How does the age at which a child develops Acute Lymphoblastic Leukemia as well as the original treatment received by the patient impact the risk of relapse in the child?

Sports, Exercise, and Health Science

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Introduction

Over the course of this paper, my goal is to determine which treatment of acute lymphoblastic leukemia, chemotherapy or allogeneic stem cell therapy. I also want to look at if a certain age group has a higher relapse rate, children younger than ten or older than ten. This will answer the question, **how does the age at which a child develops Acute Lymphoblastic Leukemia as well as the original treatment received by the patient impact the risk of relapse in the child?** To accomplish this, I will use a statistical hypothesis test to determine if the difference between each rate for each condition is statistically significant. I will also look at the basics of how blood is made, how leukemia starts, how each treatment is given, and the effects of each treatment on the child. From this information, I can justify the results of my statistical test based on the data collected.

The study of acute lymphoblastic leukemia and its cures is very important. It is the most common form of cancer that affects children. Looking at the effectiveness of treatments and how each treatment impacts relapse rate can help influence parents decisions if they are in the unfortunate circumstance of having a child with ALL. It also may impact the way that doctors decide on treatments and look at how different options change for different patients.

The question addresses age and treatment because, although there are a lot of different factors that impact relapse rates of children, these are among the most important to consider. Both of these factors are guaranteed to impact a child with ALL, and knowing how they impact a child can help inform a course of action.

Background

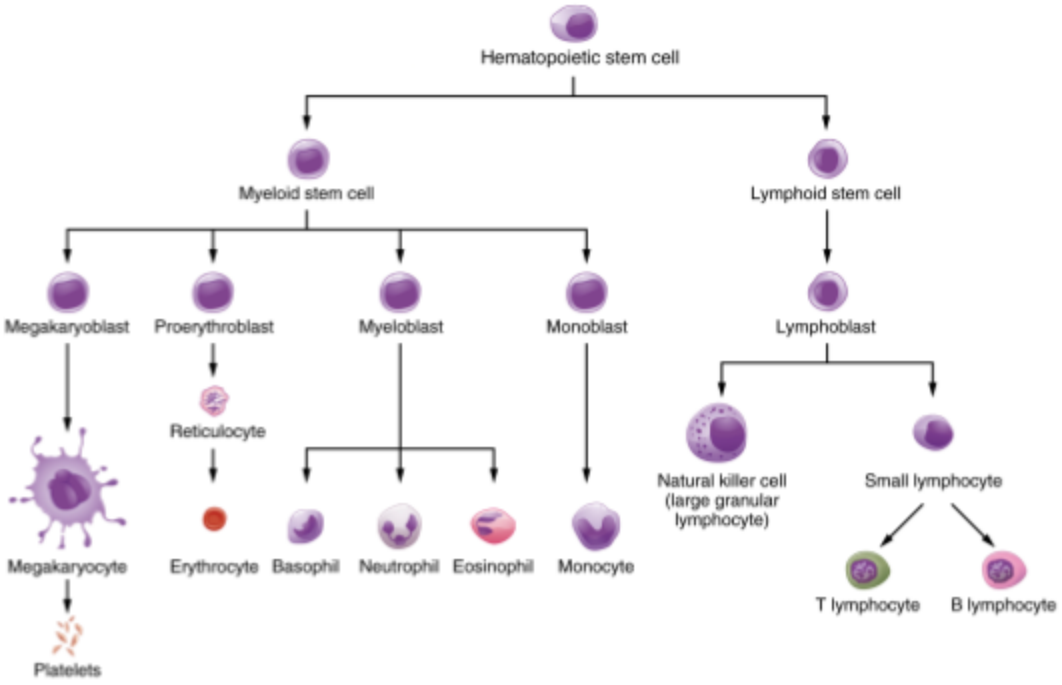
Bone Marrow and Blood Cells

Bone marrow is located inside of all bones in the body. The two types of bone marrow are known as red marrow or myeloid tissue and yellow marrow. The primary function of red marrow is hematopoiesis, or the production of blood cells that are then released into the bloodstream. Over time, this process is localized to the the major flat bones in the body such as the sternum and the pelvis. Production is also found in the vertebrae. Bone marrow's structure contains a spongy material consisting of stromal fibroblasts, or cells that are important in connective tissue and structural tissue, as well as blood cell precursors. The spongy material surrounds trabeculae or long, thin structures (McLarnon).

Hematopoiesis plays a vital role in the circulatory system. When children are young, this process is continuous and new blood cells are constantly being made in all bone marrow. The cycle slows down as the person grows older, eventually only taking place in specific flat bones. The blood cells that are made are thrombocytes, or blood platelets, which are fragments of cells that are vital in blood clotting and are produced by blood cells called megakaryocytes; erythrocytes, or red blood cells, which are the most commonly found cells in the blood and contain hemoglobin that carries oxygen and carbon dioxide to and from the lungs to the rest of the body; and leukocytes, or white blood cells. These white blood cells can be distinguished as neutrophils (which respond immediately to signs of infection or disturbance) and lymphocytes (which are separated into T lymphocytes that assist in regulating the other immune cells as well as

attack infections and tumors and B lymphocytes that are important in the production of antibodies that can target specific foreign bodies)(“Blood Basics”). The majority of these white blood cells are produced from the bone marrow. The other two types of blood cells are entirely reliant on the precursor cells in the red marrow for their production and replenishment in the bloodstream (Rogers).

The formation of the blood cells themselves begins with hematopoietic stem cells in the bone marrow. These stem cells are able to renew themselves as time goes on and as they are needed in the body. They are triggered to differentiate by either external stimuli or genetics. As the stem cell becomes more and more differentiated, it loses its potential to be any other cell. With each stage of development, alternate lineages are slowly removed and made impossible. First they are developed into either white blood cells or the precursor to both erythroid and megakaryocytic cells. Leukocytes become myeloids or common lymphoid progenitors. These eventually become the cells that were triggered to develop into (See diagram #1). The triggers involve the change of gene expression in a cell. They can be brought about by communication among cells (the extracellular matrix), cytokines, or external forces such as proteins or hormones. While not everything is understood about these processes and their triggers, they are some of the most commonly studied stem cells and advancement in technology and research are very important in the development of the knowledge surrounding them in order to impact the way problems within this process are addressed and treated (Gordon Betts).



(Betts) Diagram 1: Cell differentiation in hematopoiesis

It is also important to note that this process changes between the embryonic state and the adult state. In utero hematopoiesis occurs at a much higher rate than in adults and there are many more locations where the process is being triggered. In addition to the majority of the developed bone marrow creating blood, umbilical stem cells are also taking part in this development. This results in a much higher likelihood of error or mutation in development, which may lead to problems in the future in terms of the present blood cells in the stream.

Acute Lymphoblastic Leukemia

Acute lymphoblastic leukemia (ALL) is one of the most commonly found variants of cancer in very young children aged 0-13. The causes of it, as with most cancers, are undiscovered or unconfirmed for the most part. However, more recent studies and research indicate that many genetic and environmental factors may be involved. In

addition, the treatment of this leukemia with chemotherapy, targeted therapy, or stem cell transplants tend to be very successful, with an improving cure rate each year (Ravindranath).

Overview

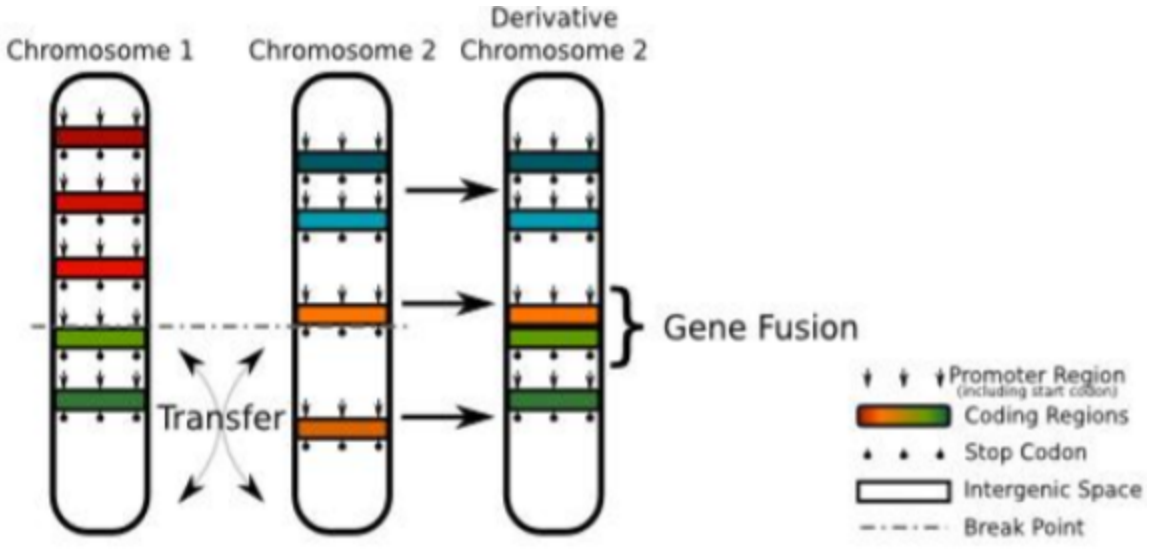
When acute leukemia occurs, there is a problem with the hematopoiesis resulting in the stem cells differentiating uncontrollably. The excess of lymphoblasts that are produced as a result of lymphoblastic leukemia, while they are still white blood cells, are very weak and are unable to function in the way that they are meant to. They are basically rendered useless as the blood becomes more and more saturated. In addition, the bone marrow is slowly displaced by these irregular cells, causing normal blood cell production to slow down or stop altogether, which is potentially fatal to the patient. The cells are forced to fight for the niche resources and alter the bone marrow's conditions, which, in turn, causes other stem cells to start overproducing white blood cells (Enciso).

Initially, there are some leukemic cells that may be present in a niche. However, if there is any sort of imbalance in hematopoiesis, then the stem cells will begin to make more of these cells, thereby starting the cycle that ultimately leads to the development of leukemia. In addition, the leukemic cells are divided into two categories. One is a set of cells that are being created very quickly and cause the spread of the abnormal cells to accelerate. The other type is slow-growing due to different genetic mutations that occur throughout this cycle of the development of cancerous cells. These cells begin to develop the very dangerous ability to self-renew, even after treatment. These are more

often the cause of relapse in a patient, since they are not targeted by chemotherapy or other treatments because they are so undetectable (Enciso).

Origins and Causes

ALL research focuses on causes with fetal origins. It has been found that a leukemic clone can be identified in the blood samples of children with the disease. These observations indicate that the mutation originates before birth, meaning that some children are predisposed to the cancer (Jan, Majeti). The source of this mutation can be linked to a variety of factors, including inheritance or the environment in prenatal stages. Stronger correlations come from studies involving genetics and the genetic predisposition of children to ALL. The genes from before birth were compared to after birth, and some of the children who developed ALL shared gene fusions (when a gene is made from two separate genes that were not meant to be together or the DNA from one chromosome moves to a different one as a result of some sort of mutation) (See diagram #2) with the MLL gene (Belson). This process shows that some cases of ALL were created in utero.



(Pruittfl) Diagram 2: Gene fusion

Another possibility is mutations in metabolizing genes that help control the processing of certain drugs or other external substances that enter the body. This inhibits the body's ability to control the transport of ingested materials. While this can result in ALL in young children, it is also commonly linked to patients who have a relapse in ALL. Many times, these mutations can be attributed to the treatments used to cure the original disease (Belson).

Genetic disorders are also considered as a precursor to acute lymphoblastic leukemia in certain cases. It has been found that there is a high correlation between the cancer and these disorders. For example, Down Syndrome is a genetic abnormality that is commonly associated with and researched with ALL. Through research and studies, it has been shown that this disorder affects the susceptibility of children to many diseases. This susceptibility occurs as a result of the weakened state of the immune systems of the children.The links between Down syndrome and acute lymphoblastic leukemia become very clear when the genetics of children with Down syndrome and acute lymphoblastic leukemia (DS-ALL) are compared with children with just ALL. Specifically, differences in the Janus kinase 2 gene on the ninth chromosome were considered as indicators of ALL in children with down syndrome. Mutations of this gene can be linked to a cause of the ALL that children with down syndrome develop (Ravindranath).

Treatment

Overview

Treatments of ALL range from chemotherapy to stem cell transplants. It commonly takes two to four years. Unlike other cancers, there is no surgical or radiation therapy options as treatments for this cancer due to how widespread and unlocalized it is. The bloodstream is constantly moving, so it would be impossible to target specific points where the leukemic cells may be present. The entirety of the treatment must be supplemented with preventive measures in order to stop the consequences that come with the side effects of the leukemia. For example, the treatment may result in a decreased blood cell counts. This may lead to many other problems, including heart failure due to the body's inability to control blood flow and circulation. However, these treatment options have a very high success rate.

Chemotherapy

Chemotherapy is the most common form of treatment for cancer. In acute lymphoblastic leukemia, there are three stages in the treatment course. The first stage is known as remission induction which aims at destroying the leukemic cells present in the bone marrow and blood stream. To do this, drugs are often prescribed in high doses to try and attack the cells. However, since it is commonly small children that are affected by this disease, doctors tend to be more careful, while still trying to be aggressive and stop the spread. Sometimes, it is required that these drugs are also sent into the spinal cord or the brain as a result of spread to these regions ("Acute Lymphoblastic Leukaemia (ALL)").

This form of treatment is often successful. Once it is determined that the patient is in remission (that the treatment has worked), they move into the second stage of the therapy, or consolidation. This is working to prevent relapse by attempting to target cells in the blood or marrow that may remain but isn't found on tests. Simply put, this is a continuation of the chemotherapy that was used in the first stage but with no known target. While this reduces the risk of relapse, it is impossible to ensure that the cancer will not come back. Finally, the third stage is maintenance or attempting to sustain the remission phase. Chemotherapy is continued at low doses for a few years following the original treatment ("Acute Lymphoblastic Leukaemia (ALL)").

One of the major problems with chemotherapy is that in the process of destroying the cancerous cells in the body, the drugs are also destroying healthy bone marrow cells and blood cells. This means that those cells that are important to a body's functions are lost and the body must learn to compensate or grow new cells to replace these roles. In addition, the effectiveness of it long term can not be confirmed and relapse is a possibility in any patient.

Stem Cell Transplants

The other most common form of treatment of acute lymphoblastic leukemia is stem cell transplants. It is often used as a post-remission treatment in order to work towards the prevention of relapse. It is used most often in the case of relapsed ALL. For patients who are children, it is usually not included in their treatment due to the effectiveness of the chemotherapy. However, in severe cases of ALL in young children, stem cell transplants are the most viable treatments ("Allo: ALL"). These children have a

form of leukemia known as Burkitt's type leukemia which is a very rare occurrence where there are several other genetic mutations of the B-cells that increase the severity of the cancer (Sato). They may also have Philadelphia Chromosome-positive ALL, or when there is a translocation of chromosome 9 and chromosome 22, causing a fusion gene that assists in the development of the cancer ("Prognosis and Survival for Acute Lymphocytic Leukemia").

The method of stem cell transplant used is known as allogeneic stem cell transplantation. For this treatment, the cells are taken from the bone marrow or peripheral blood from a donor. The T-lymphocytes are then removed from the samples of stem cells to avoid certain complications of the transplant.

The recipient undergoes intense chemotherapy before the transplant. This is meant to destroy the recipient's existing bone marrow with the goal of removing the cancerous cells. Then, stem cells are then transplanted into the patient's bloodstream. This type of transplant helps the patient regrow healthy bone marrow, thereby alleviating the side effects of chemotherapy on its own. However, the radiation that is required before transplantation can be too dangerous, especially in patients with compromised immune systems. In this case, a less aggressive chemotherapy is used and the stem cells are transplanted with the goal of the donor cells recognizing the cancerous cells as a threat and attacking them, something the original marrow could not do. This method is still being studied, but it is the best alternative currently ("Allo: ALL").

Method

In order to accomplish the ultimate goal of finding whether or not the age that a child develops acute lymphoblastic leukemia as well as whether allogeneic stem cell transplants or chemotherapy impact relapse rates of ALL in children, data will be taken from two different studies and compared to each other. The first study looked at the outcomes of 200 ALL patients between the ages of 0 and 18 years old. It was a five year retrospective cohort study conducted through the Mansoura University in Egypt. The 200 patients were all observed over five years after receiving the induction stage of chemotherapy. In addition to the outcome being recorded, the age of diagnosis was also recorded and grouped into children younger than 10 and older than 10. This data was chosen because it accurately and effectively reports information that can then be compared to the data collected in the second study used. This study consolidated Japanese nationwide registry data over five years to compare the outcomes of allogeneic stem cell transplants in two different age groups of children, younger than 10 and older than 10.

For the purposes of this study, the mortality rates and the patients included in these groups were removed from the overall samples and data. In order to compare the two data sets, first the effectiveness of each treatment overall will be considered. The proportion of relapsed patients who received chemotherapy and the proportion of patients who received allogeneic stem cell transplants will be used in a hypothesis test for two populations. This test will show if there is a difference between the two proportions at the $\alpha=0.05$ significance level. Then, the correlation between age and

relapse rate will be found for each treatment using the same method but comparing the total proportion of children under the age of 10 and children over the age of ten who relapsed from each study. If there is a statistically significant difference between the two proportions, then it can be concluded that one treatment is more effective than the other, or that one age group is more susceptible to relapse.

Data Analysis

Calculations

Chemotherapy and Allogeneic Stem Cell Transplant:

Hypotheses:

- p_1= Proportion of chemotherapy patients who relapsed
- p_2= Proportion of allogeneic stem cell transplant patients who relapsed
- H_0: $p_1 - p_2 = 0$
- H_0: $p_1 - p_2 > 0$

Test Statistic

$$z = \frac{(\bar{p}_1 - \bar{p}_2) - 0}{\sqrt{\bar{p}(1 - \bar{p})\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

p=(41+339)/(1730+152)=0.2019

$$z = \frac{(0.2697 - 0.1960) - 0}{\sqrt{(0.2019)(0.7981)\left(\frac{1}{152} + \frac{1}{1730}\right)}}$$

z=2.17

P-value

Normal distribution, probability that z>2.17

Normal cdf, lower=2.17, upper=∞, μ=0, σ=1

p=0.015

Conclusion: Since 0.015<0.05, H_0 is rejected and it can be concluded that the proportion of patients who relapse after receiving chemotherapy is greater than the relapse rate of patients who received allogeneic stem cell transplants.

Percent difference: $z=\frac{(0.2697-0.1960)}{0.1960} = 0.3760$

Age and Relapse rate:

Hypotheses:

- p_1= Proportion of patients under 10 years old who relapsed
- p_2= Proportion of patients over 10 years old who relapsed
- H_0: $p_1 - p_2 = 0$
- H_0: $p_1 - p_2 > 0$

Test Statistic

$$z= \frac{(\bar{p}_1 - \bar{p}_2) - 0}{\sqrt{\bar{p}(1-\bar{p})\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}}$$

p=(127+253)/(723+1159)=0.2019

$$z= \frac{(0.1757-0.2183) - 0}{\sqrt{(0.2019)(0.7981)(\frac{1}{723}+\frac{1}{1159})}}$$

z= - 2.239

P-value

Normal distribution, probability that z<-2.239

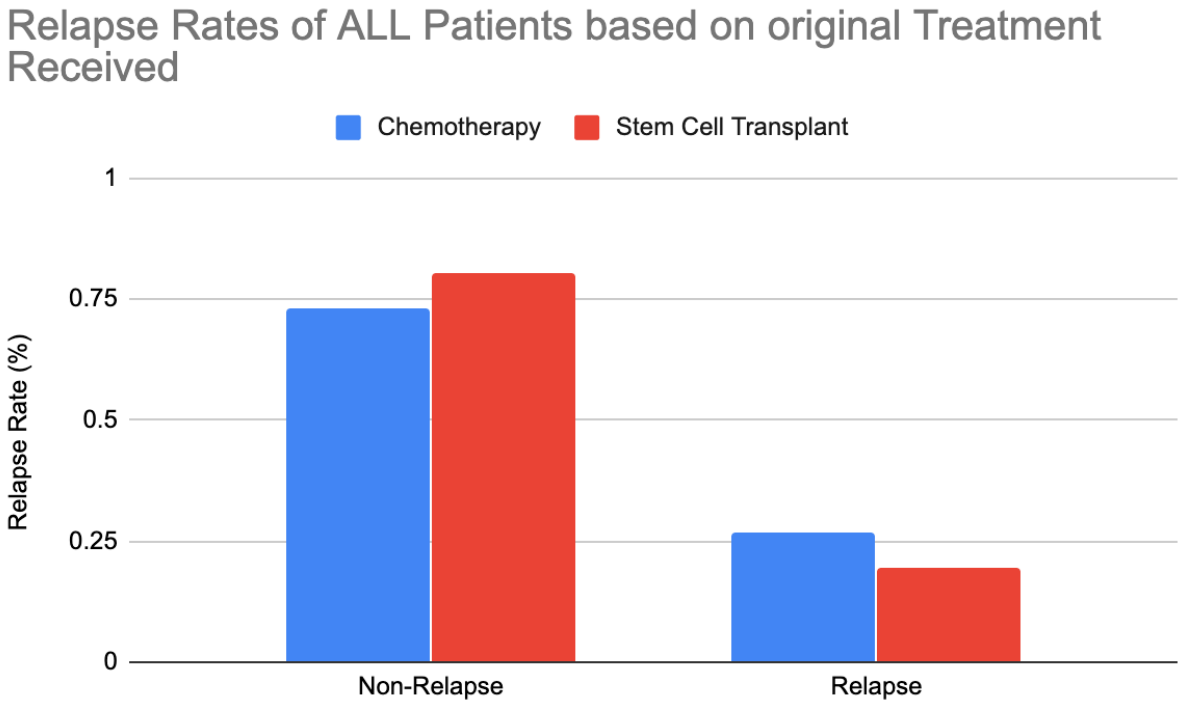
Normal cdf, lower=-∞, upper=-2.239, μ=0, σ=1

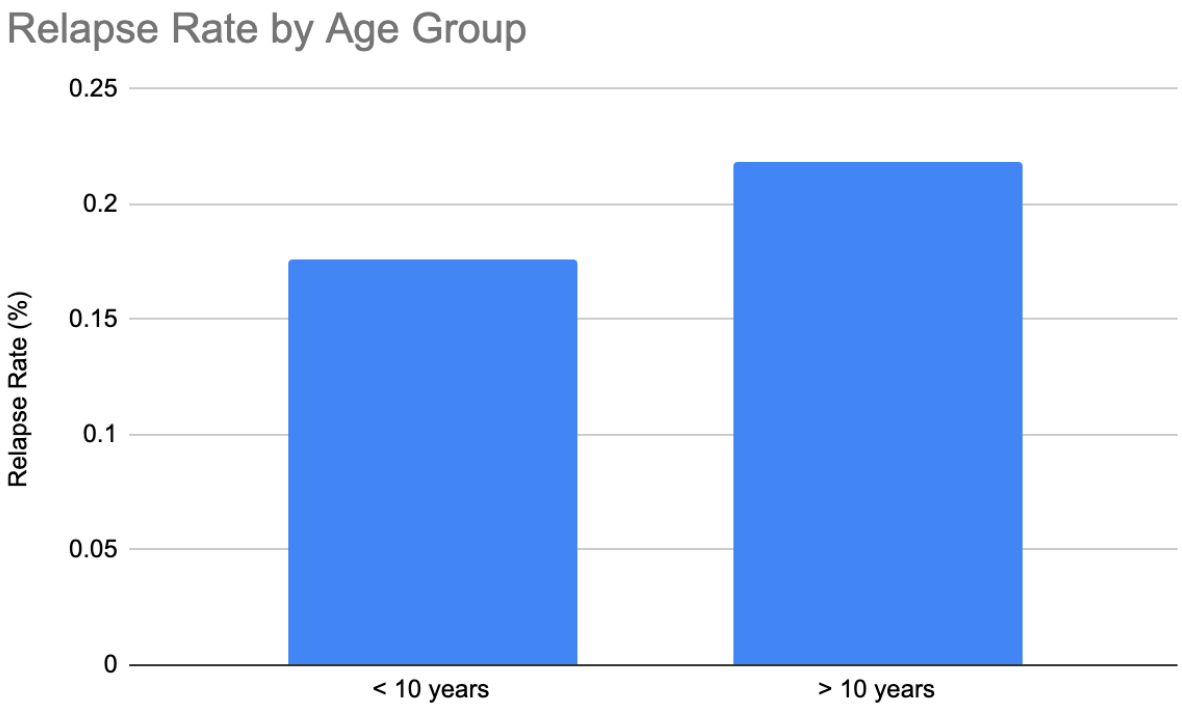
p=0.012578

Conclusion: Since 0.012578<0.05, H_0 is rejected and it can be concluded that the proportion of patients who relapse that are under 10 years old is less than the relapse rate of patients over 10 years old.

Percent Increase: $z=\frac{(0.2183-0.1757)}{0.1757} = 0.2425$

Graphs





Results

The aim of this study was to examine the correlation between the relapse rate of children with ALL and the type of treatment they received during originally as well as to determine if age has an impact on the likelihood of a patient to relapse. Data was taken from two different studies, one that found data on the outcomes of patients who received chemotherapy as well as their age groups and another that collected information about the results of allogeneic stem cell transplantation on patients and their age groups. The two were compared to see if the difference between relapse rates overall was statistically significant. Then, each study was used to see if the difference between relapse rates of each age group (younger than 10 and older than 10) was statistically significant. Each of these were completed using hypothesis tests.

In the end, it was found that there was a 37.60% increase in relapse rate of chemotherapy when compared to allogeneic stem cell transplants. It was also calculated that, in both chemotherapy and stem cell transplants, children over the age of 10 had a 24.25% increase in relapse rates than children under the age of 10. Both of these differences were statistically significant, according to the hypothesis tests.

Conclusion

Treatment

As seen by the results of the data analysis, the treatment that had a lower relapse rate in children with acute lymphoblastic leukemia was allogeneic stem cell transplants, with an over relapse rate of 19.60% compared to chemotherapy's relapse rate of 26.97%. This 37.60% increase may have occurred due to the variance in target between the two treatments. Chemotherapy is the use of drugs to try and attack cancerous blood cells that are formed as a result of ALL. In the multiple stages of chemotherapy that a patient receives, the drugs target the rapidly dividing lymphoblasts. The stem cells that have been impacted and are the source of the invasive blood cells are also targeted. However, mutated, slow growing leukemic cells are not often targeted. Having multiple rounds of chemotherapy is an attempt to target this, but this may not be enough or timed properly enough to completely remove these new cancerous cells that may result in relapse. On top of this, healthy bone marrow and stem cells are destroyed in the process of chemotherapy. This means that there is ultimately a deficiency of healthy blood cells in the bloodstream, leaving the patient

vulnerable to the concentration of leukemic cells getting too high (“Acute Lymphoblastic Leukaemia (ALL)”, Enciso).

On the other hand, allogeneic stem cell transplants are prefaced with radiation therapy to obliterate all of the bone marrow, leaving a very low chance of the leukemia returning. This is then replenished by the stem cells and the bone marrow created from it (“Allo: ALL”).

Age

The other part of the results indicated that children over the age of 10 in both treatments had a higher relapse rate (21.83%) than children under the age of 10 (17.57%). While many factors play into this 24.25% increase, one of the major considerations in the explanation of this is the development of the blood-making systems in the body. In younger children, many locations and sets of bone marrow are working towards blood making. The blood supply is constantly being replenished and having treatment of ALL wipe out healthy bone marrow has a much lower effect. However, as people grow older, hematopoiesis becomes more and more localized. This means that fewer stem cells are working towards making blood, so when healthy bone marrow is attacked, this has a more severe impact, leaving the child more vulnerable to a relapse (Rogers).

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Appendix

Data

Outcomes of Chemotherapy Grouped by Age

	Total Patients Studied (152)	Non-Relapsed ALL Patients (111, 73.03%)	Relapsed ALL Patients (41, 26.97%)
<10 years	116 (76.3%)	92 (82.88%)	24 (58.54%)
>10 years	36 (23.7%)	19 (17.12%)	17 (41.46%)

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Outcomes of Allogeneic Stem Cell Transplant Grouped by Age

	Total Patients Studied (1730)	Non-Relapsed ALL Patients (1391, 80.40%)	Relapsed ALL Patients (339, 19.60%)
<10 years	607 (35.09%)	504 (36.23%)	103 (30.38%)
>10 years	1123 (64.91%)	887 (63.77%)	236 (69.62%)

Hangai, Mayumi. “Allogeneic Stem Cell Transplantation for Acute Lymphoblastic Leukemia in Adolescents and Young Adults.” *Biology of Blood and Marrow Transplantation*, Aug. 2019, doi:10.1016/j.bbmt.2019.04.014.

Combined data based on age

	Total Patients Studied (1882)	Non-Relapsed ALL Patients (1502, 79.81%)	Relapsed ALL Patients (380, 20.19%)
<10 years	723 (38.42%)	596 (82.43%)	127 (17.57%)
>10 years	1159 (61.58%)	906 (78.17%)	253 (21.83%)