdV RNA can be detectable on surfaces for 3 months[63,64]. Monitoring of HAdV DNA in the environment after cleaning may be a way of preventing insufficient cleaning and environmental transmission of HAdV[180,181]. Houldcraft et al reported of a cluster of four patients with HAdV A31, who seemed to be part of a nosocomial transmission, which was supported by whole genome sequencing (WGS), although two of the patients had been admitted three years apart. Other HAdV A31 cases had been in the ward during this period of three years, and may have been part of the transmission, but in those cases WGS could not be performed[49]. Routine phylogenetic typing of HAdV strains in HSCT patients could be an important measure, enabling early discovery of HAdV transmission.

2. Testing for HAdV at the end of the outbreak was less frequent, and unidentified cases, with mild or no symptoms, may have contributed to the continued transmission among patients in the ward. Asymptomatic HSCT patients can have high concentrations of HAdV in stool[59].

3. It is also possible that staff members transmitted the virus between patients, although none of them reported any clinical symptoms during the outbreak. At the beginning of the outbreak, there was an excessive strain on the staff, due to several seriously ill patients in the ward, and hygiene routines may not always have been properly maintained. Short staffing has been reported to be a risk factor for transmission of pathogens between patients[182,183].

4. The ward used at the time was not optimal for infection control purposes, since some rooms did not have separate toilet and shower facilities. This has now been changed with the move to a new purpose-built transplant ward.

4.1.5 Clinical consequences

Transplantation was postponed due to HAdV, and later cancelled, in one patient. Five patients were treated with cidofovir because of high levels of viremia, but no patient developed severe disease. Seven patients had concomitant aGVHD grade II–IV, complicating the clinical picture, with difficulties discriminating symptoms of GVHD from symptoms of HAdV.

4.1.6 Conclusion

An outbreak of HAdV among HSCT patients can be difficult to control. The clinical consequences were significant for some patients, although no patient developed severe HAdV disease. Contamination of the common facilities in the ward, and unidentified asymptomatic cases, may have contributed to the prolonged outbreak.

4.2 PAPER II AND III

In paper II, 63 patients having undergone HSCT and who were diagnosed with norovirus infection 2006-2012 were studied retrospectively. The aims were to determine the clinical importance of norovirus and identify risk factors for long-term symptoms. The duration of norovirus symptoms was defined as the number of days of diarrhea and/or vomiting with the concomitant detection of norovirus. Gastrointestinal symptoms lasting > 30 days (with continued detectable norovirus) were defined as chronic symptoms of norovirus. Other possible causes of gastrointestinal symptoms such as mucositis, GVHD or other infections, were also registered. In paper III, we analyzed if either secretor-status or norovirus genotype influenced the clinical course of norovirus infection following HSCT. In total 89 HSCT patients diagnosed with norovirus infection 2006-2016 were included in paper III, of whom 63 also had been included in paper II. Clinical data were retrieved from medical records.

4.2.1 Microbiological analyses

Detection of norovirus RNA in fecal and vomit samples (paper II and III) was performed with a few different methods, as described in paper III. In patients for whom fecal samples were available, sequencing and phylogenetic analysis of the norovirus strain was performed (paper III), as previously described [184-186]. In paper III, we also investigated the possibility of detecting norovirus RNA in stored blood samples (the first available sample after the diagnosis of the norovirus infection), for further genotyping, as previously has been reported [132,187]. We chose to first analyze samples from patients with known genotype (available fecal samples), as a pilot study, and expand to test all patients if we found positive results. For the detection of norovirus RNA in blood, a multiplex TaqMan real-time PCR was used, as previously reported, with slight modifications [184,188,189]. The phylogenetic analyses and detection of norovirus in blood were performed at the Department of Clinical and Experimental Medicine, Linköping University, Linköping. The details of these methods are presented in the supplementary material of paper III.

4.2.2 Secretor-status

This analysis was also conducted at the Department of Clinical and Experimental Medicine, Linköping University. To determine the secretor-status of the HSCT recipients, we used samples collected at the time of HSCT, either stored extracted DNA, or whole blood from which DNA was extracted, as presented by Bucardo et al[190]. The extracted DNA was

analyzed for the *FUT2* G428A (rs601338) nonsense single nucleotide polymorphism (SNP), using the TaqMan® SNP Genotyping Assay (Applied Biosystems, Carlsbad, CA, USA)[190].

4.2.3 Statistics

The logistic regression method was used for analysing factors associated with chronic symptoms (>30 days). Factors with a p-value ≤0.20 in the univariate analysis were introduced into the backward elimination (study II) or stepwise selection (study III) multivariate analysis. For study II, Statistica software (StatSoft, Tulsa, Oklahoma, USA), and for paper III, R software (R Core Team, Vienna, Austria), were used. Time independent variables were analyzed separately from time dependent variables.

In paper III, the Mann-Whitney test was used for assessing differences in symptom duration and viral load between norovirus GII.4 vs norovirus non-GII.4.

4.2.4 Results and discussion

The duration of symptoms in the 89 patients was in median 9 (1-681) days, and 18/89 (20%) patients had chronic symptoms of norovirus (>30 days). SCID diagnosis, as the indication for HSCT, was associated with chronic symptoms of norovirus in paper II (OR 30.3, CI 2.5-368, $p<0.05$), which was confirmed in paper III (OR 10.7, CI 1.8-62.1, $p=0.01$). The number of patients with SCID was 5/63 patients in paper II and 8/89 patients in paper III. Further, in paper III, we found that patients with chronic symptoms of norovirus had a significantly higher probability of concurrent gastrointestinal GVHD (OR 11.0, CI 2.4-50.4, $p<0.001$). However, it was not always possible to determine if the symptoms were due to norovirus, GVHD or both. Nor can we conclude if GVHD increases the risk for chronic norovirus symptoms, or vice versa.

Patients with SCID commonly have an impaired and delayed immune reconstitution after HSCT[191,192], supporting that prolonged symptoms of norovirus are linked to poor immune status of the patient. We found no connection between chronic norovirus symptoms and ALC (study II) or lymphopenia < 0.2 X 10^9/L (study III), but detailed immunological characteristics of the patients are lacking. In a case series of HSCT children with chronic norovirus infection, reconstitution of CD3+ T-cells was linked to clearance of the infection[136], and immunological control of several other viral infections is strongly associated

with the reconstitution of T-cells[58,59,193]. Studies focusing on the significance of different immunological factors for the control of norovirus in immunocompromised patients are needed.

GVHD impairs the immune status of the patient, both directly due to the GVHD and, in addition, due to its treatment. The association between gastrointestinal GVHD and chronic norovirus symptoms found in study III may further indicate the importance of the immune-status for the course of norovirus infection. Norovirus may, on the other hand, increase the risk for development of gastrointestinal GVHD. Montfrans et al found that shedding of gastrointestinal viruses prior to HSCT predicted development of intestinal GVHD[194]. Norovirus has also been linked to chronic GVHD, although as in our study, the cause and effect relationship was unclear[137].

In paper II and III, 47/89 (53%) patients had at least one other gastrointestinal condition (mucositis, gastrointestinal GVHD or other gastrointestinal infection) concurrently with the norovirus infection (for a part of or during the whole course of the infection), which may have contributed to the symptoms. As in our study of adenovirus, we found it difficult to determine if the gastrointestinal symptoms were primarily due to norovirus infection, or to gastrointestinal GVHD, or if both conditions were causing the symptoms.

4.2.4.1 Transmission and indirect consequences

We observed an accumulation of six patients with onset of symptoms > 48 hours after hospital admission (defined as nosocomial infections), occurring within a period of three weeks, which may have constituted an outbreak. Among the first 63 patients (paper II), we registered indirect consequences of the norovirus infection, and found that HSCT was postponed in one patient, and four patients were not accepted for endoscopy (for suspected GVHD) due to the norovirus infection. One of these patients was later terminally ill, because of relapse, and was not accepted for palliative homecare due to the infection.

4.2.4.2 Secretor-status

7/89 (8%) of the patients were secretor-negative, which is a significantly smaller proportion compared to the general population (p=0.004), indicating that secretor-negative genotype protects against norovirus infection even when the immune system is severely compromised.

We found no association between secretor-status and chronic symptoms of norovirus (>30 days). However, due to the small number of secretor-negative patients, this result should be interpreted cautiously.

4.2.4.3 Norovirus genotype

Previous data suggest that norovirus GII.4 is associated with a more severe clinical picture compared to other genotypes in immunocompetent children[113]. The importance of norovirus genotype in relation to the clinical course has not been reported of in immunocompromised patients. Genotyping of norovirus was possible in 18 patients, demonstrating GII.4 in 12 of them. We compared the patients with norovirus GII.4 with those infected with other genotypes (in total ten patients, including four with GI, not genotyped), termed non GII.4. We found a possible difference in symptom duration between norovirus GII.4 and non GII.4. The symptoms lasted in median 36 (3-681) days in patients with norovirus GII.4, compared to 15 (1-94) days in patients with norovirus non GII.4 (p=0.1). All the patients with norovirus GII.4 were secretor-positive.

These results may indicate that norovirus genotype influences the clinical course also if the patient is immunocompromised. However, as the number of patients with identified norovirus genotype was small, also these results should be interpreted cautiously. Stored fecal samples were lacking in most patients, and thereby there was no opportunity for norovirus genotyping. We investigated if norovirus could be detected in blood, primarily for the possibility of obtaining the norovirus genotype in additional patients, as have been shown in a few other studies[132,187]. Blood samples from the subgroup of 18 patients with a known genotype, were all negative for norovirus RNA, and we decided not to proceed with the remaining patients.

4.2.5 Conclusions

Norovirus is an important pathogen in the HSCT setting, causing chronic symptoms in approximately 20% of the patients. Our results indicate that secretor-negative genotype protects against norovirus infection, even in immunocompromised patients. Susceptibility to norovirus may thereby depend on genetic rather than immunological factors. When infected however, HSCT patients with SCID are at risk for chronic symptoms of norovirus, which may be due to the slow immune recovery in many of these patients. Gastrointestinal GVHD was also linked to chronic norovirus symptoms, but it was not possible in all patients to determine if either, or both, conditions were causing the symptoms.

4.3 PAPER IV

HEV can cause chronic hepatitis and liver cirrhosis in immunocompromised patients. The aim of study IV was to determine the prevalence and the clinical importance of HEV infection in a Swedish cohort of HSCT recipients. Our hypothesis was that HEV infection could be responsible for abnormal liver function tests in a subset of HSCT patients and thereby possibly be misinterpreted as chronic GVHD of the liver.

We studied 236 patients who had undergone HSCT during the period 2008–2015. These were patients who had been included in a previous prospective study (with blood samples drawn at several time points after HSCT), and for whom blood samples collected at six months after HSCT were available. We chose the timepoint six months for the screening samples based on several considerations. First, we had readily access to a collection of samples from this point in time. Secondly, available data suggested that patients with HEV infection early after HSCT had a high risk of long-term HEV viremia, and HEV detection at six months could therefore be likely, even if the infection had been contracted earlier[39]. Third, this was a time when chronic GVHD was common.

The blood samples were analyzed for HEV RNA, anti-HEV IgG, and IgM antibodies. In patients who were positive for HEV RNA and/or had anti-HEV antibodies at six months, we analyzed HEV RNA and HEV serology in samples collected from the patients, and their donors, at the time of HSCT. In the HEV RNA positive patients, a series of samples were analyzed to determine the duration of viremia. If samples were not available (from the sample collection of the previous prospective study), the biobank at the laboratories of Clinical Microbiology and Immunology were queried. Serum, plasma or whole blood was used for HEV RNA detection, and serum or plasma for serology. The HEV genotype was determined by phylogenetic analysis. Cases with verified HEV infection (positive HEV RNA) were compared to matched controls. Clinical data was retrieved from the medical records, including the level of alanine amino transferase (ALT) and bilirubin at three and six months, the occurrence of active GVHD at three and six months, and the level of systemic corticosteroid treatment at 6 months after HSCT.

4.3.1 Microbiological analyses

All microbiological analyses were performed at the Department of Clinical Microbiology, Sahlgrenska University Hospital, Gothenburg. Anti-HEV IgG and IgM were analyzed using the HEV IgG/IgM test (Diapro, Milan, Italy) in line with the manufacturer's instructions, but

with modification of the cutoff levels, according to Norder et al[144]. For detection of HEV RNA, reverse transcription real-time PCR was conducted, as outlined by Roth et al[195]. Quantification of HEV RNA was performed using digital droplet PCR (ddPCR). The details of this method are presented in the supplementary material of paper IV. Briefly, the isolated RNA was subjected to reverse transcription to generate cDNA. A ddPCR reaction was set up using cDNA. An emulsion PCR reaction was performed, and the resulting droplets were counted. This method for quantification of HEV RNA was chosen since it required a small amount of blood, and the volume we could obtain from the initial samples collected at 6 months (50µl) was too small for conventional qPCR. Sequencing and phylogenetic analysis of the HEV strains were performed as previously described by Roth et al.[195]. For patients with chronic infection (> 6 months), the HEV strains in samples drawn at two different time points were sequenced.

4.3.2 Statistics

For patients with HEV infection (positive HEV RNA), two controls were randomly selected, matched for age, conditioning regimen, type of stem cell donor, and diagnosis (controls match 1). Two additional controls, for the same patients, were selected by matching for age, sex, and transplantation year (controls match 2), to evaluate if possible associations were linked to factors in match 1 (or match 2), or to the HEV infection. The controls were picked from the population who were negative for both HEV RNA and anti-HEV antibodies. Odds ratios (OR) for abnormal liver function tests and GVHD were computed for verified cases versus controls match 1 and match 2.

4.3.3 Results and discussion

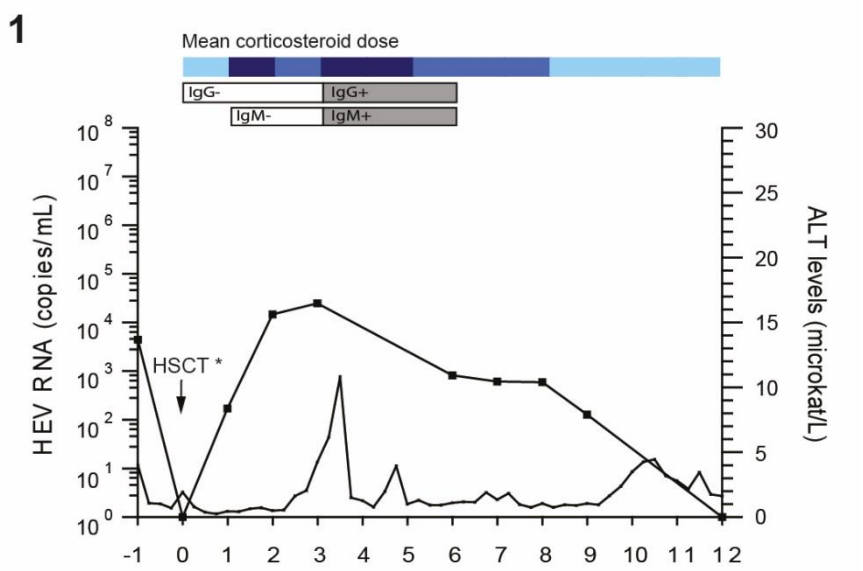
HEV RNA was detected in 8/236 (3.4%) patients 6 months after HSCT, which is in line with a number of previous reports[39,160,161]. Three of the HEV RNA-positive patients (patient 1, 2 and 3) had persistent viremia (repeatedly positive HEV RNA) and are presented in Figure 3. Two of these patients had chronic HEV infection (>6 months) and were infected already at the time of HSCT. In the remaining five patients, a low level of HEV RNA was detected at only one occasion (six months after HSCT). In two of these patients, the duration of infection could not be determined (due to death shortly after HEV RNA detection, and lack of samples, respectively), but in the other three patients, a short duration of viremia was demonstrated. In contrast, others have reported of a higher proportion of long-term viremia in HSCT patients with HEV infection[39,161]. In four patients with low-level HEV RNA, the samples were

positive in both RT-PCR and ddPCR, and in one of these strains sequencing could be performed.

4.3.3.1 The clinical picture

One patient developed lethal acute liver and multiple organ failure 8 months after HSCT, where HEV infection may have contributed to the severe course (patient 2, Figure 3). Another patient died, shortly after HEV was detected, but the death was not related to HEV. The remaining six patients with HEV RNA had cleared the infection at 7–24 (median 8.5) months after HSCT. HEV infection was associated with elevated alanine aminotransferase (ALT) > 3 upper limit of normal (ULN) at 6 months after HSCT (OR 15, 1.3–174, $p = 0.03$). In HSCT patients with liver abnormalities, microbiological testing for hepatic infections including HEV should be performed, and in unclear cases liver biopsy should be considered. Notably, we found normal ALT during several months, concurrent with high levels of HEV viremia, in the patient who later developed acute liver failure (patient 2, Figure 3), indicating that the diagnosis of HEV infection can be challenging. It is however difficult to draw conclusions from current data, whether screening (or monitoring) of HSCT patients for HEV infection should be recommended.

Active GVHD of the liver was present in 3/8 patients at the time of HEV infection, but there was no significant association between liver GVHD and HEV-infection.



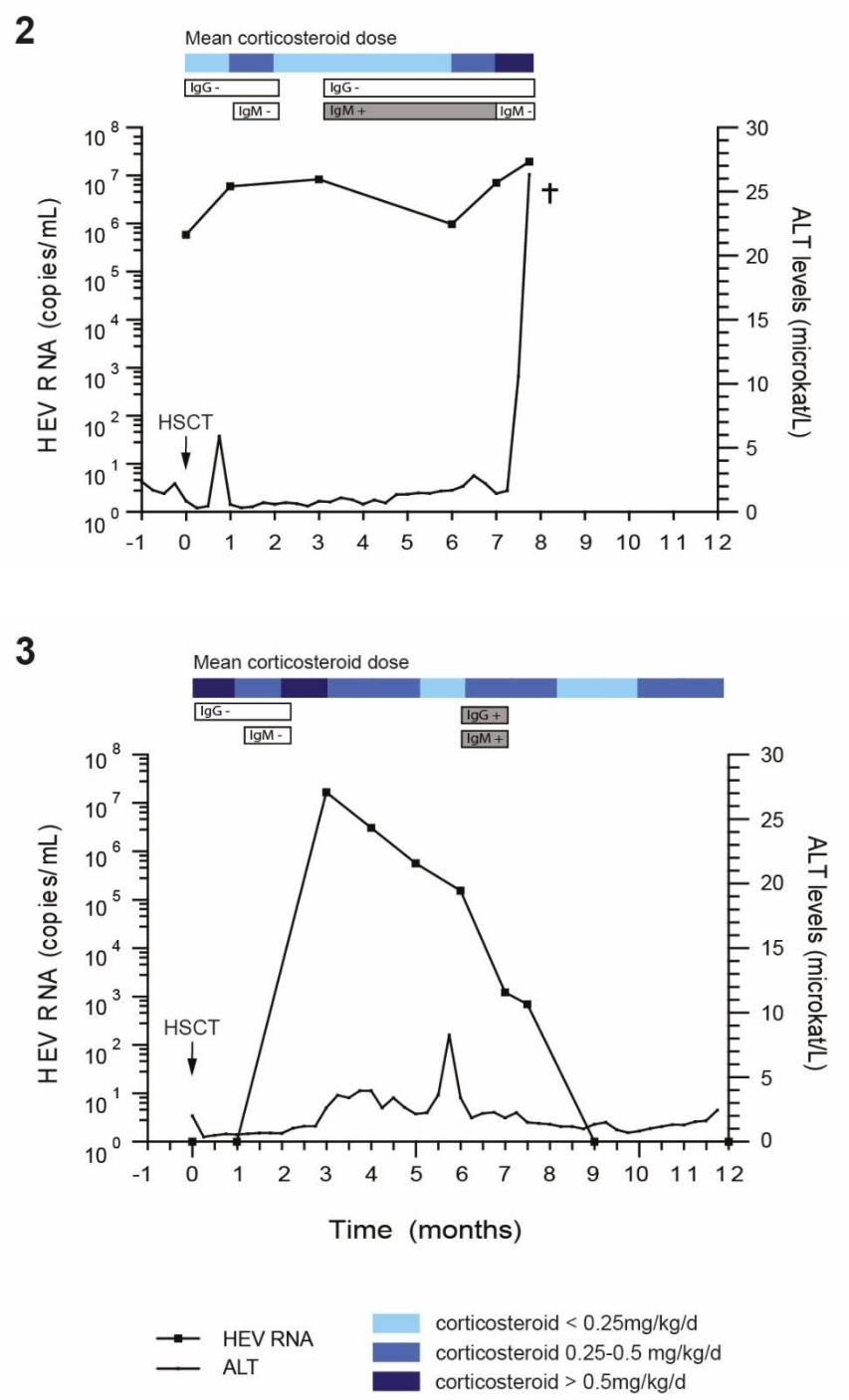


Figure 3. The course of HEV infection in the three patients with persistent viremia (patients 1, 2 and 3). The levels of HEV RNA, measured by digital droplet PCR (ddPCR), are displayed. ALT, alanine aminotransferase; HEV, hepatitis E virus; HSCT, hematopoietic stem cell transplantation. The ALT upper limit of normal is 0.76 for females (patient 2) and 1.20 for males (patient 1 and 3). Patient 1 was infected with HEV one month before HSCT. At the time of transplantation, HEV RNA was detected with real time PCR (RT-PCR), but the levels could not be assessed with ddPCR (indicated by *). Acute GVHD of the gut evolved, but HEV cleared during tapering of the corticosteroids. Patient 2 was infected with HEV at the time of HSCT. Mild chronic GVHD of the gut was diagnosed at six months, and infectious endocarditis at seven months after HSCT. Acute lethal liver and multiorgan failure developed eight months after HSCT. Patient 3 became positive for HEV RNA 1-3 months after HSCT. He had at that time acute GVHD of the gut, followed by mild chronic GVHD of the liver, but cleared HEV during reduction of the corticosteroids.

4.3.3.2 The source of HEV infection

Sequencing of the HEV strains was possible in four patients, revealing HEV3f in three patients and HEV3c/i in one patient. HEV3f and HEV3c/i have both previously been detected in Swedish blood donors[196], and HEV3f has been identified in Swedish pigs and wild boar[197], whereas HEV3c/i has been found in pigs and wild boar in other European countries[198].

Both blood products and donations of stem cells have been reported to transmit HEV[146,147]. In our study, transfusion of erythrocytes and/or thrombocytes had been given to 4/8 patients within three months prior to the first detection of HEV RNA. No blood product was available for analysis. Thus, infected blood components may have been a source of HEV infection. All donors of the patients with HEV infection were negative for HEV RNA. Although several European countries have implemented HEV screening of blood components and donors of stem cells, this has not been introduced in Sweden[150]. The reported prevalence of HEV RNA in Swedish blood donors has been 0.01%, which is 10-fold lower than in some other countries, where the prevalence has been up to 0.13%[149,150]. However, the prevalence may change over time, and a continuous surveillance of the local prevalence is important[199].

Apart from possible transmission of HEV from blood products, the patients may contract HEV by contaminated food products[139]. At our center, the patients have diet recommendations, leaving out poorly cooked meat and shell fish, but contaminated food may still have been a possible source of HEV infection.

4.3.3.3 The relevance of anti-HEV antibodies for diagnosis of HEV infection following HSCT

The prevalence of anti-HEV antibodies at 6 months after HSCT was 4.7% in our cohort, which is low compared to the HEV seroprevalence of 16% found in Swedish blood donors[144]. Interpretation of anti-HEV serology should be done cautiously in patients having undergone HSCT, especially early after the transplantation, or when the immune reconstitution is delayed. Antibodies may be passively transferred to the HSCT patient, through blood components or intravenous immunoglobulin. In addition, antibodies and antibody-producing cells may be transferred from the stem cell donor[200]. Conversely, the antibody response to HEV may be weak or absent in these patients, due to their compromised immune status.

During the first 3-12 months after HSCT, or longer, the antibody production is deficient, as the recipient's plasma cells are replaced with those of the donor, which was reported of for several other viruses[200]. Koenecke et al reported a seroprevalence of 6% in patients with unexplained ALT after HSCT, which is similar to our result[158]. In our study one patient with chronic HEV infection only developed transient anti-HEV IgM, but no anti-HEV IgG antibodies (patient 2, Figure 3), and among the patients with short term viremia, none developed anti-HEV antibodies. These results underline that diagnosis of HEV infection in HSCT patients should be done with HEV RNA detection[39].

4.3.4 Conclusions

HEV infection is an important differential diagnosis in patients with elevated liver enzymes after HSCT. Since the clinical course can be severe, and liver function tests may be only intermittently elevated, a high awareness of HEV is required.

5 SUMMARY

- An outbreak of HAdV among HSCT patients is a serious event that can be difficult to control. High strain on the staff during the early part of the outbreak, possible contamination of the facilities of the ward, and unidentified cases with sparse symptoms, may have contributed to the prolonged outbreak of HAdV A31 at our center.
- Norovirus causes chronic symptoms (> 30 days) in 20% of HSCT patients.
- SCID as indication for HSCT, and gastrointestinal GVHD, are associated with chronic symptoms in patients having norovirus infection.
- Secretor-negative genotype may protect HSCT recipients against norovirus infection.
- Difficulties to discriminate symptoms of HAdV, or norovirus, from symptoms of gastrointestinal GVHD, are a significant clinical challenge.
- HEV infection is a potentially severe differential diagnosis in patients with elevated ALT six months after HSCT.

6 CONCLUDING REMARKS AND FUTURE PERSPECTIVES

6.1 GASTROINTESTINAL INFECTION VERSUS GVHD

Gastrointestinal symptoms and liver abnormalities are common early after HSCT. Diarrhea and vomiting are usually caused by toxicity of chemotherapy or other drugs, GVHD, tube feeding, or gastrointestinal infections. Similarly, elevated liver function tests can be caused by drug toxicity, GVHD, or infections. In addition, liver affection can be a sign of veno-occlusive disease, parenteral nutrition, or hemochromatosis. In the studies of HAdV and norovirus in the present thesis, we observed difficulties to determine if the patients' symptoms were primarily due to the gastrointestinal infection, or due to gastrointestinal GVHD, when the histopathological diagnosis was inconclusive, or biopsy was not obtained. An additional complexity is that both GVHD and viral infection frequently occur concurrently, as is common for CMV gastrointestinal disease[201,202].

There may be several situations complicating the interpretation of gastrointestinal disease and concurrently detected norovirus RNA or HAdV DNA:

- Previous shedding of norovirus RNA/HAdV DNA can be identified at the emergence of gastrointestinal symptoms from other causes, such as drug toxicity, GVHD or CMV colitis, and the symptoms may then be misinterpreted as norovirus/HAdV gastroenteritis.

- Patients with shedding of norovirus/HAdV after resolution of symptoms may suffer from recurrence of gastrointestinal symptoms if they receive treatment that compromises the immune system. We observed a patient with shedding of norovirus, who experienced severe recurrence of gastrointestinal symptoms shortly after HSCT (and persisting after engraftment).

- Norovirus/HAdV infection can be misinterpreted as GVHD, with immunosuppressive treatment aggravating the infection, which may in turn induce gastrointestinal GVHD, due to tissue damage[194]. In the case of HAdV, increased immunosuppression puts the patient at risk for invasive infection.

- Gastrointestinal infection and GVHD may both cause the disease, where increasing as well as decreasing the immunosuppression may aggravate the symptoms, but for different reasons.

- A new gastrointestinal infection with another pathogen might develop so that symptoms are not related to the previously documented one.

For patients with HAdV, this differential diagnostic problem is especially serious, since HAdV can progress to lethal disseminated disease, and invasive infection (viremia) should evoke reduction or withdrawal of the immunosuppressive treatment. GVHD, on the other hand, also carries the potential to progress to lethal disease and requires increased immunosuppression. This potential problem has to our knowledge not been much reported of previously for HAdV.

Norovirus is not associated with the threat of dissemination and lethal disease in HSCT patients, as for HAdV, although there are some reports of deaths where norovirus may have contributed[120]. However, the consequence of norovirus infection can still be serious. A child in our study had chronic severe diarrhea, with partial response to heavy immunosuppression, but the symptoms never resolved, and the patient eventually died of opportunistic infections almost two years after the diagnosis of norovirus infection. It is impossible to know to what extent norovirus may have caused an exacerbation of GVHD, or if norovirus was, to some degree, misinterpreted as GVHD. For some additional patients in our study, the problems separating norovirus symptoms from GVHD were less severe, but complicated the treatment of the patients significantly, as previously described by others[120,135].

Endoscopies with histopathological diagnosis is golden standard for the diagnosis of gastrointestinal GVHD. Histopathology is also required to confirm proven HAdV disease[86]. However, the histological picture is not always conclusive with respect to GVHD, and the histological diagnosis of HAdV may be difficult.

Little is known of the histopathological picture during norovirus infection, and biopsy is not routine for diagnosis of norovirus gastroenteritis. Several authors have reported of villus blunting and epithelial infiltration of CD8+ T-lymphocytes in duodenal biopsies from immunocompromised patients with norovirus infection[132,134,203], including three HSCT patients[120]. Although the picture is not specific for norovirus infection[133], duodenal biopsy may be an important complement to colon biopsy to clarify whether GVHD or norovirus infection seems to be the dominating condition, since norovirus is a pathogen of the small intestine and GVHD might also develop in the upper gastrointestinal tract. In a recent report norovirus antigen and RNA were detected in small bowel biopsies from an immunocompromised patient, using immune histochemistry and in-situ hybridization, respectively[204]. Prospective studies investigating duodenal biopsies in HSCT patients, with and without norovirus infection, and evaluating norovirus antigen and/or RNA detection in

tissue, could provide important knowledge and a diagnostic tool in the treatment these patients.

As for HAdV and norovirus, HEV infection may be mistaken for GVHD, which can lead to chronic HEV infection and rapid development of liver cirrhosis, due to the immunosuppressive treatment[39,161]. HEV infection may also coincide with GVHD of the liver, as was shown in our study, and possibly can activate or maintain GVHD of the liver[161]. There are, however, many different causes of elevated liver function tests, so careful assessment of possible differential diagnoses is important. HEV infection can be treated with ribavirin, which is generally well tolerated, and seems to be effective, although data is still sparse in the HSCT setting[161,163,164]. Thus, the dominating issue when it comes to HEV in HSCT patients, is to identify the infection.

6.2 INDIRECT EFFECTS OF TRANSMISSIBLE VIRAL GASTROINTESTINAL INFECTIONS

Norovirus and HAdV are easily transmitted and outbreaks especially of norovirus can occur in health care facilities. These may require closing of wards and postponed therapy for both infected and non-infected patients. We have investigated an outbreak of HAdV, and in the studies of norovirus, we recognized a possible outbreak involving six patients. Indirect clinical consequences of transmissible gastrointestinal viral infections are poorly understood. In the studies of this thesis we observed that indirect effects of HAdV and norovirus may be severe. During the outbreak of HAdV, transplantation was postponed in one patient, since he contracted HAdV after arrival at the ward. After this event, the parents did not want to proceed with the HSCT. Postponing transplantation can constitute a high risk for individual patients, such as relapse or progress of malignancy. Conversely, HSCT during HAdV enteritis carries a high risk for early invasive HAdV infection[24]. We also found potentially serious indirect consequences for some patients with norovirus infection, such as postponed HSCT and problems to get endoscopy performed. This last observation underlines that it can be a challenge preventing transmission of norovirus without compromising the medical care of the infected patients.

6.3 INVESTIGATING INFECTIVITY

Immunocompromised patients can shed norovirus RNA for a prolonged time, but it is not known if the detected RNA represents infectious viral particles[123]. The recent development of a cell culture system, so called enteroids, containing multiple intestinal epithelial cell types, has provided the possibility of culturing norovirus[205]. Chan et al inoculated norovirus RNA positive fecal samples from immunocompetent individuals in enteroid cultures, showing that norovirus was cultured only when the viral load was high (Ct-value $\leq$ 30), suggesting the presence of infectious norovirus[206]. I am involved in a planned study of norovirus infectivity in immunocompromised patients, in collaboration with professor Lennart Svensson at the University of Linköping and Karolinska Institutet. We will prospectively include solid organ transplant and HSCT recipients with norovirus infection. Fecal samples collected serially will be inoculated in enteroids. The number of infected cells will be compared to the Ct-value obtained in the PCR. The study can provide information of the infectivity of norovirus, possibly related to the Ct-value, during the course of the infection in immunocompromised patients.

6.4 UNDERSTANDING NOROVIRUS IN THE HSCT POPULATION

Norovirus causes chronic symptoms in a subset of HSCT patients. In our study, SCID and concurrent gastrointestinal GVHD were associated with chronic norovirus symptoms, possibly due to impaired immune reconstitution in patients with these diagnoses. Prospective studies, investigating immunological components and norovirus genotype, in relation to the clinical symptoms, can confer important knowledge of factors linked to a chronic course of norovirus infection. To better evaluate the importance of secretor-status in this setting, prospective studies of secretor-status in HSCT patients, with and without norovirus infection, are warranted. The HSCT population can further be an interesting model for studies of the immunological control of norovirus.

6.5 TREATMENT POSSIBILITIES

The treatment options for HAdV are poor, and for norovirus treatment is lacking. Existing data on brincidofovir for HAdV seem promising, but brincidofovir is currently not available. Prospective controlled studies of new potentially active antiviral drugs against HAdV should be a high priority. Adoptive cell therapy for HAdV may prove to be an effective alternative, or can be a complement, and the development of this therapy is equally important.

For norovirus, the recent development of a cell culture system implies the opportunity to investigate antivirals. Several substances have been proposed as possible candidates, including ribavirin and nitazoxanide[207]. Therapeutic possibilities for norovirus could reduce the morbidity for HSCT patients, and other immunocompromised patients, with chronic norovirus infection.

6.6 SCREENING STRATEGIES

We found that elevated ALT six months after HSCT was associated with HEV infection. However, one of the patients in our study had normal liver function tests for a prolonged time, despite high level HEV viremia, illustrating that it may be difficult to identify some patients with HEV infection, solely on clinical suspicion. Versluis et al performed a cross-sectional study of the point prevalence of HEV infection after HSCT. In addition, HEV analysis was conducted at time-points of elevated ALT ($\geq$ 2.5 ULN). HEV RNA was detected in 8/328 (2.4%) patients, of which seven were identified in the cross-sectional analysis, and only one by screening the episodes of elevated ALT[39]. This raises the question of whether HEV screening or monitoring should be implemented. HEV infection is infrequent in the HSCT setting, and most patients in our study cleared the infection spontaneously, but the course of the infection may also be severe. It is therefore important to clarify risk factors for chronic infection, and moreover, to assess the prevalence of HEV in HSCT populations over time. Such insights can contribute to strategies for identifying patients with HEV: screening all patients depending on the local prevalence, screening identified high risk patients, monitoring all patients during a period of risk, or monitoring identified high risk patients? To gain this knowledge, larger prospective studies are warranted. Studies in areas with a high HEV prevalence may have a better chance of bringing clarity to these questions.

7 ACKNOWLEDGEMENTS

Elda, du introducerade mig i det otroligt spännande arbetet som infektionskonsult för hematologen, CAST, transplantation och barn, och var under många år ett ovärderligt stöd i kliniska frågor, alltid intresserad och klok! Det var också du som öppnade dörren till min doktorandutbildning, och axlade rollen som huvudhandledare. Jag är så tacksam för det! Du är smart, intresserad och empatisk. Du har haft tusen andra saker att göra, men ändå stöttat mig hela vägen, och på köpet har jag fått en god vän.

Per, det har varit ett stort nöje att få ha dig som handledare. Du har hela tiden varit väldigt schysst och stöttande, och en trygg garant för att jag fortskridit åt rätt håll. Utöver dina stora vetenskapliga och kliniska kunskaper har du en osviklig förmåga att alltid se en självklar väg framåt, även när det verkar omöjligt och trassligt. Jag har lärt mig mycket av dig!

Jonas, du bidrog till att det var kul att börja med detta doktorandarbete, och du har fortsatt vara positiv och stöttande, även från andra sidan atlanten.

Till alla **kollegor/vänner på infektion**, Karolinska, SöS, St Göran och Danderyd, för gott samarbete, kamratskap, och oändligt många trevliga luncher, prat och diskussioner genom åren.

Ola för alla år vi kamperat ihop, det är kul att jobba med dig. **Anna** för din underbara humor och vänskap. **Karolin** för stöd och vänskap. **Malin** för stöd i alla år vi jobbade ihop.

Karin Lindahl, du väckte mitt intresse för att göra en doktorandutbildning. **Börje** och **Ola Weiland** för att ni varit intresserade och uppmuntrande hela vägen. **Maria Norrby, Veronica, Anna-Mia, Lina** och **Katja**, för er uppmuntran och goda råd inför disputationen.

Mina chefer **Lena** och **Hedvig**, och tidigare **Calle**, samt **Nettan, Lennart** och **Bertil**, för att ni tänjt på schemat till det omöjliga så att jag har kunnat slutföra mina doktorandprojekt.

Ilona för all hjälp med prover, och **Emmi** för goda råd och virologiska synpunkter på denna bok

Barnläkarna på Huddinge och **kollegorna på transplantation** och **hematologen** för ett otroligt kul och lärorikt samarbete.

Kollegor och personal på CAST, för likaledes roligt och lärorikt samarbete. Tack särskilt till **Ksenia** för hjälp med hepatit E och norovirus-studien och **Johan** för goda råd under arbetets gång. Tack också **Lotta** och **Eva** för er alltid så vänliga hjälp.

Stephan Mielke för värdefulla synpunkter på hepatit E manuskriptet

Davide for excellent help with statistics, and not least, explaining to me patiently.

Thomas Poiret, for, also patiently, helping me with samples for the hepatitis E study

Annika Allard för lärorikt samarbete i adenovirus-studien, och **Annika Tiveljung**, **Mats Remberger** och **Mikael Sundin** för samarbetet med första norovirus studien

Rickard Nordén, Ebba Samuelsson, Helene Norder och **Johan Westin** för vårt samarbete, och allt ni lärt mig i samband med hepatit E studien

Lennart Svensson och **Johan Norgren**, för ett roligt och bra samarbete med det senare norovirus-projektet

Olle, Mona-Lisa, Mikael och **Elda** för vår fina gemenskap med kursen i pediatriska infektioner, och alla roliga kurs-utvärderingar hos Olle, till fantastiska måltider!

Mina kollegor i **”Opportunisterna”** för våra väldigt trevliga möten (= terminens höjdpunkt) sedan minst 10 år tillbaka. Jag har lärt mig massor av er alla!

Mina vänner, det går ofta lång tid mellan varven, men så glad att ni finns.

Mina kära bröder **Torbjörn, Gunnar och Janne**, och mina svägerskor **Christina, Sara** och **Johanna**. För att ni är en så bra familj.

Min kära **mamma**, för all kärlek och värme, och för din stora nyfikenhet och intresse för andra människor. Det är alltid roligt att vara med dig.

Pappa, för din positiva livssyn, kärlek, och det kloka stöd du alltid gav. Jag saknar dig.

Sist, men allra mest, min älskade familj, **Patrik, Maj, David, Felix** och **Albin**. För allt roligt, och för att ni är precis dom ni är.

8 REFERENCES

1. Weiden PL, Flournoy N, Thomas ED, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *The New England journal of medicine.* 1979;300(19):1068-1073.
2. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. *The New England journal of medicine.* 1981;304(25):1529-1533.
3. Shouval R, Fein JA, Labopin M, et al. Outcomes of allogeneic haematopoietic stem cell transplantation from HLA-matched and alternative donors: a European Society for Blood and Marrow Transplantation registry retrospective analysis. *Lancet Haematol.* 2019;6(11):e573-e584.
4. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2005;11(12):945-956.
5. Lee SJ. Classification systems for chronic graft-versus-host disease. *Blood.* 2017;129(1):30-37.
6. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation.* 1974;18(4):295-304.
7. Cahn JY, Klein JP, Lee SJ, et al. Prospective evaluation of 2 acute graft-versus-host (GVHD) grading systems: a joint Société Française de Greffe de Moëlle et Thérapie Cellulaire (SFGM-TC), Dana Farber Cancer Institute (DFCI), and International Bone Marrow Transplant Registry (IBMTR) prospective study. *Blood.* 2005;106(4):1495-1500.
8. Einsele H, Ehninger G, Hebart H, et al. Incidence of local CMV infection and acute intestinal GVHD in marrow transplant recipients with severe diarrhoea. *Bone marrow transplantation.* 1994;14(6):955-963.
9. Zeiser R, von Bubnoff N, Butler J, et al. Ruxolitinib for Glucocorticoid-Refractory Acute Graft-versus-Host Disease. *The New England journal of medicine.* 2020;382(19):1800-1810.
10. Penack O, Marchetti M, Ruutu T, et al. Prophylaxis and management of graft versus host disease after stem-cell transplantation for haematological malignancies: updated consensus recommendations of the European Society for Blood and Marrow Transplantation. *Lancet Haematol.* 2020;7(2):e157-e167.
11. Mensen A, Jöhrens K, Anagnostopoulos I, et al. Bone marrow T-cell infiltration during acute GVHD is associated with delayed B-cell recovery and function after HSCT. *Blood.* 2014;124(6):963-972.
12. Shono Y, Shiratori S, Kosugi-Kanaya M, et al. Bone marrow graft-versus-host disease: evaluation of its clinical impact on disrupted hematopoiesis after allogeneic hematopoietic stem cell transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2014;20(4):495-500.
13. Clave E, Rocha V, Talvensaari K, et al. Prognostic value of pretransplantation host thymic function in HLA-identical sibling hematopoietic stem cell transplantation. *Blood.* 2005;105(6):2608-2613.
14. Stern L, McGuire H, Avdic S, et al. Mass Cytometry for the Assessment of Immune Reconstitution After Hematopoietic Stem Cell Transplantation. *Frontiers in immunology.* 2018;9:1672.
15. Mikulska M, Del Bono V, Raiola AM, et al. Blood stream infections in allogeneic hematopoietic stem cell transplant recipients: reemergence of Gram-negative rods and increasing antibiotic resistance. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2009;15(1):47-53.

16. Blennow O, Ljungman P, Sparrelid E, Mattsson J, Remberger M. Incidence, risk factors, and outcome of bloodstream infections during the pre-engraftment phase in 521 allogeneic hematopoietic stem cell transplantations. *Transplant infectious disease : an official journal of the Transplantation Society.* 2014;16(1):106-114.
17. Bodey GP, Jadeja L, Elting L. Pseudomonas bacteremia. Retrospective analysis of 410 episodes. *Arch Intern Med.* 1985;145(9):1621-1629.
18. Averbuch D, Orasch C, Cordonnier C, et al. European guidelines for empirical antibacterial therapy for febrile neutropenic patients in the era of growing resistance: summary of the 2011 4th European Conference on Infections in Leukemia. *Haematologica.* 2013;98(12):1826-1835.
19. Kimura S, Akahoshi Y, Nakano H, et al. Antibiotic prophylaxis in hematopoietic stem cell transplantation. A meta-analysis of randomized controlled trials. *J Infect.* 2014;69(1):13-25.
20. Girmenia C, Raiola AM, Piciocchi A, et al. Incidence and outcome of invasive fungal diseases after allogeneic stem cell transplantation: a prospective study of the Gruppo Italiano Trapianto Midollo Osseo (GITMO). *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2014;20(6):872-880.
21. Maertens JA, Girmenia C, Brüggemann RJ, et al. European guidelines for primary antifungal prophylaxis in adult haematology patients: summary of the updated recommendations from the European Conference on Infections in Leukaemia. *The Journal of antimicrobial chemotherapy.* 2018;73(12):3221-3230.
22. Ljungman P, Perez-Bercoff L, Jonsson J, et al. Risk factors for the development of cytomegalovirus disease after allogeneic stem cell transplantation. *Haematologica.* 2006;91(1):78-83.
23. Uhlin M, Wikell H, Sundin M, et al. Risk factors for Epstein-Barr virus-related post-transplant lymphoproliferative disease after allogeneic hematopoietic stem cell transplantation. *Haematologica.* 2014;99(2):346-352.
24. Kosulin K, Berkowitsch B, Matthes S, et al. Intestinal Adenovirus Shedding Before Allogeneic Stem Cell Transplantation Is a Risk Factor for Invasive Infection Post-transplant. *EBioMedicine.* 2018;28:114-119.
25. Piñana JL, Martino R, García-García I, et al. Risk factors and outcome of COVID-19 in patients with hematological malignancies. *Exp Hematol Oncol.* 2020;9:21.
26. Hirsch HH, Martino R, Ward KN, Boeckh M, Einsele H, Ljungman P. Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis and treatment of human respiratory syncytial virus, parainfluenza virus, metapneumovirus, rhinovirus, and coronavirus. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2013;56(2):258-266.
27. Ljungman P, de la Camara R, Robin C, et al. Guidelines for the management of cytomegalovirus infection in patients with haematological malignancies and after stem cell transplantation from the 2017 European Conference on Infections in Leukaemia (ECIL 7). *The Lancet Infectious diseases.* 2019;19(8):e260-e272.
28. Marty FM, Ljungman P, Chemaly RF, et al. Letermovir Prophylaxis for Cytomegalovirus in Hematopoietic-Cell Transplantation. *The New England journal of medicine.* 2017;377(25):2433-2444.
29. Styczynski J, Reusser P, Einsele H, et al. Management of HSV, VZV and EBV infections in patients with hematological malignancies and after SCT: guidelines from the Second European Conference on Infections in Leukemia. *Bone marrow transplantation.* 2009;43(10):757-770.
30. Hiwarkar P, Kosulin K, Cesaro S, et al. Management of adenovirus infection in patients after haematopoietic stem cell transplantation: State-of-the-art and real-life current approach: A position statement on behalf of the Infectious Diseases Working Party of the European Society of Blood and Marrow Transplantation. *Rev Med Virol.* 2018;28(3):e1980.

31. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2009;15(10):1143-1238.
32. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. *Blood.* 2002;100(13):4358-4366.
33. Blennow O, Remberger M, Törlén J, Szakos A, Ljungman P, Mattsson J. Risk Factors for Invasive Mold Infections and Implications for Choice of Prophylaxis after Allogeneic Stem Cell Transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2016;22(9):1684-1689.
34. Maschmeyer G, Helweg-Larsen J, Pagano L, Robin C, Cordonnier C, Schellongowski P. ECIL guidelines for treatment of Pneumocystis jirovecii pneumonia in non-HIV-infected haematology patients. *The Journal of antimicrobial chemotherapy.* 2016;71(9):2405-2413.
35. Ljungman P, Cordonnier C, Einsele H, et al. Vaccination of hematopoietic cell transplant recipients. *Bone marrow transplantation.* 2009;44(8):521-526.
36. Cordonnier C, Einarsdottir S, Cesaro S, et al. Vaccination of haemopoietic stem cell transplant recipients: guidelines of the 2017 European Conference on Infections in Leukaemia (ECIL 7). *The Lancet Infectious diseases.* 2019;19(6):e200-e212.
37. Green ML, Leisenring W, Xie H, et al. Cytomegalovirus viral load and mortality after haemopoietic stem cell transplantation in the era of pre-emptive therapy: a retrospective cohort study. *Lancet Haematol.* 2016;3(3):e119-127.
38. Mallet V, van Bömmel F, Doerig C, et al. Management of viral hepatitis in patients with haematological malignancy and in patients undergoing haemopoietic stem cell transplantation: recommendations of the 5th European Conference on Infections in Leukaemia (ECIL-5). *The Lancet Infectious diseases.* 2016;16(5):606-617.
39. Versluis J, Pas SD, Agteresch HJ, et al. Hepatitis E virus: an underestimated opportunistic pathogen in recipients of allogeneic hematopoietic stem cell transplantation. *Blood.* 2013;122(6):1079-1086.
40. Lion T. Adenovirus persistence, reactivation, and clinical management. *FEBS letters.* 2019;593(24):3571-3582.
41. Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. *Clinical microbiology reviews.* 2014;27(3):441-462.
42. Carr MJ, Kajon AE, Lu X, et al. Deaths associated with human adenovirus-14p1 infections, Europe, 2009-2010. *Emerging infectious diseases.* 2011;17(8):1402-1408.
43. Savón C, Acosta B, Valdés O, et al. A myocarditis outbreak with fatal cases associated with adenovirus subgenera C among children from Havana City in 2005. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* 2008;43(2):152-157.
44. Lynch JP, 3rd, Kajon AE. Adenovirus: Epidemiology, Global Spread of Novel Serotypes, and Advances in Treatment and Prevention. *Semin Respir Crit Care Med.* 2016;37(4):586-602.
45. Binder AM, Biggs HM, Haynes AK, et al. Human Adenovirus Surveillance - United States, 2003-2016. *MMWR Morb Mortal Wkly Rep.* 2017;66(39):1039-1042.
46. Przybylski M, Rynans S, Waszczuk-Gajda A, et al. Sequence typing of human adenoviruses isolated from Polish patients subjected to allogeneic hematopoietic stem cell transplantation - a single center experience. *Hematology.* 2018;23(9):633-638.
47. Mynarek M, Ganzenmueller T, Mueller-Heine A, et al. Patient, virus, and treatment-related risk factors in pediatric adenovirus infection after stem cell transplantation: results of a routine monitoring program. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2014;20(2):250-256.
48. Lugthart G, Oomen MA, Jol-van der Zijde CM, et al. The effect of cidofovir on adenovirus plasma DNA levels in stem cell transplantation recipients without T cell reconstitution. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2015;21(2):293-299.

49. Houldcroft CJ, Roy S, Morfopoulou S, et al. Use of Whole-Genome Sequencing of Adenovirus in Immunocompromised Pediatric Patients to Identify Nosocomial Transmission and Mixed-Genotype Infection. *The Journal of infectious diseases.* 2018;218(8):1261-1271.
50. Fox JP, Hall CE, Cooney MK. The Seattle Virus Watch. VII. Observations of adenovirus infections. *American journal of epidemiology.* 1977;105(4):362-386.
51. Allard A, Albinsson B, Wadell G. Detection of adenoviruses in stools from healthy persons and patients with diarrhea by two-step polymerase chain reaction. *Journal of medical virology.* 1992;37(2):149-157.
52. Garnett CT, Talekar G, Mahr JA, et al. Latent species C adenoviruses in human tonsil tissues. *Journal of virology.* 2009;83(6):2417-2428.
53. Garnett CT, Erdman D, Xu W, Gooding LR. Prevalence and quantitation of species C adenovirus DNA in human mucosal lymphocytes. *Journal of virology.* 2002;76(21):10608-10616.
54. Speck SH, Ganem D. Viral latency and its regulation: lessons from the gamma-herpesviruses. *Cell Host Microbe.* 2010;8(1):100-115.
55. Kosulin K, Geiger E, Vécsei A, et al. Persistence and reactivation of human adenoviruses in the gastrointestinal tract. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases.* 2016;22(4):381.e381-381.e388.
56. Kosulin K, Hoffmann F, Clauditz TS, Wilczak W, Dobner T. Presence of adenovirus species C in infiltrating lymphocytes of human sarcoma. *PloS one.* 2013;8(5):e63646.
57. Veltrop-Duits LA, van Vreeswijk T, Heemskerk B, et al. High titers of pre-existing adenovirus serotype-specific neutralizing antibodies in the host predict viral reactivation after allogeneic stem cell transplantation in children. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2011;52(12):1405-1413.
58. van Tol MJ, Kroes AC, Schinkel J, et al. Adenovirus infection in paediatric stem cell transplant recipients: increased risk in young children with a delayed immune recovery. *Bone marrow transplantation.* 2005;36(1):39-50.
59. Lion T, Kosulin K, Landlinger C, et al. Monitoring of adenovirus load in stool by real-time PCR permits early detection of impending invasive infection in patients after allogeneic stem cell transplantation. *Leukemia.* 2010;24(4):706-714.
60. Hum RM, Deambrosis D, Lum SH, et al. Molecular monitoring of adenovirus reactivation in faeces after haematopoietic stem-cell transplantation to predict systemic infection: a retrospective cohort study. *Lancet Haematol.* 2018;5(9):e422-e429.
61. Jeulin H, Salmon A, Bordigoni P, Venard V. Diagnostic value of quantitative PCR for adenovirus detection in stool samples as compared with antigen detection and cell culture in haematopoietic stem cell transplant recipients. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases.* 2011;17(11):1674-1680.
62. Hara J, Okamoto S, Minekawa Y, Yamazaki K, Kase T. Survival and disinfection of adenovirus type 19 and enterovirus 70 in ophthalmic practice. *Japanese journal of ophthalmology.* 1990;34(4):421-427.
63. Gordon YJ, Gordon RY, Romanowski E, Araullo-Cruz TP. Prolonged recovery of desiccated adenoviral serotypes 5, 8, and 19 from plastic and metal surfaces in vitro. *Ophthalmology.* 1993;100(12):1835-1839; discussion 1839-1840.
64. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC infectious diseases.* 2006;6:130.
65. Kampf G. Efficacy of ethanol against viruses in hand disinfection. *The Journal of hospital infection.* 2018;98(4):331-338.
66. McCormick L, Maheshwari G. Inactivation of adenovirus types 5 and 6 by Virkon S. *Antiviral research.* 2004;64(1):27-33.

67. Rutala WA, Peacock JE, Gergen MF, Sobsey MD, Weber DJ. Efficacy of hospital germicides against adenovirus 8, a common cause of epidemic keratoconjunctivitis in health care facilities. *Antimicrob Agents Chemother.* 2006;50(4):1419-1424.
68. Sanchez JL, Binn LN, Innis BL, et al. Epidemic of adenovirus-induced respiratory illness among US military recruits: epidemiologic and immunologic risk factors in healthy, young adults. *Journal of medical virology.* 2001;65(4):710-718.
69. Faden H, Wynn RJ, Campagna L, Ryan RM. Outbreak of adenovirus type 30 in a neonatal intensive care unit. *The Journal of pediatrics.* 2005;146(4):523-527.
70. Montessori V, Scharf S, Holland S, Werker DH, Roberts FJ, Bryce E. Epidemic keratoconjunctivitis outbreak at a tertiary referral eye care clinic. *American journal of infection control.* 1998;26(4):399-405.
71. James L, Vernon MO, Jones RC, et al. Outbreak of human adenovirus type 3 infection in a pediatric long-term care facility--Illinois, 2005. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2007;45(4):416-420.
72. Jalal H, Bibby DF, Tang JW, et al. First reported outbreak of diarrhea due to adenovirus infection in a hematology unit for adults. *Journal of clinical microbiology.* 2005;43(6):2575-2580.
73. Leruez-Ville M, Chardin-Ouachee M, Neven B, et al. Description of an adenovirus A31 outbreak in a paediatric haematology unit. *Bone marrow transplantation.* 2006;38(1):23-28.
74. Mattner F, Sykora KW, Meissner B, Heim A. An adenovirus type F41 outbreak in a pediatric bone marrow transplant unit: analysis of clinical impact and preventive strategies. *The Pediatric infectious disease journal.* 2008;27(5):419-424.
75. Swartling L, Allard A, Torlen J, Ljungman P, Mattsson J, Sparrelid E. Prolonged outbreak of adenovirus A31 in allogeneic stem cell transplant recipients. *Transplant infectious disease : an official journal of the Transplantation Society.* 2015;17(6):785-794.
76. Sedláček P, Petterson T, Robin M, et al. Incidence of Adenovirus Infection in Hematopoietic Stem Cell Transplantation Recipients: Findings from the AdVance Study. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2019;25(4):810-818.
77. Ali S, Krueger J, Richardson SE, et al. The yield of monitoring adenovirus in pediatric hematopoietic stem cell transplant patients. *Pediatr Hematol Oncol.* 2019;36(3):161-172.
78. Fisher BT, Boge CLK, Petersen H, et al. Outcomes of Human Adenovirus Infection and Disease in a Retrospective Cohort of Pediatric Hematopoietic Cell Transplant Recipients. *Journal of the Pediatric Infectious Diseases Society.* 2019;8(4):317-324.
79. Feghoul L, Chevret S, Cuinet A, et al. Adenovirus infection and disease in paediatric haematopoietic stem cell transplant patients: clues for antiviral pre-emptive treatment. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases.* 2015;21(7):701-709.
80. Hubmann M, Fritsch S, Zoellner AK, et al. Occurrence, risk factors and outcome of adenovirus infection in adult recipients of allogeneic hematopoietic stem cell transplantation. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* 2016;82:33-40.
81. Sive JI, Thomson KJ, Morris EC, Ward KN, Peggs KS. Adenoviremia has limited clinical impact in the majority of patients following alemtuzumab-based allogeneic stem cell transplantation in adults. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2012;55(10):1362-1370.
82. Lee YJ, Huang YT, Kim SJ, et al. Adenovirus Viremia in Adult CD34(+) Selected Hematopoietic Cell Transplant Recipients: Low Incidence and High Clinical Impact. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2016;22(1):174-178.
83. Ohrmalm L, Lindblom A, Omar H, et al. Evaluation of a surveillance strategy for early detection of adenovirus by PCR of peripheral blood in hematopoietic SCT recipients: incidence and outcome. *Bone marrow transplantation.* 2011;46(2):267-272.

84. Lion T, Baumgartinger R, Watzinger F, et al. Molecular monitoring of adenovirus in peripheral blood after allogeneic bone marrow transplantation permits early diagnosis of disseminated disease. *Blood.* 2003;102(3):1114-1120.
85. Rustia E, Violago L, Jin Z, et al. Risk Factors and Utility of a Risk-Based Algorithm for Monitoring Cytomegalovirus, Epstein-Barr Virus, and Adenovirus Infections in Pediatric Recipients after Allogeneic Hematopoietic Cell Transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2016;22(9):1646-1653.
86. Matthes-Martin S, Feuchtinger T, Shaw PJ, et al. European guidelines for diagnosis and treatment of adenovirus infection in leukemia and stem cell transplantation: summary of ECIL-4 (2011). *Transplant infectious disease : an official journal of the Transplantation Society.* 2012;14(6):555-563.
87. Omar H, Yun Z, Lewensohn-Fuchs I, et al. Poor outcome of adenovirus infections in adult hematopoietic stem cell transplant patients with sustained adenovirus viremia. *Transplant infectious disease : an official journal of the Transplantation Society.* 2010;12(5):465-469.
88. Feuchtinger T, Lucke J, Hamprecht K, et al. Detection of adenovirus-specific T cells in children with adenovirus infection after allogeneic stem cell transplantation. *British journal of haematology.* 2005;128(4):503-509.
89. Srinivasan A, Klepper C, Sunkara A, et al. Impact of adenoviral stool load on adenoviremia in pediatric hematopoietic stem cell transplant recipients. *The Pediatric infectious disease journal.* 2015;34(6):562-565.
90. Cesaro S, Berger M, Tridello G, et al. A survey on incidence and management of adenovirus infection after allogeneic HSCT. *Bone marrow transplantation.* 2019;54(8):1275-1280.
91. Ljungman P, Ribaud P, Eyrich M, et al. Cidofovir for adenovirus infections after allogeneic hematopoietic stem cell transplantation: a survey by the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Bone marrow transplantation.* 2003;31(6):481-486.
92. Hoffman JA, Shah AJ, Ross LA, Kapoor N. Adenoviral infections and a prospective trial of cidofovir in pediatric hematopoietic stem cell transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2001;7(7):388-394.
93. Grimley MS, Chemaly RF, Englund JA, et al. Brincidofovir for Asymptomatic Adenovirus Viremia in Pediatric and Adult Allogeneic Hematopoietic Cell Transplant Recipients: A Randomized Placebo-Controlled Phase II Trial. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2017;23(3):512-521.
94. Florescu DF, Pergam SA, Neely MN, et al. Safety and efficacy of CMX001 as salvage therapy for severe adenovirus infections in immunocompromised patients. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2012;18(5):731-738.
95. Hiwarkar P, Amrolia P, Sivaprakasam P, et al. Brincidofovir is highly efficacious in controlling adenoviremia in pediatric recipients of hematopoietic cell transplant. *Blood.* 2017;129(14):2033-2037.
96. Marty FM, Winston DJ, Chemaly RF, et al. A Randomized, Double-Blind, Placebo-Controlled Phase 3 Trial of Oral Brincidofovir for Cytomegalovirus Prophylaxis in Allogeneic Hematopoietic Cell Transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2019;25(2):369-381.
97. Lee YJ, Prockop SE, Papanicolaou GA. Approach to adenovirus infections in the setting of hematopoietic cell transplantation. *Current opinion in infectious diseases.* 2017;30(4):377-387.
98. Feuchtinger T, Matthes-Martin S, Richard C, et al. Safe adoptive transfer of virus-specific T-cell immunity for the treatment of systemic adenovirus infection after allogeneic stem cell transplantation. *British journal of haematology.* 2006;134(1):64-76.

99. Leen AM, Christin A, Myers GD, et al. Cytotoxic T lymphocyte therapy with donor T cells prevents and treats adenovirus and Epstein-Barr virus infections after haploidentical and matched unrelated stem cell transplantation. *Blood.* 2009;114(19):4283-4292.
100. Feucht J, Opherk K, Lang P, et al. Adoptive T-cell therapy with hexon-specific Th1 cells as a treatment of refractory adenovirus infection after HSCT. *Blood.* 2015;125(12):1986-1994.
101. Qian C, Campidelli A, Wang Y, et al. Curative or pre-emptive adenovirus-specific T cell transfer from matched unrelated or third party haploidentical donors after HSCT, including UCB transplantations: a successful phase I/II multicenter clinical trial. *Journal of hematology & oncology.* 2017;10(1):102.
102. Kaeuferle T, Krauss R, Blaeschke F, Willier S, Feuchtinger T. Strategies of adoptive T -cell transfer to treat refractory viral infections post allogeneic stem cell transplantation. *Journal of hematology & oncology.* 2019;12(1):13.
103. Hall AJ, Lopman BA, Payne DC, et al. Norovirus disease in the United States. *Emerging infectious diseases.* 2013;19(8):1198-1205.
104. Ahmed SM, Hall AJ, Robinson AE, et al. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *The Lancet Infectious diseases.* 2014;14(8):725-730.
105. Rockx B, De Wit M, Vennema H, et al. Natural history of human calicivirus infection: a prospective cohort study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2002;35(3):246-253.
106. Gustavsson L, Andersson LM, Lindh M, Westin J. Excess mortality following community-onset norovirus enteritis in the elderly. *The Journal of hospital infection.* 2011;79(1):27-31.
107. Atmar RL, Ramani S, Estes MK. Human noroviruses: recent advances in a 50-year history. *Current opinion in infectious diseases.* 2018;31(5):422-432.
108. Thorne L, Arias A, Goodfellow I. Advances Toward a Norovirus Antiviral: From Classical Inhibitors to Lethal Mutagenesis. *The Journal of infectious diseases.* 2016;213 Suppl 1:S27-31.
109. Robilotti E, Deresinski S, Pinsky BA. Norovirus. *Clinical microbiology reviews.* 2015;28(1):134-164.
110. Nordgren J, Svensson L. Genetic Susceptibility to Human Norovirus Infection: An Update. *Viruses.* 2019;11(3).
111. De Grazia S, Lanave G, Giammanco GM, et al. Sentinel hospital-based surveillance for norovirus infection in children with gastroenteritis between 2015 and 2016 in Italy. *PloS one.* 2018;13(12):e0208184.
112. de Graaf M, van Beek J, Koopmans MP. Human norovirus transmission and evolution in a changing world. *Nat Rev Microbiol.* 2016;14(7):421-433.
113. Huhti L, Szakal ED, Puustinen L, et al. Norovirus GII-4 causes a more severe gastroenteritis than other noroviruses in young children. *The Journal of infectious diseases.* 2011;203(10):1442-1444.
114. Fraenkel CJ, Inghammar M, Söderlund-Strand A, Johansson PJH, Böttiger B. Risk factors for hospital norovirus outbreaks: impact of vomiting, genotype, and multi-occupancy rooms. *The Journal of hospital infection.* 2018;98(4):398-403.
115. Atmar RL. Noroviruses - State of the Art. *Food Environ Virol.* 2010;2(3):117-126.
116. Teunis PF, Moe CL, Liu P, et al. Norwalk virus: how infectious is it? *Journal of medical virology.* 2008;80(8):1468-1476.
117. Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. *The New England journal of medicine.* 2009;361(18):1776-1785.
118. Doshi M, Woodwell S, Kelleher K, Mangan K, Axelrod P. An outbreak of norovirus infection in a bone marrow transplant unit. *American journal of infection control.* 2013;41(9):820-823.
119. Khanna N, Goldenberger D, Graber P, Battegay M, Widmer AF. Gastroenteritis outbreak with norovirus in a Swiss university hospital with a newly identified virus strain. *The Journal of hospital infection.* 2003;55(2):131-136.

120. Schwartz S, Vergoulidou M, Schreier E, et al. Norovirus gastroenteritis causes severe and lethal complications after chemotherapy and hematopoietic stem cell transplantation. *Blood.* 2011;117(22):5850-5856.
121. Branch-Elliman W, Araujo-Castillo RV, Snyder GM, Sullivan BF, Alonso CD, Wright SB. Identification of a norovirus outbreak on a hematopoietic stem cell transplant unit and development and implementation of a novel infection prevention algorithm for controlling transmission. *Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America.* 2020:1-5.
122. Kirkwood CD, Streitberg R. Calicivirus shedding in children after recovery from diarrhoeal disease. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* 2008;43(3):346-348.
123. Schorn R, Höhne M, Meerbach A, et al. Chronic norovirus infection after kidney transplantation: molecular evidence for immune-driven viral evolution. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2010;51(3):307-314.
124. King JR, Varadé J, Hammarström L. Fucosyltransferase Gene Polymorphisms and Lewisb-Negative Status Are Frequent in Swedish Newborns, With Implications for Infectious Disease Susceptibility and Personalized Medicine. *Journal of the Pediatric Infectious Diseases Society.* 2019;8(6):507-518.
125. Günaydın G, Nordgren J, Sharma S, Hammarström L. Association of elevated rotavirus-specific antibody titers with HBGA secretor status in Swedish individuals: The FUT2 gene as a putative susceptibility determinant for infection. *Virus research.* 2016;211:64-68.
126. Knoll BM, Lindesmith LC, Yount BL, Baric RS, Marty FM. Resolution of diarrhea in an immunocompromised patient with chronic norovirus gastroenteritis correlates with constitution of specific antibody blockade titer. *Infection.* 2016;44(4):551-554.
127. Lindesmith L, Moe C, Lependu J, Frelinger JA, Treanor J, Baric RS. Cellular and humoral immunity following Snow Mountain virus challenge. *Journal of virology.* 2005;79(5):2900-2909.
128. Roos-Weil D, Ambert-Balay K, Lanternier F, et al. Impact of norovirus/sapovirus-related diarrhea in renal transplant recipients hospitalized for diarrhea. *Transplantation.* 2011;92(1):61-69.
129. Lee LY, Ladner DP, Ison MG. Norovirus infection in solid organ transplant recipients: a single-center retrospective study. *Transplant infectious disease : an official journal of the Transplantation Society.* 2016;18(6):932-938.
130. Avery RK, Lonze BE, Kraus ES, Marr KA, Montgomery RA. Severe chronic norovirus diarrheal disease in transplant recipients: Clinical features of an under-recognized syndrome. *Transplant infectious disease : an official journal of the Transplantation Society.* 2017.
131. Chong PP, van Duin D, Sonderup JL, Grant WJ, Kalil AC, Florescu DF. Predictors of persistent diarrhea in norovirus enteritis after solid organ transplantation. *Clinical transplantation.* 2016;30(11):1488-1493.
132. Frange P, Touzot F, Debre M, et al. Prevalence and clinical impact of norovirus fecal shedding in children with inherited immune deficiencies. *The Journal of infectious diseases.* 2012;206(8):1269-1274.
133. Brown JR, Gilmour K, Breuer J. Norovirus Infections Occur in B-Cell-Deficient Patients. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2016;62(9):1136-1138.
134. Woodward JM, Gkrania-Klotsas E, Cordero-Ng AY, et al. The role of chronic norovirus infection in the enteropathy associated with common variable immunodeficiency. *The American journal of gastroenterology.* 2015;110(2):320-327.
135. Roddie C, Paul JP, Benjamin R, et al. Allogeneic hematopoietic stem cell transplantation and norovirus gastroenteritis: a previously unrecognized cause of morbidity. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2009;49(7):1061-1068.

136. Saif MA, Bonney DK, Bigger B, et al. Chronic norovirus infection in pediatric hematopoietic stem cell transplant recipients: a cause of prolonged intestinal failure requiring intensive nutritional support. *Pediatric transplantation.* 2011;15(5):505-509.
137. Robles JD, Cheuk DK, Ha SY, Chiang AK, Chan GC. Norovirus infection in pediatric hematopoietic stem cell transplantation recipients: incidence, risk factors, and outcome. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2012;18(12):1883-1889.
138. Ye X, Van JN, Munoz FM, et al. Noroviruses as a Cause of Diarrhea in Immunocompromised Pediatric Hematopoietic Stem Cell and Solid Organ Transplant Recipients. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons.* 2015;15(7):1874-1881.
139. Kamar N, Izopet J, Pavio N, et al. Hepatitis E virus infection. *Nat Rev Dis Primers.* 2017;3:17086.
140. Horvatits T, Schulze Zur Wiesch J, Lütgehetmann M, Lohse AW, Pischke S. The Clinical Perspective on Hepatitis E. *Viruses.* 2019;11(7).
141. EASL Clinical Practice Guidelines on hepatitis E virus infection. *Journal of hepatology.* 2018;68(6):1256-1271.
142. Hartl J, Otto B, Madden RG, et al. Hepatitis E Seroprevalence in Europe: A Meta-Analysis. *Viruses.* 2016;8(8).
143. Olsen B, Axelsson-Olsson D, Thelin A, Weiland O. Unexpected high prevalence of IgG-antibodies to hepatitis E virus in Swedish pig farmers and controls. *Scandinavian journal of infectious diseases.* 2006;38(1):55-58.
144. Norder H, Karlsson M, Mellgren A, et al. Diagnostic Performance of Five Assays for Anti-Hepatitis E Virus IgG and IgM in a Large Cohort Study. *Journal of clinical microbiology.* 2016;54(3):549-555.
145. Donnelly MC, Scobie L, Crossan CL, Dalton H, Hayes PC, Simpson KJ. Review article: hepatitis E-a concise review of virology, epidemiology, clinical presentation and therapy. *Alimentary pharmacology & therapeutics.* 2017;46(2):126-141.
146. Matsubayashi K, Kang JH, Sakata H, et al. A case of transfusion-transmitted hepatitis E caused by blood from a donor infected with hepatitis E virus via zoonotic food-borne route. *Transfusion.* 2008;48(7):1368-1375.
147. Koenecke C, Pischke S, Beutel G, et al. Hepatitis E virus infection in a hematopoietic stem cell donor. *Bone marrow transplantation.* 2014;49(1):159-160.
148. Schlosser B, Stein A, Neuhaus R, et al. Liver transplant from a donor with occult HEV infection induced chronic hepatitis and cirrhosis in the recipient. *Journal of hepatology.* 2012;56(2):500-502.
149. Baylis SA, Gartner T, Nick S, Ovemyr J, Blumel J. Occurrence of hepatitis E virus RNA in plasma donations from Sweden, Germany and the United States. *Vox sanguinis.* 2012;103(1):89-90.
150. Boland F, Martinez A, Pomeroy L, O'Flaherty N. Blood Donor Screening for Hepatitis E Virus in the European Union. *Transfus Med Hemother.* 2019;46(2):95-103.
151. Guillois Y, Abravanel F, Miura T, et al. High Proportion of Asymptomatic Infections in an Outbreak of Hepatitis E Associated With a Spit-Roasted Piglet, France, 2013. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2016;62(3):351-357.
152. McLean BN, Gulliver J, Dalton HR. Hepatitis E virus and neurological disorders. *Pract Neurol.* 2017;17(4):282-288.
153. Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *The New England journal of medicine.* 2008;358(8):811-817.
154. Dalton HR, Bendall RP, Keane FE, Tedder RS, Ijaz S. Persistent carriage of hepatitis E virus in patients with HIV infection. *The New England journal of medicine.* 2009;361(10):1025-1027.

155. Pischke S, Peron JM, von Wulffen M, et al. Chronic Hepatitis E in Rheumatology and Internal Medicine Patients: A Retrospective Multicenter European Cohort Study. *Viruses.* 2019;11(2).
156. Ollier L, Tieulie N, Sanderson F, et al. Chronic hepatitis after hepatitis E virus infection in a patient with non-Hodgkin lymphoma taking rituximab. *Annals of internal medicine.* 2009;150(6):430-431.
157. Abravanel F, Mansuy JM, Huynh A, et al. Low risk of hepatitis E virus reactivation after haematopoietic stem cell transplantation. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* 2012;54(2):152-155.
158. Koenecke C, Pischke S, Heim A, et al. Chronic hepatitis E in hematopoietic stem cell transplant patients in a low-endemic country? *Transplant infectious disease : an official journal of the Transplantation Society.* 2012;14(1):103-106.
159. Reekie I, Irish D, Ijaz S, et al. Hepatitis E infection in stem cell and solid organ transplantpatients: A cross-sectional study: The importance of HEV RNA screening in peri-transplant period. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* 2018;107:1-5.
160. Ankcorn MJ, Ijaz S, Poh J, et al. Toward Systematic Screening for Persistent Hepatitis E Virus Infections in Transplant Patients. *Transplantation.* 2018;102(7):1139-1147.
161. Willemse SB, Bezuur DL, Blom P, et al. Hepatitis E virus infection and hepatic GvHD in allogeneic hematopoietic stem cell transplantation recipients. *Bone marrow transplantation.* 2017;52(4):622-624.
162. Carre M, Thiebaut-Bertrand A, Larrat S, et al. Fatal autochthonous fulminant hepatitis E early after allogeneic stem cell transplantation. *Bone marrow transplantation.* 2017;52(4):643-645.
163. von Felden J, Alric L, Pischke S, et al. The burden of hepatitis E among patients with haematological malignancies: A retrospective European cohort study. *Journal of hepatology.* 2019;71(3):465-472.
164. Xhaard A, Roque-Afonso AM, Mallet V, et al. Hepatitis E and Allogeneic Hematopoietic Stem Cell Transplantation: A French Nationwide SFGM-TC Retrospective Study. *Viruses.* 2019;11(7).
165. McPherson S, Elsharkawy AM, Ankcorn M, et al. Summary of the British Transplantation Society UK Guidelines for Hepatitis E and Solid Organ Transplantation. *Transplantation.* 2017.
166. Kamar N, Garrouste C, Haagsma EB, et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology.* 2011;140(5):1481-1489.
167. Kamar N, Lhomme S, Abravanel F, et al. An Early Viral Response Predicts the Virological Response to Ribavirin in Hepatitis E Virus Organ Transplant Patients. *Transplantation.* 2015;99(10):2124-2131.
168. Abravanel F, Lhomme S, Rostaing L, Kamar N, Izopet J. Protracted fecal shedding of HEV during ribavirin therapy predicts treatment relapse. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2015;60(1):96-99.
169. Kamar N, Abravanel F, Behrendt P, et al. Ribavirin for Hepatitis E Virus Infection After Organ Transplantation: A Large European Retrospective Multicenter Study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2020;71(5):1204-1211.
170. Remberger M, Ackefors M, Berglund S, et al. Improved survival after allogeneic hematopoietic stem cell transplantation in recent years. A single-center study. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2011;17(11):1688-1697.
171. Torlen J, Ringden O, Garming-Legert K, et al. A prospective randomized trial comparing cyclosporine/methotrexate and tacrolimus/sirolimus as graft-versus-host disease prophylaxis after allogeneic hematopoietic stem cell transplantation. *Haematologica.* 2016;101(11):1417-1425.

172. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2008;14(6):641-650.
173. Raiola AM, Dominietto A, di Grazia C, et al. Unmanipulated haploidentical transplants compared with other alternative donors and matched sibling grafts. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2014;20(10):1573-1579.
174. Locatelli F, Merli P, Pagliara D, et al. Outcome of children with acute leukemia given HLA-haploidentical HSCT after αβ T-cell and B-cell depletion. *Blood.* 2017;130(5):677-685.
175. Ljungman P. Molecular monitoring of viral infections after hematopoietic stem cell transplantation. *International journal of hematology.* 2010;91(4):596-601.
176. Omar H, Hagglund H, Gustafsson-Jernberg A, et al. Targeted monitoring of patients at high risk of post-transplant lymphoproliferative disease by quantitative Epstein-Barr virus polymerase chain reaction. *Transplant infectious disease : an official journal of the Transplantation Society.* 2009;11(5):393-399.
177. Gustafson I, Lindblom A, Yun Z, et al. Quantification of adenovirus DNA in unrelated donor hematopoietic stem cell transplant recipients. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* 2008;43(1):79-85.
178. Heim A, Ebnet C, Harste G, Pring-Akerblom P. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *Journal of medical virology.* 2003;70(2):228-239.
179. Allard A, Albinsson B, Wadell G. Rapid typing of human adenoviruses by a general PCR combined with restriction endonuclease analysis. *Journal of clinical microbiology.* 2001;39(2):498-505.
180. Cloutman-Green E, Canales M, Pankhurst L, et al. Development and implementation of a cleaning standard algorithm to monitor the efficiency of terminal cleaning in removing adenovirus within a pediatric hematopoietic stem cell transplantation unit. *American journal of infection control.* 2015;43(9):997-999.
181. Pankhurst L, Cloutman-Green E, Canales M, D'Arcy N, Hartley JC. Routine monitoring of adenovirus and norovirus within the health care environment. *American journal of infection control.* 2014;42(11):1229-1232.
182. Grundmann H, Hori S, Winter B, Tami A, Austin DJ. Risk factors for the transmission of methicillin-resistant Staphylococcus aureus in an adult intensive care unit: fitting a model to the data. *The Journal of infectious diseases.* 2002;185(4):481-488.
183. Vicca AF. Nursing staff workload as a determinant of methicillin-resistant Staphylococcus aureus spread in an adult intensive therapy unit. *The Journal of hospital infection.* 1999;43(2):109-113.
184. Nordgren J, Bucardo F, Dienus O, Svensson L, Lindgren PE. Novel light-upon-extension real-time PCR assays for detection and quantification of genogroup I and II noroviruses in clinical specimens. *Journal of clinical microbiology.* 2008;46(1):164-170.
185. Kojima S, Kageyama T, Fukushi S, et al. Genogroup-specific PCR primers for detection of Norwalk-like viruses. *Journal of virological methods.* 2002;100(1-2):107-114.
186. Loisy F, Atmar RL, Guillon P, Le Cann P, Pommepuy M, Le Guyader FS. Real-time RT-PCR for norovirus screening in shellfish. *Journal of virological methods.* 2005;123(1):1-7.
187. Lemes LG, Correa TS, Fiaccadori FS, et al. Prospective study on Norovirus infection among allogeneic stem cell transplant recipients: prolonged viral excretion and viral RNA in the blood. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* 2014;61(3):329-333.
188. Oluwatoyin Japhet M, Adeyemi Adesina O, Famurewa O, Svensson L, Nordgren J. Molecular epidemiology of rotavirus and norovirus in Ile-Ife, Nigeria: high prevalence of G12P[8] rotavirus strains and detection of a rare norovirus genotype. *Journal of medical virology.* 2012;84(9):1489-1496.

189. Kageyama T, Kojima S, Shinohara M, et al. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *Journal of clinical microbiology.* 2003;41(4):1548-1557.
190. Bucardo F, Nordgren J, Reyes Y, Gonzalez F, Sharma S, Svensson L. The Lewis A phenotype is a restriction factor for Rotateq and Rotarix vaccine-take in Nicaraguan children. *Scientific reports.* 2018;8(1):1502.
191. Myers LA, Patel DD, Puck JM, Buckley RH. Hematopoietic stem cell transplantation for severe combined immunodeficiency in the neonatal period leads to superior thymic output and improved survival. *Blood.* 2002;99(3):872-878.
192. Pai SY, Logan BR, Griffith LM, et al. Transplantation outcomes for severe combined immunodeficiency, 2000-2009. *The New England journal of medicine.* 2014;371(5):434-446.
193. Kim YJ, Guthrie KA, Waghmare A, et al. Respiratory syncytial virus in hematopoietic cell transplant recipients: factors determining progression to lower respiratory tract disease. *The Journal of infectious diseases.* 2014;209(8):1195-1204.
194. van Montfrans J, Schulz L, Versluys B, et al. Viral PCR positivity in stool before allogeneic hematopoietic cell transplantation is strongly associated with acute intestinal graft-versus-host disease. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2015;21(4):772-774.
195. Roth A, Lin J, Magnius L, et al. Markers for Ongoing or Previous Hepatitis E Virus Infection Are as Common in Wild Ungulates as in Humans in Sweden. *Viruses.* 2016;8(9).
196. Norder H, Galli C, Magnil E, et al. Hepatitis E Virus Genotype 3 Genomes from RNA-Positive but Serologically Negative Plasma Donors Have CUG as the Start Codon for ORF3. *Intervirology.* 2018;61(2):96-103.
197. Norder H, Sundqvist L, Magnusson L, et al. Endemic hepatitis E in two Nordic countries. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin.* 2009;14(19).
198. Boxman ILA, Jansen CCC, Hagele G, Zwartkruis-Nahuis A, Tijsma ASL, Vennema H. Monitoring of pork liver and meat products on the Dutch market for the presence of HEV RNA. *International journal of food microbiology.* 2019;296:58-64.
199. Thom K, Gilhooly P, McGowan K, et al. Hepatitis E virus (HEV) in Scotland: evidence of recent increase in viral circulation in humans. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin.* 2018;23(12).
200. Wahren B, Gahrton G, Linde A, et al. Transfer and persistence of viral antibody-producing cells in bone marrow transplantation. *The Journal of infectious diseases.* 1984;150(3):358-365.
201. Martínez C, Rosales M, Calvo X, et al. Serial intestinal endoscopic examinations of patients with persistent diarrhea after allo-SCT. *Bone marrow transplantation.* 2012;47(5):694-699.
202. Cho BS, Yahng SA, Kim JH, et al. Impact of cytomegalovirus gastrointestinal disease on the clinical outcomes in patients with gastrointestinal graft-versus-host disease in the era of preemptive therapy. *Annals of hematology.* 2013;92(4):497-504.
203. Westhoff TH, Vergoulidou M, Loddenkemper C, et al. Chronic norovirus infection in renal transplant recipients. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association.* 2009;24(3):1051-1053.
204. Green KY, Kaufman SS, Nagata BM, et al. Human norovirus targets enteroendocrine epithelial cells in the small intestine. *Nat Commun.* 2020;11(1):2759.
205. Ettayebi K, Crawford SE, Murakami K, et al. Replication of human noroviruses in stem cell-derived human enteroids. *Science.* 2016;353(6306):1387-1393.
206. Chan MC, Cheung SKC, Mohammad KN, Chan JCM, Estes MK, Chan PKS. Use of Human Intestinal Enteroids to Detect Human Norovirus Infectivity. *Emerging infectious diseases.* 2019;25(9):1730-1735.
207. Netzler NE, Enosi Tuipulotu D, White PA. Norovirus antivirals: Where are we now? *Med Res Rev.* 2019;39(3):860-886.

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