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Prevalence of Norovirus among Rehabilitation Inpatients with Neurogenic Bowel Dysfunction due to Spinal Cord Injury or Traumatic Brain Injury: A Pilot Study

By

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Master of Public Health

Epidemiology

_____

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By

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B.S.A
The University of Georgia
2013

Thesis Committee Chair: Amy Kirby PhD, MPH

An abstract of
A thesis submitted to the Faculty of the
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Abstract

Prevalence of Norovirus among Rehabilitation Inpatients with Neurogenic Bowel Dysfunction due to Spinal Cord Injury or Traumatic Brain Injury: A Pilot Study

By Katherine Reece

Bowel dysfunction is a common condition among spinal cord injury and disease (SCI/D), traumatic brain injury (TBI), and stroke patients and can result in a lower quality of life. Diarrhea episodes are commonly attributed to neurogenic bowel dysfunction (NBD), and infectious agents are typically not considered to cause gastroenteritis in this population. Norovirus (NoV) is a leading cause of gastrointestinal illness worldwide and has been related to many outbreaks in health-care settings. This pilot study evaluated the prevalence of NoV (genogroups I and II) among rehabilitation inpatients with NBD due to a SCI/D, TBI, or stroke. Following a diarrhea or vomiting episode, stool samples, medical information, and demographic information were collected from 25 rehabilitation inpatients from the Shepherd Center in Atlanta, GA during the months of November 2014 through March 2015. No patients were found to be infected with NoV GI or GII. This provides evidence that there was not an outbreak of NoV at Shepherd Center during the 2014-2015 season. However, we cannot conclude that NoV does not affect this population due to small sample size and low study power. Other infectious agents affected the majority of study patients (92%), suggesting further analyses should be done to evaluate the immune response following an SCI/D or TBI. There should be future studies designed with enough power to detect the prevalence of NoV infections among SCI/D and TBI rehabilitation inpatients. Ultimately, such evaluations may lead to improvements of care and thus increased quality of life among SCI/D and TBI patients.

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Chapter I: Literature Review

Spinal Cord Injury/Disease and Traumatic Brain Injury

Spinal cord injury and disease (SCI/D) affect a substantial number of individuals in the United States. In 2014, it was calculated that the highest prevalence of traumatic SCI in the world was in the United States at 906 cases per one million individuals [1]. The total prevalence of SCI in 2013 was estimated to be between 238,000 and 332,000 people and the estimated incidence for the 1990's was 12,000 new cases a year in the United States [2]. The estimated crude annual incidence in 2014 was highest in Alaska, compared to other North American states and provinces, at 83 per one million people [1]. In 2012 the average age of SCI patients was 42.6 years old and the majority of patients were male (80.7%)[2]. The majority of patients from 2005-2012 were Caucasian (67%), followed by African American (24.4%) and Asian (2.1%)[2]. Most injuries occur as a result of a motor vehicle collision (36.5%) and the remaining injuries result mostly from falls and violence[2]. In 2012 the majority of SCI patients had incomplete tetraplegia (formally known as quadriplegia) (40.6%), 18% had complete paraplegia, 18.7% had incomplete paraplegia, and the remaining patients had complete tetraplegia[2]. In 2012, life expectancy was lower for SCI patients compared to the average life expectancy of the same age, non-injury individuals and the lowest life expectancy was with ventilator dependent and high tetraplegia injury patients [2].

Traumatic brain injuries (TBI) and stroke are both leading causes of morbidity and mortality in the United States. The Centers for Disease Control and Prevention estimate that 1.7 million people sustain a TBI each year in the United States[3]. Children

0 to 4 years old, youth 15 to 19 years old, and adults 65 years and older are most likely to experience a TBI[3]. In the United States, the incidence of stroke is estimated to be 795,000 cases annually, with 185,000 of these being recurrences[4]. Approximately 55,000 more women than men have a stroke each year in the United States[4] and although incidence of stroke among whites has decreased in recent years, this trend is not seen among African Americans[5].

Common diseases of the spinal cord include conditions that can result in partial or complete limb paralysis. Some examples of spinal cord diseases include multiple sclerosis (MS), neuromyelitis optica, and cancer. MS is a relapsing and remitting autoimmune disease that can lead to episodes of paraplegia. MS is the most common neurologic disease in young adults with the peak age of onset between 20 and 40 years[6]. Neuromyelitis optica results when the patient's immune system attacks the optic nerves and spinal cord, which may lead to chronic spinal paraplegia. Tumors, or cancer, of the spinal cord can lead to neurological symptoms such as myelopathy and surgical procedures to remove such tumors can lead to paralysis[7]. Paralysis resulting from such conditions can have an impact on individuals' daily lives and everyday bodily functions.

The spinal cord is a thin tubular structure that is contained within the spinal canal and serves as the center for most motor and sensory function, making injury or disease to the spinal cord a potentially devastating condition. There are 31 segments to the spinal cord and four main sections where damage can occur: cervical (C1-C8), thoracic (T1-T12), lumbar (L1-L5), and sacral (S1-S5). Lesions on the cervical cord, the highest level, usually result in tetraplegia and weakness of the diaphragm[8]. Lesions on the thoracic cord usually lead to leg weakness and disturbances of bladder and bowel function and

lumbar cord lesions are known to cause weak leg, foot, and ankle extension[8]. Lesions at the sacral level are most commonly associated with symptoms of bladder and bowel dysfunction and impotence[8].

Spinal cord injuries (SCI) are evaluated based on Frankel Scores, which are part of the international standard for classification created by the American Spinal Cord Association. Complete SCI is labeled as Grade A, which results in no motor or sensory function below the injury[9]. Incomplete SCI fall on a range from Grade B-D. Grade B indicates preserved sensation function but no motor function and Grade C corresponds to an injury that results in some motor function but none that is of practical use to the individual[9]. Grade D injury results in impaired, but intact, motor function[9]. There are many types of disorders that can result following a spinal cord injury (SCI) which include, but are not limited to; central cord syndrome, anterior cord syndrome, spinal shock, and autonomic dysreflexia. Central cord syndrome is the most common and often occurs in the elderly who have a preexisting injury and it is associated with bladder dysfunction and greater impairment in the upper extremities[10]. Spinal shock is a condition that lasts for hours to several weeks and is characterized by motor paralysis, loss of sensation, and the lack of bladder and bowel control[10]. Autonomic dysreflexia results sometimes after an injury above the T6 segment and leads to symptoms of headaches, sweating, facial erythema, and urinary retention[10].

The leading cause of death among SCI/D patients in the past was renal failure but in recent years infections (pneumonias and septicemias) have been common causes of death[2]. SCI/D may alter the sympathetic nervous system (SNS), which coordinates immune suppression. It has been stated many times that SCI/D patients are highly

susceptible to infection and researchers have labeled this immune suppression, SCI-immune depression syndrome, but there is little study on the immune response of patients following a SCI/D[11]. Findings also suggest that TBI and stroke patients are particularly susceptible to infection[12-14]. Decreased function and loss in T-cells are common changes found in stroke patients and there is developing clinical and experimental data, which propose SCI may lead to immune suppression and systematic autoimmunity [15, 16]. Studies have evaluated the presence and activities of immune cells such as CD14+ monocytes and CD3+ T-lymphocytes. One study found that following SCI, levels of these immune cells decreased during the first 24 hours and remained low for up to a week[17]. There is evidence SCI that occurs lower on the spinal cord, specifically T9, activates a normal immune response but high-level injuries, specifically T3 SCI, lead to an impaired immune response to exogenous antigens [18]. These data suggest that SCI/D may impair the immune response of a patient exposed to infectious agents, such as norovirus (NoV).

SCI/Ds and TBIs are usually life long conditions and thus the long term care of affected patients is of great concern. The median length of stay in the acute care setting for SCI patients in 2010 to 2012 was 11 days and median days in rehabilitation was 36 days[2]. Due to the complexity of SCI/Ds, there are many associated direct costs, such as inpatient stays, physician fees, medications, physical therapy and home modification[19]. These costs can lead to an increase in monetary demand on patients and families. It was estimated in 2012 that the lifetime cost for a patient who was injured at 25 years old and had a high tetraplegia injury was $44,633,137 and a paraplegic lifetime cost was $2,265,584[2]. This same report also estimated that even though 57.1% of patients were

employed at the time of injury, only 11.8% were employed one year following the injury, which could increase the monetary burden placed on a patient's family [2]. One major contributor to the cost of care is treatment of neurogenic bladder and bowel. Complications of these conditions are some of the most common problems of SCI/D patients and are associated with increased rates of illness, physician visits, and consumption of supplies[20].

A major concern in SCI/D patients is how the condition, treatment of the condition, and complications affect their well-being. Although patients lose sensory function following a SCI/D, many still experience chronic pain that is sufficient to reduce their quality of life[8]. Due to the uncertainty and life-long, extensive treatment needed, many SCI/D patients have decreased quality of life scores [8, 21]. One study among veterans, through surveys and questionnaires, found that individuals with chronic SCI have significantly lower quality of life scores than other individuals[21]. The decreased quality of life could be attributed to the loss of independence and loss of privacy that happen because of the required care and treatment, specifically regarding bowel function and management[22].

Neurogenic Bowel Dysfunction

Neurogenic bowel dysfunction (NBD) is a common, persistent complication in patients with SCI/D. It has been reported that it affects nearly half (46.9%) of SCI/D patients[23]. The control of bowel function depends on the autonomic and somatic nervous system, which can be damaged following a SCI/D, TBI, or stroke. Patients with NBD present with impaired gastrointestinal motility, fecal incontinence, or other long-

term medical conditions. The two types of NBD are upper motor dysfunction and lower motor dysfunction[24]. Upper motor dysfunction results from damage above the conus (the lower-end of spinal cord near L1 and L2) and symptoms can include reflex bowel contractions, absence of sensations, and loss of sphincter control while lower motor dysfunction occurs due to damage below the conus and can cause loss of sphincter control and anocutaneous reflex[24]. Bowel function can be very unpredictable following a SCI/D or TBI and many studies have found that there is a desire to regain this function among paraplegics and tetraplegics [22, 25]. One study found 30% of patients were more dissatisfied with colorectal dysfunction than either bladder or sexual dysfunction[26]. It was reported that defecation was abnormal in 68% of cases and that time spent defecating was more than 30 min in 24% cases[27]. Another study found that fecal incontinence was experienced by 75% of patients, but that most of these patients only had a few episodes of fecal incontinence each month or year[26]. These few episodes could be due to infectious causes because they are not encountered during usual routine. It has been reported that increasing time since injury, episodes of fecal incontinence decrease while constipation increases [28]. Using a NBD score to measure severity, it was found that the overall severity in SCI/D patients did not change significantly over time since injury [28].

There have been a number of studies which evaluate the effect NBD has on the quality of life of individuals and the majority of these studies used the generic Medical Outcomes Study SF-36 as the quality of life measurement tool[29]. Other quality of life measurement tools have been used in studies but the majority of studies, regardless of tool used, found a decreased quality of life among SCI/D patients suffering from NBD[22, 26, 28, 29]. Nanigian et al. used a specific fecal incontinence quality of life

measurement and observed a statistically significant difference in quality of life in regards to bowel management program, dietary management, symptoms, travel and socialization, family relationships, caregiver emotional impact, and financial impact ($P < 0.0001– 0.033$) [30]. NBD can affect the relationships of SCI/D and TBI patients. Due to the fear of incontinence, a reluctance to pursue and develop new relationships was found among SCI/D patients [22]. Many SCI patients who suffer from NBD often describe feeling humiliated or embarrassed due to incontinence[22].

There are limited treatment options available to patients who suffer from NBD. Treatments can involve life long bowel management programs, which are commonly multifaceted approaches. Bowel management programs involve high fiber diets [31], digital rectal stimulation[32], electric and magnetic stimulation[33], transanal irrigation[34], suppositories[35], abdominal massage[36], oral laxatives[37], or a combination of these treatments. These conservative methods are reported successful in 67% of SCI/D patients and when conservative management is ineffective, surgical interventions are another option[38]. Surgical options included sacral nerve stimulation, colostomy, and Malone antegrade continence enema[33]. Many treatments involve a diet change that promotes soft stool to reduce constipation [24]. However, it has been reported that some patients choose to eat and diet in a way to stay constipated in order to avoid accidents[24]. Over half of patients report providing their own bowel management, which includes suppositories, digital stimulation, and other aids[39]. There have been many reported problems, however, with bowel programs such as hemorrhoids, involuntary movements, gas, and more [39].

The effects these bowel management options have on patients' quality of life are poorly described[29]. One study found an effective decrease in time spent on bowel management when stoma formation was implemented (average decrease from 10.3 hours to 1.9 hours per week), which in turn could improve patients' quality of life[40]. Overall, patients who are more satisfied with their bowel management programs report a higher quality of life scores, making it important to find bowel management programs that please SCI/D patients[39].

There are many studies on the infections that arise due to the treatment of neurogenic bladder, another condition SCI/D often suffer from[20, 37, 38]. *Clostridium difficile*, a common nosocomial infection that causes diarrhea in susceptible individuals, has also been investigated and studied among SCI/D patients[41, 42]. There are, however, no evaluations on acute gastroenteritis in NBD patients, which could be due to agents such as NoV.

Norovirus

Norovirus (NoV), or more commonly named the "stomach flu," is a known leading cause of gastrointestinal disease worldwide. It has been estimated to be responsible for 19- 21 million cases of acute gastroenteritis in the United States each year [43]. The incubation period for NoV-associated gastroenteritis is between 12 and 48 hours, with one study finding a mean incubation period of 37 hours [44]. A NoV infection causes a sudden illness characterized by diarrhea, vomiting, and other adverse intestinal symptoms for 24 to 48 hours. Severity of disease is increased among the very young and the old, but the incidence of NoV infections does not vary across

demographics of age, sex, and race[45]. In Australia, it was found that only 0.6% of NoV infections required hospitalization[46]. Although hospitalization is rare with NoV infections, gastroenteritis hospitalizations have been increasing in recent years and it is estimated that NoV causes 10% of the cause unspecified illnesses and 7% of all gastroenteritis hospitalizations[47]. Outbreaks of NoV are known to occur throughout the year but 80% of outbreaks occur between November and April in the US[48].

NoV is an RNA virus that consists of a major structural protein and a minor capsid protein. There are many NoV strains and distinguishing these strains involves classification at three levels; genogroup, genotype and strain/variant [49]. The human NoV genome is compromised of a linear, 7.6 kB, positive-sense RNA [50]. There are three open reading frames that encode eight viral proteins (VP). The virion is composed of viral protein 1 (VP1) and viral protein 2 (VP2). NoVs are classified into 6 genogroups (genogroup I (GI) to GVI) and >40 genotypes based on the VP1 amino acid sequences [51]. The most common genogroups associated with outbreaks of gastrointestinal illness in humans are genogroup I (GI) and genogroup II (GII). GII.4 has been confirmed in 62% of worldwide NoV outbreaks, making it the most common genotype [52]. Genetic drift of the VP1 gene leads to the emergence of new strains and GII.4 has been found to have a pandemic cycle with new variants emerging every 2-3 years [53].

Outbreaks of acute gastroenteritis attributable to NoV demonstrate the multiple transmission pathways of the virus. It has been demonstrated that infected food-handlers can transfer the virus to ready to eat food[54]. Outbreaks have also been traced back to contaminated food sources such as raspberries[55] and oysters[56]. Groundwater, which was consumed as drinking water in a particular investigation, has also been linked to

outbreaks[57]. Three NoV GI.4 outbreaks in Korea were traced back to kimchi, which was prepared with contaminated groundwater[58]. When considering hospitals, schools, and other institutionalized facilities; the shared environments can become contaminated, increasing the risk of an outbreak. The environment can become contaminated through infected individuals' aerosolized vomitus [59] and contaminated hands touching surfaces after medical personnel care for affected patients[60].

NoV is an extremely contagious virus. It is easily transmitted from person to person and infected individuals can shed up to 10^{11} viral particles per one gram of stool [61]. Asymptomatic individuals have also been found to shed viral particles and thus can contribute to the spread of NoV unknowingly [61]. The dose of NoV needed to cause infection is estimated to be between 1 and 100 viruses and thus can easily lead to illness in individuals[62]. In addition to this, NoV has been found to persist in the environment for a long period of time, for months and possibly years[62]. These factors make it a particular concern in the healthcare and rehabilitation setting, where SCI/D patients spend a substantial amount of time.

Due to its high infectivity, individuals with decreased immunity could be more susceptible to illness, and particularly severe illness, compared to healthy individuals. The majority of NoV illnesses last for 24 to 48 hours, but long term/chronic infections have been documented in immunocompromised individuals[52, 63, 64]. One study found, through molecular information combined with demographic data, the direction of transmission was most likely from the chronic patients to other hospitalized patients[63]. It has been found that these long term infections can lead to individuals shedding the virus for extended time periods[65]. One case was documented with chronic diarrhea and

NoV shedding for more than two years[66]. Long-term infections have also been associated with virus evolution and there is evidence that these infections may be a source of emerging viral variants[66, 67]

Institutionalized populations, such as those in a hospital or rehabilitation setting, are at an increased risk of contracting NoV due to shared resources; which include nursing staff, equipment, and food[68]. Immunocompromised individuals are usually included in these long-term health care populations. Because of these factors, there is a particular concern for outbreaks of NoV in institutionalized settings. GII.4 (genogroup II, genotype 4) viruses have been detected as the most predominant strain of outbreaks that occurred in a healthcare or rehabilitation setting [69-71]. One such study found that over a five year period, 91% of patients in health care settings with NoV-associated gastroenteritis were infected with NoV GII.4[71]. Although the disease usually has a short duration, outbreaks in a health-care setting were found to last an average of 15.6 days and 13.1 days in a nursing home environment[70], with one outbreak lasting 63 days[72]. The length of these outbreaks may be due to improper or ineffective infection control practices.

NoV and other common nosocomial infections, such a *Clostridium difficile,* have been found to be resistant to many common disinfectants such as alcohol [73, 74]. Alcohol-based hand sanitizer was found to be relatively ineffective in reducing NoV cDNA while antibacterial liquid soap with water produced the greatest reduction[75]. Total inactivation of human NoV was found with sodium hypochlorite-based disinfectant with a contact time of 10 min while no reduction was found with either of the commonly used disinfectants; ethoxylated alcohol or quaternary ammonium[74]. During a NoV

outbreak in a rehabilitation facility, it was found that even though disinfectant use appeared to be sufficient, environmental samples of toilet seats, a door handle, and ultrasound equipment were contaminated with the same strain that was detected in patients[76]. These data reveal that NoV is of great concern in hospital and healthcare setting and infections may have a greater effect on this vulnerable population.

Human NoVs cannot be grown in cell culture for detection, therefore methods for detecting viral RNA or antigens are commonly used. According to the Centers for Disease Control and Prevention, the options used for detection are Real Time Quantitative Polymerase Chain reaction (RT-qPCR) Assays, conventional RT-PCR assays for genotyping, and enzyme immunoassays. Electron microscopy may also be used and has the ability to detect multiple pathogens, but it is costly and has low sensitivity[77]. Immunochromatographic (ICG) lateral flow assays are other rapid tests that have 100% specificity, but low sensitivity (35-53%)[77]. RT-qPCR is the most commonly used technique and the preferred method because of high sensitivity, detecting as few as 10 to 100 NoV copies per reaction[78]. Conventional RT-PCR is also used for detection due to its lower cost, although it has decreased sensitivity compared to RT-qPCR. One study, however, estimated RT-PCR sensitivity to be 97.3% (36/37) in stool sampling and rectal swab testing [79]. Enzyme Immunoassays, particularly rapid commercial assays have recently been created but many of these kits also have very low sensitivity (50%) and should not be used in place of molecular techniques in detecting NoV in an outbreak situation [78].

The high burden of NoV disease has motivated researchers to develop a vaccine to protect against illness and reduce transmission. Expression of the NoV capsid protein

VP1 leads to formation of virus-like particles (VLPs), which have emerged as possible candidates for vaccines[80]. There is evidence supporting the effectiveness of nasally and orally administered vaccines [81, 82]. In recent years there has been progress in the development of a NoV intramuscular vaccine. During a randomized double blind study, an intramuscular NoV virus-like particle (VLP) vaccine was found to be immunogenic and provided evidence of a rapid immune response to a single dose, offering potential beneficial control options during an outbreak situation[80]. Future vaccine development may provide preventative options for healthcare workers, family members of immunocompromised patients, military personnel, travelers, and children. Due to the common occurrence of NoV outbreaks on cruise ships, a vaccine would be of particular interest to cruise ship workers and vacationers. A future vaccine may decrease the overall burden of disease worldwide because of the multiple populations affected by NoV.

No studies have been done to evaluate the particular concern of NoV infection among SCI/D or TBI rehabilitation inpatients who experience NBD. NBD is common in this population and has been associated with lower quality of life[8, 22, 25, 30]. This pilot study aims to demonstrate the need for further study on infectious causes of gastroenteritis among SCI/D patients. If diarrheal and vomiting episodes are due to NoV in this study population, treatment and infection control programs can be better adapted to respond to, and prevent, potential NoV outbreaks in a rehabilitation facility such as the Shepherd Center. Ultimately, such evaluations may lead to improvements of care and thus increased quality of life among SCI/D patients.

Chapter II: Manuscript

Title

Prevalence of Norovirus among Rehabilitation Inpatients with Neurogenic Bowel Dysfunction due to Spinal Cord Injury or Traumatic Brain Injury: A Pilot Study

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Abstract

Bowel dysfunction is a common condition among spinal cord injury and disease (SCI/D) traumatic brain injury (TBI), and stroke patients and can result in a lower quality of life. Diarrhea episodes are commonly attributed to neurogenic bowel dysfunction (NBD), and infectious agents are typically not considered to cause gastroenteritis in this population. Norovirus (NoV) is a leading cause of gastrointestinal illness worldwide and has been related to many outbreaks in health-care settings. This pilot study evaluated the prevalence of NoV (genogroups I and II) among rehabilitation inpatients with NBD due to a SCI/D, TBI, or stroke. Following a diarrhea or vomiting episode, stool samples, medical information, and demographic information were collected from 25 rehabilitation inpatients from the Shepherd Center in Atlanta, GA during the months of November 2014 through March 2015. No patients were found to be infected with NoV GI or GII. This provides evidence that there was not an outbreak of NoV at Shepherd Center during the 2014-2015 season. However, we cannot conclude that NoV does not affect this population due to small sample size and low study power. Other infectious agents

affected the majority of study patients (92%), suggesting further analyses should be done to evaluate the immune response following an SCI/D or TBI. There should be future studies designed with enough power to detect the prevalence of NoV infections among SCI/D and TBI rehabilitation inpatients. Ultimately, such evaluations may lead to improvements of care and thus increased quality of life among SCI/D and TBI patients.

Introduction

Spinal Cord Injury and Disease (SCI/D) affect many individuals worldwide. The highest prevalence of traumatic SCI in the world was estimated in the United States at 906 cases per one million individuals[1]. In 2012, 40.6% of SCI patients in the United States had incomplete tetraplegia (formerly known as quadriplegia), 22.7% of patients had complete tetraplegia, 18.7% had incomplete paraplegia, and 18% had complete paraplegia [2].

Bowel dysfunction is a common condition among SCI/D patients. Patients with neurogenic bowel dysfunction (NBD) can present with impaired gastrointestinal motility, fecal incontinence, or other long-term medical conditions. It has been reported that defecation is abnormal in 68% of SCI patients and that many patients spend a substantial amount of time on their bowel management program[27]. Living with these conditions can increase monetary demands and have an impact on an individual's diet, employment, relationships, social participation, and emotions [9, 22, 26, 28, 29]. Infectious agents are not commonly considered as a possible cause of bowel dysfunction among SCI patients. Alternatively, diarrheal episodes are usually attributed to NBD and are treated accordingly.

NoV, also known as the "stomach flu," is a leading cause of gastrointestinal disease worldwide that affects all age groups [45, 48]. NoV infection has a short incubation period, between 12 and 48 hours, and causes a sudden illness characterized by diarrhea, vomiting, and other adverse intestinal symptoms for 24 to 48 hours. NoV is an extremely contagious virus with observed attack rates >50% in outbreaks[61, 83-85]. NoV is a particular concern in healthcare settings due to high viral titers shed by infected

individuals, up to 10^{11} viral particles per one gram of stool [61] [62], low infectious dose (10-100 virions)[73], long persistence in the environment[73, 86], and resistance to common disinfectants[74, 75].

Most human NoV infections are due to viruses in genogroup I (GI) and genogroup II (GII)[52]. GII.4 (genogroup II, genotype 4) viruses are the most common and have been detected in 62% of worldwide NoV outbreaks[52]. GII.4 have also been detected as the most predominant strain in outbreaks that occur in a healthcare or rehabilitation setting [69, 70]. Outbreaks of NoV are known to occur throughout the year but 80% of outbreaks occur between November and April in the US[48].

This study aims to determine if NoV is present and potentially causing gastrointestinal illness among SCI/D patients with NBD who are admitted to the inpatient rehabilitation unit at Shepherd Center in Atlanta, GA between November 2014 and March 2015. If diarrheal and vomiting episodes are due to NoV in this study population, treatment and infection control programs can be better adapted to respond to, and prevent, potential NoV outbreaks in a rehabilitation facility such as Shepherd Center.

Materials and Methods

Study Design

Spinal Cord Injury or Disease (SCI/D) rehabilitation inpatients at the Shepherd Center diagnosed with NBD were enrolled into the study following a diarrheal or vomiting episode. Samples were de-identified prior to transportation from Shepherd Center and ineligible subject's samples were destroyed. Shepherd Center's Institutional Review Board approved the study protocol.

Diarrhea was defined as an episode of spontaneous defecation, which produced a stool with a grade of 5 or higher on the Bristol stool scale. NBD was defined as any loss of bowel control following a SCI/D. SCI/D was defined as partial or complete limb paralysis following a traumatic injury or disease such as multiple sclerosis.

Study patients were admitted to the Shepherd Center between November 2014 and March 2015 and individuals were recruited one of two ways. A patient was identified when a stool sample was sent by physician's request to the clinical laboratory for diagnostic testing following a diarrheal episode or a healthcare provider collected a stool sample from patients following an episode of vomiting. Patients were then enrolled into the study after obtaining a signed or verbal consent. Samples were excluded from the study if they belonged to patients who tested positive for norovirus in the preceding two-week period. Samples were stored at -20°C in Shepherd Center's diagnostic laboratory, then collected and transferred once a week to Emory University. Samples were stored in an Emory University laboratory at -80°C until testing.

After inclusion into the study, patient charts were reviewed at Shepherd Center and the following data were collected: age, gender, injury level, bowel management, co-

infections, medications, bowel history, admission date, and, if applicable, discharge date. With this information, patients were assigned a NBD score using the questionnaire developed by Krogh et al[87].

Microbiological Methods

Samples were tested for the presence of norovirus GI and norovirus GII genomic RNA using protocols explained in greater detail elsewhere[88, 89]. Following the creation of 20% stool vol/vol suspensions with sterile water for each sample, viral particles were separated using Vertrel XF (DuPont, Wilmington, DE). RNA extraction was performed using the QiaAmp Viral RNA kit (Qiagen, Germantown, MD). Following extraction, real-time reverse transcription polymerase chain reaction (RT-PCR) for GI and GII were performed and all samples were tested in duplicate. Broadly reactive primer and probe sets were used to detect GI or GII RNA viruses using the Qiagen One Step RT-PCR kit (Qiagen, Germantown, MD) [89]. GI and GII standards were used as assay controls. Analyses were performed on a Bio-Rad CFX96 qPCR machine (Bio-Rad, Hercules, CA). RNA concentrations were calculated using CFX Manager Software (Bio-Rad) and samples with duplicate C_t values greater than or equal to 41 and within 2 C_t values of each other were considered positive.

Statistical Analysis

The outcome was dichotomized into the presence (GI or GII) or absence of NoV and the exposure was categorized into 2 groups: thoracic injury or cervical injury and traumatic brain injury or stroke. Patients NBD scores were classified using available data as the following: 0-6 as very minor, 7-9 as minor, 10-13 as moderate, and 14 or more as severe bowel dysfunction[87]. Descriptive statistics were calculated to describe the

demographic characteristics between the exposure categories. Results were expressed as the mean (range) or as a proportion of the total number of samples. Differences in means between injury level groups were compared using T-test procedures and proportions were compared using a Fisher's exact test. Power and sample size calculations were performed using a precision of 5%. All tests of significance are 2-tailed with α set at 0.05.

Data entry and data cleaning were performed in Microsoft Excel 2011 and data analyses were done using Statistical Analysis System (SAS, version 9.4) software.

Results and Discussion

There were a total of 418 patients admitted to Shepherd Center for inpatient rehabilitation and 63 samples submitted to the laboratory between November 1, 2014 and April 1, 2015 (table 1). Twelve patients were discharged before consent was obtained. Nineteen patients were unable to consent to the study, which is a common issue when working with vulnerable populations, such as TBI patients[90]. Of the remaining 25 inpatients enrolled, 4 individuals had 2 samples submitted during the study period, resulting in 29 stool samples eligible for analysis. During the study period, the majority of eligible samples were submitted between mid-January and mid-February, with the least number of samples submitted in March (figure 1). This trend closely follows the one seen for all 63 submitted samples, suggesting there was no temporal bias in the consent process (data not shown).

The study population was 76% male and 84% white (table 2). The average age was 37.8 years (range 16-73, table 2). The demographics of the study sample similarly represent the demographics of SCI patients in the United States with approximately 80% male, but the study sample has a higher percentage of white compared to the 67% National SCI/D population [2]. The average age of the study patients were slightly younger than the national average of 42.6[2]. The median length of inpatient care for the 15 patients with discharge information was 63 days (mean 66.9, range 36-153, table 2), which is higher that the national median days of a SCI/D rehabilitation (36 days) [2]. The extended length of stay for Shepherd Center rehabilitation inpatients could be reflecting the severity of injuries that this facility regularly treats compared to other rehabilitation hospitals in the United States.

Reported conditions in the study population included; TBI (n=10), stroke (n=2), cervical spine injury (n=8), and thoracic spine injury (n=5). Among the SCI inpatients who experienced cervical spine injuries, 62.5% were documented with complete tetraplegia, 25.0% with incomplete tetraplegia, and the remaining with incomplete paraplegia (table 2). Among the SCI inpatients who suffered a thoracic spine injury, 80% were documented with complete paraplegia while the remaining with incomplete paraplegia (table 2).

The average NBD score for the 21 study subjects with relevant information available was 10.9 (range 4-20, table 3). A total of 16 patients had a history of diarrhea and one patient had a history of constipation the week prior to sample collection (table 3). For the treatment of NBD, one patient had a colostomy, one was treated with an enema, 4 had rectal tube placements within the week before sample collection, and 9 were administered oral or IV medications (table 3). Specifically among SCI patients, bowel management programs involved digital rectal stimulation for 8 subjects and suppository use for 5 subjects (table 3). There was a significant difference between the two injury groups for the use of digital rectal stimulation ($p=0.002$) and suppository use ($p=0.039$) because no TBI or stroke patients had documented use of these treatments for NBD (table 3). Complete chart abstraction could only be done on discharged patients and the patient electronic medical records did not have all the necessary data to compute NBD scores. The reported NBD scores, therefore, are not precise but provide the lowest value possible. Patients' true NBD scores are as high or higher than what is reported. Sixty-two percent of patients experienced moderate to severe NBD (table 4), reflecting the typically high burden of disease experienced by SCI/D and TBI inpatients[27, 39].

The goal of this study was to evaluate whether NoV was prevalent and causing gastrointestinal illness between SCI/D and TBI rehabilitation inpatients at Shepherd Center in Atlanta, GA. Neither NoV GI nor GII strains were detected in the 29 stool samples submitted from 25 study subjects. Although this provides evidence that NoV is not affecting inpatients at Shepherd Center, our study size was small and only constitutes 6% of the total admitted patients and 40% of the total stool samples submitted during the study period (table 1). Based off of a previous study, which found the prevalence of NoV in sporadic cases of gastrointestinal illness to be 9.3%, we predicted a prevalence of 10% among inpatients at Shepherd Center during the study period[91]. With this prediction, a 5% precision, 95% confidence level, and with the knowledge that there were 418 patients admitted during the study period we would have needed a sample size of 105 in order to have enough power to detect a 10% prevalence of NoV among the inpatients at Shepherd Center. Thus, this study size was not sufficient to adequately test the hypothesis. In addition to the lack of an adequate sample size, it is important to note that patients can potentially be carriers with no symptoms and yet unknowingly contribute to the spread of infection [61].

Selection bias is also a major limitation of this study because the sample is a convenience sample. Physicians only submitted stool samples to the Shepherd laboratory for *Clostridium difficile* after symptoms of diarrhea lasted for multiple days or weeks. NoV causes a sudden illness with gastrointestinal symptoms lasting 24-48 hours. Thus, the shorter duration NoV infections were most likely missed if they were present in this population. In addition to this, the sample is probably not fully representative of the admitted inpatient population during the study period and may not entirely represent the

United States population of individuals who have experienced SCI/D or TBI due to the high acuity of cases seen at Shepherd Center. Therefore, it would be beneficial for future studies to consent every admitted patient at Shepherd Center during the study period and obtain multiple stool and vomitus samples during the course of the NoV season. It would also be beneficial to collect samples from healthcare workers and visitors of the facility to see if there is potential exposure to the virus from outside the hospital.

We cannot conclude that no patients had gastrointestinal illness caused by NoV at Shepherd Center during the 2014-2015 season, but because we have samples spread out over the course of the study months, there is evidence to suggest that no outbreak occurred at the rehabilitation facility. This is encouraging as it provides evidence of proper and effective infection control practices by the healthcare providers and facility staff.

There is little study on the immune response of patients following a SCI/D. However, there is developing clinical and experimental data, which propose SCI may lead to immune suppression and systematic autoimmunity [15, 16, 18]. Infections the week prior to sample collection were documented in 92% of study subjects (table 3). Infections documented on inpatients included; pneumonia (n=6), urinary tract infection (n=6), MRSA (n=6), C*lostridium difficile* (n=6), and fungal infections (n=2). Among patients with infections, four had two or more. There was no significant difference in infection status between TBI/stroke patients compared to SCI patients (p=0.480). These data further suggest SCI/D and TBI may have a role in negatively affecting the immune response of a patient exposed to infectious agents, such as NoV. Better understanding the prevalence of NoV and other infectious agents in this population will prompt further

study into the immune susceptibility of SCI patients. These data suggest that if an outbreak was to occur in a rehabilitation facility such as Shepherd Center, patients could be more severely affected compared to their healthy counterpart.

If an outbreak of acute gastroenteritis were to occur at Shepherd Center or similar facility there is beneficial information that should be gathered that was not in this study. Inpatients at Shepherd Center often have rehabilitation in locations other than their assigned treatment rooms and travel to the shared gym and cafeteria space. These locations can provide an environment in which NoV can spread rapidly. It would be useful to gather information on the number of shared resources, how often individuals visit specific locations, and surface contaminates on equipment identified through environmental swabs, which have been linked as the source of NoV outbreaks in the past [68].

Conclusion

To the authors' knowledge, this is the first study to look at NoV infections among SCI/D and TBI rehabilitation inpatients. This pilot study provides evidence that there was not an outbreak of NoV among rehabilitation inpatients who suffer from SCI/D and TBIs during the 2014-2015 season, but does not have enough study power to conclude these patients are not susceptible to NoV infection. Further studies should take into account the limitations of this pilot study to direct and plan thorough study recruitment and data collection to evaluate the effect NoV and other infectious agents have on SCI/D and TBI rehabilitation inpatients.

Figures and Tables

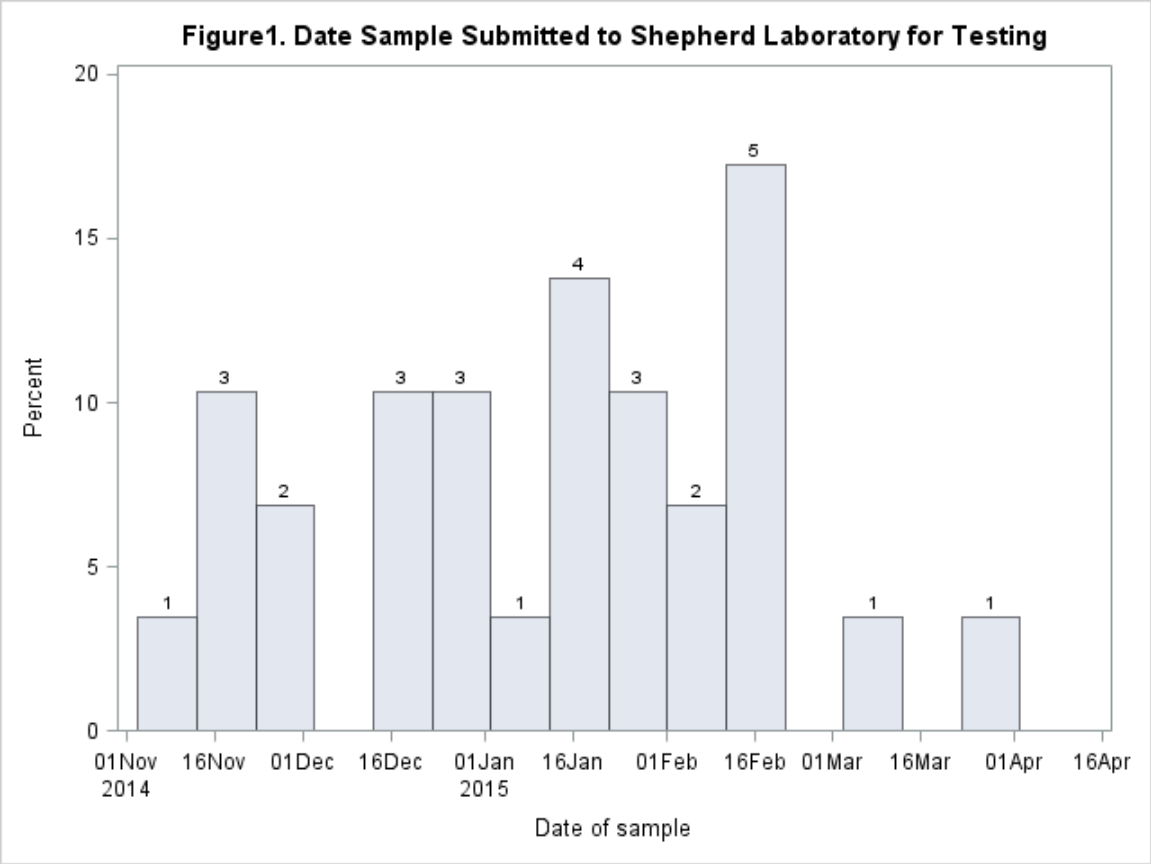

Table 1. Admitted rehabilitation inpatients, submitted stool samples, and enrolled patients between November 1, 2014 and March 31, 2015 at Shepherd Center in Atlanta, GA

	No.
Total admitted patients	418
Total samples submitted to Shepherd Center Laboratory	63
Patients with samples submitted[1]	56
Patients enrolled[2]	25
Patients discharged before consent obtained	12
Patients who could not consent	19
Samples analyzed for Norovirus GI/GII	29

[1] This indicates the total number of patients who had samples submitted to the lab. One patient had three submitted samples while five had two submitted samples each.

[2] Patients were enrolled into the study after obtaining a signed or verbal consent.

Table 2. Characteristics of Spinal Cord Injury, Traumatic Brain Injury and Stroke Patients at Shepherd Center November 2014-March 2015. n=25

	No.	%
Stroke	2	8
Traumatic Brain Injury	10	40
Spinal Cord Injury Level	17	68
Cervical	8	32
Complete[1] tetraplegia	5	62.5
Incomplete[2] tetraplegia	2	25
Complete paraplegia	1	12.5
Thoracic	5	20
Complete paraplegia	4	80
Incomplete paraplegia	1	20
Sex		
Male	19	76
Race		
White	21	84
Black	3	12
Hispanic	1	4
	Mean	**Range**
Age (years)	37.8	16-73
Days in Hospital (n=15)	66.9	36-153

[1] Complete SCI is defined as an injury where no motor or sensory function is preserved.

[2] Incomplete SCI is defined as an injury where sensory function is intact, but little to no motor function is preserved.

Table 3. Characteristics between Spinal Cord Injury and Traumatic Brain injury/Stroke Patients at Shepherd Center November 2014-March 2015.

	Total n=25		TBI or Stroke n=12		Thoracic or Cervical Injury Level n=13		p-value[1]
	No.	**%**	**No.**	**%**	**No.**	**%**	
Sex							
Male	19	76.0	8	66.7	11	84.6	0.378
Race							1.000
White	21	84.0	11	91.7	10	76.9	
Black	3	12.0	1	8.3	2	15.4	
Hispanic	1	4.0	0	0	1	7.7	
Treatment of NBD[2]							
Digital Rectal Stimulation	8	32.0	0	0.0	8	61.5	0.002
Suppository Use	5	20.0	0	0.0	5	38.5	0.039
Medication	9	36.0	6	50.0	3	23.1	0.226
Rectal Tube	4	16.0	1	8.3	3	23.1	0.593
Colostomy	1	4.0	0	0.0	1	7.7	1.000
Enema	1	4.0	0	0.0	1	7.7	1.000
Co-infection	23	92.0	12	100.0	11	84.6	0.480
Clostridium difficile	6	24.0	3	25.0	3	23.1	1.000
MRSA	6	24.0	2	16.7	4	30.8	0.645
Pneumonia	6	24.0	2	16.7	4	30.8	0.645
UTI	6	24.0	2	16.7	4	30.8	0.645
Fungal	2	8.0	1	8.3	1	7.7	1.000
Antibiotic Use	21	84.0	10	83.3	11	84.6	1.000
NBD Symptoms							
Diarrhea[3]	16	64.0	9	75.0	7	53.8	1.000
Missing	6	24.0	4	33.3	2	15.4	
Constipation	1	4.0	0	0.0	1	7.7	0.421
	Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**	
Age (years)	37.8	16-73	31.6	16-53	43.5	19-73	0.084
NBD Score	10.9 (n=21)	4-20	10.4 (n=8)	4-17	11.2 (n=13)	4-20	0.745
Days in Hospital	66.9 (n=15)	36-153	52.4 (n=7)	36-69	79.5 (n=8)	40-153	0.086

[1] p-values were calculated using Fisher's Exact Test for comparing proportions and T-test for comparing mean values between TBI/stroke and SCI inpatients.
[2] Neurogenic Bowel Dysfunction (NBD) is defined as any loss of bowel control secondary to a central nervous system disease or injury.
[3] Diarrhea was defined as any episode of spontaneous defecation which produced a stool with a grade of 5 of higher on the Bristol stool scale

Table 4. Neurogenic Bowel Dysfunction Severity between Spinal Cord Injury and Traumatic Brain injury/Stroke Patients at Shepherd Center November 2014-March 2015.

	Total n=21		TBI or Stroke n=8		Thoracic or Cervical Injury Level n=13	
	No.	**%**	**No.**	**%**	**No.**	**%**
NBD Severity[1,2]						
Very Minor	7	33.3	2	25.0	5	38.5
Minor	1	4.8	1	12.5	0	0.0
Moderate	7	33.3	3	37.5	4	30.8
Severe	6	28.6	2	25.0	4	30.8

[1] Severity determined using criteria described by Krog et al.

[2] Neurogenic Bowel Dysfunction (NBD) is defined as any loss of bowel control secondary to a central nervous system disease or injury.

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Appendix A: Shepherd Center IRB Approval Letter

Shepherd Center
2020 Poachtree Road, NW Atlanta, GA 30309-1465 404-352 2020 shepherd.org

Project#: 632
Event#: 653656-1

DATE: September 11, 2014

TO: *Amy Kirby, PhD*

FROM: Michael L. Jones, Ph.D.
Chair, Shepherd Center Research Review Committee

RE: Project# 632- (653656-1] Norovirus Infection in Rehabilitation Inpatients With Neurogenic Bowel Dysfunction: A Pilot Study; PI Amy Kirby, PhD

Request for Approval of the Use of Human Subjects in Research

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This is to inform you that the Shepherd Center Research Review Committee has reviewed and approved the above referenced proposal at its meeting on September 11, 2014. Approval for this research is granted effective for one year. One month before expiration on September 11, 2015, you will be reminded to inform the Research Review Committee of the status of this project. Re-approval must be granted before the expiration date or the project will automatically be "suspended". Failure to receive a notification that it is time to renew does not relieve you of your responsibility to provide the RRC with a request for "Continuation Approval" In time for the request to be processed and approved before your expiration date.

The Shepherd Center Research Review Committee has the following recommendation for this study:

1) Revise the informed consent process to use a single process and form both for the use of specimens already collected and for obtaining a specimen.

The Principal Investigator must report to this office, in writing, within 10 days, any unanticipated problems involving risks to the subjects or others, such as serious adverse reactions to biological drugs, radio- isotopes or to medical devices. Records pertaining to research must be retained for at least three years after completion of the research.

You are responsible for notifying all parties about the approval of this project, including your Co-PIs and department head. If you have any questions, please feel free to call me at 404-350-7595.

Appendix B: Consent Document

SHEPHERD CENTER
RESEARCH PATIENT INFORMATION AND CONSENT FORM WITH AUTHORIZATION FOR RELEASE OF PROTECTED HEALTH INFORMATION

Norovirus Infection in Rehabilitation Inpatients with Neurogenic Bowel Dysfunction: A Pilot Study

Principal investigator: Amy E. Kirby, PhD, MPH **Project: #632**

Subject's Name: _____ MR#:_____
ID# *(Leave Blank – assigned by investigator):* _____

Background: Norovirus (or the stomach flu) is the most common cause of diarrhea and vomiting in the US. It is estimated that 21 million people get norovirus every year in the US. People with neurogenic bowel dysfunction due to spinal cord injuries or disease can also have diarrhea and vomiting. These symptoms are believed to be due to the neurogenic bowel dysfunction but it they could also be due to norovirus infection. There have not been any studies on norovirus in patients with neurogenic bowel dysfunction, so we do not know how often bowel symptoms are due to norovirus infection versus neurogenic bowel dysfunction. Knowing the cause of bowel symptoms is important because it might change the treatment.

Purpose: The purpose of this study is to determine whether norovirus infection can cause diarrhea and vomiting in patients with neurogenic bowel dysfunction.

Duration and Scope: This study will last 8 months and enroll 120 participants.

Procedures: If you have diarrhea or vomiting during your stay at Shepherd Center and you have agreed to participate in this study, a stool sample will be collected by the nursing staff. The sample will be collected within one week of your symptoms. If you have diarrhea and your doctor orders a stool test, a portion of that sample will be used for this study. The sample will be sent to the research laboratory at Emory University for norovirus testing.

If your stool sample is tested for norovirus, your Shepherd medical chart will be reviewed to collect the following information: age, gender, date of admission, injury level, bowel management program, and recent bowel history.

You will not receive any medical treatment in this study.

Risks: There are no health risks for participating in this study. There is a risk that your personal information may be released.

Right of Investigator to Withdraw: The investigator has the right to withdraw you from the study at any time.

Benefits: There are no direct benefits to you for participating in this study. The results of this study may be used improve the treatment of patients with neurogenic bowel dysfunction.

Confidentiality: Information that could identify you will be removed from your stool samples and medical data before it is released to Emory University. At Emory, the study data will be stored on a secure network maintained by Emory University. Paper records will be stored in a locked file cabinet in an office with controlled access. Only authorized study personnel and regulatory offices will have access to the study records.

Cost/Compensation: There is no cost to participate in this study. You will not be compensated for your participation in this study.

Voluntary Participation/Withdrawal: You can withdraw from this study at any time. To withdraw, please contact Amy E. Kirby at 404-712-8164 or aekirby@emory.edu.

Authorization to Use and Disclose Information for Research Purposes

Federal regulations give you certain rights related to your Protected Health Information (PHI). These include the right to know who will be able to get the information and why. The researchers and providers must get your authorization (permission) to use or give out any health information that might identify you.

The term "researchers and providers" will be used to include the group of people who may get personal information about you. These include the:

- doctor
- study staff
- Hospital or clinic (involved with a study procedure)
- Other health care providers involved in your care during the study

What information may be used and given to others?

If you choose to be in this study, the study doctor will get personal information about you. The study doctor may also get information about your health including:

- Medical and research records identifying you and describing your medical condition
- Records of physical exams
- Laboratory, x-ray, and other test results
- Records about your medications

Who might get this information?

The researchers and providers may give your information to the sponsor of this research, NoroCORE and the USDA. "Sponsor" includes any persons or companies that are working for or with the sponsor, or are owned by the sponsor. Your health information, your name, and other information that could be used to identify you will be coded. Information about you and your health, which might identify you, may also be given by the researchers or the providers to a third party including the following:

- The U.S. Food and Drug Administration (FDA)
- Department of Health and Human Services (DHHS) agencies
- The Institutional Review Board (IRB)
- Other parties as required by law

How will this information be used?

Your health information will be used to determine if there are any risk factors for norovirus infection in patients
with neurogenic bowel dysfunction.

What if I decide not to give permission to use and give out my health information?

If you do not give permission to use your health information, you cannot participate in this study.

May I withdraw or revoke (cancel) my permission?

You can withdraw your permission at any time by contacting Amy E. Kirby at 404-712-8164 or aekirby@emory.edu.

May I review or copy the information obtained from me or created about me?

The research records will not be available for your review.

Source of Funding: Funding for this research study will be provided by NoroCORE and the US Department of
Agriculture (USDA).

Patient's Rights Questions: If you have any questions about your rights as a patient in this study, please contact Michael L. Jones, Ph.D., Chair, Shepherd Research Review Committee, 2020 Peachtree Road NW, Atlanta, Georgia 30309, (404) 350-7595.

Consent:
I have read the information in this consent form (or it has been read to me). All my questions about the study and
my participation in it have been answered. I freely consent to participate in this Research Study . I authorize the use and disclosure of my health information to the parties listed in the authorization section of this consent for the
purposes described above. By signing this consent form, I have not waived any of the legal rights, which I
otherwise would have as a patient in a research.

Patients 18 years and older must sign & date on the patient line:

_____ _____
Signature of Patient **Date**

_____
Printed Name of Patient

Signature of staff person conducting consent discussion:_____ Date:____
Printed name of staff person conducting consent discussion:_____

Use the following only if applicable

* *If the patient is minor under 18 years of age or unable to consent, consent must be provided by the Legally Authorized Representative: (if patient is under 18, also complete assent signatures in box below)*

_____ _____
Signature of Patient's Legally Authorized Representative Date

_____
Printed Name of Patient's Legally Authorized Representative

**For patients under the age of 18, assent signatures must be provided:*
Minor Assent Statement: I am a minor and this research has been explained to me and I agree to be in this study.

_____ _____ _____
Minor Patient's Signature for Assent Date Age (years)

Signature of staff person: I confirm that I have explained the research to the extent compatible with the patient's age and understanding, and that the minor patient has agreed to be in the study.

_____ _____
Signature of Staff Person Conducting Assent Discussion Date

**If this consent form is read to the patient because the patient (or legally authorized representative) is unable to read the form, an impartial witness not affiliated with the research must be present for the consent and sign the following statement:* I confirm that the information in the consent form and any

other written information was accurately explained to, and apparently understood by, the *patient* (or the *patient*'s legally authorized representative). The *patient* (or the *patient*'s legally authorized representative) freely consented to participate in the research study.

_____ _____

Signature of Impartial Witness Date