The Effects of Chemotherapy and Radiation Therapy Treatment on Influenza Vaccine Antibody Response of Cancer Survivors

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ABSTRACT

The immune response of cancer patients is depressed in comparison to healthy individuals. Previous studies have shown that many components of B and T cell immunity are deficient during the active phase of cancer treatment, and some cancer patients are at twice the risk of being infected with influenza virus in comparison to the general population. In this study, the antibody response of cancer patients to H1N1 influenza virus was analyzed and compared to non-cancer participants matched by race, age, and BMI. Serum samples were collected from study participants pre and post influenza vaccination. Hemagglutination inhibition assay was performed to determine pre vaccination antibody titer, post vaccination antibody titer, fold increase of antibodies, and percent of participants protected from influenza virus. It was determined that cancer survivors who were exposed to chemotherapy and radiation therapy in the past had significantly lower H1N1 influenza virus antibody response in comparison to their non-cancer matches. Post vaccination antibody titers and fold increase of cancer survivors showed no significant difference in comparison to the control group. However, cancer survivors still had difficulty reaching an antibody titer considered protective against influenza virus, as half of this group was unable to develop an adequate antibody immune response post vaccination. This suggests that dysregulation in the immune system of cancer survivors caused by previous chemotherapy and radiation therapy treatment and/or cancer itself could be inhibiting the ability of cancer survivors to develop adequate protection from influenza vaccination.
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INTRODUCTION

Influenza Virus

Each year human influenza A and B viruses are routinely spread among people and lead to seasonal flu epidemics. Influenza A viruses are classified into strains by their expression of surface proteins, hemagglutinin (HA) and neuraminidase (NA). The current strains of influenza virus found in people are virus subtypes influenza A (H1N1) and influenza A (H2N2). Influenza B viruses can be further classified by lineage and strains. The current circulating influenza B viruses are derived from lineages B/Yamagata and B/Victoria.¹

Through the processes of antigenic shift and antigenic drift, the circulating influenza virus changes with each season. Antigenic drift consists of small changes in the genome of influenza viruses that result from the virus continually replicating over time. These small changes to the virus genetic material accumulate over time and can lead to virus strains that are antigenically different and possibly unrecognized by the immune system. Antigenic shift is a significant and abrupt change in the influenza A viruses that results in new hemagglutinin or neuraminidase proteins on the virus’s surface. This drastic change leads to a new influenza A subtype or a virus with a different hemagglutinin and neuraminidase combinations derived from an animal population. When antigenic shift occurs, people usually have little or no protection against the new virus strain. Influenza A viruses can undergo both antigenic drift and antigenic drift, while influenza B viruses can only undergo antigenic drift.²

Influenza Virus Infection and Flu Response

Influenza virus spreads mainly though small droplets containing influenza virus. When an infected individual sneezes, coughs or talks, these tiny droplets can be expelled into the air. Droplets can then land near the mouths or noses of other people, potentially leading to the spread of influenza virus. Once in the body, the virus attaches to cells in the nasal passage and throat. In the
respiratory tract, the virus’s HA surface proteins can bind to sialic acid receptors on the surface of human cells. This binding allows the virus to enter the cell, leading to influenza virus infection.³

Upon infection, the immune system responds by developing innate and adaptive immune responses that work to counter the infection. The innate immune system is the first line of defense against infection and consists of components, such as mucus, that work to prevent infection of the epithelial cells along the respiratory tract. Innate cellular immune responses also play a role in preventing replication of virus. Adaptive immunity is the second line of defense against influenza virus and consists of virus-specific antibodies and T cells. Infection of influenza virus leads to the development of virus-specific antibodies. Antibodies specific for HA and NA surface proteins are important for protection against influenza virus, leading to protective immunity. Virus-specific T cell responses occur after infection, including CD4+ T helper cells and CD8+ cytotoxic cells. Activation of CD4+ T cells occurs after recognition of virus-derived MHC class II-associated peptides on APCs. Once infected by virus, CD8+ T cells are activated by lymphoid tissues and recruited to the sight of infection; they work to eliminate influenza virus infected cells and prevent replication of virus.⁴

The majority of healthy adults have the ability to infect others with influenza virus one day before symptoms appear and up to seven days after infection. Children can spread the virus longer than seven days after. Symptoms usually appear one to four days after infection.⁵ Infection can lead to mild to severe illness, and potentially death in extreme cases. Common symptoms of individuals infected with influenza virus include fever, cough, sore throat, runny or stuffy nose, muscle and body aches, headaches, fatigue, vomiting, and diarrhea. However, some people can still be infected with influenza virus and show no physical symptoms.⁶

Most individuals infected with influenza virus recover in a few days to less than two weeks after infection. Some people develop complications as a result of the influenza infection, such as pneumonia, bronchitis, and sinus and ear infections.⁶ Risk factors that may increase one’s risk of developing influenza
include age, strength of immune system, chronic illnesses, pregnancy and obesity. Older adults and younger children have been shown to be at greater risk of influenza infection. Cancer treatments, corticosteroids, and HIV/AIDS can weaken the immune system, making it easier for infection to occur. Chronic illnesses, such as asthma and diabetes, can increase the risk of influenza infection. Pregnant women are more likely to develop influenza complications, especially towards the end of their pregnancy. It has also been shown that obese individuals with a BMI of 30 or more have an increased risk of developing complications from influenza virus.7

**Influenza Vaccine**

Influenza vaccination helps provide protection against influenza infection. It is recommended that all individuals from six months old to elderly adults get vaccinated each year, optimally before the onset of influenza activity within a community. Influenza vaccines either contain inactivated virus or particles designed to look like the virus to the immune system. Trivalent vaccines protect against three different influenza viruses, including two influenza A viruses and an influenza B virus, that are likely to cause disease in the upcoming flu season. Quadrivalent vaccines protects against four different viruses, including two influenza A viruses and two influenza B viruses. On average it takes two weeks for the immune system to develop protection after vaccination, and protection should last throughout the flu season.8

Vaccination increases the resistance to influenza virus by training the immune system to respond to specific virus strains. The vaccination presents weakened virus or virus particles and the immune system responds by producing antibodies against the virus. Thus, if a vaccinated individual encounters the virus in the future, the immune system should be able to recognize and destroy the virus before illness occurs. Some vaccines also contain adjuvant, which is a secondary agent that further stimulates an immune response.9
Cancer and Immune System

The immune response of cancer patients in comparison to healthy individuals in the population of similar age is almost constantly depressed. Previous studies have shown that many components of B and T cell immunity are deficient during the active phase of cancer treatment. However, the extent of dysregulation in the immune system depends on numerous factors such as type of disease and status, age, type and time of specific therapies, nutritional status and more. Heterogeneity between different types of cancers makes it challenging to translate results from one cancer subpopulation to another. This challenge stresses the importance of conducting more prospective studies in well-defined cancer populations.10

Cancer and Vaccination

Effective vaccination of cancer patients is vital since this population has an increased risk of getting a community infection in comparison to healthy individuals. The risk of getting influenza infection is higher in cancer patients; some cancer patients are at twice the risk of being infected by influenza virus. This challenge presents a unique paradox: though cancer patients are the individuals with higher needs of protection, they are also the individuals with a lowered immune response to vaccines.10

Some studies have observed the effectiveness of vaccination in child and adolescent cancer patients. A study conducted in 2014 found that intensive chemotherapy in children with cancer results in long-term impairment of humoral immunity. Children and adolescents who had undergone chemotherapy treatment for cancer lost protective humoral immunity against vaccine-preventable diseases, including measles, mumps, rubella, and chicken pox. This study also suggests post-chemotherapy revaccination of childhood cancer survivors may improve immune response. The reason for the loss in antibodies is not fully understood, but some studies suggest this loss could be the result of chemotherapy-induced alterations to the immune system.11
Studies observing immunization in cancer patients have been limited by small sample size and lack of significant clinical benefit. While some studies have observed the effects of cancer treatment among children, few studies have examined the vaccination effectiveness and antibody response of adult cancer survivors. More studies need to be conducted that observe optimal timings of immunization in cancer patients, and evaluating cancer patient vaccine response. It would also beneficial to study alternatives to immunization in cancer patients that have little or no response to vaccination.\textsuperscript{10}
SPECIFIC AIM AND HYPOTHESIS

Specific Aim
To determine if immunity dysregulation of adult cancer survivors could potentially impair their ability to produce an adequate antibody immune response to H1N1 influenza vaccination.

Hypothesis
Cancer survivors are more likely to have a reduced antibody response to influenza virus due to a dysregulated immune system, resulting from chemotherapy or radiation therapy treatment.
METHODS

Study Design

This study observed participants from an ongoing, prospective, observational study conducted at the Family Medicine Center of the University of North Carolina. Participants were adults (≥ 18 years of age) who received the 2014-2015 trivalent, inactive seasonal flu vaccine. During enrollment, height and weight of subjects was recorded, and a baseline blood sample was collected. A post-vaccination blood sample was obtained 25-28 days after the first influenza vaccine administration. The subjects selected for this subset study were cancer survivors who had undergone chemotherapy or radiation treatment. Some of the cancers patients suffered from include breast, colon and prostate cancer; and cases of basal cell carcinoma and squamous cell carcinoma were excluded. The cancer participants were matched by age, race and BMI.

Serum Collection

Pre and post vaccination serum samples from study participants were collected at time of enrollment and 25-28 days after administration influenza vaccination, respectively. The blood collected from study participants was allowed to clot for 30-60 minutes at room temperature, and then samples were refrigerated. Blood samples were centrifuged at 800 x g for 10 minutes at 4°C, and serum was aliquoted into 500µL volumes, and stored in an -80°C freezer.

HAI

Hemagglutination inhibition assay was performed to determine antibody titers of the study participants in response to H1N1 influenza virus. Two fold dilutions of influenza virus were prepared to determine virus concentration at which hemagglutination with red blood cells(RBC) ends. The first row of wells contained a 1:10 ratio of virus to PBS. Two fold virus dilutions were performed on the consecutive rows, up to a 1:1280 dilution. A 0.5% turkey RBC solution was added to each of the wells and the plate incubated for 30 minutes at room
temperature. The highest dilution of virus resulting in complete agglutination was observed, the HAU titer was determined, and a virus dilution of 8HAU/50µL was created.

The virus dilution was then tested by performing two fold dilutions of the solution and adding 0.5% turkey RBC solution; and allowing plate to incubate for 30 minutes at room temperature. Serum samples were treated with receptor destroying enzyme. Serial dilutions of sera were performed to determine antibody titers of serum samples. Each plate for the hemagglutination assay consisted of serum samples, a positive control of a serum known to contain antibodies, and a negative control that contained no serum. Two fold dilutions of serum samples and controls were performed, and the virus solution was added to each well. The plate was incubated for 15 minutes at room temperature, allowing interaction between the virus and serum. After incubation, 0.5% turkey RBC was added to all wells, and the plate incubated for an additional 30 minutes at room temperature. The highest dilution of serum resulting in complete agglutination was used to determine antibody titers of subjects to H1N1 influenza virus.
RESULTS

Demographics of Study Population

Study participants were classified into two groups: cancer and control. The cancer group included all cancer survivors from the 2014-2015 flu study year, excluding those diagnosed with basal cell carcinoma and squamous cell carcinoma. Each cancer participant was matched to a control individual who did not have a history of cancer, and was of similar race, age and BMI.

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Table 1. Demographic distribution of all participants in this study. The mean age and BMI of the cancer group was 55.5 and 29.0, respectively; and the mean age and BMI of the control group was 54.4 and 28.4, respectively.

Antibody Titers for Pre Vaccination and Post Vaccination

The null hypothesis for pre and post vaccination antibody titers is that there was no significant difference in the antibody response of cancer and control individuals for H1N1 influenza virus.
Pre vaccination antibody titers were determined for each group through hemagglutination inhibition assay. As shown in Figure 1, cancer survivors had an average H1N1 antibody HAI titer of 8.75, with a standard deviation of 8.35. The control group had an average H1N1 antibody HAI titer of 70.0, with a standard deviation of 62.34. The sample size of each group was 8 individuals. Significance was determined by performing a paired t-test ($\alpha=0.05$). Cancer survivors had a significantly lower pre vaccination antibody titer for H1N1 in comparison to the control group, with a p-value of 0.0381. Thus, the null hypothesis for pre vaccination antibody response was rejected.

![HAI Titer for PH1N1](image)

**Figure 1.** Pre vaccination HAI antibody titer for PH1N1 influenza virus of cancer survivors and controls during the 2014-2015 flu season.

Post vaccination antibody titers were determined for each group through hemagglutination inhibition assay (Figure 2). Post vaccination serum samples were collected 25-28 days after initial influenza vaccine administration. Cancer survivors had an average H1N1 antibody HAI titer of 232.5, with a standard deviation of 437.0. The control group had an average H1N1 antibody HAI titer of 162.5, with a standard deviation of 109.3. The sample size of each group was 8 individuals. Significance was determined by performing a paired t-test ($\alpha=0.05$).
Cancer survivors did not have a significantly different post vaccination antibody titer for H1N1 in comparison to the control group, with a p-value of 0.693. Thus, the null hypothesis for post vaccination antibody response was accepted.

Figure 2. Post vaccination HAI antibody titer for PH1N1 influenza virus of cancer survivors and controls during the 2014-2015 flu season.

Antibody increase of cancer and control participants was determined by calculating the fold increase from pre vaccination antibody titers to post vaccination antibody titers. The null hypothesis for fold increase is that there was no significant difference in the antibody fold increase of cancer and control individuals for H1N1 influenza virus.

Cancer survivors had an average antibody titer fold increase of 26.25 and the control group had an average H1N1 antibody fold increase of 10.0 (Figure 3). The sample size of each group was 8 individuals. Significance was determined by performing a paired t-test ($\alpha=0.05$). Cancer survivors did not have a significantly different antibody titer fold increase for H1N1 influenza virus in
comparison to the control group, with a p-value of 0.241. Thus, the null hypothesis for antibody titer fold increase was accepted.

![HAI Titer for PH1N1](image)

**Figure 3.** Antibody titer fold increase for PH1N1 influenza virus of cancer survivors and controls during the 2014-2015 flu season.

This study also observed if participants were able to produce a protective antibody response against H1N1 influenza virus 25-28 days after vaccine administration. Previous studies have shown a hemagglutination inhibition antibody titer of 40 or greater is considered protective against influenza virus. Based off this criterion, it was determined that half of the cancer survivor participants were not protected against H1N1 influenza virus 25-28 days after vaccine administration. In comparison, only one of eight control participants were not protected against H1N1 influenza virus 25-28 days after vaccine administration.
Mean post vaccination HAI antibody titer and percent of participants with a protective titer (≥40 HAI titer) for cancer survivors and control groups.

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<tr>
<td>Mean post vaccination HAI antibody titer</td>
<td>232.5</td>
<td>162.5</td>
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<tr>
<td>Percent of participants with a protective titer (≥40 HAI titer)</td>
<td>50%</td>
<td>87.5%</td>
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**Table 2.** Mean post vaccination HAI antibody titers against H1N1 influenza virus and percent of participants who produced a protective antibody titer 25-28 days post vaccination for cancer survivors and control groups.
DISCUSSION

Conclusion

In this study, the immune response of cancer survivors to influenza virus vaccination was observed by looking at pre and post vaccination antibody titers, fold increase, and percent of participants protected post vaccination. It was hypothesized that cancer survivors would be more likely to have a reduced antibody response to influenza virus due to a dsyregulated immune system, resulting from previous chemotherapy or radiation therapy treatment. Previous studies have demonstrated that many components of B and T cell immunity are deficient during the active phase of cancer treatment. However, the extent of dsyregulation in the immune system depends on numerous factors such as type of disease and status, age, type and time of specific therapies, nutritional status and more.\(^\text{10}\)

This study concluded that cancer survivors that were exposed to chemotherapy and radiation therapy treatment in the past had significantly lower H1N1 influenza virus antibody response in comparison to their non-cancer matches. This suggests that cancer survivors who are exposed to chemotherapy and radiation therapy treatment may have difficulty maintaining antibody levels due to dysregulation of the immune system. Post vaccination antibody titers and fold increase of cancer survivors showed no significant difference in comparison to the control group. Though the cancer survivors initially had a significantly lower titer before vaccination, these post vaccination results suggest the vaccine was still beneficial for helping cancer survivors produce a greater antibody response 25-28 days after influenza vaccine administration. Thus, despite dysregulation in the immune system of cancer patients, influenza vaccination could provide cancer survivors with some protection against influenza virus.

Cancer survivors still had difficulty reaching an antibody titer considered protective against influenza virus. Previous studies have shown a hemagglutination inhibition antibody titer of 40 or greater is considered protective against influenza virus.\(^\text{12}\) Half of the cancer survivors were not able to produce a
protective level of antibodies 25-28 days post vaccination. This suggests that the 2014-2015 trivalent, inactive seasonal flu vaccine was unable to provide adequate protection to some cancer survivors, thus making them more susceptible to influenza infection. All cancer survivors who did not reach a protective titer were up-to-date on influenza vaccinations for previous flu seasons, excluding the possibility that this lack of protection could be the result of not receiving previous influenza vaccinations.

One control participant did not produce a protective level of antibodies 25-28 days post vaccination. However, it was found that this participant began showing symptoms of cancer one year after their serum was collected for the study. Three months later, a physician officially diagnosed this participant with cancer. This finding may suggest that even in the absence chemotherapy and radiation therapy treatment, the presence of cancer alone causes enough dysregulation in the immune system to potentially inhibit an individual from developing an adequate immune response post influenza vaccination.

This study concludes that cancer survivors had a significantly lower H1N1 influenza virus antibody response in comparison to their non-cancer matches. While vaccination did help increase overall antibody titers to H1N1 influenza virus, half of the cancer survivors were still unable to receive adequate protection from influenza vaccination. These outcomes could be the result of dysregulation in the immune system of cancer survivors caused by previous chemotherapy and radiation therapy treatment.

Limitations and Recommendations for Further Studies

This study experienced multiple limitations. One of the most significant limitations was a small sample size, of 8 cancer survivors and 8 matches. There was difficulty in trying to maintain a larger sample size since only 8 participants of the original influenza study classified as cancer survivors who received chemotherapy and radiation therapy treatment in the past. The low sample size decreased the power of this study and decreases the ability to produce more
significant results. Further studies could be conducted observing a larger group of cancer survivors.

Another limitation of this study is that there was limited information on cancer history of the cancer survivors. The original study from which participants were selected from did not focus on cancer. Thus, limited information was provided on types of chemotherapy and radiation therapy treatment, timelines of cancer progression and treatment, and additional details about the cancer diagnosis. It would be beneficial to conduct studies in the future that observed the effects of different kinds of cancer, various stages of cancer, and types of cancer treatment on the immune system and vaccination effectiveness.

This study only observed antibody responses to influenza virus of cancer survivors. It would be important for future studies to also observe dysregulation of the immune system by measuring T cells, such as CD4 and CD8, and serum inflammation markers, such as the cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6.

This study concluded that only half of the cancer survivors were able to reach a protective antibody response after vaccination. Future studies should also consider how to provide better protection for cancer survivors. Studies could observe how the antibodies are maintained after 30 days since vaccine administration and if multiple vaccinations within a single flu season could provide increased protection for cancer survivors.
REFERENCES


