MAJOR ARTICLE

The Natural History of Influenza Infection in the Severely Immunocompromised vs Nonimmunocompromised Hosts

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Introduction. Medical advances have led to an increase in the world's population of immunosuppressed individuals. The most severely immunocompromised patients are those who have been diagnosed with a hematologic malignancy, solid organ tumor, or who have other conditions that require immunosuppressive therapies and/or solid organ or stem cell transplants.

Materials and methods. Medically attended patients with a positive clinical diagnosis of influenza were recruited prospectively and clinically evaluated. Nasal washes and serum were collected. Evaluation of viral shedding, nasal and serum cytokines, clinical illness, and clinical outcomes were performed to compare severely immunocompromised individuals to nonimmunocompromised individuals with influenza infection.

Results. Immunocompromised patients with influenza had more severe disease/complications, longer viral shedding, and more antiviral resistance while demonstrating less clinical symptoms and signs on clinical assessment.

Conclusions. Immunocompromised patients are at risk for more severe or complicated influenza induced disease, which may be difficult to prevent with existing vaccines and antiviral treatments. Specific issues to consider when managing a severely immunocompromised host include the development of asymptomatic shedding, multidrug resistance during prolonged antiviral therapy, and the potential high risk of pulmonary involvement.

Clinical trials registration. ClinicalTrials.gov identifier NCT00533182.

Keywords. influenza A; influenza; immunocompromised host; stem cell transplant.

During the past half-century, medical advances have led to an increase in the world's population of immunosuppressed individuals. This includes those receiving immunosuppressive therapies, those with acquired immunosuppressive diseases such as the 34 million worldwide with human immunodeficiency virus (HIV) and AIDS [1], and individuals living longer with any of the over 150 known primary immunodeficiencies [2]. The most severely immunocompromised are those who have been diagnosed with a hematologic malignancy, solid organ tumor, or who receive other immunosuppressive therapies such as chemotherapy and/or solid organ or stem cell transplants.

Over 100 000 individuals annually receive solid organ transplants (SOT) worldwide [3] and more than 50 000 with hematologic malignancies and other diseases are treated with hematopoietic stem cell transplants (HSCT) annually [4], leaving most of these individuals immunosuppressed for long periods. Even larger numbers of individuals are receiving immunosuppressive chemotherapies making immunocompromised individuals a larger part of the population than

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during any of the influenza pandemics of the twentieth century.

Epidemiologic studies have shown that severe immunosuppression is a major comorbidity that places individuals at the highest risk of severe morbidity and mortality due to influenza infection. Patients with AIDS have increased duration of disease due to influenza and higher incidence of pneumonia leading to increased mortality [5–7]. A study of hospitalized patients with leukemia and influenza reported 80% with pneumonia and 33% mortality [8]. More recently during the 2009 pandemic, studies reported similarly high levels of lower respiratory tract disease and need for hospitalization in those with hematologic malignancies and solid tumors undergoing chemotherapy [9].

Similarly, in a large retrospective study of HSCT recipients, 1.3% of all patients developed influenza infection and 29% developed pneumonia [10]. A more recent study of HSCT patients demonstrated that up to 75% developed pneumonia after influenza infection with mortality as high as 43% [11]. In SOT, nosocomial outbreaks [12] and severe complications of influenza such as myocarditis [13] and severe pneumonitis [14] have been reported, even in those previously vaccinated. Multicenter studies of A(H1N1)pdm09 infection in SOT recipients have reported that 30%–40% of individuals infected developed pneumonia, 16%–17.5% required intensive care, with mortality as high as 7% [15]. Influenza following SOT leads to higher morbidity and mortality rates [16], with increased rates of influenza pneumonia following lung transplantation, and a >20% mortality rate observed in SOT recipients infected with influenza [17, 18].

In addition to increased morbidity and mortality following infection, severely immunocompromised patients have been reported to show prolonged influenza shedding [19–25]. This has been associated with intrahost viral evolution, including antigenic drift within a single immunosuppressed host [24], the development of antiviral resistance after therapy [19, 21–23, 25, 26], and simultaneous coinfection with 2 influenza subtypes [27].

Rigorous vaccination programs and improved pharmacotherapy have decreased the impact of influenza on the general population; however, influenza still remains a serious threat to severely immunocompromised individuals. The 2009 pandemic was a reminder that it is still unclear how well current vaccination strategies and current pharmacotherapy can perform in preventing and mitigating illness in immunocompromised individuals.

The primary goal of this study was to compare the natural history of influenza infection in those who were severely immunocompromised to individuals who were not immunocompromised. Careful examination of symptoms and signs of infection, virological measurements, immunological studies, and clinical parameters were performed to investigate the natural pathogenesis of influenza in this group of severely immunocompromised hosts.

MATERIALS AND METHODS

Clinical Protocol

Subjects were recruited at the National Institutes of Health (NIH) Clinical Center in Bethesda, MD, and Washington Hospital Center in Washington, DC, under an IRB approved protocol "Influenza in Nonimmunocompromised and Immunocompromised Hosts" (Clinicaltrials.gov identifier NCT00533182). All participants or legal guardian signed informed consent. Medically attended patients with a diagnosis of influenza were recruited. History and physical was performed, initial diagnostic specimen collected, and further nasal washes and blood samples collected every 2 days during the first 7 days postdiagnosis. Nasal washes were performed using the method described by Neclerio [28]. Follow-up examinations and sample collection at both 2 weeks and 4 weeks were performed. Subjects who shed virus for an extended period of time were followed throughout the course of their infection, and sampling was performed until the infection resolved. Clinical data were compiled and statistical analyses performed using GraphPad Prism software.

Viral Isolation and Identification

Initial diagnosis was made by the clinical laboratories at the facility where samples were collected. Further samples were tested using a matrix one-step Taqman real-time reverse transcription polymerase chain reaction (RT-PCR) assay as described elsewhere [29] and/or the Luminex Multiplex Respiratory Viral Panel kit (Luminex Corp. Austin, TX). Viral isolation used standard MDCK culture methods. Type and subtype were determined by RT-PCR and sequencing of the hemagglutinin (HA) and neuraminidase (NA) genes and comparison to known influenza sequences in Genbank.

Sequencing and Genotype Identification

Total nucleic acid was extracted from samples using the bioMérieux NucliSENS easyMAG system. Viral complementary DNA (cDNA) was reverse transcribed using the influenza specific uni-12 primer, and PCR was performed using custom primers to amplify the viral gene segments (Table 1). Sanger sequencing was performed on all PCR products.

Immunologic Assays

Serum and nasal wash cytokines were measured using the Bio-Plex Pro Human Cytokine 17-plex assay on a Bio-Rad Bio-Plex 200 (Bio-Rad Hercules, Ca). The following cytokines were measured: interleukin 1β, interleukin 2, interleukin 4, interleukin 5, interleukin 6 (IL-6), interleukin 7, interleukin 8 (IL-8), interleukin 10, interleukin 12, interleukin 13, interleukin 15, interleukin 17, granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), interferon γ, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1β (MIP-1β), and tumor

Table 1. Primers Used to Sequence Viral Isolates

	Name	Sequence
Seasonal H3N2 HA	sH3N2HA-1F	AGCAAAAGCAGGGGATAA
	sH3N2HA-645F	GACCAAATCTTCCTGTATGCTCAA
	sH3N2HA-1200F	ATCAAATCAATGGGAAGC
	sH3N2HA-1725R	AAATGTTGCACCCTAATG
	sH3N2HA-1325R	GTCCTCAACATATTTCTCAAG
	sH3N2HA-800R	ATGTCTCCCGGTTTTACTATTGTC
Seasonal H3N2 NA	sH3N2NA-5F	AGCAAAAGCAGGAGTAA
	sH3N2NA-700F	CAGGAGTCAGAATGCGTTTGTATC
	sH3N2NA-1435R	ATATAGGCATGAAGATTGA
	sH3N2NA-800R	TTTTCCCCTCCTCAATG
	sH3N2MP-5F	AAAAGCAGGTAGATATTGAAAGA
A(H1N1)pdm09 HA	HA-60F	GTAAAACGACGGCCAGAGGTTATCATGCGAACAATTCA
	HA-710R	CAGGAAACAGCTATGACCCTCACTTTGGGTCTTAT
	HA-580F	GTAAAACGACGGCCAGGGGCATTCACCATCCATCTACT
	HA-1100R	CAGGAAACAGCTATGACTACCATCCATCTACCATCCCTGTC
	HA-970F	GTAAAACGACGGCCAGTAAAAAGCACAAAATTGAGACTGG
	HA-1560R	CAGGAAACAGCTATGACCTTTACCCCATCTATTTCTTCTCT
	HA-1380F	GTAAAACGACGGCCAGTATATGAAAAGGTAAGAAGC
	HA-1740R	CAGGAAACAGCTATGACCATGCTTCTGAAATCCTAA
A(H1N1)pdm09 NA	NA-1F	GTAAAACGACGGCCAGATGAATCCAAACCAAAAG
	NA-720R	CAGGAAACAGCTATGACTACAGTAAAGCAAGAACCA
	NA-550F	GTAAAACGACGGCCAGATGGCATCAATTGGCTAACA
	NA-1200R	CAGGAAACAGCTATGACTGTTAGCCAATTGATGCCAT
	NA-1050F	GTAAAACGACGGCCAGTTCAAATACGGCAATGG
	NA-1400R	CAGGAAACAGCTATGACCCATTGCCGTATTTGAA
Seasonal H1N1 HA	sH1N1HA-20F	GTAAAACGACGGCCAGAGTAGCGAAAGCAGGGGAAAATA
	sH1N1HA-710R	CAGGAAACAGCTATGACTGAAGACACTACAGAAACATAAGC
	sH1N1HA-600F	GTAAAACGACGGCCAGCAAACAACAAAGAAAAAGAAGTCC
	sH1N1HA-1240R	CAGGAAACAGCTATGACTTCACCTTGTTTGTAATCC
	sH1N1HA-1230F	GTAAAACGACGGCCAGATGGTAGATGGTTGGTATGGTTAT
	sH1N1HA-1760R	CAGGAAACAGCTATGACTTCTGAAATTCTGGTCTTA
Seasonal H1N1 NA	sH1N1NA-50F	GTAAAACGACGGCCAGTAATGTTGCAAATAGGAAATA
	sH1N1NA-650R	CAGGAAACAGCTATGACTATTTTAGTACAGCCACAGC
	sH1N1NA-550F	GTAAAACGACGGCCAGAAAGTTCGAATCAGTTGC
	sH1N1NA-1180R	CAGGAAACAGCTATGACATCACTGTCGGTATTTGTC
	sH1N1NA-1000F	GTAAAACGACGGCCAGTCCGCGTCCCGAAGATG
	sH1N1NA-1400R	CAGGAAACAGCTATGACCAATGGTGAACGGCAACT

necrosis factor α (TNF-α). Controls used were collected from healthy volunteers. Results were compiled and statistical analysis performed using GraphPad Prism software.

RESULTS

Eighty-six influenza patients were enrolled over a 3-year period between 2008 and 2011. Of these, 32 (37.2%) were classified as severely immunocompromised, primarily due to a malignancy or condition being treated with either a recent HSCT or immunosuppressive therapy. The remaining 54 patients (62.8%) were classified as nonimmunocompromised and included individuals both with and without underlying comorbidities. The demographics of these individuals are summarized in Table 2.

Of note, 46.5% of the individuals in the study had been vaccinated within 1 year of becoming infected with influenza. A majority of the nonimmunocompromised individuals were overweight or obese (81.4%). Although most patients in the study were nonsmokers, 22.2% of nonimmunocompromised participants were current smokers, whereas there were no current smokers in the immunocompromised group.

Hematologic conditions were the most common underlying diagnosis (78.1%) and 59.4% of patients had undergone HSCT within 1 year of influenza infection in the immunocompromised

Table 2. Patient Demographics

	Immunocompromised		NonImmunocompromised		Total	
No. of participants	32	(37.2%)	54	(62.8%)	86	
Sex						
Male	22	(68.8%)	26	(48.2%)	48	(55.8%)
Female	10	(31.2%)	28	(51.8%)	38	(44.2%)
Age, years						
<18	1	(3.1%)	7	(13.0%)	8	(9.3%)
18–65	31	(96.9%)	40	(74.0%)	71	(82.6%)
>65	0	(0.00%)	7	(13.0%)	7	(8.1%)
Race						
White	17	(53.1%)	17	(31.5%)	34	(39.5%)
Black	7	(21.9%)	29	(53.7%)	36	(41.9%)
Asian	1	(3.1%)	5	(9.2%)	6	(7.0%)
Hispanic	7	(21.9%)	1	(1.9%)	8	(9.3%)
Multirace	0	(0.00%)	2	(3.7%)	2	(2.3%)
BMI						
Underweight <18.50	2	(6.25%)	0	(0.00%)	2	(2.33%)
Normal 18.50–24.99	19	(59.38%)	10	(18.52%)	29	(33.72%)
Overweight ≥25.00	6	(18.75%)	34	(62.96%)	40	(46.51%)
Obese ≥30.00	5	(15.63%)	10	(18.52%)	15	(17.44%)
Tobacco use						
Current use	0	(0.00%)	12	(22.2%)	12	(14.0%)
Past use (≤10 y prior)	2	(6.2%)	6	(11.1%)	8	(9.3%)
No use (or >10 Y prior)	30	(93.8%)	36	(66.7%)	66	(76.7%)
Vaccinated within 1 y						
Vaccinated	8	(25.0%)	32	(59.3%)	40	(46.5%)
Unvaccinated	24	(75.0%)	22	(40.7%)	46	(53.5%)

Abbreviation: BMI, body mass index.

group. Most had some form of graft-vs-host disease (GVHD; Table 3). Those who did not undergo HSCT (21.9%) were undergoing immunosuppressive therapy (Table 3). In the nonimmunocompromised group 17 patients (31.5%) had chronic comorbidities including hypertension, diabetes mellitus, coronary artery disease, asthma or chronic obstructive pulmonary disease, or well-controlled HIV infection.

A full symptom evaluation at the time of diagnosis was performed in 80 of 86 patients (Table 4). The most common symptoms noted were dry cough (90%), fever (83.8%), and headache (82.5%). Chills, coryza, productive cough, and sweats were also common. A range of other symptoms were observed including shortness of breath and chest pain. Of interest was the prevalence of multiple gastrointestinal and neurologic symptoms.

The majority of symptoms were more prevalent in the nonimmunocompromised group including those that were statistically higher: dry cough, chills, sweats, myalgia, shortness of breath, chemosis, and neurologic symptoms. Gastrointestinal symptoms were slightly more common in the immunocompromised group with decreased appetite observed as the only symptom statistically more prevalent (Table 4).

Fifty-seven of the patients had complete physical exams documented on diagnosis, and an overall trend of physical exam abnormalities was observed more in the nonimmunocompromised group, with pulmonary abnormalities in particular being more statistically prevalent (Table 5). Pulmonary abnormalities on exam were the most common finding (44% overall). Cardiac abnormalities were noted in 14% of patients, mostly consisting of tachycardia. Other abnormalities were minimal, and many individuals had no significant findings.

All of the immunocompromised patients required hospitalization upon diagnosis. Six patients (18%) in the immmunocompromised group required intensive care and mechanical ventilation. In contrast, only 16 patients (29.6%; $P = .0001$) of the nonimmunocompromised group required hospitalization, and none required intensive care treatment ($P = .0019$). There was 1 death observed in the immunocompromised group (3%). The immunocompromised patients also exhibited a significantly longer length of illness ($P = .0466$) with a mean shedding

Table 3. Underlying Conditions in the Immunocompromised Group

Immunocompromised Total	32
Stem-cell transplant	19 (59.4%)
No transplant	13 (40.6%)
Hematologic condition	25 (78.1)
Multiple myeloma	1
Acute lymphoblastic leukemia	1
Hodgkin's lymphoma	2
Peripheral T-cell lymphoma	1
Diffuse large B cell lymphoma	3
Severe aplastic anemia	9
Myelodysplastic syndrome	2
Mantle cell lymphoma	1
Chronic myelogenous leukemia	3
Chronic lymphocytic leukemia	2
Other	7 (21.9%)
Ewing's sarcoma	2
Rectal cancer	2
Metastatic melanoma	1
Breast cancer	2

of 19.0 days, whereas nonimmunocompromised patients were observed to shed for a mean of 6.4 days (Figure 1).

Consistent with these differences were the radiologic examinations observed. Chest computed tomography (CT) or chest radiograph (CXR) was performed at the time of diagnosis on 58 of the 86 patients recruited. A majority of immunocompromised patients (87.5%) had radiologic studies performed, whereas less (55.5%) were performed on nonimmunocompromised patients. Of these 58 radiologic examinations, 23 (39.7%) showed new abnormalities at the time of diagnosis. The immunocompromised patients were significantly more likely to have new imaging abnormalities with 16 of 28 (57.1%) compared to 7 of 30 (23.3%) in the nonimmunocompromised group ($P = .015$).

Viral diagnoses of the 86 patients enrolled are summarized in Table 6. Of the 80 influenza A virus strains identified, 6% were seasonal H1N1, 19.7% H3N2, and 66.3% A(H1N1)pdm09 subtypes. Of the 80 patients identified as having influenza A infection, 9 (11.3%) of these patients either were infected with a neuraminidase inhibitor resistant virus or developed resistance during the course of infection and treatment. When adamantane resistance is considered, 100% of the influenza A isolates collected in this study were resistant to at least one class of antiviral drug, and 3 patients (4%) had viral infections developing resistance to both available classes of antivirals (Table 6). The A (H1N1)pdm09 virus was the most prevalent subtype of influenza A virus isolated (66.3%), and as expected, all of these viruses were resistant to the adamantanes as were the H3N2 viruses. All 6 of the seasonal H1N1 isolates identified contained the H275Y NAI resistance mutation conferring resistance to oseltamivir and possibly peramivir [22]. This mutation was noted at

Table 4. Clinical Symptoms

Symptom	Immunocompromised	Percent	Nonimmunocompromised	Percent	*P* Value	ODDS RATIO	Total	Percent
Dry cough	21	77.8%	51	96.2%	**.016**	0.137	72	90.0%
Fever	23	85.2%	44	83.0%	1.000	1.176	67	83.8%
Headache	19	70.4%	47	88.7%	.061	0.303	66	82.5%
Chills	14	51.9%	43	81.1%	**.009**	0.250	57	71.3%
Coryza	20	74.1%	36	67.9%	.616	1.349	56	70.0%
Productive Cough	16	59.3%	36	67.9%	.467	0.687	52	65.0%
Sweats	10	37.0%	41	77.4%	**.001**	0.172	51	63.8%
Myalgia	11	40.7%	36	67.9%	**.030**	0.325	47	58.8%
Shortness of breath	10	37.0%	33	62.3%	**.037**	0.357	43	53.8%
Sore throat	10	37.0%	32	60.4%	.060	0.386	42	52.5%
Chest pain	10	37.0%	29	54.7%	.161	0.487	39	48.8%
Nausea	14	51.9%	19	35.9%	.230	1.927	33	41.3%
Arthralgia	9	33.3%	19	35.9%	1.000	0.895	28	35.0%
Vomiting	8	29.6%	16	30.2%	1.000	0.974	24	30.0%
Chemosis	2	7.4%	21	39.6%	**.003**	0.187	23	28.8%
Diarrhea	4	14.8%	19	35.9%	.068	0.311	23	28.8%
Neurologic symptoms	2	7.4%	20	37.7%	**.004**	0.132	22	27.5%
Abdominal pain	4	14.8%	11	20.8%	.763	0.664	15	18.8%
Fatigue	4	14.8%	11	20.8%	.763	0.664	15	18.8%
Decreased appetite	6	22.2%	3	5.7%	**.054**	4.762	9	11.3%
Skin rash	0	0.00%	4	7.6%	.294	0.200	4	5.0%

Bold values indicate statistical significance.

Table 5. Physical Exam Findings

	Total Abnormal Exams	%	Immunocompromised	%	Non immunocompromised	%	*P* Value	Odds Ratio
Cardiac	7	14.0%	3	11.1%	4	17.4%	.688	0.5938
Pulmonary	22	44.0%	7	25.9%	15	65.2%	**.0095**	0.1867
ENT	12	24.0%	6	22.2%	6	26.1%	1	0.8095
Skin	3	6.0%	0	0.0%	1	4.4%	.46	0.727
GI	1	2.0%	0	0.0%	1	4.4%	1	2.66
Musculoskeletal	1	2.0%	0	0.0%	1	4.4%	.46	0.2727
Neurologic	0	0.0%	0	0.0%	0	0.0%		
Lymph nodes	1	2.0%	1	3.7%	0	0.0%	1	2.66
Extremities	0	0.0%	0	0.0%	0	0.0%		
Total exams	50	100.0%	27	100.0%	23	100.0%		

Bold values indicate statistical significance.

Abbreviations: ENT, ear, nose, and throat; GI, gastrointestinal.

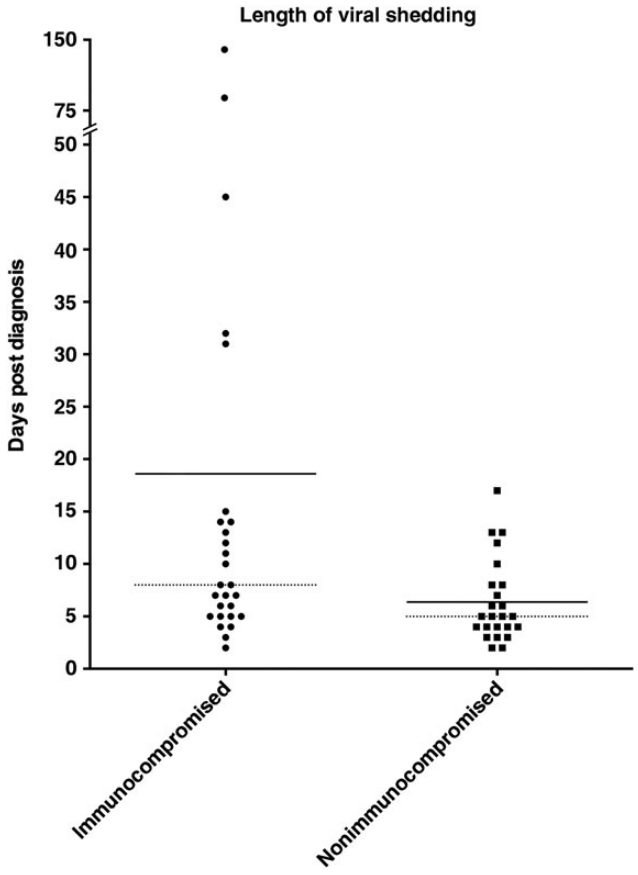

Figure 1. Length of viral shedding. Patients were sampled daily until they had a negative clinical diagnostic test. Significantly longer shedding was detected in the immunocompromised group. Each dot represents an individual patient's length of shedding. Solid lines represent the mean (immunocompromised 19.04 days vs nonimmunocompromised 6.38 days; *P* value .0466). Dotted lines represent the median (immunocompromised 8.0 days vs nonimmuncompromised 5.0 days; *P* value .0130).

the time of initial identification of virus and remained stable in all consecutive isolates collected from those patients.

Three immunocompromised patients were infected with influenza viruses that developed neuraminidase inhibitor resistance during treatment. One patient with an H3N2 infection developed a novel deletion mutation in the NA gene. The other 2 cases were A(H1N1)pdm09 infections that developed H275Y mutations. In all of these cases the mutations arose after treatment with oseltamivir and in one case peramivir during a prolonged clinical course [21–23].

Cytokine response during acute infection was measured both from serum and nasal washes. Of the cytokines measured IL-6, IL-8, TNF-α, G-CSF, GM-CSF, MCP-1, and MIP-1b were found to be elevated in serum and/or nasal wash compared to controls during infection (Figures 2 and 3). Overall, no significant differences were noted in these responses between the immunocompromised and non-immunocompromised groups.

DISCUSSION

Influenza infection has a major effect on the population in all regions of the world, differing from season to season and emerging unpredictably during a pandemic. The patient population identified in this study reflects the population of the Washington, DC, metropolitan region with the majority of subjects identifying themselves as either white or black. Given the nature of a prospective study of immunocompromised patients, it was not possible to have extremely well matched comparator groups, but they were reasonably well matched (Table 2). Most participants were between the ages of 18 and 65 with a mean age of 25.55. This mean age of approximately 25 is consistent with the fact that the 2009 A(H1N1)pdm09 virus was the most prevalent virus identified in this study, and that much of this

Table 6. Characteristics of Viruses Identified

Type	Subtype	Immunocompromised	Nonimmunocompromised	Total
Influenza A		30	50	80 (93%)
	A(H1N1)pdm09	18	39	57 (66.3%)
	Seasonal H3N2	8	9	17 (19.7%)
	Seasonal H1N1	4	2	6 (7%)
Influenza B		1	1	2 (2.3%)
Unidentified subtype		1	3	4 (4.7%)
Coinfection		0	2 (RSV, Coronavirus)	2 (2.3%)

Abbreviation: RSV, Respiratory syncytial virus.

A(H1N1)pdm09: 2 patients developed H275Y.

Seasonal H1N1: 100% resistance H275Y.

Seasonal H3N2: 1 patient with deletion mutation.

study was performed during the 2009 pandemic and post-pandemic period when a slightly younger population was more affected by influenza than during a typical influenza season [30]

A number of significant differences were observed between the severely immunocompromised and nonimmunocompromised groups. The immunocompromised patients experienced more hospitalizations, longer lengths of influenza virus shedding, and more severe disease and complications requiring intensive care and mechanical ventilation. These differences highlight that not only are these patients an important at-risk group for enhanced morbidity and mortality but also should be considered a group that could contribute to viral spread in the general population. The immunocompromised patients in this study shed virus for a mean of approximately 19 days, likely making them contagious for a significant length of time. Many of these patients were asymptomatic for a significant portion of the time in which they were shedding. Importantly, in a number of these cases the viruses they were shedding had developed drug resistance mutations while maintaining their transmissibility [21, 23], making it possible that these individuals could also be a source for community spread of drug resistant viral strains.

Although many of the immunocompromised patients were asymptomatic during some portion of the time they were infected, most exhibited some typical influenza symptoms during the first few days of infection. This was consistent with the cytokine measurements observed, as the acute innate immune response is likely principally responsible for many of the acute symptoms observed.

Cytokine measurements in nasal wash specimens demonstrated similar cytokine responses in both the immunocompromised and nonimmunocompromised groups. These cytokine elevations were consistent with what has been reported previously during influenza infection [31]. In serum, similar elevations were noted in both groups as well, although to a somewhat lesser degree when compared to noninfected controls.

Despite the similarity in acute innate response between the 2 groups, important differences were noted in overall symptoms between the immunocompromised and nonimmunocompromised groups. Fewer overall symptoms were noted in the immunocompromised group, and all influenza symptoms were found more commonly in the nonimmunocompromised group except for gastrointestinal symptoms, that were likely related to underlying illnesses or therapeutics as many of these immunocompromised individuals suffered from GVHD of the gut or were receiving a toxic chemotherapeutic agent. The overall trend of the more inflammatory and systemic symptoms occurred more often in the nonimmunocompromised group suggesting that although the innate immune response is similar, the clinical manifestations may not be, and the possibility of a blunted illness should be taken into account when evaluating immunocompromised patients with an influenza-like illness.

A significantly higher number of radiographic abnormalities were noted in the immunocompromised group, pointing to their increased risk of pulmonary involvement and complications. These radiologic changes were often in the absence of pulmonary abnormalities on physical exam, which were more prevalent in the nonimmunocompromised group. This is important to consider clinically as the presence or absence of symptoms and signs may not be adequate indicators of disease, especially in immunocompromised individuals who are likely at higher risk of pneumonia, secondary bacterial infections, and other pulmonary complications.

Vaccination remains the cornerstone of prevention for influenza infection and its complications. In this study, approximately 47% of all individuals recruited had been vaccinated within the

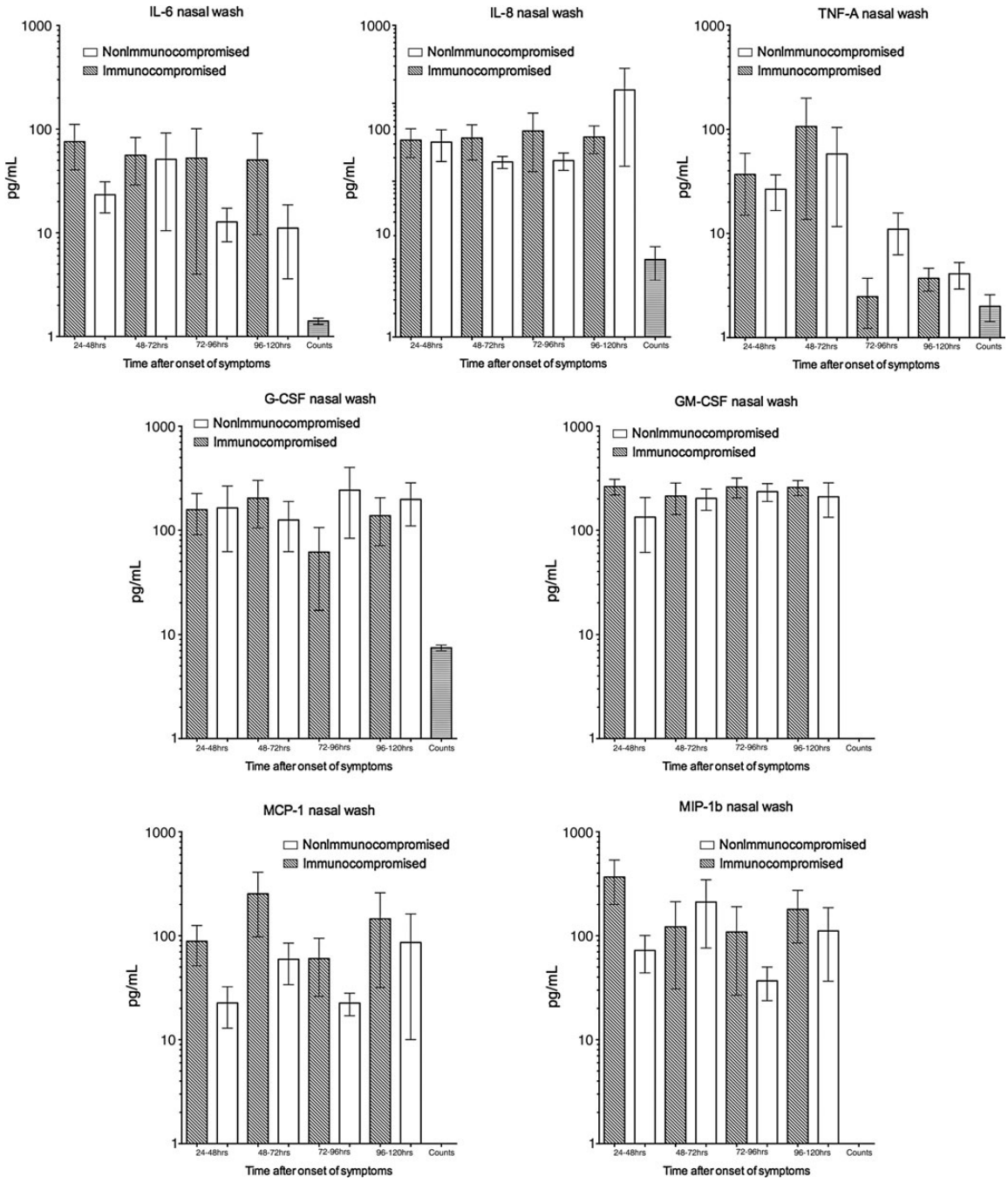

Figure 2. Cytokines levels detected in nasal wash. Seventeen cytokines were measured in nasal washes, and the 7 represented here were elevated when compared to a mean of 3 healthy controls. Error bars represent the standard error of the mean.

last year prior to infection yet still became ill with influenza. A larger number of the nonimmunocompromised (59%) were vaccinated compared to only 25% of the immunocompromised. This fact makes this observation even more concerning as a significant number of immunocompetent individuals became infected despite a vaccine that closely matched the infecting virus. The observations of vaccination made in this study are somewhat consistent with recent observations by the CDC that during recent years the effectiveness of the influenza vaccine, especially in certain groups of individuals has been moderate to poor [32].

Clearly influenza vaccines have variable efficacy that is hard to predict, especially when factoring in emerging viruses and

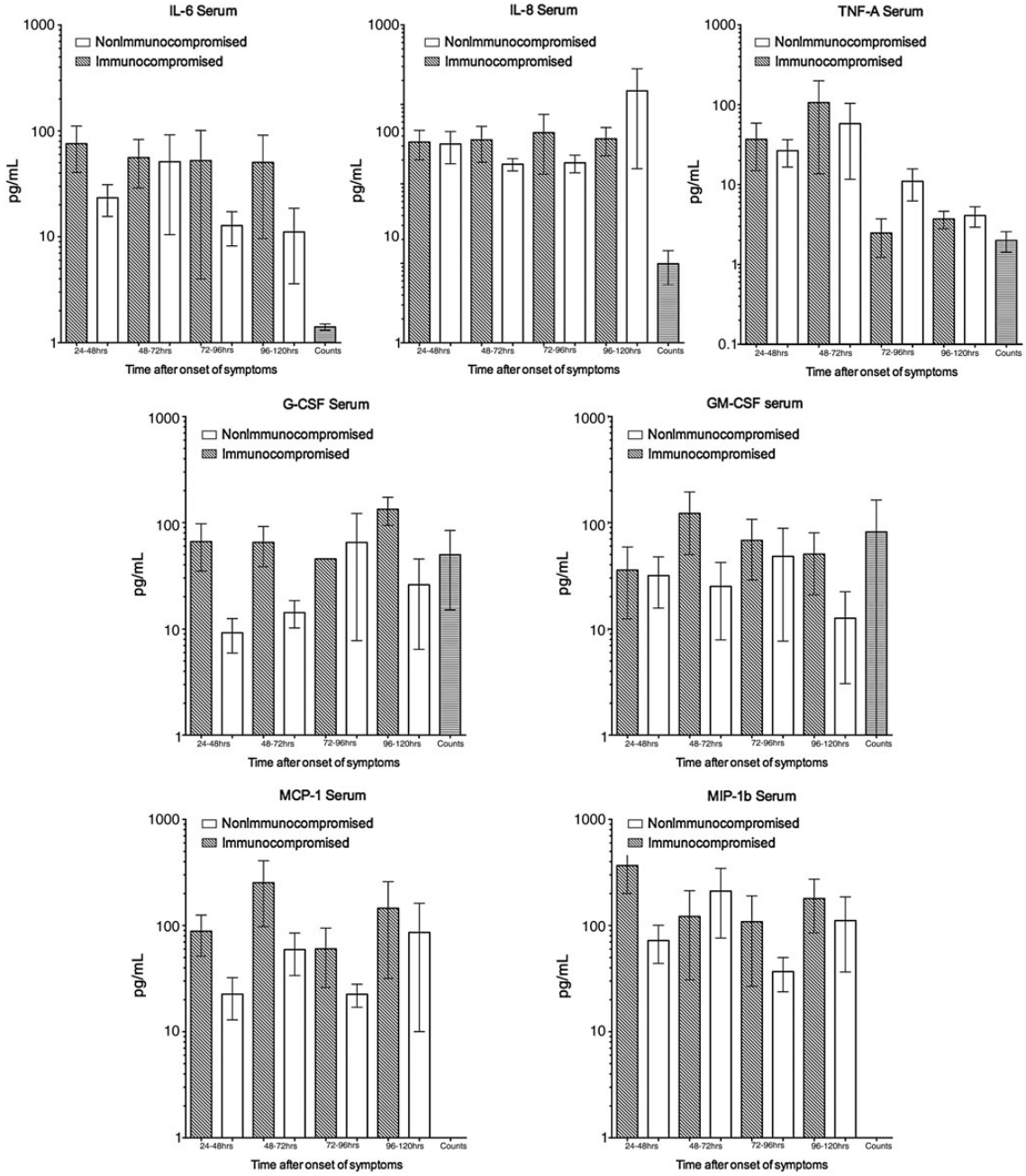

Figure 3. Cytokines detected in serum. Seventeen cytokines were measured in serum, and the 7 represented here were elevated when compared to a mean of 3 healthy controls. Error bars represent the standard error of the mean.

special populations. This highlights that further work needs to be undertaken to find novel vaccine/prevention strategies to reduce the disease burden in the general healthy population and especially in special populations such as the severely immunocompromised, where vaccination is even further hampered by the inability of the host to respond. This may require reconsidering prevention of disease as our goal and shifting our focus to reducing severe disease and complications such as the prolonged shedding and severe illness observed in the immunocompromised patients in this study.

When vaccines do not prevent infection or are not given, the last step in preventing morbidity and mortality in influenza is therapeutic treatment, of which antivirals are the current gold standard. The prevalence of antiviral drug resistance found in this and other studies is a warning that should not be ignored. Consistent with recent observations, all influenza viruses

identified in this study were resistant to at least one class of antiviral. In this group of patients, 4% of viruses were resistant to both major classes of antivirals including some isolates of H3N2 and A(H1N1)pdm09. The majority of these viruses were identified in immunocompromised patients after they had received prolonged courses (beyond 5 days) of antiviral therapy. These data in conjunction with observations that these viruses are transmissible and not attenuated in animal models [21, 23] suggest that the development of multi-drug resistance is a significant concern and that immunocompromised patients may be at risk of acting as hosts for the development and community spread of resistance. Further research, the development of novel therapies, and reevaluation of how immunocompromised individuals are treated for influenza should be undertaken.

CONCLUSION

Study of the natural history of influenza is extremely difficult because in almost all cases it is impossible to identify exactly when a patient was infected. Although a relatively small cohort was observed in this study, it is one of the largest cohorts of severely immunocompromised individuals with influenza infection studied prospectively to date. The comparison of these individuals with nonimmunocompromised individuals during influenza infection demonstrated that the immunocompromised patients are at risk of more severe or complicated disease, which may be difficult to prevent with current vaccines and treat with current antivirals. Specific issues to consider when managing severely immunocompromised hosts include the development of asymptomatic shedding that could increase the risk of transmission, the development of multi-drug resistance during prolonged antiviral therapy, and the potential high risk of pulmonary involvement of their disease leading to secondary infections or complications.

A better understanding of the basic human pathogenesis of influenza will be necessary if we are to address how to prevent and treat influenza in a more individualized way, especially for those who are immunocompromised. It is clear that current antivirals are not adequate in all circumstances, current vaccine strategies must be improved, and careful evaluation of each individual case may be necessary to tailor treatment and prevention for not only severely immunocompromised individuals but likely those in other important at-risk populations.

Notes

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