HYDROXYUREA THERAPY OUTCOMES IN SICKLE CELL CHILDREN WITH HISTORY OF STROKE AT THE UNIVERSITY TEACHING HOSPITAL- ZAMBIA

By

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DECLARATION

I **Racheal Sikabalu** declare that this Dissertation represents my own work and that all the sources I have quoted have been indicated and acknowledged by means of complete references. I further declare that this Dissertation has not previously been submitted for Degree, Diploma or other qualifications at this or another University. It has been prepared in accordance with the guidelines for Masters in Clinical Pharmacy of the University of Zambia.

Signed……..……..…on the…….……..day of ……...…….

CERTIFICATE OF APPROVAL

The University of Zambia approves this dissertation on Hydroxyurea therapy outcomes in sickle cell children with history of stroke at the University Teaching Hospital-Zambia in partial fulfilment for the requirements for the award of Masters in Clinical Pharmacy-Paediatrics

Signature for examiner one……....Date…….

Signature for examiner two……....Date…….

Signature for examiner three……..Date……..

Signature for Supervisor ……Date ……

Father and Mother

I dedicate this work to mum and dad for the wonderful things they have done in my life.

I will forever appreciate their love, care, support and guidance.

With love and thanks

ABSTRACT

Background

Sickle cell disease (SCD) is the most common debilitating genetic disorder among people of African descent. The most devastating neurologic manifestation of SCD is stroke. Therapeutic studies of hydroxyurea performed in children include investigations indicating hematologic response, lack of significant toxicity, decreases in vaso-occlusive episodes and possible prevention of secondary strokes. However, most treatment recommendations for the management of SCD are based on studies conducted in resource-rich countries and not the resource limited regions which are most affected. The objective of the study was to assess the hydroxyurea (HU) therapy outcomes in SCD children with history of stroke at University Teaching Hospital (UTH)-Zambia.

Design and site

Retrospective cohort study conducted at the UTH-Zambia.

Methods

Clinical and laboratory data was analyzed in 34 patients. Changes in hematological parameters during HU therapy were abstracted from the patient files. Vaso occlusive crisis (VOC) episodes, number of hospital inpatient days and stroke episodes 6 months before and 6 months after initiation of HU were also captured.

Results

The mean dose of HU was 10.45 mg/kg/day. There was no significant increase in the red blood cell indices at 6 months of therapy. Mean hemoglobin changed from 7.18 g/dl to 7.11g/dl, P = 0.8443 and the mean MCV (mean capsular volume) changed from 92.51fl to 95.08 fl, P = 0.2982.

There were however, significant reductions in the number of vaso occlusive episodes, number of hospital stay and number of stroke episodes after initiation of HU therapy. The ratio of VOC reduced from 0.337/day to 0.093/day, P=0.00001, the ratio of hospital stay reduced from 5.012 to 0.578, P = 0.0004 where as the stroke incidences reduced from 0.149/day to 0.005/day, P = 0.00001 after initiation of HU therapy.

There was no significant decrease in the mean white blood cell (WBC) and platelet count at 6 months on HU therapy. Mean WBC changed from 22.63 x 10^9/l to 22.35 x 10^9/l, P = 0.9479 and mean platelets from 434.74 x 10^9/l to 386.94 x 10^9 /l, P = 0.2634.

A number of positive correlations were found between dose and therapeutic response. The pearson's correlation coefficient between HU dose of <15mg/kg/day and change in hospital inpatient days was 0.0564 where as between HU dose of <15mg/kg/day and stroke recurrence is 0.1665. The pearson's correlation coefficient between HU dose of 15-30mg/kg/day and change in hospital inpatient day was 0.1197.

Conclusion

The study shows that at the mean dose of 10.45mg/kg/day, sickle cell children with history of stroke at the University Teaching Hospital presented with significant reductions in the number of inpatient hospital days and in the number of stroke recurrences. The study results reviewed no HU hematological toxicity, however, at this mean HU dose; there was no hematological therapeutic response. The study results also indicated a positive correlation between dose and the HU therapeutic response. Beneficial effects of HU therapy are achieved with HU dose of <15mg/kg/day although hematological therapeutic response is not achieved at this dose.

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LIST OD ABBREVIATIONS

i.	Hb	:	Haemoglobin
ii.	HbF	:	Fetal haemoglobin
iii.	HU	:	Hydroxyurea
iv.	MCV	:	Mean corpuscular Volume
v.	MTD	:	Maximum Tolerated Dose
vi.	Plat	:	Platelets
vii.	SCD	:	Sickle Cell Disease
viii.	UTH	:	University Teaching Hospital
ix.	VOC	:	Vaso occlusive crises
x.	WBC	:	White Blood Count
xi.	SCA	:	Sickle cell Anaemia

LIST OF DEFINITIONS

- **Children** – persons aged below 16 years of age
- **Cerebral Vascular Accident/stroke** - defined as an acute neurologic syndrome secondary to occlusion of an artery or hemorrhage resulting in ischemia and neurologic symptoms or signs.
- **Sickle cell disease/anemia** - The child has most or all of the normal hemoglobin (HbA) replaced with the sickle hemoglobin (HbS)
- **Sickle cell trait** - The child is carrying the defective gene, HbS, but also has some normal hemoglobin, HbA
- **Vaso occlusive crisis** -pain caused when the flow of blood is blocked to an area because the sickled cells have become stuck in the blood vessel
- **Therapeutic response** –Increases in hemoglobin increases, mean capsular volume, white blood cell and reduction in both vaso occlusive crisis and incidences of stroke.

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Sickle cell disease (SCD) is an autosomal recessive inherited haemoglobinopathy. It is the most common and potentially devastating condition that results in the vasoocclusive phenomena and hemolysis (American Academy of Pediatrics 2002; Bunn 1997). The most devastating neurologic complication of SCD is stroke. The incidence of primary stroke in children with SCD is 0.6-0.8 events per 100 patient-years; with a cumulative incidence of 7.8% by age 14 years in the Jamaican cohort and 11% by age 20 years in the United States Cooperative Study of Sickle Cell Disease. (Ohene-Frempong 1998; Balkaran et al 1992)

Long-term observational studies have shown that chronic transfusion largely decreases the risk of stroke recurrence by approximately 80–90% compared with no intervention. This treatment choice however is limited by several factors including transmission of infectious agents, erythrocyte alloantibody and autoantibody formation, and iron overload (Wang & Dwan, 2013). In developing countries, where availability of blood for the management of acute emergencies is limited, and where the treatment costs associated with the long-term effects of chronic transfusions (i.e. chelation for iron overload) pose a significant challenge, the alternative therapy being employed in these settings is hydroxyurea therapy.

The actual mechanism through which HU exerts its clinical response is not fully understood. However, multiple beneficial effects of hydroxyurea in SCD might include (1) Fetal hemoglobin induction through soluble guanylyl cyclase activation and altered erythroid kinetics; with a concomitant reduction in the intracellular concentration of sickled hemoglobin (HbS) , which affects the polymerization of deoxygenated HbS (2) lower neutrophil and reticulocyte counts from ribonucleotide reductase inhibition and marrow cytotoxicity; (3) decreased adhesiveness and improved rheology of circulating neutrophils and reticulocytes; (4) reduced hemolysis through improved erythrocyte hydration, macrocytosis, and reduced intracellular sickling; and (5) Nitric oxide (NO) release with potential local vasodilatation and improved vascular response. (Silva-Pinto et al, 2013)

Hydroxyurea has been proved clinically to significantly reduce the number of painful vaso-occlusive events, blood transfusions, episodes of acute chest syndrome, and hospitalizations. The multicenter phase I/II safety trial of hydroxyurea therapy for school-aged children severely affected with SCD showed significant increases in hemoglobin concentration mean corpuscular volume, and fetal hemoglobin parameters, and decreases in white blood cell, neutrophil, platelet, and reticulocyte counts. Hydroxyurea has also proved to help prevent stroke recurrence in children with previous cerebrovascular accident. (Thomas et al 1999)

In one case series study, hydroxyurea was used to determine if it could prevent recurrent stroke. Of the 5 patients investigated, 4 initially had infarctive stroke and one had a transient ischemic attack (TIA). Four patients took HU at a dose of 40 mg/kg/d while one patient at 30 mg/kg/d. Results from the study indicated that none of the patients had recurrent stroke and pain crises during 42–112 months of observation. In all the participants, fetal hemoglobin (HbF) increased significantly and was maintained above 14.7% during treatment. The total Hb concentration increased by 1.95 g/dL (median) above the value before treatment. None of the five children had leukopenia or thrombocytopenia during therapy. (Sumoza et al 2002)

This however might not be the clinical picture at UTH due to a number of factors; among which could be attributed to lower dosages being employed (most patients on 10mg/kg/d HU dose), adherence factors and genetic variability. This study will therefore endeavor to provide evidence based information on the therapeutic outcomes of HU in SCD children with history of stroke. Data from this study will be important in promoting and formulating credible pharmaceutical care plans that will not only optimize patient safety but therapeutic response as well.

1.2 **STATEMENT OF THE PROBLEM**

Statistics show three quarters of the 300 000 SCD children born worldwide every year, occur in sub-Saharan Africa (Diallo & Tchemia, 2002; World Health Organisation, 2006). However, most studies that have looked at the effects of hydroxyurea (HU) therapy in children with SCD with history of stroke are particularly from the developed countries, with only few studies from this most affected region. To this effect, most treatment recommendations for the management are based on studies

conducted in resource-rich countries. Rahimy et al (2009) reported significant differences in SCD mortality between developed and developing countries; with as low as 0.5-1.0 per 100,000 children in developing countries and as high as 15.5 per 1,000 children (or 1,550 per 100,000 children) in Benin, a developing country.

Sumoza et al (2002) indicated that at high doses of HU of 30-40 mg/kg/d, there were minimal or no recurrent strokes; decreased painful crisis and increased hemoglobin with minimal side effects of leucopenia and thrombocytopenia. However, the current practice at UTH might not be achieving these desired HU therapy outcomes.

1.3 **STUDY RATIONALE**

Because public health implications of sickle-cell disease are quite significant, there is need to ensure effective monitoring and management of the disease. This can be achieved through evidence based practice.

There is however no local published study that has assessed/evaluated the hydroxyurea therapy outcomes in SCD children; what the benefits and adverse effects have been in this age group.

This study will therefore endeavor to highlight the hydroxyurea therapy outcomes experienced by children who previously had a stroke at UTH-Zambia. It will provide evidence based information on the therapeutic response, drug toxicity and the appropriate interventions that can be employed to get optimal benefits from the HU therapy. Physicians will be able to rationally prescribe effective therapies and be aware of the necessary interventions to make. Pharmacists will be more alert in identifying and monitoring of the common toxicities for the HU drug they are supplying. The study will also serve to provide basic information for further researches on hydroxyurea use in children in Zambia that will consequently improve both the quality and quantity of life for SCD patients

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1.4 **RESEARCH QUESTION**

Does current HU therapy at the University Teaching Hospital-Zambia benefit SCD children with history of stroke and is there a correlation between dose and response to therapy?

1.5 **AIM**

To assess the hydroxyurea therapy outcomes in children with sickle cell disease with history of stroke at the University Teaching Hospital-Zambia.

1.6 **OBJECTIVES**

i. Determine the therapeutic response of hydroxyurea therapy in terms of hematological parameters in SCD children with history of stroke at the University Teaching Hospital.

ii. Determine the extent to which hydroxyurea therapy prevents vaso occlusive crisis and recurrent strokes in SCD children with history of stroke at the University Teaching Hospital.

iii. Determine the toxicity of hydroxyurea therapy in terms of hematological parameters in SCD children with history of stroke at the University Teaching Hospital

iv. Investigate the relationship between dose and the therapeutic outcomes of hydroxyurea therapy (hematological and clinical parameters) in SCD children who previously had a stroke at the University Teaching Hospital.

CHAPTER 2: LITERATURE REVIEW

This review of literature explored the main concerns centered on hydroxyurea therapy outcomes in sickle cell children. The review of literature focused mainly on objectives 1, 2, and 3 as set out in chapter one. (Objective 4 was achieved as a result of findings from objectives 1, 2 and 3). The objectives were;

i. Determine the therapeutic response of hydroxyurea therapy in terms of hematological parameters in SCD children with history of stroke at the University Teaching Hospital.
ii. Determine the extent to which hydroxyurea therapy prevents vaso occlusive crisis and recurrent strokes in SCD children with history of stroke at the University Teaching Hospital.
iii. Determine the toxicity of hydroxyurea therapy in terms of hematological parameters in SCD children with history of stroke at the University Teaching Hospital
iv. Investigate the relationship between dose and the therapeutic outcomes of hydroxyurea therapy in SCD children who previously had a stroke at the University Teaching Hospital.

Sickle cell disease (SCD) is unevenly distributed worldwide. Approximately 70% of the SCD births occur in the sub-Saharan and less commonly seen in those of Mediterranean, Latino, East Indian, and Arab descent. (Angastiniotis et al 1995)

In Africa, the prevalence of the sickle-cell trait ranges between 10% and 40% across equatorial Africa and decreases to between 1% and 2% on the north African coast and <1% in South Africa. In West African countries such as Ghana and Nigeria, the frequency of the trait is 15% to 30% whereas in Uganda it shows marked tribal variations, reaching 45% among the Baamba tribe in the west of the country. In some areas of Sub-Saharan Africa, up to 2% of all children are born with sickle cell disease (WHA 59/9, 2006).

There is however no recent data on the prevalence of sickle cell trait in Zambia though it has long been known that 18% of the population of the Zambia Copperbelt carry the sickle cell trait. (Barclay GP 1971)

There are a number of studies that have looked at the effects of hydroxyurea (HU) therapy in children with SCD with history `of stroke particularly in the developed countries. Therapeutic studies of hydroxyurea that have been performed in children include investigations indicating hematologic response, lack of significant toxicity (Scott JP et al, 1996, Zimmerman SA et al, 2004), decreases in vaso-occlusive episodes (Jayabose S et al, 1996) and possible prevention of secondary strokes (Ware RE et al, 2004)

In the HUG-KIDS study, a phase I/II clinical trial study, eighty-four children with sickle cell disease aged between 5 and 15 years were enrolled between December 1994 and March 1996 and started with hydroxyurea drug at 15 mg/kg/d and escalated to 30 mg/kg/d unless the patient experienced laboratory toxicity. Sixty-eight children reached maximum tolerated dose (MTD) and 52 were treated at MTD for 1 year. Patients were monitored by 2-week visits to assess compliance, toxicity, clinical adverse events, growth parameters, and laboratory efficacy associated with HU treatment. By 6 months of HU treatment, there were statistically significant increases in the hemoglobin concentration, MCV, mean corpuscular hemoglobin (MCH), Hb F level, and percentage of F cells and significant decreases in the reticulocyte count, WBC count, absolute neutrophils count, platelet count and total bilirubin compared with baseline values ($P = 0.0001$). When this study ended (24 months) the significant hematologic changes included; increases in hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, and fetal hemoglobin parameters, and decreases in white blood cell, neutrophil, platelet, and reticulocyte counts. Laboratory toxicities typically were mild, transient, and were reversible upon temporary discontinuation of HU. The clinical trial shows that HU therapy is safe for children with sickle cell anemia when treatment was directed by a pediatric hematologist though these were only short term effects and could not reflect on the long term effects of hydroxyurea therapy. (Thomas et al, 1999)

In a longitudinal non randomized interventional study by Susanna et al (2011), at the sickle cell unit, University of West Indies in Jamaica, an assessment of stroke recurrence was conducted in SCD children following their first clinical stroke. Of the forty-four children enrolled; one died at that presentation. Forty-three children were therefore followed for 111 person-years, of whom 10 (23.3%) agreed to start HU. The average HU dose at maximum tolerated dose (MTD) was 25.4 ± 3.4 (mg/kg)/day

(median 25.4; range: 18.0–29.7 (mg/kg)/day). Only one child in the HU group, incidence rate 2/100 person-years, had clinical stroke recurrence, compared to 20/33 in the non-HU group, incidence rate 29/100 person-years. When the groups were compared, in the non-HU group, four died against zero in the HU group. Thirteen (53%) in the non-HU group had moderate–severe physical disability compared to 1 (10%) in the HU group (P =0.017). Twelve (44%) in the non-HU group required special education or were too disabled to attend school against 2 (20%) in the HU group. Though this data support the role of HU as a useful intervention for prevention of stroke recurrence in SCD when transfusion programs are not available or practical, the sample size might have been inadequate to make inferences to a large population on the therapy outcomes of HU in SCD children with history of stroke.

In another study, they determined the clinical and hematologic effects of hydroxyurea in children with sickle cell anemia. The results from this study indicated that HU increased hemoglobin by 1.9g/dl, mean capsular value increased by 22% and there was a reduction in painful crisis by 65%. (Jayabose et al 1996). This study was however, an open-label pilot study hence inferences cannot be drawn from the study results. VOCs episodes that were not severe enough to require hospitalization were not considered as VOCs in the study hence not included in the analysis of the study results. Adherence monitoring was also not exhaustively done as most participants were unable to complete their drug diaries which could have otherwise compromised the study results.

In a cohort study conducted at Duke, the study looked at the initiation of HU with abrupt cessation on transfusion. With the duration of follow up of 219 patient years at the median period of 0.9 years, 10 of 35 patients (29%) had recurrent stroke after switching to hydroxyurea; seven were previously reported and three new strokes occurred during extended follow-up. The overall secondary stroke event rate was 4.6 per 100 patient-years. It should however be noted that sample selection was bias. The participants had variable time on transfusion prior to initiating HU; ranging from 7-130 months. Most patients on transfusion therapy are likely to develop a recurrent stroke within the first 3 years but for this study however, most of the patients were beyond the high-risk period for having a stroke within 3 years, thus biasing the results towards a lower stroke rate compared with blood transfusion therapy (Greenway 2011).

Lefe`vre (2008) reported that there was an average decrease in Transcranial Doppler (TCD) velocity from 235 to 202 cm/second in those treated with hydroxyurea versus an average increase from 148 to 172 cm/second in those untreated. It was further observed that a low rate of stroke 0.36 per 100 patient-years in the children treated for abnormal TCD and also a low rate of recurrence (2.9 per 100 patient-years) in those treated with hydroxyurea after a first stroke. These reports indicate other than chronic blood transfusion; HU is also beneficial at preventing recurrent strokes.

Dosing of HU is usually varied depending on patient response and tolerance. Escalation of HU dose is usually limited by its hematological adverse effects (neutropenia, but also by reticulocytopenia, and more rarely by thrombocytopenia) which are dose related. There has been no direct comparison of fixed dose to Maximum Tolerated Dose (MTD) in children with SCD. However, the indirect comparison of multiple studies that escalated HU therapy to MTD compared to fixed dose or escalation to clinical effect supports greater improvement in beneficial laboratory indices (increased total hemoglobin (Hb) concentration, fetal Hb in children treated at the MTD. The MTD, measured in mg/kg/day, is typically established within 6 months, but should be assigned only after tolerating a particular dose for at least 8 weeks. The MTD of hydroxyurea should not exceed 35 mg/kg/day (or 2,500 mg/day) because failure to achieve marrow suppression at these doses strongly suggests non-adherence. Hydroxyurea toxicity guidelines include thresholds for hepatic or renal toxicity (e.g., transaminases >3–5X the upper limit of normal or a doubling of creatinine) but such organ toxicity is almost never related to hydroxyurea treatment. Indeed, significant increases in ALT or creatinine without accompanied hematological toxicity should prompt investigations for alternative etiologies.

(Zimmerman SA et al ,2004; McGrann et al 2011; Ware et al 2004 & 2009; Thomas et al 1999).

As indicated from literature discussed above, most studies have assessed the benefits of HU therapy in children at the MTD. This might however not be the scenario at the University Teaching Hospital; most patients are receiving lower doses (10-15 mg/kg/d). This study will thus establish evidence whether the SCD children with history of stroke are getting the optimal benefit from HU therapy.

CHAPTER THREE: METHODOLOGY

This chapter includes the following: study design, study site , study population, study population, sampling technique, inclusion/exclusion criteria, variables, data collection/data collection tools, data consolidation/analysis/interpretation and ethical considerations.

The general objective of this research was to assess the hydroxyurea therapy outcomes in sickle cell children with history of sickle cell at the University Teaching Hospital in Lusaka, Zambia.

3.1 **STUDY DESIGN**

This study was a retrospective cohort study. This study design enabled the researcher to assess a number of hydroxyurea therapeutic outcomes (study variables) in a short period of time. HU therapy was used as a pharmaceutical intervention in the management of SCD patients with history of stroke at the University Teaching Hospital. Participants were followed up retrospectively for a period of 6 months before and 6 months after initiation of HU to compare the therapy outcomes on therapeutic response and drug toxicity using patients as their own control.

The research design was able to assess a number of HU treatment outcomes of sickle cell disease. HU treatment outcomes that were analyzed included; hematological responses and non physiological responses (i.e. number of recurrent strokes, vaso-occlusive crisis)

3.2 **STUDY SITE**

The study was conducted at the University Teaching Hospital, Paediatric hematology/oncology unit. This is the only institution in the country that manages SCD patients on hydroxyurea therapy. The institution provides health care services, teaching and research. Patient files were obtained and used for data collection from the Hematology clinic (Clinic 4), the Hematology ward (A06) and general paediatric wards.

3.3 **TARGET POPULATION**

The target population in the study included SCD children with history of stroke receiving HU therapy at the University Teaching Hospital. There was no available data indicating the actual number of SCD patients with history of stroke on HU therapy at the institution however, the UTH paediatric pharmacy records showed that 86 patients had been supplied with the drug since 2005. For the purpose of this study, the target population therefore was 86.

3.4 **SAMPLE SIZE**

Considering a small target population and in order to achieve a desirable level of precision, the entire population was used as the study sample. Of the 86 patients captured in the hospital pharmacy records, a sample size of 34 participants was enrolled in the study. It was thus difficult to account for the other files (i.e. died, defaulted, stopped due to adverse effects etc) as the patient files are not kept at the institution hence the researcher enrolled all the patients meeting the study criteria who visited the in institution during the period of data collection.

3.5 **SAMPLING TECHNIQUE**

All SCD patients with history of stroke who have been initiated on therapy since 2007 and have been on hydroxyurea therapy for at least 6 months at UTH hematology/oncology Paediatric department were enrolled for the study. Patients had varying duration on therapy at the time of the study hence only the first 6 months on therapy were considered in this study.

3.6 **INCLUSION CRITERIA**

- Children 15 years of age and below
- Children with SCD who had a stroke and are on hydroxyurea therapy for at least 6 months
- Children seen at UTH in the last five years.

3.7 **EXCLUSION CRITERIA**

- Children who previously had a stroke and on hydroxyurea therapy less than 6 months.

3.8 **DEPENDANT VARIABLES**

Two drug therapy outcomes were studied; the desired therapeutic response and drug adverse effects. The efficacy of hydroxyurea in the treatment of sickle cell disease is generally attributed to its ability to boost the levels of fetal hemoglobin ($\alpha 2\gamma 2$). The adverse effects of HU are due to its bone marrow suppression. In the study, for therapeutic responses, both hematological (hemoglobin and mean capsular value) and non physiological responses (vaso occlusive crisis and stroke incidences) were assessed. Hematological parameters (platelet and white blood cell counts) were used to assess the toxicity of hydroxyurea therapy. Below are the reference values that were used to determine both therapeutic response and drug toxicity of hydroxyurea therapy (Strouse JJ et al 2008; Silva Pinto et al 2013; Ohene-Frempong 1998).

Therapeutic response	**Acceptable values**
• Hemoglobin	Increase greater than 1g/dl
• MCV	Increase greater than 14%
• Vaso-occlusive episodes	56% - 87% decline
• Stroke episodes	Less than 12%
Hematological drug toxicity	
• Neutrophils	Less than 2000 cells/mm^3
• Platelets	Less than 80 000/mm^3
• White blood cells	Less than 3 x 10^9/l

3.9 **INDEPENDENT VARIABLES**

Dosing has some effect on therapy outcomes. The current labeled dosing of hydroxyurea for sickle cell disease calls for the administration of an initial dose of 15 mg/kg/day in the form of a single dose, with monitoring of the patient's blood count every 2 weeks. If the blood counts are in an acceptable range, the dose may be

increased by 5 mg/kg/day every 12 weeks until the MTD of 35 mg/kg/day are reached. Adapted from the British National Formulary for Children, 2013.

Dosage of hydroxyurea

For the purpose of this study, the following dose categories were used.

- Below 15mg/kg/day — Low dose
- 15-35mg/kg/day — Acceptable dose
- More than 35mg/kg/day — Over dose

3.10 DATA COLLECTION TOOL

Data collection tool used was developed to capture study data from patient record files. Three steps in capturing of data were employed; patient demographic data, therapeutic response and drug toxicity (see Data collecting tool in the Appendix A).

Hemoglobin, mean capsular volume, episodes of vaso-occlusive crisis and stroke was used for therapeutic response analysis. Platelet count and white blood cell count was used for drug toxicity analysis.

3.11 DATA COLLECTION TECHNIQUE

Data collection was done by the researcher for the duration of 3 months. Data collection was conducted on Mondays, Wednesdays and Fridays during routine ward rounds and routine hospital visits/patient clinic appointments.

Patient files were the sole source of information during data collection and data was retrieved from patient files using the developed data collection tool**.** Study data was solely dependent on the information available in the files at the time of the study.

The hematological therapeutic response of patients with SCD on hydroxyurea (hemoglobin and mean corpuscular levels) were captured as recorded in the patient files during their hospital clinical appointments on quarterly basis.

Vaso-occlusive crisis episodes included in the study were those severe enough to require hospitalization. Vaso-occlusive crisis episodes will be assessed by the number of inpatient days for each patient during the period of treatment and compare it with the number of inpatient days for the period before the patient was initiated on

hydroxyurea therapy, using the subject as his own control subject. This information will be captured during the first six months of HU therapy. The ratio of VOCs to the period of follow up before and after therapy was used due to missing information particularly before commencement of HU therapy;

$$\text{The ratio} = \frac{\text{Total number of VOCs}}{\text{Total period of follow up}}$$

Basis for the diagnosis of stroke was entirely clinical. Sub clinical silent strokes were not captured in the study. Episodes of stroke were assessed before and after the patient were initiated on HU, using the subject as his own control subject. The ratio of stoke incidences used before and after therapy is as shown below:

$$\text{The ratio} = \frac{\text{Total number of stroke episodes}}{\text{Total period of follow up}}$$

The drug toxicity was monitored using the hematological parameters (decrease platelets, neutrophils and white blood cell values below acceptable levels) during therapy.

The study was conducted after approval from the University of Zambia, Biomedical Research and Ethics committee in December, 2013.

3.12 DATA PROCESSING/ANALYSIS

The data was extracted manually from the patient records and entered into the data master sheet, coded and categorized. Thereafter the quantitative data was analysed and presented into tables, graphs and charts using the Starter Package for Social Sciences software, version 11.0

Using the hematological mean values from the study, the paired sample t-test was used to show significance in therapeutic response and toxicity.

Regression method was conducted to study the relationship of dosage to the therapeutic outcomes of hydroxyurea therapy.

The missing information was defined and treated as missing data during analysis and thus did not affect the results.

The confidence interval of 95% and p value of less than 5% was used to show significance change.

3.13 DATA DISSEMINATION

Data will be disseminated in accordance with the University of Zambia requirements to the relevant departments.

3.14 ETHICAL CONSIDERATIONS

Authorisation

Although the study did not directly involve patients but the use of patient record files for data collection, authorization was sought from the University of Zambia, Ethics committee for clearance. Authorisation for approval to conduct the study at UTH Paediatric Haematocolgy/Oncology was also sought from the UTH management.

Confidentiality

Patients enrolled in the study were guaranteed that information extracted from their patient record files shall be confidential. No name of individuals was mentioned in the report. Data will be kept in a de-identified file for 1-2 years in case of disputes or until publication of this study.

Beneficence

Data from this study is beneficial to the clinicians, pharmacists and the patients. The study highlighted hydroxyurea therapy outcomes in SCD pediatric patients with history of stroke that is hoped to promote effective and optimal usage of HU dosages and the appropriate recommendations aimed at improving the quantity and quality the SCD children.

CHAPTER FOUR: RESEARCH FINDINGS

This chapter provides study results on hydroxyurea therapeutic response in terms of hematological and clinical response, hydroxyurea hematological toxicity and whether drug response is associated to dose.

Thirty four SCD children aged 15 years and below were enrolled in the study. Of these, 17 were males and 7 patients had chronic co-morbidities (2 were HIV positive, 2 had pulmonary tuberculosis, 2 had congestive heart failure and 1 had renal impairment).

4.1 Hematological therapeutic response

At 3 months of HU therapy, mean hemoglobin changed from 7.19 g/dl to 7.47g/dl (SD = 1.63 and 1.47 respectively) with P = 0.4969 vs. baseline value, by paired t test. Between 3 and 6 months of HU therapy, the mean hemoglobin changed from 7.47 g/dl to 7.11 g/dl (SD =1.47 and 1.42 respectively) with P = 0.3273, by paired t test. At 6 months of therapy, the mean hemoglobin was 7.11 g/dl from baseline value of 7.19 g/dl (SD=1.42 and 1.63 respectively) with P = 0.8443, by paired t test.

For the mean corpuscular volume (MCV), at 3 months of therapy, the mean MCV changed from 92.51 fl to 94.76 fl (SD = 10.85 and 10.75 respectively) with P = 0.4066 vs. baseline value, by paired t test. The mean MCV between 3 and 6 months of HU therapy changed from 94.76 fl to 95.08 fl (SD = 10.75 and 9.31 respectively) with P = 0.9030, by paired t test. At the end of this study, the mean MCV was 95.08 fl from baseline value of 92.5 fl (SD = 9.32 and 10.85 respectively) with P = 0.2982 vs. baseline value, by paired t test as illustrated in table 1 below.

Table 1. Changes in hematological parameters at various periods of therapy in SCD children with history of stroke

Duration of Therapy (months)	Number of observation	Hematological parameters during HU therapy			
		Hb (g/dl)	MCV (fl)	WBC (x 109/l)	Plt (x 109/l)
Baseline	34	7.19 (1.63)	92.51 (10.85)	22.63 (18.52)	434.79 (162.67)
3	30	7.47 (1.47)	94.77 (10.75)	18.96 (14.74)	435.14 (160.58)
6	34	7.11 (1.42)	95.08 (9.31)	22.36 (16.70)	386.98 (181.49)

Hb; hemoglobin, MCV; mean corpuscular volume, WBC; white blood cells, Plt; platelet. The number of observation is less at 3 months due to missing values in patient files. The variable changes in the table were not significant, $P > 0.05$, by paired t test. Values are means $\pm$ SD.

4.2 Effects of HU on vaso occlusive crisis and recurrent strokes

The ratio of the total number of hospital in-patient days (VOC) during the period of follow up before therapy was 0.02 ± 0.01 where as the ratio during HU therapy was 0.002 ± 0.004, with the P-value of 0.00001, by paired t test.

The ratio of the total number of stoke episodes during the period of follow up before therapy was 0.26 $\pm$ 0.04 strokes/day where as the ratio during HU therapy was 0.0002 $\pm$ 0.0002 strokes/day, P =0.0004, by paired t test as shown in table 2.

Table 2. Changes in clinical picture before and after therapy in SCD children with history of stroke

Duration	Number of obs	Hosp inpatient days (/day)	Strokes (/day)
Before HU therapy	26	0.02 (0.01)	0.26 (0.04)
After HU therapy	34	0.002* (0.004)	0.0002* (0.0002)

HU, hydroxyurea. The number of observation before therapy is less due to missing values in patient files. Values are means $\pm$ SD. Obs, observation; VOC, vaso occlusive crisis.
* where P < 0.05 vs. the 'before" value, by paired t test.

4.3 Hematological drug toxicity

The mean WBC at 6 months of therapy changed from 22.63 x 10^9/l to 22.35 x 10^9/l (SD = 18.51 and 16.21 respectively) with P = 0.9479 vs. the baseline value, by paired t test. At 3months of HU therapy, the mean WBC changed from 22.63 x 10^9 /l to 18.96 x 10^9/l (SD = 18.51 and 14.71 respectively) with P =0.2533 vs. baseline value, by paired t test. Between 3 months and 6 months of therapy, the mean WBC count was 18.96 x 10^9/l from baseline value of 22.35 x 10^9/l (SD= 14.71 and 16.21 respectively) with P =0.4179, by paired t test.

The mean Plt count at 6 months of HU therapy was 386.98 x 10^9/l from baseline value of 434.79 x 10^9/l (SD = 181.49 and 162.68 respectively), P = 0.2634, by paired t test. At 3 months of HU therapy, the mean Plt count changed from 434.79 x 10^9/l to 435.14 x 10^9/l

(SD = 162.68 to 160.58 respectively), P =0.9933 vs. baseline value, by paired t test. Between 3 months and 6 months of therapy, the mean Plt count changed from 435.14 x 10^9/l to 386.98 x 10^9/l (SD = 160.58 and 181.49 respectively), P = 0.2790, by paired t test. Refer to table 1 that shows hematological changes during hydroxyurea therapy.

4.4 Effect of HU dose on therapeutic response

The pearson's correlation coefficient between HU dose of <15mg/kg/day and change in hospital inpatient days was 0.0564 where as between HU dose of <15mg/kg/day and stroke recurrence is 0.1665. The pearson's correlation coefficient between HU dose of 15-30mg/kg/day and change in hospital inpatient day was 0.1197.

CHAPTER FIVE: DISCUSSION OF RESULTS

This study provides a detailed discussion of the results. It tries to interpret the findings to the pharmacokinetics (generally attributed to its ability to boost the levels of fetal hemoglobin ($\alpha 2\gamma 2$) and its ability to suppress the bone marrow suppression) and relates them to the findings from other similar studies. Justification of the study results in relation to other similar studies was also done.

5.1 Hematological therapeutic response

The study shows that there is no therapeutic response of hydroxyurea therapy in terms of hematological parameters in SCD children with history of stroke.

Evidence is that there is no significant increase in the red blood cell indices which included the mean hemoglobin and mean MCV during the first 6 months of HU therapy. At the end of the study the mean hemoglobin changed from 7.19 g/dl to 7.11 g/dl (SD = 1.63 and 1.42 respectively) with P = 0.8443 vs. baseline value, by paired t test where as the mean MCV changed from 92.51 fl to 95.08 fl (SD = 9.32 and 10.85 respectively) with P = 0.2982 vs. baseline value, by paired t test.

This is in contrast with a number of study reports that have shown an average significant HU-induced increase in the volume of the red blood cells. There is strong evidence presented in observational studies of hemoglobin increase, usually +1 g/dl. (Ware R.E and Banu Aygun, 2009).

The lack of significant increase in red blood cell indices in the study could be attributed to the comparatively low mean dose used (at 10.45mg/kg/day) than the recommended dose of an initial dose of 15mg/kg/day and increased by 5mg/kg/day every 12 weeks according to patient response to the maximum dose of 35mg/kg/day (British National Formulary for Children, 2013). Zimmerman et al (2004) also did show that additional beneficial changes are obtained when HU is used at maximum tolerated dose. This therefore entails that at the mean dose of 10.45 mg/kg/day of HU, there is no therapeutic response of HU therapy in terms of hematological response in SCD children with history of stroke.

The other cause for insignificant increase in red blood cell indices in this study could be due to the inconsistent and low availability of the HU drug at UTH. This is evidenced from pediatric pharmacy records where the drug availability for the year 2013 was at 25%.

This could significantly affect response as not all patients can manage to buy HU from retail outlets

The duration of follow up could also have been too short to sufficiently assess hematological response to HU therapy.

The other reason could be due to compliance invariables that were not captured in this study.

In this study, insignificant increase in the mean MCV could have also been attributed to unknown factors such as α-thalassemia which is frequent and often associated to SCD as indicated by Falusi & OLatunji (1994).

5.2 Effects of HU on vaso occlusive crisis and recurrent strokes

The study shows that hydroxyurea therapy reduces vaso occlusive crisis and recurrent strokes in SCD children with history of stroke.

Evidence is that there is significant decrease in the ratio of hospital inpatient days (VOC) and ratio in stroke incidences after patients were initiated on HU therapy (see table 2).

This is similar to other studies that showed strong evidence that HU therapy reduces pain episodes and hospitalizations. (Ware R & Aygun B 2009). These study results therefore indicate HU response (reduction in vaso occlusive crisis and recurrent strokes) do occur at low doses (mean 10.45mg/kg/day)

This study has shown increased clinical improvements possibly due to the study design used. This study did include only the VOC episodes that lead to hospitalization. Stroke diagnosis was solely clinical and could have missed sub clinical silent strokes.

The duration of follow up (6 months) of follow up compared to other studies was relatively too short to fully assess these variables.

5.3 Hematological HU toxicity

This study shows that there is no toxicity of hydroxyurea therapy in terms of hematological parameters in SCD with history of stroke.

Evidence is that there is no reduction in the white blood cell below 3×10^9/l and Plt below $80 \times 10^{9/]}$ (Strouse JJ et al, 2008)].

A number of studies have shown that HU therapy is relatively safe in children; however, these studies have also shown that hematological toxicity occurs. Neutropenia, reticulocytopenia, rarely thrombocytopenia occur with escalation of HU dose as the HU hematological toxicities are dose related (Zimmerman et al 2004; McGrann et al 2011; Ware et al 2004 & 2009; and Thomas et al 1999)

The reason why there was no HU hematological toxicity observed in the study would be due to low HU doses, inconsistent availability of the drug at the institution, compliance invariability and the short duration of follow up as earlier mentioned (See 5.1).

The study results also show that the mean WBC (18.98×10^{9} /l) is relatively high. This could be attributed to frequent infections and other unknown factors which this study did not capture. According to Okpala (2004), a raised white blood cell count was identified as a marker of severe SCD and, specifically, as a risk factor for early death, stroke, acute chest syndrome and nephropathy. Whether the same will be true in this population is, as yet, unknown.

5.4 Effect of HU dose on therapeutic response

The study shows that there was a correlation between dose and therapeutic response [i.e. change in number of hospital in patient days (VOC episodes) with a positive correlation coefficient of .0564 and change in stroke recurrence with pearson's correlation coefficient of 0.1665.

However, the duration of follow up in this study could have been too short to fully appreciate the effects of dose on HU therapy response.

CHAPTER SIX: CONCLUSIONS & RECOMMENDATIONS.

6.1 CONCLUSION

At the mean HU dose of 10.45 mg/kg/day), the study findings show that there is HU therapeutic response in terms of reductions in the vaso occlusive crisis (number of hospital inpatient days) and stroke recurrence. The study results show that no hematological toxicity was observed in the study. However, in this study, we provided evidence that there is no HU hematological therapeutic response. Our results also show that there was a positive correlation between dose and the HU therapeutic response. Beneficial effects of HU therapy are achieved with HU dose of <15mg/kg/day although hematological therapeutic response is not achieved at this dose.

6.2 RECOMMENDATIONS

The recommendations of this study are as outlined below:

- More prospective studies to assess if the use of HU at 15-30 mg/kg/day will increase both hematological and clinical therapy outcomes without increasing toxicity risks in this resource limited setting. A prospective study will also overcome challenges of missing information in patient files.
- The Ministry of Health, through the procurement department to improve availability of hydroxyurea drug at the University Teaching Hospital as not every patient can afford to buy the expensive drug. Inconsistent availability of drugs can result in poor patient response to hydroxyurea.
- Clinicians and pharmacists to formulate local guidelines on use of hydroxyurea in sickle cell disease and that these guidelines are included in the Standard Treatment Guidelines (STG) and in the Zambian National Formulary (ZNF) to enhance uniform and effective management of the disease. Reference of these guidelines can be adopted from the British National Formulary for Children and from study literature highlighted in this study.
- Clinician to improve on the documentation of clinical patient characteristics for easy tracking of patient information in the patient files.
- UTH to keep patient files at the institution for safety and easy accessibility of patient data and patients to be provided with patient care cards on which vital patient data is indicated. They should also improve on the record keeping and update the sickle cell patient register at the institution.

- UTH to ensure that each patient has one file for all the health services provided at the institution to easy access of a more complete clinical profile of the patients and allow for easy tracking of patient information.

6.3 STUDY LIMITATIONS

During this study, there were a number of obstacles and constraints that were incurred. The limitations/constraints to the study included:

a) The study was retrospective hence certain information needed was not available in the patient files hence not captured in the study. In the study, the rate and not the actual number of VOCs and stroke episodes was used for analysis.
b) VOCs episodes that were not severe enough to require hospitalization were not considered as VOCs in the study and diagnosis of stroke was clinically based with no confirmatory tests. This might have lead to the study not reflecting the actual episodes at the institution.
c) Diagnosis of stroke was clinical. Sub clinical silent strokes were not included in this study.
d) Difficulties in accessing study data due to lack availability of patient files at the institution. Patient files are kept by patients' parents and guardians hence the researcher only accessed study data when patients visited the institution. . Out of the 86 patients files captured in the UTH pharmacy records, only 34 files were accessed. In the study, the data collection was extended from the initial 2 months to 3 months in order to have a statistically significant sample size.
e) Lack of updated records of the sickle cell disease patient register by the institution was another challenge in determining the study population. In the study, the target population was used as the study population due to no proper records/registers for the patients
f) Inconsistent monitoring of hematological parameters by the institution e.g. investigation of drug toxicity on neutrophils count was not done due to inconsistent monitoring of the parameter. In the study, some variables were not captured and these were treated as missing values during analysis.

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APPENDICES

APPENDIX A - DATA COLLECTION TOOL.

PATIENT DEMOGRAPHIC DATA

1. **Age**
   - 1.1 0 – 5 years [1]
   - 1.2 6 – 10 years [2]
   - 1.3 11 – 15 years [3]
2. **Gender**
   - 2.1 Female [1]
   - 2.2 Male [2]
3. **HIV/AIDS status**
   - 3.1 Positive [1]
   - 3.2 Negative [2]
   - 3.3 Unknown [3]
4. **Any other chronic infectious disease**
   - 4..1 None 1
   - ` 4.2 Co-infected 2
5. **Hemoglobin (g/dl)**
   - 5.1 Hemoglobin at baseline ……
   - 5.2 Hemoglobin at 3 months …….
   - 5.3 Hemoglobin at 6 months ……
6. **Mean Capsular Volume (MCV)**
   - 6.1 MCV at baseline ……

6.2 MCV at 3 months …….

6.3 MCV at 6 months ……

7. Vaso-Occlusive Crisis (VOCs) episodes

7.1 Before therapy

7.1.1 Number of VOCs …….

7.1.2 Number of inpatient days ……..

7.1.3 Duration of follow-up ……..

7.2 During therapy

7.2.1 Number of VOCs …….

7.2.2 Number of inpatient days ……..

7.2.3 Duration of follow-up ……..

8. Stroke episodes

8.1 Before therapy

8.1.1 Number of strokes …….

8.1.2 Duration of follow-up ……..

8.2 During therapy

8.2.1 Number of strokes ……..

8.2.2 Duration of follow-up …….

9. Platelet count

9.1 Platelets at baseline ……..

9.2 Platelets at 3 months ……..

9.3 Platelets at 6 months ……..

10. Neutrophil count

10.1 Neutrophils at baseline …….

10.2 Neutrophils at 3 months …….

10.3 Neutrophils at 6 months …….

11. White blood cell count

11.1 White blood cell count at baseline ……..

11.2 White blood cell count at 3 months ……..

11.3 White blood cell count at 6 months ……..

12. Dosage in mg/kg/day

12.1 Dosage at baseline …….

12.2 Dosage at 3 months …….

12.3 Dosage at 6 months …….

DATA OUTPUT

**********objective one
. ***using t test
. *hemoglobin
. ttest hemoglobin1 == hemoglobin2, unpaired unequal welch

Two-sample t test with unequal variances

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
hemogl~1	34	7.185294	.2797099	1.630975	6.61622	7.754368
hemogl~2	30	7.47	.267863	1.467146	6.922159	8.017841
combined	64	7.31875	.1938396	1.550717	6.931392	7.706108
diff		-.2847059	.3872831		-1.058398	.4889857

diff = mean(hemoglobin1) - mean(hemoglobin2) t = -0.7351
Ho: diff = 0 Welch's degrees of freedom = 63.9801

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.2325 Pr(T > t) = 0.4649 Pr(T > t) = 0.7675

. ttest hemoglobin2 == hemoglobin3, unpaired unequal welch

Two-sample t test with unequal variances

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
hemogl~2	30	7.47	.267863	1.467146	6.922159	8.017841
hemogl~3	34	7.112059	.2443573	1.424836	6.61491	7.609207
combined	64	7.279844	.1805663	1.44453	6.919011	7.640677
diff		.3579412	.3625756		-.3667217	1.082604

diff = mean(hemoglobin2) - mean(hemoglobin3) t = 0.9872
Ho: diff = 0 Welch's degrees of freedom = 62.5005

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.8363 Pr(T > t) = 0.3273 Pr(T > t) = 0.1637

. ttest hemoglobin1 == hemoglobin3, unpaired unequal welch

Two-sample t test with unequal variances

Variable Obs Mean Std. Err. Std. Dev. [95% Conf. Interval]

hemogl~1 34 7.185294 .2797099 1.630975 6.61622 7.754368
hemogl~3 34 7.112059 .2443573 1.424836 6.61491 7.609207

combined 68 7.148676 .18437 1.520354 6.780672 7.516681

diff .0732353 .3714137 -.6681586 .8146291

diff = mean(hemoglobin1) - mean(hemoglobin3) t = 0.1972
Ho: diff = 0 Welch's degrees of freedom = 66.7597

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.5779 Pr(T > t) = 0.8443 Pr(T > t) = 0.4221

. ttest hemoglobin1 == hemoglobin2, unpaired

Two-sample t test with equal variances

Variable Obs Mean Std. Err. Std. Dev. [95% Conf. Interval]

hemogl~1 34 7.185294 .2797099 1.630975 6.61622 7.754368
hemogl~2 30 7.47 .267863 1.467146 6.922159 8.017841

combined 64 7.31875 .1938396 1.550717 6.931392 7.706108

diff -.2847059 .3898856 -1.064076 .4946643

diff = mean(hemoglobin1) - mean(hemoglobin2) t = -0.7302
Ho: diff = 0 degrees of freedom = 62

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.2340 Pr(T > t) = 0.4680 Pr(T > t) = 0.7660

. ttest hemoglobin2 == hemoglobin3, unpaired

Two-sample t test with equal variances

Variable Obs Mean Std. Err. Std. Dev. [95% Conf. Interval]

hemogl~2 30 7.47 .267863 1.467146 6.922159 8.017841
hemogl~3 34 7.112059 .2443573 1.424836 6.61491 7.609207

combined 64 7.279844 .1805663 1.44453 6.919011 7.640677

```
diff           .3579412    .3619026           -.3654918    1.081374

diff = mean(hemoglobin2) - mean(hemoglobin3)                  t =   0.9891
Ho: diff = 0                     degrees of freedom =       62

Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
Pr(T < t) = 0.8368         Pr(T > t) = 0.3265         Pr(T > t) = 0.1632
```

```
. ttest hemoglobin1 == hemoglobin3, unpaired
```

```
Two-sample t test with equal variances

Variable     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]

hemogl~1      34    7.185294    .2797099    1.630975    6.61622    7.754368
hemogl~3      34    7.112059    .2443573    1.424836    6.61491    7.609207

combined      68    7.148676     .18437    1.520354    6.780672    7.516681

diff           .0732353    .3714137           -.6683161    .8147866

diff = mean(hemoglobin1) - mean(hemoglobin3)                  t =   0.1972
Ho: diff = 0                     degrees of freedom =       66

Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
Pr(T < t) = 0.5779         Pr(T > t) = 0.8443         Pr(T > t) = 0.4221
```

```
. *mean capsular value
. ttest meancapsularvalue1 == meancapsularvalue2, unpaired unequal welch
```

```
Two-sample t test with unequal variances

Variable     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]

meanca~1      34    92.50588    1.860045    10.84583    88.72159    96.29017
meanca~2      30    94.76667    1.964146    10.75807    90.74954     98.7838

combined      64    93.56563    1.347365    10.77892    90.87313    96.25812

diff          -2.260784    2.705113           -7.666294    3.144727

diff = mean(meancapsularva~1) - mean(meancapsularva~2)        t =  -0.8357
Ho: diff = 0                     Welch's degrees of freedom =  63.1353

Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
Pr(T < t) = 0.2032         Pr(T > t) = 0.4065         Pr(T > t) = 0.7968
```

. ttest meancapsularvalue1 == meancapsularvalue2, unpaired unequal

Two-sample t test with unequal variances

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
meanca~1	34	92.50588	1.860045	10.84583	88.72159	96.29017
meanca~2	30	94.76667	1.964146	10.75807	90.74954	98.7838
combined	64	93.56563	1.347365	10.77892	90.87313	96.25812
diff		-2.260784	2.705113		-7.669756	3.148188

diff = mean(meancapsularva~1) - mean(meancapsularva~2) t = -0.8357
Ho: diff = 0 Satterthwaite's degrees of freedom = 61.1319

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.2033 Pr(T > t) = 0.4066 Pr(T > t) = 0.7967

. ttest meancapsularvalue2 == meancapsularvalue3, unpaired unequal

Two-sample t test with unequal variances

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
meanca~2	30	94.76667	1.964146	10.75807	90.74954	98.7838
meanca~3	34	95.07647	1.596571	9.309528	91.82822	98.32472
combined	64	94.93125	1.241828	9.934626	92.44965	97.41285
diff		-.3098041	2.531187		-5.376888	4.75728

diff = mean(meancapsularva~2) - mean(meancapsularva~3) t = -0.1224
Ho: diff = 0 Satterthwaite's degrees of freedom = 57.8059

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.4515 Pr(T > t) = 0.9030 Pr(T > t) = 0.5485

. ttest meancapsularvalue1 == meancapsularvalue3, unpaired unequal

Two-sample t test with unequal variances

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
meanca~1	34	92.50588	1.860045	10.84583	88.72159	96.29017

meanca~3 34 95.07647 1.596571 9.309528 91.82822 98.32472

combined 68 93.79118 1.226555 10.11443 91.34296 96.23939

diff -2.570588 2.451287 -7.466839 2.325663

diff = mean(meancapsularva~1) - mean(meancapsularva~3) t = -1.0487
Ho: diff = 0 Satterthwaite's degrees of freedom = 64.5179

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.1491 Pr(T > t) = 0.2982 Pr(T > t) = 0.8509

. ttest meancapsularvalue1 == meancapsularvalue2, unpaired

Two-sample t test with equal variances

Variable Obs Mean Std. Err. Std. Dev. [95% Conf. Interval]

meanca~1 34 92.50588 1.860045 10.84583 88.72159 96.29017
meanca~2 30 94.76667 1.964146 10.75807 90.74954 98.7838

combined 64 93.56563 1.347365 10.77892 90.87313 96.25812

diff -2.260784 2.706509 -7.671019 3.149451

diff = mean(meancapsularva~1) - mean(meancapsularva~2) t = -0.8353
Ho: diff = 0 degrees of freedom = 62

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.2034 Pr(T > t) = 0.4067 Pr(T > t) = 0.7966

. ttest meancapsularvalue2 == meancapsularvalue3, unpaired

Two-sample t test with equal variances

Variable Obs Mean Std. Err. Std. Dev. [95% Conf. Interval]

meanca~2 30 94.76667 1.964146 10.75807 90.74954 98.7838
meanca~3 34 95.07647 1.596571 9.309528 91.82822 98.32472

combined 64 94.93125 1.241828 9.934626 92.44965 97.41285

diff -.3098041 2.508201 -5.323627 4.704019

diff = mean(meancapsularva~2) - mean(meancapsularva~3) t = -0.1235
Ho: diff = 0 degrees of freedom = 62

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.4510 Pr(T > t) = 0.9021 Pr(T > t) = 0.5490

. ttest meancapsularvalue1 == meancapsularvalue3, unpaired

Two-sample t test with equal variances

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
meanca~1	34	92.50588	1.860045	10.84583	88.72159	96.29017
meanca~3	34	95.07647	1.596571	9.309528	91.82822	98.32472
combined	68	93.79118	1.226555	10.11443	91.34296	96.23939
diff		-2.570588	2.451287		-7.46474	2.323563

diff = mean(meancapsularva~1) - mean(meancapsularva~3) t = -1.0487
Ho: diff = 0 degrees of freedom = 66

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 0.1491 Pr(T > t) = 0.2982 Pr(T > t) = 0.8509

*******objecive two
* vaso occlusive incidences

*number of VOCs before and after therapy

number of VOCs before therapy	Freq.	Percent	Cum.
1	9	34.62	34.62
2	10	38.46	73.08
3	6	23.08	96.15
4	1	3.85	100.00
Total	26	100.00	

. ta avocs1 if avocs1 != 0

number of VOCs after therapy	Freq.	Percent	Cum.
1	9	69.23	69.23
2	3	23.08	92.31
3	1	7.69	100.00

```
------------+-----------------------------------
      Total |        13      100.00
```

*number of inpatient days before and after therapy

```
. ta bvocs2 if  bvocs2 != .
```

number of VOCs in-patient before therapy	Freq.	Percent	Cum.
3	1	3.85	3.85
5	3	11.54	15.38
7	1	3.85	19.23
9	1	3.85	23.08
10	2	7.69	30.77
11	1	3.85	34.62
12	1	3.85	38.46
14	1	3.85	42.31
15	1	3.85	46.15
16	2	7.69	53.85
17	2	7.69	61.54
18	1	3.85	65.38
19	2	7.69	73.08
20	1	3.85	76.92
22	1	3.85	80.77
24	1	3.85	84.62
27	1	3.85	88.46
35	1	3.85	92.31
42	1	3.85	96.15
59	1	3.85	100.00
Total	26	100.00	

```
. ta avocs2 if  avocs2 != 0
```

number of VOCs in-patient after therapy	Freq.	Percent	Cum.
2	2	15.38	15.38
3	1	7.69	23.08
4	3	23.08	46.15

```
         5 |        1       7.69      53.85
         7 |        1       7.69      61.54
        10 |        2      15.38      76.92
        12 |        1       7.69      84.62
        18 |        1       7.69      92.31
        37 |        1       7.69     100.00
------------+-----------------------------------
     Total |       13     100.00
```

```
. *number of follow ups before and after therapy
. ta bvocs3 if  bvocs3 != .
```

```
duration of |
 follow ups |
   of VOCs |
    before |
   therapy |      Freq.     Percent        Cum.
------------+-----------------------------------
        30 |        2       7.69       7.69
        60 |        2       7.69      15.38
        90 |        2       7.69      23.08
       180 |       20      76.92     100.00
------------+-----------------------------------
     Total |       26     100.00
```

```
. ta avocs3 if  avocs3 != 0
```

```
duration of |
 follow ups |
   of VOCs |
     after |
   therapy |      Freq.     Percent        Cum.
------------+-----------------------------------
         6 |        1       2.94       2.94
        11 |        1       2.94       5.88
       180 |       32      94.12     100.00
------------+-----------------------------------
     Total |       34     100.00
```

```
. label var change_vocs1 "change between number of vocs before and after therapy"
```

```
. ta change_vocs1

    change |
```

```
   between |
  number of |
vocs before |
  and after |
   therapy |      Freq.     Percent        Cum.
------------+-----------------------------------
        -4 |          1        3.85        3.85
        -3 |          3       11.54       15.38
        -2 |          8       30.77       46.15
        -1 |         10       38.46       84.62
         0 |          3       11.54       96.15
         1 |          1        3.85      100.00
------------+-----------------------------------
     Total |         26      100.00
```

```
. ttest bvocs1 == avocs1, unpaired

Two-sample t test with equal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  bvocs1 |      26    1.961538    .1707969    .870897    1.609776    2.313301
  avocs1 |      34    .5294118    .1350731    .7876045    .2546036     .80422
---------+--------------------------------------------------------------------
combined |      60        1.15    .1402681    1.086512    .8693242    1.430676
---------+--------------------------------------------------------------------
    diff |           1.432127     .214813                1.002132    1.862122
------------------------------------------------------------------------------
    diff = mean(bvocs1) - mean(avocs1)                            t =   6.6669
Ho: diff = 0                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 1.0000         Pr(|T| > |t|) = 0.0000          Pr(T > t) = 0.0000
```

```
. ttest bvocs1 == avocs1, unpaired unequal
```

```
Two-sample t test with unequal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  bvocs1 |      26    1.961538    .1707969    .870897    1.609776    2.313301
  avocs1 |      34    .5294118    .1350731    .7876045    .2546036     .80422
---------+--------------------------------------------------------------------
combined |      60        1.15    .1402681    1.086512    .8693242    1.430676
---------+--------------------------------------------------------------------
    diff |           1.432127    .2177529                .9949594    1.869294
```

```
------------------------------------------------------------------------------
   diff = mean(bvocs1) - mean(avocs1)                            t =   6.5768
Ho: diff = 0                     Satterthwaite's degrees of freedom =  50.9517

   Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 1.0000         Pr(|T| > |t|) = 0.0000          Pr(T > t) = 0.0000
```

. label var change_vocs2 "change between number in patient of vocs before and after therapy"

. ta change_vocs2

change between number in patient of vocs before and after therapy	Freq.	Percent	Cum.
-57	1	3.85	3.85
-42	1	3.85	7.69
-27	1	3.85	11.54
-24	1	3.85	15.38
-23	1	3.85	19.23
-22	1	3.85	23.08
-20	1	3.85	26.92
-19	1	3.85	30.77
-17	3	11.54	42.31
-16	1	3.85	46.15
-14	1	3.85	50.00
-12	1	3.85	53.85
-11	1	3.85	57.69
-10	2	7.69	65.38
-9	2	7.69	73.08
-8	2	7.69	80.77
-7	1	3.85	84.62
-5	1	3.85	88.46
-1	1	3.85	92.31
0	1	3.85	96.15
7	1	3.85	100.00
Total	26	100.00	

. ttest bvocs2 == avocs2, unpaired

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  bvocs2 |      26    17.57692    2.437636    12.42956    12.55652    22.59733
  avocs2 |      34    3.470588    1.254749     7.31638    .9177827    6.023394
---------+--------------------------------------------------------------------
combined |      60    9.583333     1.55507    12.04552    6.471645    12.69502
---------+--------------------------------------------------------------------
    diff |            14.10633    2.566516                8.968894    19.24378
------------------------------------------------------------------------------
    diff = mean(bvocs2) - mean(avocs2)                            t =   5.4963
Ho: diff = 0                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 1.0000         Pr(|T| > |t|) = 0.0000          Pr(T > t) = 0.0000
```

```
. ttest bvocs2 == avocs2, unpaired unequal
```

```
Two-sample t test with unequal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  bvocs2 |      26    17.57692    2.437636    12.42956    12.55652    22.59733
  avocs2 |      34    3.470588    1.254749     7.31638    .9177827    6.023394
---------+--------------------------------------------------------------------
combined |      60    9.583333     1.55507    12.04552    6.471645    12.69502
---------+--------------------------------------------------------------------
    diff |            14.10633    2.741617                8.556138    19.65653
------------------------------------------------------------------------------
    diff = mean(bvocs2) - mean(avocs2)                            t =   5.1453
Ho: diff = 0     Satterthwaite's degrees of freedom =  37.9829

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 1.0000         Pr(|T| > |t|) = 0.0000          Pr(T > t) = 0.0000
```

```
. label var change_vocs3 "change between duration follow ups of vocs before and after therapy"
```

```
. ta change_vocs3

    change |
   between |
  duration |
 follow ups |
   of vocs |
```

```
 before and |
    after |
  therapy |      Freq.     Percent        Cum.
------------+-----------------------------------
        0 |         20       76.92       76.92
       90 |          2        7.69       84.62
      120 |          2        7.69       92.31
      150 |          2        7.69      100.00
------------+-----------------------------------
    Total |         26      100.00
```

```
. ttest bvocs3 == avocs3, unpaired
```

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  bvocs3 |      26    152.3077    10.38205    52.93828    130.9255    173.6899
  avocs3 |      34    169.9118    7.025338    40.96441    155.6186    184.2049
---------+--------------------------------------------------------------------
combined |      60    162.2833    6.060062    46.94103    150.1572    174.4095
---------+--------------------------------------------------------------------
    diff |           -17.60407    12.11576               -41.85639    6.648249
------------------------------------------------------------------------------
    diff = mean(bvocs3) - mean(avocs3)                            t =  -1.4530
Ho: diff = 0                                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0758         Pr(|T| > |t|) = 0.1516          Pr(T > t) = 0.9242
```

```
. ttest bvocs3 == avocs3, unpaired unequal
```

```
Two-sample t test with unequal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
  bvocs3 |      26    152.3077    10.38205    52.93828    130.9255    173.6899
  avocs3 |      34    169.9118    7.025338    40.96441    155.6186    184.2049
---------+--------------------------------------------------------------------
combined |      60    162.2833    6.060062    46.94103    150.1572    174.4095
---------+--------------------------------------------------------------------
    diff |           -17.60407    12.53564               -42.83919    7.631049
------------------------------------------------------------------------------
    diff = mean(bvocs3) - mean(avocs3)                            t =  -1.4043
Ho: diff = 0                     Satterthwaite's degrees of freedom =  45.8532
```

```
    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0835         Pr(|T| > |t|) = 0.1670          Pr(T > t) = 0.9165
```

```
. * stroke incidences
. * number of strokes before and after therapy
. ta bstrokes1 if  bstrokes1 != 0

  number of |
    strokes |
     before |
    therapy |      Freq.     Percent        Cum.
------------+-----------------------------------
          1 |         26       92.86       92.86
          2 |          2        7.14      100.00
------------+-----------------------------------
      Total |         28      100.00

. ta astroke1 if astroke1 != 0

  number of |
    strokes |
      after |
    therapy |      Freq.     Percent        Cum.
------------+-----------------------------------
          1 |          1      100.00      100.00
------------+-----------------------------------
      Total |          1      100.00

. *duration of follow ups before and after therapy
. ta bstrokes2 if bstrokes2 != 0

duration of |
 follow ups |
 of strokes |
     before |
    therapy |      Freq.     Percent        Cum.
------------+-----------------------------------
          7 |          1        2.94        2.94
          9 |          1        2.94        5.88
         10 |          1        2.94        8.82
         11 |          1        2.94       11.76
         12 |          2        5.88       17.65
         15 |          1        2.94       20.59
         30 |          2        5.88       26.47
```

```
       60 |        2       5.88      32.35
       90 |        2       5.88      38.24
      180 |       21      61.76     100.00
-----------+-----------------------------------
    Total |       34     100.00
```

. ta astroke2 if astroke2 != 0

```
duration of |
 follow ups |
 of strokes |
     after |
   therapy |      Freq.     Percent        Cum.
-----------+-----------------------------------
       6 |          1        2.94        2.94
      180 |         33       97.06      100.00
-----------+-----------------------------------
    Total |         34     100.00
```

. label var change_strokes1 "change in number of strokes before and after therapy"

. ta change_strokes1

```
  change in |
  number of |
    strokes |
 before and |
     after |
   therapy |      Freq.     Percent        Cum.
-----------+-----------------------------------
       -2 |          2       5.88        5.88
       -1 |         26      76.47       82.35
        0 |          5      14.71       97.06
        1 |          1       2.94      100.00
-----------+-----------------------------------
    Total |         34     100.00
```

. ttest bstrokes1 == astroke1, unpaired

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
bstrok~1 |      34    .8823529    .0819189    .4776651    .7156877    1.049018
astroke1 |      34    .0294118    .0294118    .1714986   -.0304269    .0892504
```

```
---------+--------------------------------------------------------------------
combined |     68    .4558824    .0676776    .5580837    .3207973    .5909674
---------+--------------------------------------------------------------------
    diff |          .8529412    .0870388                .6791625     1.02672
------------------------------------------------------------------------------
    diff = mean(bstrokes1) - mean(astroke1)                       t =   9.7995
Ho: diff = 0                                     degrees of freedom =       66

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 1.0000         Pr(|T| > |t|) = 0.0000          Pr(T > t) = 0.0000
```

```
. ttest bstrokes1 == astroke1, unpaired unequal
```

```
Two-sample t test with unequal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
bstrok~1 |     34    .8823529    .0819189    .4776651    .7156877    1.049018
astroke1 |     34    .0294118    .0294118    .1714986   -.0304269    .0892504
---------+--------------------------------------------------------------------
combined |     68    .4558824    .0676776    .5580837    .3207973    .5909674
---------+--------------------------------------------------------------------
    diff |          .8529412    .0870388                .6772103    1.028672
------------------------------------------------------------------------------
    diff = mean(bstrokes1) - mean(astroke1)                       t =   9.7995
Ho: diff = 0                     Satterthwaite's degrees of freedom =  41.3688

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 1.0000         Pr(|T| > |t|) = 0.0000          Pr(T > t) = 0.0000
```

```
. label var change_strokes2 "change in duration of follow ups of strokes before and after therapy"
```

```
. ta change_strokes2

  change in |
duration of |
 follow ups |
 of strokes |
 before and |
     after |
   therapy |      Freq.     Percent        Cum.
------------+-----------------------------------
       -174 |          1        2.94        2.94
          0 |         20       58.82       61.76
         90 |          2        5.88       67.65
```

```
      120 |        2       5.88      73.53
      150 |        2       5.88      79.41
      165 |        1       2.94      82.35
      168 |        2       5.88      88.24
      169 |        1       2.94      91.18
      170 |        1       2.94      94.12
      171 |        1       2.94      97.06
      173 |        1       2.94     100.00
------------+-----------------------------------
    Total |       34     100.00
```

```
. ttest bstrokes2 == astroke2, unpaired
```

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
bstrok~2 |      34         124    12.79483    74.60604    97.96872    150.0313
astroke2 |      34    174.8824    5.117647    29.84075    164.4704    185.2943
---------+--------------------------------------------------------------------
combined |      68    149.4412    7.511752    61.94349    134.4477    164.4347
---------+--------------------------------------------------------------------
    diff |        -50.88235    13.78035               -78.3957    -23.369
------------------------------------------------------------------------------
    diff = mean(bstrokes2) - mean(astroke2)                   t =  -3.6924
Ho: diff = 0                     degrees of freedom =       66

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0002         Pr(|T| > |t|) = 0.0005          Pr(T > t) = 0.9998
```

```
. ttest bstrokes2 == astroke2, unpaired unequal
```

```
Two-sample t test with unequal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
bstrok~2 |      34         124    12.79483    74.60604    97.96872    150.0313
astroke2 |      34    174.8824    5.117647    29.84075    164.4704    185.2943
---------+--------------------------------------------------------------------
combined |      68    149.4412    7.511752    61.94349    134.4477    164.4347
---------+--------------------------------------------------------------------
    diff |        -50.88235    13.78035               -78.66759   -23.09711
------------------------------------------------------------------------------
    diff = mean(bstrokes2) - mean(astroke2)                   t =  -3.6924
Ho: diff = 0                     Satterthwaite's degrees of freedom =  43.2953
```

```
   Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0003         Pr(|T| > |t|) = 0.0006          Pr(T > t) = 0.9997
```

```
. ****** Objective three ***
. *white blood cells
. * white blood cells at baseline and 3 months
. sdtest wbc1 == wbc2
```

```
Variance ratio test
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    wbc1 |      34    22.63176    3.175743    18.51761    16.17067    29.09286
    wbc2 |      24    18.95792    3.003616    14.71465    12.74446    25.17137
---------+--------------------------------------------------------------------
combined |      58    21.11155    2.233065    17.00652    16.63992    25.58319
------------------------------------------------------------------------------
    ratio = sd(wbc1) / sd(wbc2)                 f =   1.5837
Ho: ratio = 1                     degrees of freedom =   33, 23

    Ha: ratio < 1               Ha: ratio != 1                 Ha: ratio > 1
  Pr(F < f) = 0.8734         2*Pr(F > f) = 0.2533          Pr(F > f) = 0.1266
```

```
. ttest wbc1 == wbc2, unpaired
```

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    wbc1 |      34    22.63176    3.175743    18.51761    16.17067    29.09286
    wbc2 |      24    18.95792    3.003616    14.71465    12.74446    25.17137
---------+--------------------------------------------------------------------
combined |      58    21.11155    2.233065    17.00652    16.63992    25.58319
---------+--------------------------------------------------------------------
    diff |            3.673848    4.547911               -5.436713    12.78441
------------------------------------------------------------------------------
    diff = mean(wbc1) - mean(wbc2)                t =   0.8078
Ho: diff = 0                     degrees of freedom =       56

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.7887         Pr(|T| > |t|) = 0.4226          Pr(T > t) = 0.2113
```

```
. *white blood cells at 3 months and six months
. sdtest wbc2 == wbc3
```

```
Variance ratio test
------------------------------------------------------------------------------
Variable |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    wbc2 |      24    18.95792    3.003616    14.71465    12.74446    25.17137
    wbc3 |      34     22.355     2.780313    16.21187    16.69841    28.01159
---------+--------------------------------------------------------------------
combined |      58    20.94931    2.044244     15.5685    16.85578    25.04284
------------------------------------------------------------------------------
    ratio = sd(wbc2) / sd(wbc3)                 f =   0.8238
Ho: ratio = 1                     degrees of freedom =   23, 33

    Ha: ratio < 1               Ha: ratio != 1                Ha: ratio > 1
  Pr(F < f) = 0.3178         2*Pr(F < f) = 0.6357         Pr(F > f) = 0.6822
```

```
. ttest wbc2 == wbc3, unpaired
```

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
Variable |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    wbc2 |      24    18.95792    3.003616    14.71465    12.74446    25.17137
    wbc3 |      34     22.355     2.780313    16.21187    16.69841    28.01159
---------+--------------------------------------------------------------------
combined |      58    20.94931    2.044244     15.5685    16.85578    25.04284
---------+--------------------------------------------------------------------
    diff |           -3.397084    4.162863               -11.7363    4.942133
------------------------------------------------------------------------------
    diff = mean(wbc2) - mean(wbc3)                t =  -0.8160
Ho: diff = 0                     degrees of freedom =       56

    Ha: diff < 0                Ha: diff != 0                 Ha: diff > 0
  Pr(T < t) = 0.2090         Pr(|T| > |t|) = 0.4179          Pr(T > t) = 0.7910
```

```
. *white blood cells at baseline and six months
. sdtest wbc1== wbc3
```

```
Variance ratio test
------------------------------------------------------------------------------
Variable |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    wbc1 |      34    22.63176    3.175743    18.51761    16.17067    29.09286
    wbc3 |      34     22.355     2.780313    16.21187    16.69841    28.01159
---------+--------------------------------------------------------------------
combined |      68    22.49338    2.094679    17.27317    18.31239    26.67438
```

```
------------------------------------------------------------------------------
   ratio = sd(wbc1) / sd(wbc3)                   f =   1.3047
Ho: ratio = 1                    degrees of freedom =   33, 33

   Ha: ratio < 1           Ha: ratio != 1           Ha: ratio > 1
 Pr(F < f) = 0.7755      2*Pr(F > f) = 0.4490      Pr(F > f) = 0.2245
```

```
. ttest wbc1 == wbc3, unpaired
```

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
   wbc1 |      34    22.63176    3.175743    18.51761    16.17067    29.09286
   wbc3 |      34     22.355    2.780313    16.21187    16.69841    28.01159
---------+--------------------------------------------------------------------
combined |      68    22.49338    2.094679    17.27317    18.31239    26.67438
---------+--------------------------------------------------------------------
   diff |           .2767644    4.220839           -8.150413    8.703942
------------------------------------------------------------------------------
   diff = mean(wbc1) - mean(wbc3)                t =   0.0656
Ho: diff = 0                     degrees of freedom =       66

   Ha: diff < 0            Ha: diff != 0            Ha: diff > 0
 Pr(T < t) = 0.5260      Pr(|T| > |t|) = 0.9479      Pr(T > t) = 0.4740
```

```
. * Platelets
. * Platelets at baseline and three months
. sdtest platelet1 ==  platelet2
```

```
Variance ratio test
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
platel~1 |      34    434.7941    27.89871    162.6761    378.0338    491.5545
platel~2 |      29    435.1379    29.81915     160.581    374.0562    496.2197
---------+--------------------------------------------------------------------
combined |      63    434.9524    20.20956    160.4084    394.5541    475.3507
------------------------------------------------------------------------------
   ratio = sd(platelet1) / sd(platelet2)         f =   1.0263
Ho: ratio = 1                    degrees of freedom =   33, 28

   Ha: ratio < 1           Ha: ratio != 1           Ha: ratio > 1
 Pr(F < f) = 0.5242      2*Pr(F > f) = 0.9516      Pr(F > f) = 0.4758
```

```
. ttest  platelet1 == platelet2, unpaired

Two-sample t test with equal variances
------------------------------------------------------------------------------
Variable |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
platel~1 |      34    434.7941    27.89871    162.6761    378.0338    491.5545
platel~2 |      29    435.1379    29.81915     160.581    374.0562    496.2197
---------+--------------------------------------------------------------------
combined |      63    434.9524    20.20956    160.4084    394.5541    475.3507
---------+--------------------------------------------------------------------
    diff |         -.3438134    40.87799            -82.08442    81.39679
------------------------------------------------------------------------------
    diff = mean(platelet1) - mean(platelet2)                  t =  -0.0084
Ho: diff = 0                     degrees of freedom =       61

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.4967         Pr(|T| > |t|) = 0.9933          Pr(T > t) = 0.5033

. * platelets at three and six months
. sdtest  platelet2 == platelet3

Variance ratio test
------------------------------------------------------------------------------
Variable |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
platel~2 |      29    435.1379    29.81915     160.581    374.0562    496.2197
platel~3 |      32    386.9844    32.08325    181.4903    321.5502    452.4186
---------+--------------------------------------------------------------------
combined |      61     409.877    22.04312    172.1623    365.7842    453.9699
------------------------------------------------------------------------------
    ratio = sd(platelet2) / sd(platelet3)                     f =   0.7829
Ho: ratio = 1                    degrees of freedom =   28, 31

    Ha: ratio < 1                Ha: ratio != 1                Ha: ratio > 1
  Pr(F < f) = 0.2579         2*Pr(F < f) = 0.5157          Pr(F > f) = 0.7421

. ttest  platelet2 == platelet3, unpaired

Two-sample t test with equal variances
------------------------------------------------------------------------------
Variable |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
platel~2 |      29    435.1379    29.81915     160.581    374.0562    496.2197
platel~3 |      32    386.9844    32.08325    181.4903    321.5502    452.4186
---------+--------------------------------------------------------------------
```

```
combined |     61    409.877    22.04312    172.1623    365.7842    453.9699
---------+--------------------------------------------------------------------
    diff |          48.15356    44.06848            -40.02726    136.3344
------------------------------------------------------------------------------
    diff = mean(platelet2) - mean(platelet3)                  t =   1.0927
Ho: diff = 0                     degrees of freedom =       59

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.8605         Pr(|T| > |t|) = 0.2790          Pr(T > t) = 0.1395
```

```
. * platelets at baseline and six months
. sdtest platelet1 ==  platelet3
```

```
Variance ratio test
------------------------------------------------------------------------------
Variable |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
platel~1 |      34    434.7941    27.89871    162.6761    378.0338    491.5545
platel~3 |      32    386.9844    32.08325    181.4903    321.5502    452.4186
---------+--------------------------------------------------------------------
combined |      66    411.6136    21.22186    172.4072    369.2306    453.9966
------------------------------------------------------------------------------
    ratio = sd(platelet1) / sd(platelet3)                     f =   0.8034
Ho: ratio = 1                    degrees of freedom =   33, 31

    Ha: ratio < 1                Ha: ratio != 1                Ha: ratio > 1
  Pr(F < f) = 0.2684         2*Pr(F < f) = 0.5367          Pr(F > f) = 0.7316
```

```
. ttest  platelet1 == platelet3, unpaired
```

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
Variable |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
platel~1 |      34    434.7941    27.89871    162.6761    378.0338    491.5545
platel~3 |      32    386.9844    32.08325    181.4903    321.5502    452.4186
---------+--------------------------------------------------------------------
combined |      66    411.6136    21.22186    172.4072    369.2306    453.9966
---------+--------------------------------------------------------------------
    diff |          47.80974    42.37433            -36.84272    132.4622
------------------------------------------------------------------------------
    diff = mean(platelet1) - mean(platelet3)                  t =   1.1283
Ho: diff = 0                     degrees of freedom =       64

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.8683         Pr(|T| > |t|) = 0.2634          Pr(T > t) = 0.1317
```

```
. sdtest ratio_before_therapy == ratio_after_therapy

Variance ratio test
------------------------------------------------------------------------------
Variable |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
r~efor~y |      26    .1696581    .0371392    .1893734    .0931685    .2461477
r~fter~y |      34     .019281    .0069708    .0406466    .0050988    .0334633
---------+--------------------------------------------------------------------
combined |      60    .0844444    .0190468    .1475361    .0463318    .1225571
------------------------------------------------------------------------------
    ratio = sd(ratio_before_t~y) / sd(ratio_after_th~y)          f =  21.7065
Ho: ratio = 1                                degrees of freedom =   25, 33

    Ha: ratio < 1            Ha: ratio != 1                 Ha: ratio > 1
  Pr(F < f) = 1.0000      2*Pr(F > f) = 0.0000          Pr(F > f) = 0.0000

. ttest ratio_before_therapy == ratio_after_therapy, unpaired unequal

Two-sample t test with unequal variances
------------------------------------------------------------------------------
Variable |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
r~efor~y |      26    .1696581    .0371392    .1893734    .0931685    .2461477
r~fter~y |      34     .019281    .0069708    .0406466    .0050988    .0334633
---------+--------------------------------------------------------------------
combined |      60    .0844444    .0190468    .1475361    .0463318    .1225571
---------+--------------------------------------------------------------------
    diff |           .1503771    .0377877                .0728115    .2279426
------------------------------------------------------------------------------
    diff = mean(ratio_before_t~y) - mean(ratio_after_th~y)        t =   3.9795
Ho: diff = 0                     Satterthwaite's degrees of freedom =  26.7673

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9998         Pr(|T| > |t|) = 0.0005          Pr(T > t) = 0.0002

. dis bvocs2_sum/ bvocs3_sum

. dis bvocs2_sum
```

```
. dis bvocs3_sum
```

```
. dis 457/3960
```

```
. dis avocs2_sum/ avocs3_sum
```

```
. dis avocs2_sum
```

```
. dis avocs3_sum
```

```
. dis 118/5777
```

```
. sdtest ratio_bvocs1_bvocs3 == ratio_avocs1_avocs3

Variance ratio test
------------------------------------------------------------------------------
Variable |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
r~bvocs3 |      26    .0160256    .0020747    .0105791    .0117526    .0202987
r~avocs3 |      34    .0029412    .0007504    .0043756    .0014145    .0044679
---------+--------------------------------------------------------------------
combined |      60    .0086111    .0012967    .0100441    .0060164    .0112058
------------------------------------------------------------------------------
    ratio = sd(ratio_bvocs1_b~3) / sd(ratio_avocs1_a~3)          f =   5.8456
Ho: ratio = 1                              degrees of freedom =   25, 33

    Ha: ratio < 1               Ha: ratio != 1                 Ha: ratio > 1
  Pr(F < f) = 1.0000         2*Pr(F > f) = 0.0000          Pr(F > f) = 0.0000
```

```
. ttest ratio_bvocs1_bvocs3 == ratio_avocs1_avocs3, unpaired unequal

Two-sample t test with unequal variances
------------------------------------------------------------------------------
Variable |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
r~bvocs3 |      26    .0160256    .0020747    .0105791    .0117526    .0202987
r~avocs3 |      34    .0029412    .0007504    .0043756    .0014145    .0044679
```

```
---------+--------------------------------------------------------------------
combined |     60    .0086111    .0012967    .0100441    .0060164    .0112058
---------+--------------------------------------------------------------------
    diff |          .0130845    .0022063                .008588     .017581
------------------------------------------------------------------------------
    diff = mean(ratio_bvocs1_b~3) - mean(ratio_avocs1_a~3)       t =   5.9306
Ho: diff = 0                     Satterthwaite's degrees of freedom =  31.5595

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 1.0000         Pr(|T| > |t|) = 0.0000          Pr(T > t) = 0.0000
```

```
. dis bvocs1_sum

. dis bvocs3_sum

. dis 52/138

. dis avocs1_sum

. dis avocs3_sum

. dis 19/204
```

```
. sdtest ratio_bstrokes1_bstrokes2 == ratio_astroke1_astroke2
```

```
Variance ratio test
------------------------------------------------------------------------------
Variable |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
ratio~s2 |      34    .0261565    .0066535    .0387964    .0126198    .0396932
ratio~e2 |      34    .0001634    .0001634    .0009528    -.000169    .0004958
---------+--------------------------------------------------------------------
combined |      68     .01316    .0036647    .0302196    .0058452    .0204747
------------------------------------------------------------------------------
    ratio = sd(ratio_bstrokes~2) / sd(ratio_astroke1~2)          f =  1.7e+03
Ho: ratio = 1                              degrees of freedom =   33, 33

    Ha: ratio < 1                Ha: ratio != 1                Ha: ratio > 1
  Pr(F < f) = 1.0000         2*Pr(F > f) = 0.0000          Pr(F > f) = 0.0000
```

. ttest ratio_bstrokes1_bstrokes2 == ratio_astroke1_astroke2, unpaired unequal

Two-sample t test with unequal variances

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
ratio~s2	34	.0261565	.0066535	.0387964	.0126198	.0396932
ratio~e2	34	.0001634	.0001634	.0009528	-.000169	.0004958
combined	68	.01316	.0036647	.0302196	.0058452	.0204747
diff		.0259931	.0066555		.012453	.0395333

diff = mean(ratio_bstrokes~2) - mean(ratio_astroke1~2) t = 3.9055

Ho: diff = 0 Satterthwaite's degrees of freedom = 33.0398

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0

Pr(T < t) = 0.9998 Pr(|T| > |t|) = 0.0004 Pr(T > t) = 0.0002

	change strokes1	change vocs1	change vocs2	change vocs3	lowdose1	lowdose2	lowdose3	accept~1	accep
change_str~1	1.0000								
change_vocs1	-0.0471	1.0000							
change_vocs2	-0.017	0.7999	1.0000						
change_vocs3	-0.0535	0.4875	0.3025	1.0000					
lowdose1	-0.1841	-0.1667	0.0564	0.0103	1.0000				
lowdose2	-0.2241	-0.0365	0.0767	0.1927	0.2762	1.0000			
lowdose3	-0.1388	0.219	0.2071	0.2023	0.2016	0.1693	1.0000		
acceptdose1	0.2527	0.1305	0.1197	0.2753	-0.6252	-0.2007	-0.2251	1.0000	
acceptdose2	0.0482	-0.1299	-0.0218	-0.2246	0.1251	-0.3423	-0.3839	-0.0517	1.0
acceptdose3	0.1213	-0.192	-0.1603	-0.1757	0.0831	-0.1498	-0.8085	-0.1089	

. corr change_strokes2 change_vocs1 change_vocs2 change_vocs3 lowdose1 lowdose2 lowdose3 acceptdose1 ac