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Molecular epidemiology of a Parainfluenza Type 3 virus outbreak: Informing infection control measures on adult hematology wards

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ABSTRACT

Objectives: Parainfluenza virus type 3 (PIV3) outbreaks among hematology patients are associated with high morbidity and mortality. Prompt implementation of infection prevention (IP) measures has proven to be the most efficacious approach for controlling PIV3 outbreaks within this patient population. The most suitable IP measures can vary depending on the mode of virus transmission, which remains unidentified in most outbreaks. We describe the molecular epidemiology of an outbreak of PIV3 among hematology patients and the development of a new method that allows for the differentiation of outbreak and community strains, from which a closed outbreak could be inferred.

Methods: Patients were screened for respiratory viruses using multiplex-PCR. PIV3 positive samples with a cycle threshold (Ct)-value of <31 underwent a retrospective characterization via an in-house developed sequence analysis of the hemagglutinin-neuraminidase (HN) gene.

Results: Between July and September 2022, 31 hematology patients were identified with PIV3. Although infection control measures were implemented, the outbreak persisted for nine weeks. Sequencing the HN gene of 27 PIV3 strains from 27 patients revealed that all outbreak strains formed a distinct cluster separate from the control strains, suggestive of a nosocomial transmission route.

Conclusions: Sequencing the HN gene of PIV3 strains in an outbreak setting enables outbreak strains to be distinguished from community strains. Early molecular characterization of PIV3 strains during an outbreak can serve as a tool in determining potential transmission routes. This, in turn, enables rapid implementation of targeted infection prevention measures, with the goal of minimizing the outbreak's duration and reducing associated morbidity and mortality.

1. Introduction

Parainfluenza 3 (PIV3) is an enveloped, single-stranded, negative-sense RNA virus, which belongs to the *Paramyxoviridae* family [1]. In immunocompetent individuals, infection with PIV3 is associated with a mild clinical course and a short duration of viral shedding, however, PIV3 infections in immunocompromised patients can lead to serious morbidity and mortality and prolonged viral shedding [2]. Patients with a hematological malignancy are at particular risk of serious lower-respiratory tract infections with associated mortality [2]. PIV3 is known to be highly contagious and is easily transmitted through direct contact, large-particle aerosols and fomites [3]. In northern Europe, PIV3 infections show seasonality with peaks of infection between May and September [4]. The highly contagious nature of PIV3 and ease of transmission has resulted in regular outbreaks of PIV3 among hematology and stem-cell transplant (SCT) patients worldwide [5–12].

Treatment options for PIV3 infections are limited. Ribavirin is sometimes prescribed for the treatment of PIV3 in hematology patients, however there is no evidence to suggest that ribavirin has a significant effect on reducing mortality [13]. Therefore, the most important

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strategy for controlling an outbreak of PIV3 among hematology patients is the rapid implementation of appropriate infection prevention measures early in the outbreak [2]. In order to implement the most effective infection prevention measures, it is important to know whether new infections are due to nosocomial transmission of one viral strain or whether new viral strains are being introduced from the community via patients, hospital staff or visitors. This is possible through the molecular characterization of virus strains.

We describe an outbreak of PIV3 infections on the adult hematology ward of a tertiary referral hospital in the Netherlands. PIV3 sequence analysis of the hemagglutinin-neuraminidase (HN) gene was developed using previously published primers and used to sequence PIV3 outbreak strains, mostly retrospectively. Phylogenetic analysis was performed to assess if the main route of transmission was nosocomial or whether new PIV3 viruses were being introduced from the community.

2. Description of outbreak

2.1. Patients and setting

From July until the end of September 2022 there was an outbreak of PIV3 infections among inpatients on the adult hematology unit of the University Medical Centre Groningen (UMCG), a tertiary referral hospital in the Netherlands. There are two in-patient hematology wards in the UMCG (D1 and E2), which include four single person rooms each with a personal toilet, two rooms with two beds with a shared toilet and two rooms with four beds with a shared toilet (Fig. 1). A total of 31 patients aged 21 to 79 years (12 female, 19 male) tested positive, all patients had a hematological malignancy and/or hematopoietic stem-cell transplantation.

2.2. Rationale for testing patients

There were two indications for testing patients. Firstly, throughout the outbreak period, clinical assessment of all patients with respiratory symptoms and / or fever included testing by means of a multiplex polymerase chain reaction panel, which could detect 19 respiratory viruses (including PIV3) from a nasopharyngeal swab.

Secondly, all patients (both patients with respiratory symptoms and patients without respiratory symptoms) were periodically screened using a specific PIV3 polymerase chain reaction to detect asymptomatically infected patients.

All patients were tested by means of a nasopharyngeal swab.

2.3. Course of the outbreak

The outbreak took place over a period of 9 weeks. The index patient tested positive for PIV3 at the beginning of the first week (22nd July2022) on ward D1 (Fig. 2). The patient was tested due to mild respiratory symptoms. The first positive case was not recognized as the start of the outbreak because it occurred during the summer when parainfluenza virus cases are expected [14]. The patient was isolated and infection prevention measures were implemented. At the end of the first week, the second case was detected in a patient who shared a room with the index (Fig. 2). Two more patients were diagnosed with a PIV3 infection during week two. All positively tested patients from week one and two had been inpatients for at least 48 h before diagnosis. The incubation period of PIV3 is 2–6 days [15] meaning that all positively tested patients could have contracted the infection nosocomially. In the third week, three more patients tested positive and all PIV3 positive patients were moved to another hematology ward (E2) and placed in a cohort. The original ward (D1) was closed to new admissions. In week five, all patients on ward E2 were screened using a qualitative real time polymerase chain reaction assay (qPCR) whereby four new positive patients were found. The number of cases continued to increase during week five and six (Fig. 2), and patients were separated into three cohorts; patients positive for PIV3, exposed and unexposed patients. In addition, patients, staff and visitors were instructed to wear a face mask at all times (to replace the previous policy of patients, staff and visitors being required to wear a face mask only if within 1.5 m of somebody) and all patients were screened for PIV3 every two to three days with qPCR.

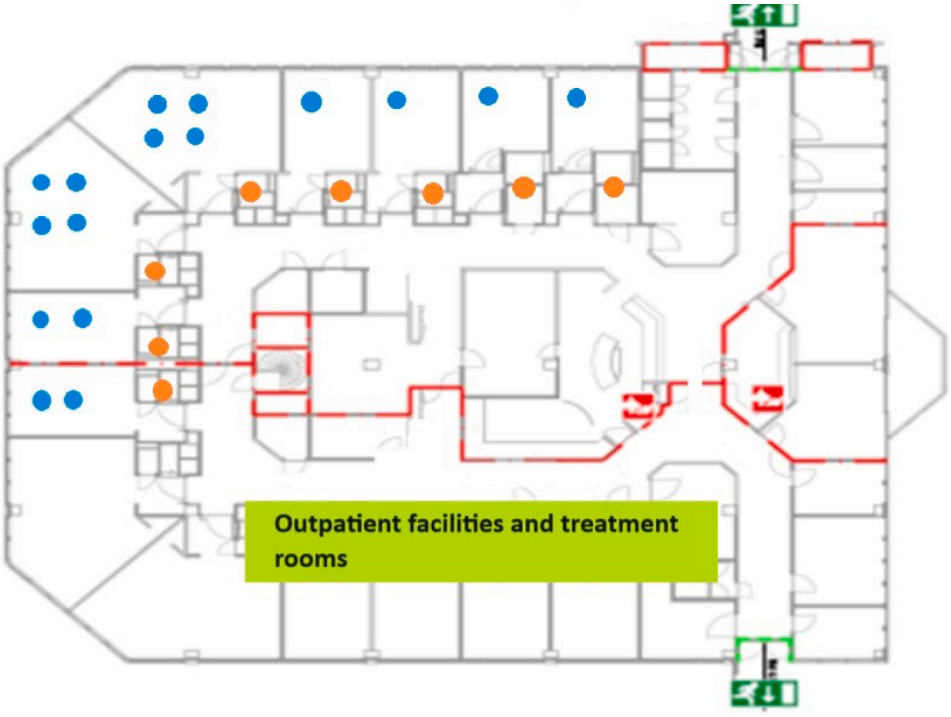

Fig. 1. Layout of the hematology wards D1 and E2, which both have the same layout as represented here, at the University Medical Centre Groningen during an outbreak of PIV3 from July – September 2022. Blue circles represent beds and orange circles represent toilet facilities.

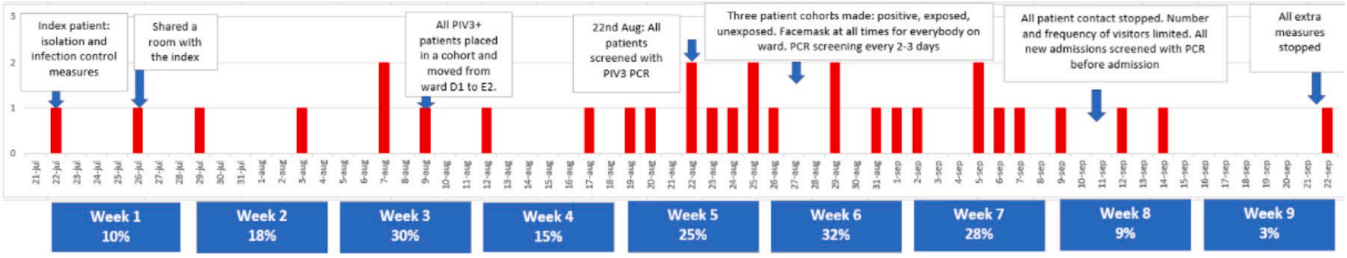

Fig. 2. Epi curve depicting the course of the outbreak of PIV3 within the hematology ward at the University Medical Center Groningen from July until September 2022. The week number of the outbreak and the percentage of positive patients per week are reported in the blue boxes. After the last case on 22nd September 2022, there were no further cases detected for 12 days (twice the incubation time) and the outbreak was declared over.

Despite the control measures, five new cases of PIV3 were identified in week seven. Further measures were taken including patients avoiding all contact with each other outside their rooms, limiting the number of visitors and the frequency of visits and screening all new patients for PIV3. The outbreak was considered over when there were no new cases for 12 days (twice the incubation period). Thirty-one patients out of a total of 137 patients (23 %) were infected with PIV3.

Patients experienced a range of symptoms from mild upper respiratory tract symptoms to serious lower respiratory tract infections and some patients were asymptomatic.

At 120 days, of the 31 patients infected with PIV3, 6 patients had died. The contribution of PIV3 infection to the cause of death was hard to determine in this vulnerable patient group with multiple morbidities. Healthcare workers and visitors were not tested for PIV3.

3. Materials and methods

3.1. Laboratory diagnosis of PIV-3

Nasopharyngeal swabs from patients were placed in Universal Transport Medium (UTM – Copan Diagnostics Inc., California, United States of America) and UTM from all swabs was stored at −80 °Celsius. Diagnosis of PIV3 was established through the detection of viral RNA in UTM from nasopharyngeal swabs collected from patients using qPCR.

Throughout the outbreak, patients with respiratory symptoms were tested with the FilmArray Torch Respiratory Panel (BioFire Diagnostics, Salt Lake City, USA), a multiplex RT-PCR, which can detect 19 respiratory viruses including adenovirus, coronavirus 229E, coronavirus HKU1, coronavirus OC43, coronavirus NL63, human metapneumovirus, human rhinovirus/enterovirus, influenza A (including influenza A Hx, H1, H1–2009, H3), influenza B, parainfluenza 1–4, respiratory syncytial virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV).

Later in the outbreak, all patients (patients with respiratory symptoms and asymptomatic patients) were periodically screened for PIV3 as an infection prevention measure using a PIV3 specific qPCR. The RNA was extracted from190ul sample with the addition of 10ul internal control (Phocine Distemper Virus) using the eMag/Nuclicense EasyMag (bioMérieux, Lyon, France). The qPCR was performed with a multiplex of RSV-B and PIV 3 amplifying 169 bp of the hemagglutinin-neuraminidase (HN) gene using 1xTaqMan® Fast Virus 1-Step Master (Thermofisher, Foster City, CA, USA) in a total reaction volume of 25ul. The reaction mixture contained 100 nM probe (FAM-ATCTGYAACA-CAACTGGRTGTCCYGGGAA-BHQ), 900 nM reverse primer (TTGAC-CATCCTYCTRTCTGAA) and 900 nM forward primer (GGACCAGGGATATACTAYAAA). The ABI PRISM 7500 (Life technologies, USA) was used for the amplification and detection by the following profile: 2 min 50 °C, 20 s 95 °C, 45 cycles of 3 s 95 °C and 32 s 60 °C.

3.2. Description of sequencing methods

The RNA was extracted as described above. cDNA was produced using the LunaScript RT SuperMix kit (Bioke, the Netherlands) 2 min 25 °C and 20 min 55 °C. The PCR amplified a 1725 bp segment of the HN gene from PIV3 using 2x Phire HS II PCR mastermix (Thermofisher, Foster City, CA, USA) with 600 nM primer HNS (AAA TCG AGT GGA TCA AAA TGA TAA GCC) and 600 nM 3HN (ACA GTG CCA TTG TTA GAT TCA G) [16] and the following thermocycler protocol: 30 s 98 °C, 34 cycles of 5 s 98 °C, 5 s 62 °C and 25 s 72 °C, 1 min 72 °C. Clean up of the PCR product was done according to the manufacturer's instructions using the QIAquick PCR Purification kit (Qiagen, USA). Sequencing was performed with the Miseq illuminia platform using Nextera XT DNA Library preparation kit and Miseq reagent kit v2 (500 cycli) (Illuminia, USA).

Trimming was performed before mapping to the reference PIV3 sequence NC_001796 and consensus sequence was generated using CLC Genomics Workbench 22.0.2 (Qiagen, Denmark). The quality trimming settings were default with the exception of quality limit (0.05). For quality metrics the following parameters were used: coverage >15000x and reads mapped >50,000. The phylogenetic tree was created with Ridom Seqsphere 8.4.2 (Ridom, Germany) using a neighbor joining algorithm. It is based on a SNP analysis of 322 targets of the HN gene from the reference strain NC_001796. The raw datasets generated in the current study are available in the European Nucleotide Archive (ENA) repository under Bioproject PRJEB72055 (hhtp://www.ebi.ac.uk/ena). Nucleotide accession numbers are listed in Supplementary Table 1.

The outbreak strains were compared with previously published references sequences from NCBI [17] and unrelated control samples from our hospital including: samples from previous years, samples from the same time period as the outbreak but from a different ward and samples from the same ward from an earlier time period.

4. Results

4.1. PCR

Twenty-five of the 31 patients were tested using the FilmArray Torch Respiratory Panel multiplex RT-PCR because they had respiratory symptoms. In addition to PIV3, one patient tested positive for human herpes virus 6, one patient tested positive for SARS-CoV-2 and two patients tested positive for rhinovirus.

4.2. Sequencing and phylogenetic analysis of PIV-3 PCR amplicons

PIV3 amplicons of the HN gene were generated in total from 38 patient nasopharyngeal samples, 27 outbreak samples and 11 unrelated control samples. It was not possible to perform sequence analysis on four of the outbreak samples due to poor sequence quality.

Fig. 3 shows the phylogenetic tree for the PIV-3 HN sequences from the patient samples compared with that from the unrelated control samples. Twenty-six of the 27 outbreak strains were identical. One of the 27 outbreak strains differed from the other 26 outbreak strains by 1 base pair. The outbreak strain differed from the unrelated control strains.

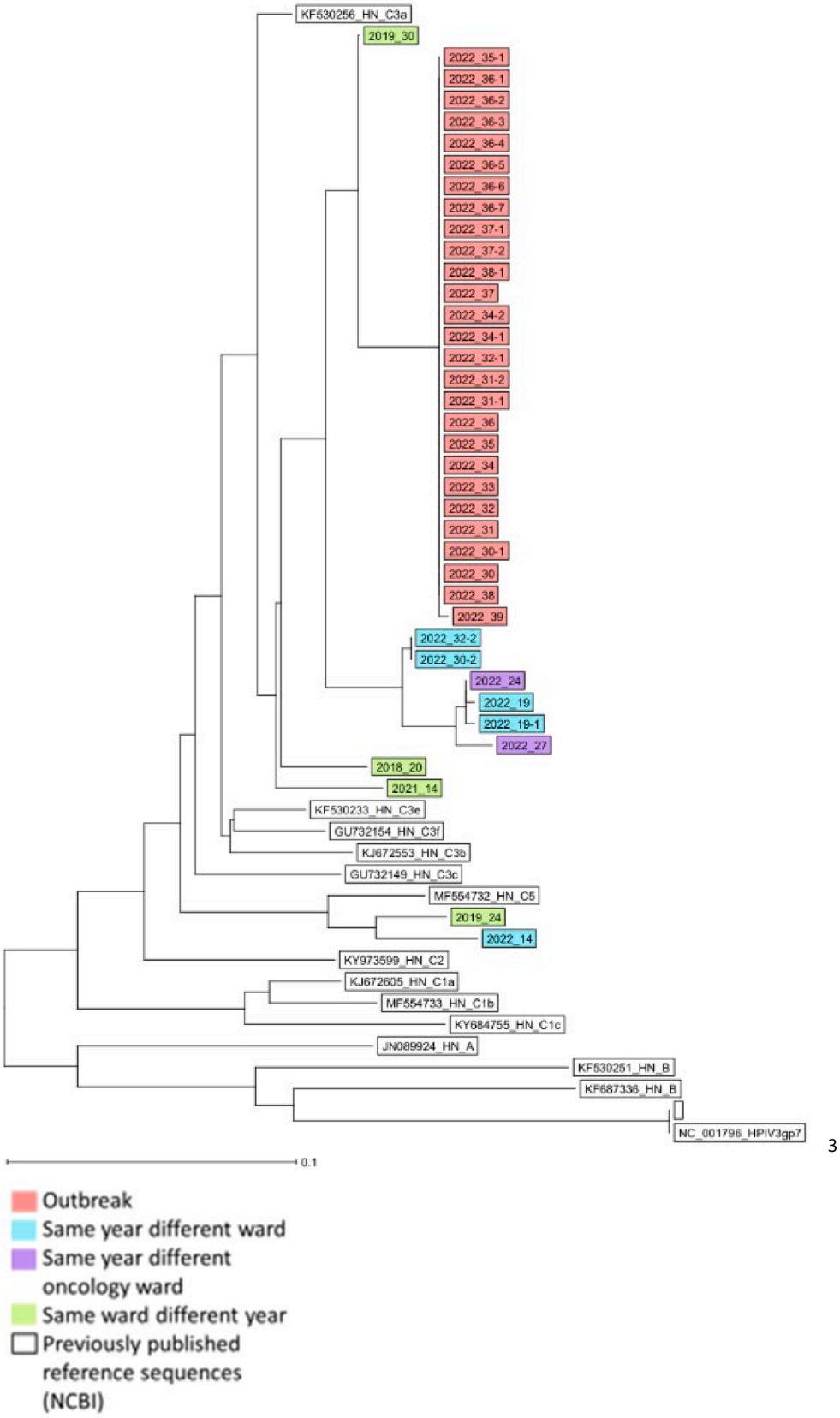

Fig. 3. Neighbour joining tree of PIV3 strains was created with Ridom Seqsphere 8.4.2 (Ridom, Germany) using a neighbor joining algorithm. The scale bar presents the% difference among 322 nucleotides of HN gene, using PIV3 strain NC_001796 as a reference. Viral strains are numbered with the year and week number that the virus strain was isolated and the respective sequence accession codes are presented in Supplementary Table 1.

5. Discussion

Here we describe the molecular investigation of a PIV3 outbreak among hematology and SCT patients admitted to a hematology ward in a tertiary referral hospital in the Netherlands.

Unfortunately, the initial cluster of PIV3 infections at the outbreak's onset was not recognised as a nosocomial outbreak, even though the time interval between the initial cases aligned with the incubation period for PIV3. The uncertainty surrounding whether this cluster represented an outbreak or repeated introductions of PIV3 strains resulted in a delay in implementing the most appropriate infection control measures. The rapid implementation of infection control measures is known to be the most effective strategy for bringing an outbreak of PIV3 under control in this patient population [2,18]. Infection prevention measures are likely to differ depending on the mode of transmission. We believe that had the initial cluster of PIV3 cases been identified as an outbreak earlier, through the implementation of sequence-based analysis of viral strains from the initial patients, more stringent and targeted infection prevention measures could have been promptly implemented. Such actions might have curtailed the outbreak's duration, reduced the number of affected patients, and potentially mitigated both morbidity and mortality.

Determining the best time for conducting sequence-based analysis of viral strains in a suspected outbreak setting poses challenges. However, in the outlined outbreak, we propose that an opportune moment for molecular analysis would have been early in the outbreak, following the cohorting of the initial group of patients. We suggest that the molecular characterisation of viruses should be integrated within the traditional epidemiological approach of managing an outbreak. Particularly in outbreaks of viruses that circulate concurrently within the general population and among hospitalized patients. In such outbreaks, molecular data can give insights into transmission pathways, including nosocomial spread versus external introductions. Such information can be used to enhance the precision of infection control strategies.

Using the PIV3 strains from the outbreak, we developed a method to characterize PIV3 strains through sequencing the HN gene that makes it possible to distinguish outbreak strains from concurrently circulating community strains of PIV3. The HN gene of PIV3 has been shown to have a high level of sequence diversity within this highly conserved region [12]. This region does not accumulate mutations over time, resulting in genetically heterogeneous viruses that circulate in the same time period [19]. By using this region, the finding of homologous strains of PIV3 is highly suggestive of the transmission of a single strain [12,20].

The results of sequence analysis of 27 PIV3 strains from the outbreak demonstrated that a single strain of PIV3 was responsible and therefore nosocomial spread between patients was the most likely route of transmission. The time intervals between the onset of symptoms of patients who successively tested positive during the outbreak were also in agreement with the incubation period of PIV3 (2 to 6 days) in keeping with nosocomial transmission. Twenty-six of the 27 outbreak strains were identical. Interestingly, one of the 27 outbreak strains differed from the other 26 outbreak strains by 1 base pair, which could be explained by virus evolution, in keeping with the known mutation rate of PIV3 of 7 base pairs per year [17] or it could represent a sequence error.

Targeted sequencing and whole-genome sequencing (WGS) each offer advantages and disadvantages for investigating virus transmission. Whole genome sequencing (WGS) enables comprehensive examination of the viral genome, allowing for the identification of genetic variations, including recombination events and mixed infections [21]. However, WGS is resource-intensive, expensive and is not available in all settings. We used targeted sequencing to differentiate between PIV3 strains during the outbreak. Targeted sequencing amplifies a target gene resulting in high sensitivity and specificity in identifying sequence variations [22]. This approach is cost-effective and can provide rapid results, which is advantageous during outbreak investigations. However, targeted sequencing can result in overlooking novel or unexpected genetic changes outside the predefined regions of interest, and mixed infections.

Infection prevention measures that have been associated with stopping the development of larger outbreaks of PIV3 among hematology and SCT patients include the timely isolation of the infected patient and patients who have been in contact with the infected patient for the length of the incubation period of PIV3 (6 days) and intensified hand hygiene measures by patients, staff and visitors [2,18]. Further, the use of a sensitive screening method to screen all new and admitted patients to identify symptomatic and asymptomatic patients and allow early cohort isolation is an effective method [18] as well as the use of a universal face mask strategy for those in contact with hematology or SCT patients [23]. In hindsight, we would have started isolation of exposed patients earlier, together with limiting contacts between patients. The limitation of visitors, which has high impact on the wellbeing of patients, might then have been prevented.

Recent studies have suggested that hematology outpatient facilities and waiting areas play an important role in nosocomial outbreaks of PIV3 on hematology wards [24,25]. Gürtler et al. [24] found that 46 % of patients had been seen in outpatient clinics during and around a nosocomial PIV3 outbreak. In the UMCG, the hematology outpatient facilities are in close proximity to the inpatient facilities. In an outbreak situation, screening of patients visiting the hematology outpatient clinic and sequencing of PIV3 strains from those patients would give useful information as to the role of the outpatient department in a nosocomial PIV3 outbreak and could guide the implementation of appropriate infection prevention in the outpatient department.

A limitation of this study is that we only screened patients for PIV3 positivity. It is probable that healthcare workers and visitors played a role in the transmission of PIV3 during the outbreak. Further studies investigating the role of staff and visitors in the transmission of PIV3 among hematology and SCT patients are needed. This information would contribute to optimal infection control strategies for outbreaks of PIV3 in this group of patients.

Furthermore, hematology patients can exhibit prolonged shedding of PIV3 due to their immunocompromised status, which can present challenges in outbreak management. Following patients longitudinally could offer insights into the significance of "prolonged shedders" during outbreaks. However, this study is constrained by the absence of longitudinal patient follow-up.

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CRediT authorship contribution statement

Laura Hughes: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Lilli Gard:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Monika Fliss:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Martijn Bakker:** Writing – review & editing, Investigation, Data curation. **Carin Hazenberg:** Writing – review & editing, Investigation, Data curation. **Xuewei Zhou:** Writing – review & editing, Investigation, Data curation. **Paulien Vierdag:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Karin von Eije:** Writing – review & editing, Investigation, Data curation. **Andreas Voss:** Writing – review & editing, Investigation. **Mariëtte Lokate:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Marjolein Knoester:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

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

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2024.105677.

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