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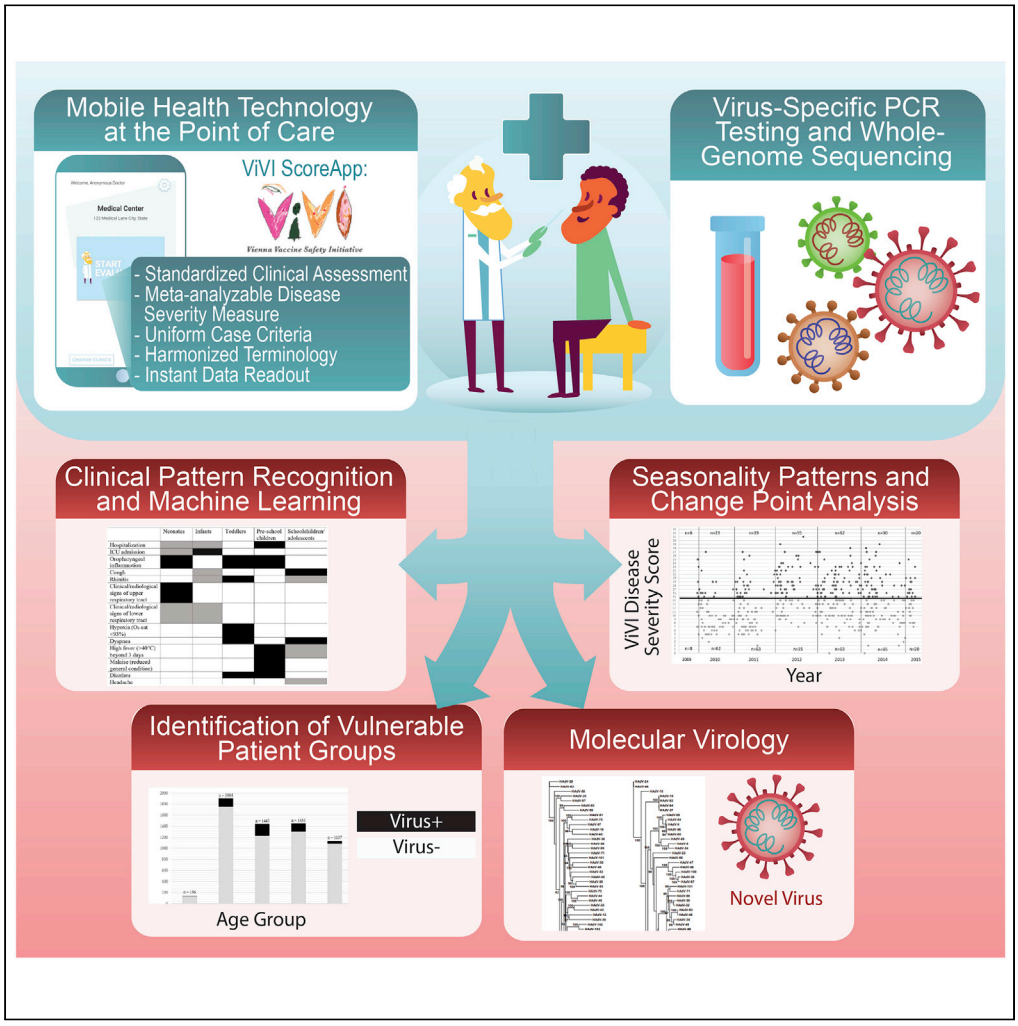

Patrick E. Obermeier, Albert Heim, Barbara Biere, ..., Tim Conrad, Brunhilde Schweiger, Barbara A. Rath

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Highlights

We used mobile health technology to enable clinical pattern recognition

The ViVI ScoreApp provided precision data for cross-cohort meta-analysis

Neonates with adenovirus infection are at risk of severe or fatal disease outcomes

In one neonate with disseminated disease, we found a new adenovirus: HAdV-D80

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Linking digital surveillance and in-depth virology to study clinical patterns of viral respiratory infections in vulnerable patient populations

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SUMMARY

To improve the identification and management of viral respiratory infections, we established a clinical and virologic surveillance program for pediatric patients fulfilling pre-defined case criteria of influenza-like illness and viral respiratory infections. The program resulted in a cohort comprising 6,073 patients (56% male, median age 1.6 years, range 0–18.8 years), where every patient was assessed with a validated disease severity score at the point-of-care using the ViVI ScoreApp. We used machine learning and agnostic feature selection to identify characteristic clinical patterns. We tested all patients for human adenoviruses, 571 (9%) were positive. Adenovirus infections were particularly common and mild in children ≥1 month of age but rare and potentially severe in neonates: with lower airway involvement, disseminated disease, and a 50% mortality rate (n = 2/4). In one fatal case, we discovered a novel virus: HAdV-80. Standardized surveillance leveraging digital technology helps to identify characteristic clinical patterns, risk factors, and emerging pathogens.

INTRODUCTION

Precision medicine aims to "classify individuals into subpopulations that differ in their susceptibility to a particular disease" (Committee on a Framework for Developing a New Taxonomy of Disease; National Research Council, 2011). The identification of patterns in disease presentation is particularly compelling for the early identification of new and emerging viral pathogens, e.g. in virus epi-/pandemics. Thus, health authorities advocate the implementation of precision methodology and use of artificial intelligence, including machine learning in both, clinical care and research of viral respiratory infectious diseases. The goal is to improve outbreak detection, in-depth epidemiological analysis, and the quality of patient care (Collins and Varmus, 2015; Gwinn and MacCannell, 2018).

For the identification of patient groups with a high risk of a particularly severe clinical course, a well-established outcome measure is needed, where the risk of interrater variability and bias is at a minimum. To this end, the Vienna Vaccine Safety Initiative (ViVI), an international non-profit research institute focusing on innovation in infectious diseases & vaccines, has developed and validated a standardized ViVI Disease Severity Score (ViVI Score, https://score.vi-vi.org), for instant clinical assessments at the point-of-care. The ViVI Score is available via mobile application capturing the patient's complex clinical presentation in real time. Based on a validated set of clinical parameters in line with World Health Organization criteria of uncomplicated and complicated influenza-like illness (ILI), a composite score is generated within 3 min, ranging from 0 to 48, and reflecting increasing disease severity with increasing values. The ViVI Score has since been validated in a 6,000 children cohort in Germany (Rath et al., 2017), a multicenter project in Europe (Rath et al., 2019), and in community clinics in the United States comprising both adults and children (Rath and Seng, 2020). The studies have shown that the ability to obtain an instant, well-standardized measure of clinical severity allows for rapid cross-cohort comparison and the identification of outliers in real time (Rath et al., 2017). This will enable the timely stratification of "individuals into subpopulations" (Committee on a Framework for Developing a New Taxonomy of Disease; National Research Council, 2011), as would be the requirement for precision medicine.

Young children may be affected by a number of viral respiratory infections with varying susceptibility and disease severity in different subgroups (Glynn and Moss, 2020). This has been studied extensively for

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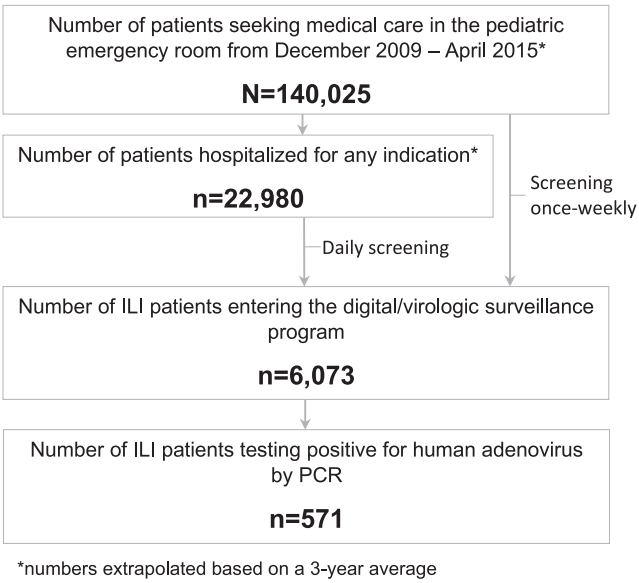

Figure 1. Precision screening flow chart

influenza virus (Wang et al., 2020b), human metapneumovirus (Wang et al., 2021a), parainfluenza virus (Wang et al., 2021b), or respiratory syncytial virus (RSV) infection (Li et al., 2021), for example. To neonates and infants, RSV infection is known to pose a major health threat (Li et al., 2020) while other respiratory viruses such as SARS-CoV-2 might usually be associated with milder disease in young children (Panetta et al., 2020; Glynn and Moss, 2020). However, one of the most prevalent respiratory viruses, human adenoviruses (HAdV), has been poorly studied in healthy young children. HAdV infection of the respiratory tract is usually considered mild and self-limiting. In certain high risk groups, such as immunocompromised individuals, HAdV infection can rapidly evolve into disseminated and fatal disease. Anecdotal reports and retrospective chart review which tend to be prone to observer bias and/or incomplete data (Obermeier et al., 2016) suggest that HAdV infection may also be serious in the very young, i.e. neonates. It has been associated with significant morbidity and mortality (Abzug and Levin, 1991; Ronchi et al., 2014; Elnifro et al., 2005; Henquell et al., 2009; Debast et al., 1996; Chiou et al., 1994; Baserga and Chan, 2018; Censoplano et al., 2018; Kelley, 2010; Montone et al., 1995; Rieger-Fackeldey et al., 2000; Auletta et al., 2019; Kim et al., 1997; Bajanowski et al., 1996; Castelli et al., 2011; Kinney et al., 1994; Angella and Connor, 1968; Moallem et al., 2016), but systematic prospective studies of HAdV incidence and severity in healthy children are lacking.

With this precision medicine study, we aim to investigate characteristic clinical patterns and disease severity of HAdV infection in children presenting with ILI based on a real-world dataset obtained during a 6-year digital/virologic surveillance program combining standardized patient assessments and disease severity scoring using mobile health (m-Health) technology for machine learning and pattern recognition analysis with in-depth virological testing in all patients.

The current study provides a proof of concept for improving quality of care and disease management through precision data and artificial intelligence. In addition, we exemplify the potential of point-of-care clinical severity assessments for the early identification of novel/emerging respiratory viral pathogens.

RESULTS

A total of 6,073 ILI patients participated in the digital syndromic and virologic surveillance program, 571 (9%) of which tested HAdV-positive (Figure 1), with the majority (65%) being <5 years of age. In detail, 4 neonates (0–28 days of age), 156 infants (29 days–12 months of age), 214 toddlers (13–24 months of age), 150 pre-school children (3–5 years of age), and 47 schoolchildren or adolescents (6–18 years of age) tested HAdV-positive (see Figure 2 for age distributions). In 44% of all HAdV-positive patients (n = 249/571), no other viral pathogen was detected. Table 1 gives an overview of baseline patient characteristics. Table 2 provides a descriptive list of co-infections.

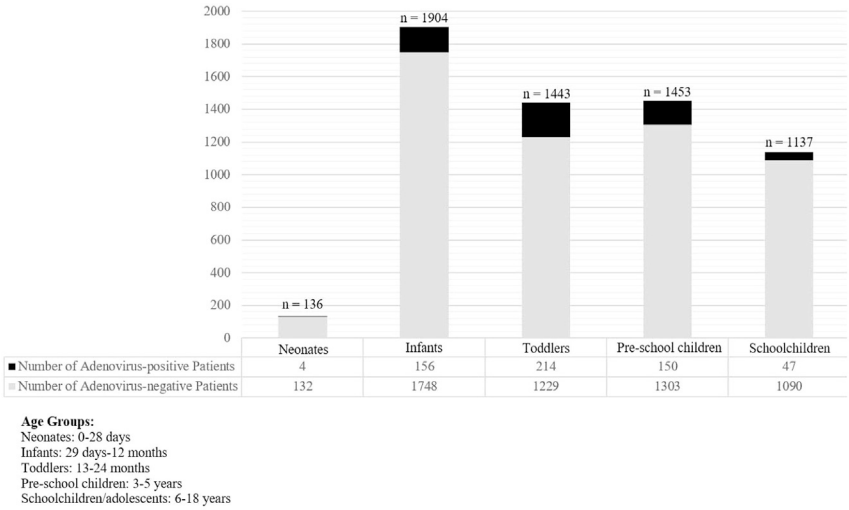

Figure 2. Age distribution of HAdV-positive (dark gray) and HAdV-negative (light gray) patients within the digital/virologic surveillance cohort

Age groups: Neonates: 0–28 days, infants: 29 days–12 months, toddlers: 13–24 months, pre-school children: 3–5 years, schoolchildren/adolescents: 6–18 years.

Above-average disease severity in HAdV-positive neonates and co-infections

Among all 6,073 ILI patients, the mean baseline ViVI Score value reflecting disease severity was 14.5 (Rath et al., 2017), representing the 50th percentile of the Cohort whereas the mean baseline ViVI Score value of all 571 HAdV-positive patients was 13.8 (≈46th percentile), thus indicating below-average disease severity in HAdV-positive patients in general. Table 3 depicts ViVI Score percentiles of the Cohort.

A total of 255/571 HAdV-positive patients (44%, mean age: 2 years, range: 0–16 years, 60% male, mean ViVI Score 19.2, range: 15–34) yielded above-average ViVI Scores, i.e. ≥15. We detected HAdV-positive cases with above-average ViVI Scores over the entire study period. In 2010, we identified the smallest proportion of HAdV-positive cases with ViVI Scores ≥15 (27%, 23/85 cases) compared to around 50% of HAdV-positive cases in 2013 and 2015. Figure 3 shows a scatter plot of ViVI Scores in HAdV-positive patients.

Except for young age (≤2 years), co-infection with another virus was associated with above-average disease severity: HAdV-positive patients with at least one viral co-infection (n=322/571) yielded a mean baseline ViVI Score of 14.6, slightly exceeding the ≈50th percentile of the Cohort. Among co-infected patients, disease severity ranked highest for HAdV/ human parainfluenzavirus (HPIV) co-infection (n=22/322), yielding a mean ViVI Score of 16.4 (≈60th percentile of the Cohort, Table 2).

Among all HAdV-positive patients (n=571), mean ViVI Scores were below-average in HAdV mono-infections (n=249, mean baseline ViVI Scores of 12.8, ≈40th percentile). Patients with HAdV mono-infection also had the lowest rates of need for hospitalization, critical care, and oxygen supplementation compared to the overall cohort average and patients with co-infection(s) (Table 1).

Comparing disease severity among different age groups of HAdV-positive patients, neonates were the only group presenting with an above-average baseline ViVI Score of 14.8 (≈57th percentile) and HAdV-positive neonates were the only HAdV-infected patient group where case fatalities occurred (see clinical vignettes below, **Neonate #1** and **#2**). The case fatality rate of HAdV-positive neonates was the highest of the entire cohort (0.3% vs. 0.1% overall case fatality rate).

Clinical vignettes of fatal HAdV infection

Neonate #1

In September 2014, a male previously healthy full-term neonate presented on day-of-life 13 with cough/rhinitis and T_{max} 39.4 °C, rapidly progressing to generalized compartment syndrome, acute respiratory

Table 1. Baseline patient characteristics

	Total (n = 6,073)	HAdV-positive (n = 571, 9%)			HAdV-negative (n = 5,502, 91%)
Median Age (range)	1.6 years (0–18.8 years)	1.5 years (0–17.1 years)			1.6 years (0–18.8 years)
		Overall (n = 571)	HAdV mono-infection[a] (n = 249)	Viral co-infection detected[a] (n = 322)	
Gender					
Male	3,399 (56%)	314 (55%)	139 (56%)	175 (54%)	3,085 (56%)
Female	2,674 (44%)	257 (45%)	110 (44%)	147 (46%)	2,417 (44%)
Outpatient	2,033 (33%)	239 (42%)	116 (47%)	123 (38%)	1,794 (33%)
Neonates[b]	9 (0%)	0 (0%)	0 (0%)	0 (0%)	9 (1%)
Infants[c]	496 (24%)	55 (23%)	24 (10%)	31 (10%)	441 (25%)
Toddlers[d]	474 (23%)	82 (34%)	36 (15%)	46 (14%)	392 (22%)
Pre-school children[e]	592 (29%)	80 (33%)	45 (18%)	35 (11%)	512 (29%)
Schoolchildren/Adolescents[f]	462 (23%)	22 (9%)	11 (4%)	11 (3%)	440 (25%)
Inpatient	4,040 (67%)	332 (58%)	133 (53%)	199 (62%)	3,708 (67%)
Neonates[b]	127 (3%)	4 (1%)	1 (0%)	3 (1%)	123 (3%)
Infants[c]	1,408 (35%)	101 (30%)	39 (16%)	62 (19%)	1,307 (35%)
Toddlers[d]	969 (24%)	132 (40%)	42 (17%)	90 (28%)	837 (23%)
Pre-school children[e]	861 (21%)	70 (21%)	36 (15%)	34 (14%)	791 (21%)
Schoolchildren/Adolescents[f]	675 (17%)	25 (8%)	15 (6%)	10 (3%)	650 (18%)
O_2 therapy	1,513 (25%)	98 (17%)	29 (12%)	69 (21%)	1,415 (26%)
Neonates[b]	41 (3%)	2 (2%)	1 (0%)	1 (0%)	39 (3%)
Infants[c]	529 (35%)	28 (29%)	8 (3%)	20 (6%)	501 (35%)
Toddlers[d]	405 (27%)	40 (41%)	10 (4%)	30 (9%)	365 (26%)
Pre-school children[e]	365 (24%)	23 (23%)	9 (4%)	14 (4%)	342 (24%)
Schoolchildren/Adolescents[f]	173 (11%)	5 (5%)	1 (0%)	4 (1%)	168 (12%)
Need for ICU[g] admission	1,303 (21%)	71 (12%)	25 (10%)	46 (14%)	1,232 (22%)
Neonates[b]	75 (6%)	2 (3%)	1 (0%)	1 (0%)	73 (6%)
Infants[c]	531 (41%)	19 (27%)	3 (1%)	16 (5%)	512 (42%)
Toddlers[d]	240 (18%)	26 (37%)	10 (4%)	16 (5%)	214 (17%)
Pre-school children[e]	238 (18%)	16 (23%)	6 (2%)	10 (3%)	222 (18%)
Schoolchildren/Adolescents[f]	219 (17%)	8 (11%)	5 (2%)	3 (1%)	211 (17%)
Fatal outcome	13 (0.1%)	2 (0.3%)	1 (0%)	1 (0%)	11 (0.2%)
Neonates[b]	2 (15%)	2 (100%)	1 (0%)	1 (0%)	0 (0%)
Infants[c]	5 (38%)	0 (0%)	0 (0%)	0 (0%)	5 (45%)
Toddlers[d]	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pre-school children[e]	2 (15%)	0 (0%)	0 (0%)	0 (0%)	2 (18%)
Schoolchildren/Adolescents[f]	4 (31%)	0 (0%)	0 (0%)	0 (0%)	4 (36%)
Mean baseline ViVI Score (range)	14.5 (0–34)	13.8 (0–34)	12.8 (2–34)	14.6 (0–29)	14.6 (0–33)
Neonates[b]	14.0 (2–25)	14.8 (10–22)	10 (10)	16.3 (12–22)	13.9 (2–25)

(*Continued on next page*)

Table 1. *Continued*

	Total (n = 6,073)	HAdV-positive (n = 571, 9%)			HAdV-negative (n = 5,502, 91%)
Infants[c]	14.6 (0–33)	13.7 (2–27)	12.3 (4–27)	14.6 (2–27)	14.7 (0–33)
Toddlers[d]	15.3 (0–32)	14.3 (0–31)	13.5 (5–31)	14.8 (0–29)	15.4 (0–32)
Pre-school children[e]	14.6 (0–32)	13.6 (0–28)	13.0 (2–28)	14.3 (0–28)	14.7 (0–32)
Schoolchildren/Adolescents[f]	13.4 (0–34)	12.7 (3–34)	11.3 (3–34)	14.3 (6–25)	13.4 (0–33)

[a]PCR testing performed for influenza A/B virus, respiratory syncytial virus, rhinovirus, metapneumovirus, bocavirus, parainfluenza, and betacoronavirus in addition to HAdV.
[b]Neonates: 0–28 days.
[c]Infants: 29 days–12 months.
[d]Toddlers: 13–24 months.
[e]Pre-school children: 3–5 years.
[f]Schoolchildren/adolescents: 6–18 years.
[g]Intensive care unit.

distress syndrome (ARDS), and encephalitis with fatal outcome in week 5 of life, despite intensive care, extracorporeal membrane oxygenation (ECMO), and ventricular-peritoneal shunt. ViVI Scores peaked at 33 (99th percentile). Intravenous immunoglobulin and cidofovir (1 mg/kg body weight, combined with probenecid) were administered on hospital days 6 and 14. Tracheobronchial lavage samples on day 4 tested positive for HAdV species D (Cq value 24). No alternative pathogens were detected. HAdV species D was also detected in blood, stool, cerebrospinal fluid, and urine. Figure 4A illustrates disease progression with continuously increasing ViVI Score values reflecting progressive disease, as well as intensifying pulmonary opacification and abdominal distension on babygram images. Figure 4B shows virus load dynamics in different body compartments with a plateau in blood samples and decreasing virus loads in urine after cidofovir administration.

Neonate #2

In November 2011, a female pre-term neonate with persistent ductus arteriosus and left ventricular dysfunction developed ARDS requiring ECMO following delivery. The ViVI Score was 22 (90th percentile). Nasopharyngeal swabs tested positive for HAdV (Cq value 33) and human bocavirus (Cq value 35); an external ear swab grew *Listeria monocytogenes* serotype 4b. The patient expired on day 2 of life despite maximum life support, high-dose intravenous corticosteroids, and catecholamines.

Severe lower airway infection is characteristic of HAdV-positive neonates

Feature selection analysis was performed to identify clinical characteristics associated with HAdV infection in different age groups. Feature selection confirmed that HAdV-positive neonates have a distinctive clinical presentation compared to older children. Severe lower respiratory tract infections, often requiring critical care, are hallmarks of adenovirus infection in this vulnerable age group (relative importance levels $\geq$80 and $\geq$60, respectively). Each of the neonatal cases in the digital/virologic surveillance program showed significant lower airway disease leading to respiratory distress and hospitalization. Children >2 years of age were more likely to experience mild upper respiratory or systemic symptoms such as fever and headache, rarely requiring hospitalization. Figure 5 depicts relative variable importance levels for top-5 clinical features of HAdV-positive patients in different age strata.

Molecular characterization of HAdV-D80—a novel adenovirus

A tracheobronchial lavage sample from **Neonate #1** underwent whole genome sequencing at the German Reference Laboratory for Adenoviruses, leading to the discovery of a novel adenovirus genotype which was designated HAdV-D80 (P19,23H28F22/2014/DEU) by the Adenovirus Working Group (GenBank accession number: KY618679).

Whole genome phylogenetic analysis revealed HAdV-D22 as the closest relative of the novel HAdV-D80 genotype (Figure 6A). See Figures 6 and 7 for phylogenetic clustering of the novel HAdV-D80 gene regions with other HAdV-D types.

Table 2. Descriptive list of co-infections (N = 322)

	HAdV + HBoV (n = 132)	HAdV + HCoV (n = 36)	HAdV + FLU A (n = 15)	HAdV + FLU B (n = 3)	HAdV + HMPV (n = 20)	HAdV + HPIV (n = 22)	HAdV + HRV (n = 142)	HAdV + RSV (n = 64)
Gender								
Male	76 (58%)	23 (64%)	10 (67%)	0	10 (50%)	7 (32%)	79 (56%)	33 (52%)
Female	56 (42%)	13 (36%)	5 (33%)	3 (100%)	10 (50%)	15 (68%)	63 (44%)	31 (48%)
Outpatient	52 (49%)	14 (39%)	9 (60%)	2 (66%)	9 (45%)	8 (36%)	53 (37%)	23 (36%)
Inpatient	80 (61%)	22 (61%)	6 (40%)	1 (33%)	11 (55%)	14 (64%)	89 (63%)	41 (64%)
O_2 therapy	26 (20%)	6 (17%)	5 (33%)	0	6 (30%)	8 (36%)	26 (18%)	22 (34%)
Need for ICU admission	19 (14%)	5 (14%)	1 (7%)	1 (33%)	0	4 (18%)	21 (15%)	10 (16%)
Fatal outcome	1 (1%)	0	0	0	0	0	0	0
Mean baseline ViVI Score (range)	14.5 (0–29)	14.5 (4–27)	14.7 (7–25)	12.7 (6–18)	14.5 (7–27)	16.4 (5–28)	14.3 (0–29)	15.3 (0–27)

HAdV, human adenovirus; HBoV, human bocavirus; HCoV, human coronovirus; FLU A/B, influenza A/B virus; HMPV, human metapneumovirus; HPIV, human parainfluenzavirus; HRV, human rhinovirus; ICU, intensive care unit; RSV, respiratory syncytial virus.

Epidemiology of HAdV infections on the German national level

For comparison, we analyzed HAdV genotypes detected within the same study period all over Germany: During nation-wide surveillance, 420 HAdV-positive diagnostic specimens from sites all over Germany were typed at the German Reference Laboratory in Hannover between 10/2009 and 04/2015. Those specimens originated from adults and children, but neonates and infants <6 months were not represented. From infants aged 6–12 months, 48 HAdV-positive samples (11.4%) had been collected. Species C predominated in this age group (n = 26/48, 54%) with 11 positives for HAdV-C1, 11 for HAdV-C2, and 4 for HAdV-C5. Other species included Species A (n = 7/48, 14.5%, all type HAdV-A31) and F (n = 15/48, 31.2%, all type HAdV-F41), whereas Species D was not detected between 6 and 12 months of age. Species D was detected more commonly beyond the infant age group with 165 samples originating from patients >1 year of age, i.e. 15 (9.1%) in children between 3 and 18 years and 150 (90.9%) in adults. The majority of Species D (93.3%) were detected in eye swabs of patients with (kerato-)conjunctivitis (types HAdV-D8, -D37, -D53, -D56, and -D64). All others (types HAdV-D9, -D20, -D22, -D29, -D42, and -D67) were detected in respiratory tract specimens, feces, and blood.

DISCUSSION

We report a precision medicine study combining systematic virological testing with real-time digital clinical assessments, enabling the first standardized and fully meta-analyzable investigation of clinical characteristics and disease severity associated with HAdV infection using a previously validated disease severity score via mobile application in a pediatric ILI cohort. This nested approach allowed us to eliminate reporting bias while performing comprehensive machine learning and pattern recognition analysis, and to determine the real-world impact of specific respiratory viral infections.

Arriving at precision diagnoses using m-Health technology

We established a standard operating procedure for the prospective precision screening (Wang et al., 2020a) of all consecutive pediatric patients presenting to one of the busiest pediatric hospitals in Europe, during an observation period of six years (Tief et al., 2016; Rath et al., 2017). The rigorous use of precision medicine methodology and pre-defined entry criteria (WHO, 2009) helped to diminish reporting bias, with a known denominator of 6,073 patients with ILI. Enabling precision analysis, each patient was assessed using the ViVI Score mobile application at the point-of-care. The app allows the user to assess the most critical items depicting individual risk and disease severity in patients with respiratory viral infections. The severity score has been described elsewhere (Rath et al., 2017), and is based on systematic literature review and prospective validation in more than 10,000 patients to date (Rath et al., 2017, 2019; Rath and Seng, 2020).

Key parameters reflecting the patient's clinical presentation were collected, requiring the user to determine the presence and absence of specific signs and symptoms at the time of assessment. This allows

Table 3. ViVI Score (0–48) percentiles of the Cohort

ViVI-Score	Percentile of the Cohort
3	1st
5	2nd–4th
6	5th–8th
7	9th–13th
8	14th–18th
9	19th–23rd
10	24th–29th
11	30th–34th
12	35th–39th
13	40th–45th
14	46th–51st
15	52nd–56th
16	57th–62nd
17	63rd–67th
18	68th–72nd
19	73rd–78th
20	79th–82nd
21	83rd–86th
22	87th–89th
23	90th–92nd
24	93rd–94th
25	95th–96th
26	97th
27	98th
28	99th

capturing pertinent negative data with precision, e.g. the *absence* of a specific sign or symptom as well as its presence. This provides a significant advantage over data mining from routine care medical records, which do not always allow the distinction between the absence of a finding versus non-assessment. By forcing the user to make this discernment at the bedside, the ViVI Score Mobile App eventually eliminated indeterminate data (Obermeier et al., 2016; Rath et al., 2017; Alchikh et al., 2019). In addition, the app verified whether a patient fulfilled a simplified ILI case definition (body temperature $\geq$38 °C and $\geq$1 respiratory symptom/s) (Alchikh et al., 2019; Tief et al., 2016; Rath et al., 2017). Verification of case criteria at the point-of-care has been shown to be particularly useful in prospective surveillance, providing highly standardized datasets while avoiding selection and ascertainment bias (Cameron et al., 2020). Of note, the resulting precision dataset facilitates downstream applications, such as machine learning and pattern recognition analysis, as well as the testing of cases against alternative case definitions by the US Centers for Disease Control and Prevention (CDC) or the European CDC, thus facilitating meta-analyzability of data (Alchikh et al., 2019).

In the acute care setting, use of the ViVI ScoreApp can help to grade disease severity in real time if implemented into the medical documentation workflow, thereby aiding physicians to allocate scarce intensive care beds based on evidence and in a transparent way, which has become a key concern during the COVID-19 pandemic (Frej et al., 2021).

Honing in on emerging pathogens - adenovirus infection in patients with ILI

Interdisciplinary linkage of digital syndromic and virologic surveillance allowed for the investigation of the true clinical impact of novel and emerging viruses in their original epidemiological context: Using predefined precision algorithms, this study focused on patients with ILI who tested positive for HAdV. Specifically, we aimed to investigate disease severity and characteristic clinical patterns associated with HAdV

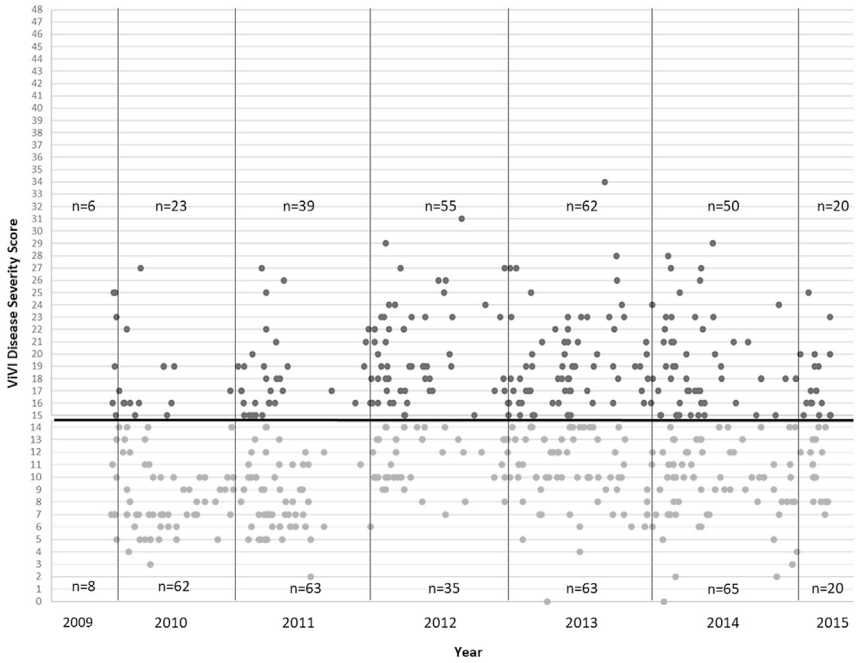

Figure 3. Scatterplot of ViVI Scores measured in HAdV-positive patients (N = 571)

ViVI Score values may range from 0–48, reflecting increasing disease severity with increasing ViVI Scores. The vertical bold black line marks the cohort average ViVI Score of 14.5, i.e. the 50th percentile in the overall cohort. Dark gray dots indicate cases with above-average ViVI Scores and light gray dots indicate cases with below-average ViVI Scores. Numbers (n) indicate the number of cases below the cohort average ViVI Score and above the cohort average, respectively.

infection and to delineate potential age strata at risk of severe clinical manifestation because systematic evidence for healthy children was lacking as opposed to other frequent respiratory viruses.

Human adenoviruses are emerging pathogens which can cause mild to severe or even fatal disease. In healthy individuals and especially in children, HAdV infection is usually deemed common and mild (Gaunt et al., 2011). Therefore, testing might be performed rarely in routine care (Alchikh et al., 2019). Reference data on HAdV genotypes and detection rates from nation-wide passive surveillance within the same time frame as our study emphasized that children below the age of 6 months might be rarely tested for HAdV, in particular.

With rigorous PCR testing for HAdV of any patient with ILI regardless of the doctor's clinical suspicion within our inception cohort, we specified that respiratory HAdV infection is most common in children aged 1–5 years, but rare in neonates.

Our understanding of disease severity associated with HAdV infection in young children presenting with ILI symptoms usually comes from anecdotal or epidemiologic reports and retrospective chart review (Wu et al., 2020; Cai et al., 2020; Gaunt et al., 2011; Goikhman et al., 2020), potentially influencing healthcare experts' willingness to test for HAdV in otherwise healthy infants and neonates, as might be seen from nation-wide surveillance data.

To measure disease severity, case fatality rates or surrogate parameters such as "need for hospitalization/critical care", or "oxygen requirement" have traditionally been used. These, however, tend to be coarse (fatal vs. non-fatal) or biased (Rath et al., 2017). To enable precision disease severity scoring, we used the ViVI Disease Severity Score at the point-of-care. We found out that HAdV infection in hospitalized children in general was associated with below-average disease severity. In neonates though, HAdV infection led to above-average disease severity (57th percentile) with a mortality rate of 50%. Our study is the first to provide a precision measure of disease severity associated with HAdV infection in pediatric patients with ILI, delineating neonates at increased risk of severe HAdV infection.

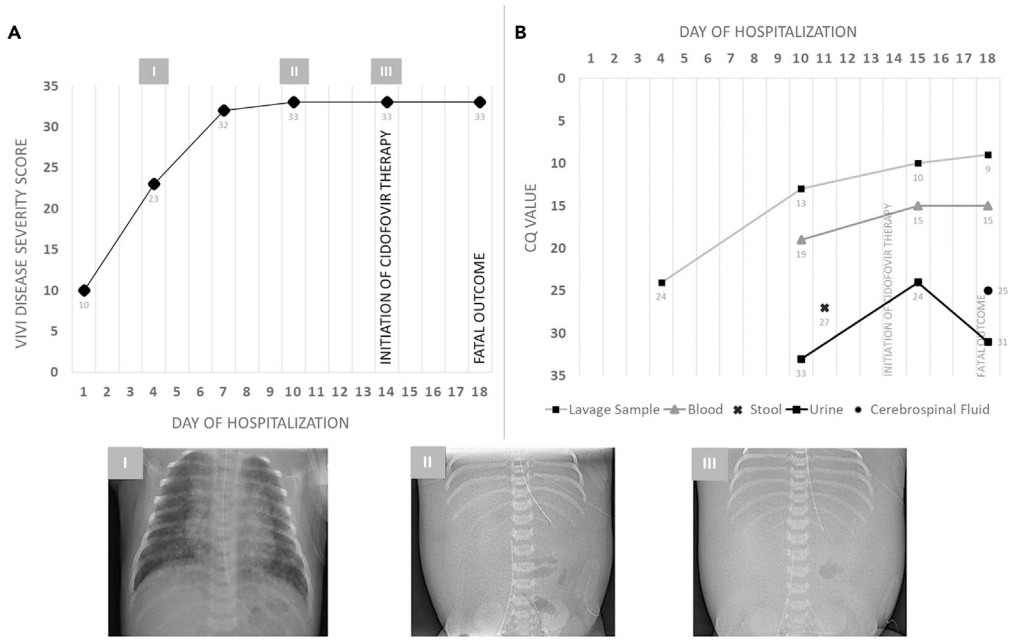

Figure 4. ViVI Score dynamics illustrating disease progression in the HAdV-D80 index case

Initial X-ray on hospital day 4 (I) showed diffuse patchy lung infiltrates, corresponding to respiratory distress syndrome grade I. Follow-up imaging on hospital day 10 (II) showed homogenic lung opacity due to diffuse bilateral atelectasis ("white lungs"), corresponding to respiratory distress syndrome grade IV. Follow-up imaging on hospital day 14 (III) revealed generalized compartment syndrome with ongoing abdominal swelling, pleural, and pericardial effusion. (B) Adenovirus load kinetics in different body compartments over time: Cq values (inverted vertical axis) during PCR-testing were used to estimate virus load. Abbreviations: ViVI – Vienna Vaccine Safety Initiative, Cq-Quantitation Cycle.

Use of the ViVI ScoreApp offered the opportunity of portraying a vivid picture of what HAdV infection would typically look like in different age strata. Using a machine learning methodology suitable for small sample sizes in our study, we showed that severe lower respiratory tract infection was characteristic of HAdV-positive neonates. Fever was a distinctive feature among older HAdV-positive children but rarely present in neonates. Given our findings, adenovirus diagnostics should be performed in afebrile neonates with lower respiratory tract infection or acute respiratory distress, especially if no other pathogen is found.

We detected co-infections more frequently than HAdV mono-infection in our Cohort. Overall, the presence of at least one more virus in addition to HAdV led to slightly increased disease severity as reflected by above-average ViVI Disease Severity Scores. As previously reported, this effect seemed to depend on the specific co-infection (Rath et al., 2017) and was most obvious for HAdV/HPIV co-infection. However, these findings need further investigation and may require evaluation on a case-by-case basis, including the investigation of host factors.

Use of the ViVI Score also enabled standardized assessment of disease severity during a specific time frame, independent of the absolute number of cases identified in the inception cohort. The current pandemic exemplified that it is of critical importance to specify what is meant when discussing a "heavy season", i.e. whether "heavy" refers to case burden (absolute numbers or prevalence) or disease burden (average disease severity/case). Using the ViVI ScoreApp at the point-of-care provides immediate measures of severity in relation to the number of confirmed cases at any time throughout a season or wave, with regards to a specific respiratory virus such as influenza viruses, HAdV, or coronaviruses. For HAdV, we detected no clear temporal clustering of severe cases over the 6-year study period.

Discovery of a novel virus - a note on HAdV-D80

In-depth virological analysis of HAdV strains has previously proven to be of clinical relevance in that certain species and genotypes may be associated with distinct clinical pictures and/or disease severity, e.g. HAdV-B7 accounting for severe respiratory tract infection (Gray et al., 2007; Lee et al., 2010; Cai et al., 2020). In our

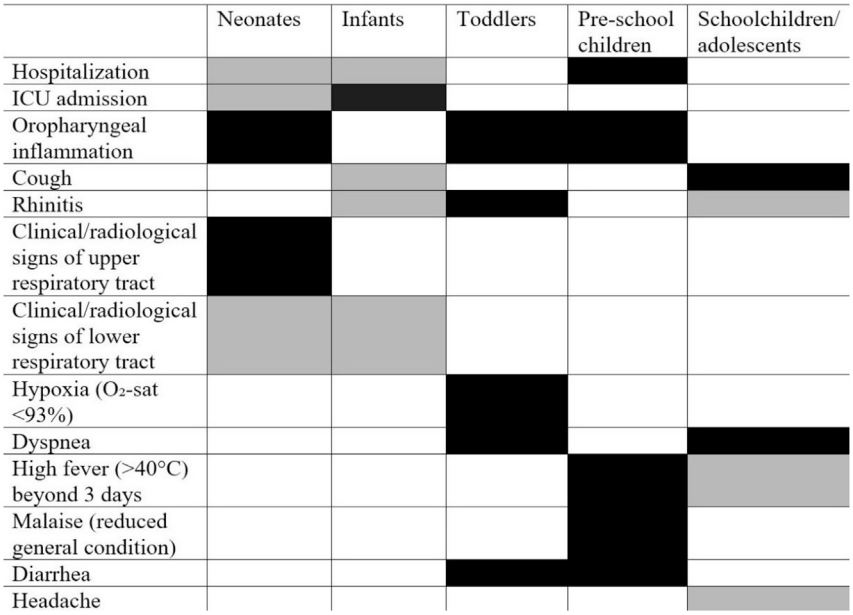

Figure 5. Relative variable importance analysis for top-5 clinical features of HAdV-positive patients (n = 571) using the permutation feature importance measure

The relative feature importance of the most important feature is set to 100. The values of the other features are scaled accordingly relative to the most important feature. Level of importance of top-5 features is reflected as follows: white indicates importance <33, gray indicates importance ≥33 and <66, black indicates importance ≥66.

study, whole genome sequencing of the clinical isolate of one deceased HAdV-positive neonate led to the discovery of a novel HAdV genotype, according to the Human Adenovirus Working Group: HAdV-D80.

HAdV-D80 belongs to species (D) and shows recombination events "hijacking" neutralization determinants, which may contribute to immune evasion and increased virulence (Walsh et al., 2009). HAdV-D80 provides a novel E4 region sequence, whereas most of its genomic backbone is closely related to HAdV-D22. E4 gene products have multiple functions, some of which relating to virulence. E4 gene products also play a role in DNA replication and hijacking of cellular protein synthesis, leading to accumulation of late viral mRNAs and proteins thus potentially promoting resistance to interferon-based immune response driven by virus-related factors (Weitzman, 2005). Indeed, E4 gene products might be of prominent clinical relevance as to be seen from adenovirus vectors of the second generation, benefiting from deletion of E4 region sequences, leading to impaired viral gene expression (Kreppel and Hagedorn, 2021).

Disease severity and virus dissemination as seen in the neonatal HAdV-D80 index case presented herein may also be driven by deviant immune responses (Adkins et al., 2004), lack of maternal antibodies, and the timing of virus transmission (Glynn and Moss, 2020). Our findings affirm previous studies that the host's age may have considerable impact on infectious disease outcomes, thus corroborating the investigation of vulnerable patient groups, e.g. neonates with HAdV infection.

Our study illustrates the need for licensed adenovirus treatment options. By now, no antivirals are licensed for HAdV treatment in the neonatal age bracket. Currently, cidofovir is the only licensed adenovirus treatment, but (off-label) use in children remains limited due to its nephrotoxicity. Brincidofovir, a cidofovir ester with favorable bioavailability and safety profiles may become an alternative(Ison and Hayden, 2016) but has only been tested in subjects aged ≥2 months of age (ClinicalTrials.Gov, 2021 [Internet]. Bethesda (MD): National Library of Medicine (US)).

Handling real-world patient data

Within this digital surveillance study, we successfully used m-Health technology to gather real-world patient data. Real-world data have been leveraged increasingly, especially during the coronavirus infectious disease 2019 (COVID-19) pandemic. Use of real-world evidence has become common practice for those

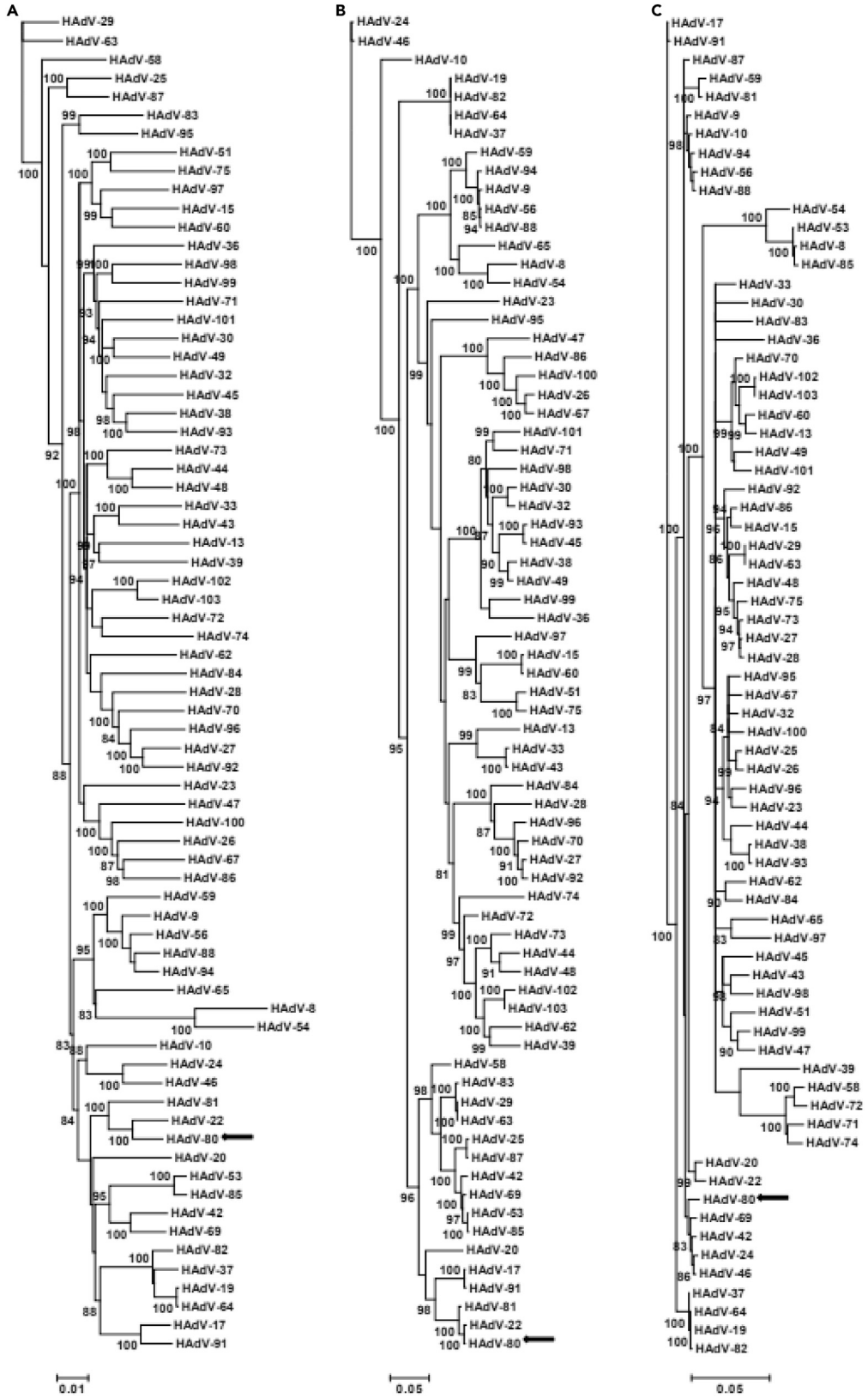
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Figure 6. HAdV-D80 phylogenetic clustering (whole genome, E4, E3)

Phylogenetic clustering of the HAdV-D80 (A) whole genome, (B) early gene region 4 (E4), and (C) early gene region 3 (E3) sequence with all other HAdV-D prototype sequences.

Bootstrap values > 80% were considered robust. Arrow indicates the clustering position of HAdV-D80.

aiming to improve pandemic preparedness and response (Drury and O'Connor, 2021; Pillai, 2021). Regulatory agencies and stakeholders from both, academia and industry, endorse compliance with framework conditions for real-world data to become actionable (Pillai, 2021; Berger et al., 2017; Dolgin, 2020; Drury and O'Connor, 2021; Jarow et al., 2017). In a contemporary opinion article, Pillai outlined key premises and avenues toward meaningful real-world data and systems in the context of pandemic preparedness (Pillai, 2021). The ViVI Score Mobile App was designed to facilitate timely and accurate diagnoses in real time and to enable comprehensive clinical assessments and decision support, including a standardized disease severity measure. Data harmonization and compliance with international data standards were among the key concerns in the development process. Not only is the ViVI Score comparable and meta-analyzable across different sites but also the app is constructed in full compliance with Clinical Data Interchange Standards Consortium (CDISC) standards (Souza et al., 2007), allowing for swift data interoperability and instant readouts to regulatory agencies issuing the same standards(Rath et al., 2017), e.g. the United States Food and Drug Administration (FDA) (U.S. Food and Drug Administration, 2017).

Outlook

Use of the methodologies described herein at multiple sites in both children and adults and including patient-generated data will be continued within the partnering for enhanced digital surveillance of influenza-like disease and the effect of antivirals and vaccines (PEDSIDEA) project (Rath et al., 2019). This will allow additional investigation of factors influencing disease severity associated with viral respiratory infections, e.g. biomarkers such as single-nucleotide polymorphisms of the host (Ghafouri-Fard et al., 2020; Forbester and Humphreys, 2021). Research is underway investigating the implementation of the ViVI Score into machine learning models to predict certain respiratory viruses and the risk of severe disease.

Also, further clarification of the role of emerging pathogens such as HAdV-D80 is warranted, including investigation of its role at the opposite extreme of age, i.e. the elderly. Future studies will focus on the importance of potential virus-virus interactions in case one individual has been infected by multiple viruses (Nickbakhsh et al., 2019). In this regard, use of novel virus detection techniques, e.g. agnostic metagenomic next-generation sequencing will soon find its way into the diagnostic workup, thus allowing to diminish bias in the laboratory context and enable detection of pathogens that have so far been unknown as has been the case with MERS-CoV or SARS-CoV-1 and -2.

Conclusion

We expect the precision medicine approach combining the use of m-Health technology at the bedside with machine learning and pattern recognition analysis and in-depth virology to be useful for virus outbreak situations by facilitating timely signal detection and clinical decision support.

By the example of respiratory HAdV infection, we identified neonates being at risk of life-threatening disease based on a real-world patient dataset. In this vulnerable patient group, rapid-turnaround diagnostics and effective antivirals are needed. Whole genome sequencing leads to the discovery of a novel virus: HAdV-D80.

Limitations of the study

The project had several strengths and limitations. First, the number of HAdV-positive neonates was very low. Albeit this being a finding itself, it impedes statistical analysis. For this reason, we refrained from performing classical significance testing but applied biostatistics analysis and machine learning methodology which is commonly considered suitable for very small case numbers. Ongoing surveillance leading to larger case numbers may further refine analysis results. Second, only the HAdV-D80 prototype isolate underwent deep sequencing allowing for exact phylogenetic analysis. However, this isolate was the only sample characterized as species (D) by fluorescence melting curve analysis (FMCA), thereby attracting special attention in a set of samples from patients with respiratory disease. Genotyping was not performed on all samples. For reference and comparison, we included genotyping results from nation-wide surveillance conducted at the Germany National Reference Laboratory collected and analyzed within the same period of time.

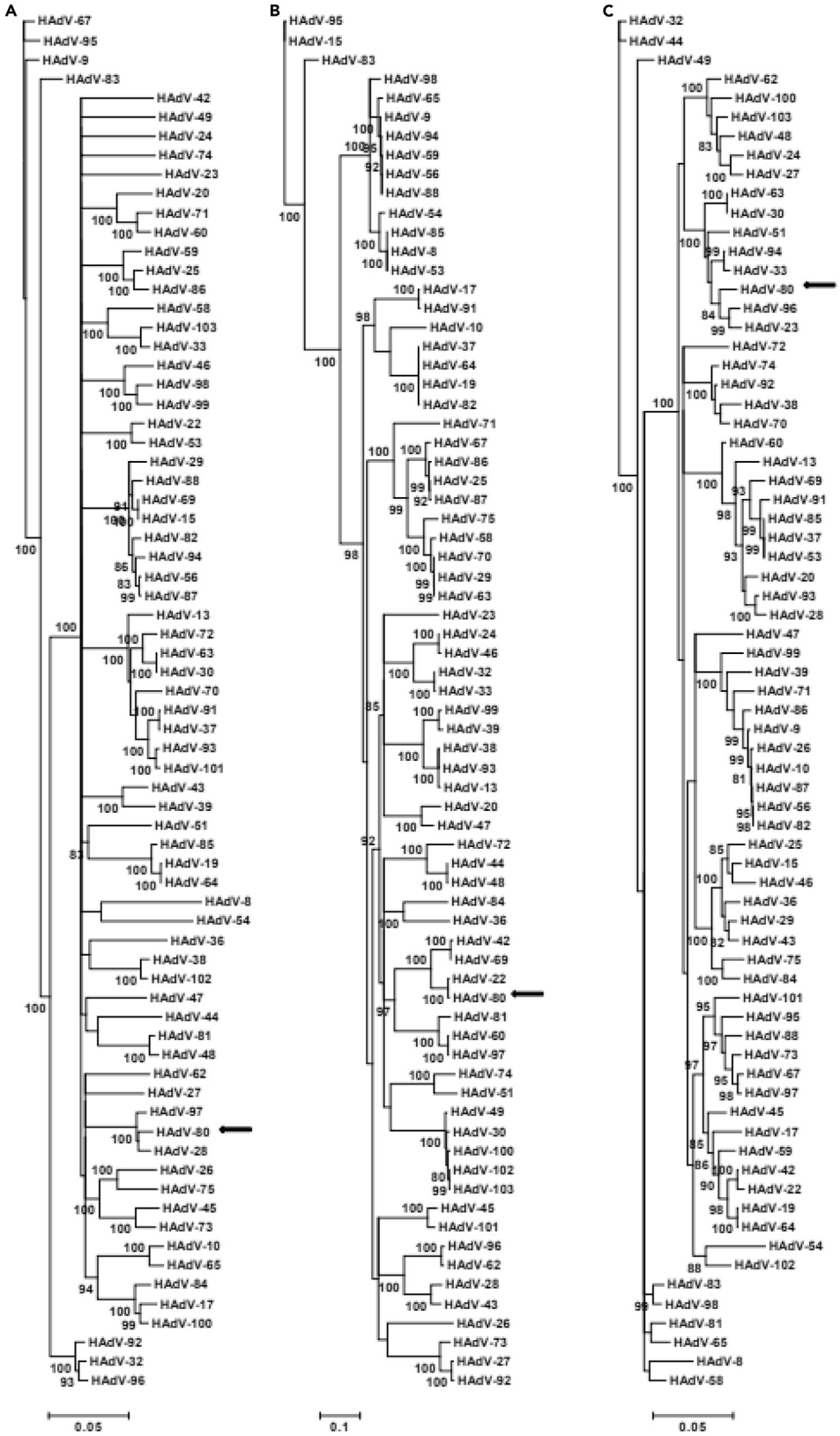
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Figure 7. HAdV-D80 phylogenetic clustering (hexon, fiber, penton)
Phylogenetic clustering of the HAdV-D80 (A) hexon gene, (B) fiber gene, and (C) penton base gene sequence with all other HAdV-D prototype sequences.
Bootstrap values > 80% were considered robust. Arrow indicates the clustering position of HAdV-D80.

Third, we applied conventional molecular virology testing, i.e. specific single PCR assays, to test for a total of nine major respiratory viruses, including HAdV. Thus covering the majority of usual viruses, it still remained unclear whether other viruses, e.g. human herpes- or enteroviruses, might have contributed to disease severity too. While agnostic molecular testing such as metagenomic next-generation sequencing might have detected a broader range of pathogens, PCR assays usually yield higher test accuracy (Chiu and Miller, 2019).

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Materials availability
  - Data and code availability
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- QUANTIFICATION AND STATISTICAL ANALYSIS

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AUTHOR CONTRIBUTIONS

Conceptualization and supervision of the work: BR, BS. Development of methodology: BR, BS. Conducting formal analysis: BR, BS, AH, BB, TC, MA, EH, PO. Provision of study materials, reagents, materials, patients, laboratory samples, computing resources, and other analysis tools: BR, BS, AH, TC. Data curation: BR, BS, AH, PO. Writing of the original draft: PO. Review and editing of the manuscript draft: all authors. Project administration: BR. Funding (in-kind): BR, BS.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
Human mastadenovirus D isolate human/DEU/Berlin/2014/80[P19/23H28F22]	GenBank	KY618679.1, https://www.ncbi.nlm.nih.gov/nuccore/KY618679.1
Biological samples		
Clinical samples	Vienna Vaccine Safety Initiative (ViVI), https://www.vi-vi.org	N/A
Chemicals, peptides, and recombinant proteins		
MiSeq Reagent Kit v3 (600-cycle)	Illumina	MS-102-3003, https://emea.illumina.com/products/by-type/sequencing-kits/cluster-gen-sequencing-reagents/miseq-reagent-kit-v3.html
Nextera XT DNA Library Preparation Kit	Illumina	FC-131-1096, https://emea.illumina.com/products/by-type/sequencing-kits/library-prep-kits/nextera-xt-dna.html
Deposited data		
Human mastadenovirus D isolate human/DEU/Berlin/2014/80[P19/23H28F22]	GenBank	KY618679.1, https://www.ncbi.nlm.nih.gov/nuccore/KY618679.1
Experimental models: Cell lines		
A549	National Reference Center for Influenza	JNCI: Journal of the National Cancer Institute, Volume 51, Issue 5, November 1973, Pages 1417–1423, https://doi.org/10.1093/jnci/51.5.1417, https://academic.oup.com/jnci/article-abstract/51/5/1417/962555?redirectedFrom=fulltext
Oligonucleotides		
Oligonucleotides for hAdV species A-F detection	National Reference Center for Influenza	Barbara Chmielewicz, Andreas Nitsche, Brunhilde Schweiger, Heinz Ellerbrok, Development of a PCR-Based Assay for Detection, Quantification, and Genotyping of Human Adenoviruses, Clinical Chemistry, Volume 51, Issue 8, 1 August 2005, Pages 1365–1373, https://doi.org/10.1373/clinchem.2004.045088, https://academic.oup.com/clinchem/article/51/8/1365/5629946
Oligonucleotides for hAdV species genotyping by FMCA	National Reference Center for Influenza	Barbara Chmielewicz, Andreas Nitsche, Brunhilde Schweiger, Heinz Ellerbrok, Development of a PCR-Based Assay for Detection, Quantification, and Genotyping of Human Adenoviruses, Clinical Chemistry, Volume 51, Issue 8, 1 August 2005, Pages 1365–1373, https://doi.org/10.1373/clinchem.2004.045088, https://academic.oup.com/clinchem/article/51/8/1365/5629946

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
ViVI Disease Severity Score	Vienna Vaccine Safety Initiative (ViVI), https://www.vi-vi.org	Rath, B., Conrad, T., Myles, P., Alchikh, M., Ma, X., Hoppe, C., Tief, F., Chen, X., Obermeier, P., Kisler, B. & Schweiger, B. 2017. Influenza and other respiratory viruses: standardizing disease severity in surveillance and clinical trials. Expert Rev Anti Infect Ther, 15, 545-568, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7103706/; https://score.vi-vi.org
SPSS Statistics	IBM Corp. Armonk, NY	RRID:SCR_019096, https://www.ibm.com/products/spss-statistics
R	R Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2019	https://www.r-project.org/
CLC Genomics Workbench	Qiagen	832,494, https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-clc-genomics-workbench/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact Barbara Rath (barbara.rath@vi-vi.org, @Vi_VIorg).

Materials availability

The ViVI Disease Severity Score Mobile App can be downloaded via https://score.vi-vi.org/or from the App Store or Google Play store.

Data and code availability

Data

Clinical data in this Quality Improvement Program are by definition subject to patient data privacy protection, thus not made public. Qualified researchers may contact the Lead Investigator with any questions or requests. The full HAdV-D80 prototype genome sequence is publicly available under GenBank accession number: KY618679.

Code
This paper does not report original code.
All other Items
Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

METHOD DETAILS

From 12/2009-04/2015, 6,073 pediatric in- and outpatients (median age: 1.6 years, range 0–18.8 years, 56% male) with ILI (fever $\geq$38 °C and $\geq$1 respiratory symptom/s and/or physician diagnosis of ILI) participated in a prospective surveillance and quality management (QM) program at the Charité Department of Pediatrics in Berlin, Germany (institutional review board (IRB) number: EA4/008/10) in collaboration with the National Reference Center for Influenza at the Robert Koch-Institute (Tief et al., 2016; Rath et al., 2017). The ratio of males to females was almost 1:1 in all major subgroups of patients, i.e. in the overall cohort, amongst HAdV-positive/-negative patients, patients with viral co-infections and patients testing negative for any virus (Table 1).

The study was performed in compliance with the ethical standards of the Declaration of Helsinki and according to German laws. Written informed consent was waived by the IRB (EA4/008/10) for the purpose of quality improvement and infection control given the observational nature of the study. Verbal informed consent was obtained from all patients and/or parents or caretakers of underage patients.

An independent QM team used a mobile application that was designed based on the user-centered principles of design-thinking to assess patients in a standardized manner, computing the ViVI (Vienna Vaccine Safety Initiative) Disease Severity Score in real-time (Tief et al., 2016; Rath et al., 2017; Rath, 2015, 2017). The ViVI Score is a published and validated 22-item weighed composite clinical score ranging from 0-48, with increasing values reflecting increasing disease severity (Rath et al., 2017; Ma et al., 2018). ViVI Score parameters are weighed in accordance with whom criteria for uncomplicated, complicated, and progressive disease (WHO, 2009).

In all patients, nasopharyngeal and/or tracheobronchial specimens were collected and delivered to the Robert Koch-Institute for PCR-testing for human adenoviruses as well as influenza virus A/B, respiratory syncytial virus (RSV), human rhinovirus, metapneumovirus, bocavirus, parainfluenza, and betacoronaviruses, irrespective of routine care (Ma et al., 2018; Tief et al., 2016; Rath et al., 2017). When disseminated disease was suspected clinically, blood, cerebrospinal fluid, bronchial lavage, and urine samples were also tested, if available. In severely ill patients, follow-up clinical assessments and repeated PCR-testing were performed every other day until resolution of symptoms and/or hospital discharge.

Age groups were defined as follows

- Neonates: 0-28 days
- Infants: 29 days-12 months
- Toddlers: 13-24 months
- Pre-school children: 3-5 years
- Schoolchildren/adolescents: 6-18 years.

The Cohort was developed in compliance with the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) statement (von Elm et al., 2007).

Molecular testing and phylogenetic analysis

In all patients, real-time PCR-testing for HAdV was performed with a set of oligonucleotides capable of detecting species A-F (Chmielewicz et al., 2005), with modified reaction conditions at the Robert Koch-Institute. Quantitation cycle (Cq) values during PCR-testing were used to estimate virus load. Cq values are reversely proportional to the logarithm of the virus load, i.e. low Cq values indicate high virus load (Chen et al., 2014). In patients with disseminated disease, the PCR amplicon was genotyped by Fluorescence Melting Curve Analysis (FMCA) (Chmielewicz et al., 2005). Virus culture was performed on A549 cells (Giard et al., 1973) in Minimum Essential Medium in a closed system. Cells were cultured in 25 cm^2 flasks and infected with 50–200 μL of sterile filtered sample material. Following cytopathic effect, cells were freeze-thawed and harvested for further analysis.

For whole-genome sequencing, HAdV clinical isolates were transferred to the Reference Laboratory for Adenoviruses in Hannover, Germany. A genomic library was prepared using the Nextera XT DNA sample preparation kit (Illumina, San Diego, CA) (Hage et al., 2015). A total DNA input of 1 ng extracted from cell culture supernatant was simultaneously fragmented and ligated to adaptors using a transposon-based approach. The library was subjected to quality control prior to sequencing on an Illumina MiSeq, generating 300 bp paired-end reads. Fastq-file quality was tested before *de novo* assembly using CLC Genomics Workbench (Qiagen, version 8, Aarhus, Denmark). Phylogenetic analysis was performed in Mega7 (Kumar, Stecher, and Tamura, 2015). Neighbor-joining trees were constructed applying a Kimura 2-parameter model approach and bootstrapping was performed with 500 replicates.

QUANTIFICATION AND STATISTICAL ANALYSIS

Descriptive analyses were performed using SPSS Statistics 22 (IBM Corp. Armonk, NY). Clinical features were analyzed using the Correlation-based Feature Selection Measure to explore distinctive disease

patterns associated with HAdV infection (Hall, 1998). Variable importance was computed following the permutation principle of the mean decrease in accuracy importance. We used the Permutation Feature Importance Measure to compare significance levels across clinical variables. This method has advantages over other measures in the case of small patient groups since it works directly on the data and does not have any underlying model assumptions (Strobl et al., 2007). The method measures the importance of a feature by calculating the increase of the model's prediction error after the feature values have been permuted. A feature's importance increases proportionally with the increase of the model error (or decrease of the accuracy) after the permutation, since in this case the model is sensitive to this feature for the prediction and it is thus important. The relative importance of the most important feature is set to 100 (non-dimensional); values of other features are scaled accordingly.

The main idea of the method is that if the predictor variable Xj is randomly permuted, its original association with the response variable Y is broken. If one now finds a significant decrease in the prediction accuracy, then the original variable Xj was important for modelling the response Y. This means, one could use the difference in prediction accuracy before and after permuting Xj as a measure for variable importance. The main advantage of this strategy (e.g. in a random forest setting) is that the impact of each predictor variable can be measured individually as well as in multivariable interactions that include other predictor variables. In our context, we used this idea in connection with a random forest algorithm, which is an ensemble method combining several classification trees for the final decision. The evaluation of the importance of the individual variables is done using only a small random subset of all possible predictor variables. This way, even datasets with small number of cases can be analyzed in a meaningful way since many different small subsets of predictor variables are evaluated – in contrast to a high number of predictor variables, which might result in unreliable results.