HAEMATOLOGICAL MANIFESTATIONS IN CYANOTIC HEART DISEASES AT KENYATTA NATIONAL HOSPITAL AND MATER HOSPITAL, NAIROBI

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DECLARATION

I declare that this dissertation is my original work and has not been published elsewhere or presented for a degree program in any other university.

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I certify that this dissertation has been presented to the University of Nairobi with the approval of my supervisors.

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DEDICATION

This dissertation is dedicated to my wife Michelle and our lovely son Christopher
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<tr>
<td>APTT</td>
<td>Activated Partial Thromboplastin Time</td>
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<tr>
<td>CCF</td>
<td>Congestive Cardiac Failure</td>
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<td>CCHD</td>
<td>Congenital Cyanotic Heart Disease</td>
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<td>CHD</td>
<td>Congenital Heart Disease</td>
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<td>CPB</td>
<td>Cardiopulmonary Bypass</td>
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<td>DIC</td>
<td>Disseminated Intravascular Coagulation</td>
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<td>HB</td>
<td>Haemoglobin</td>
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<tr>
<td>HCT</td>
<td>Haematocrit</td>
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<tr>
<td>IQR</td>
<td>Inter quartile range</td>
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<tr>
<td>ID</td>
<td>Iron Deficiency</td>
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<tr>
<td>IDA</td>
<td>Iron Deficiency Anaemia</td>
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<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
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<tr>
<td>MCH</td>
<td>Mean Corpuscular Haemoglobin</td>
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<tr>
<td>MCV</td>
<td>Mean Corpuscular Volume</td>
</tr>
<tr>
<td>MH</td>
<td>Mater Hospital</td>
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<tr>
<td>PBF</td>
<td>Peripheral Blood Film</td>
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<tr>
<td>PT</td>
<td>Prothrombin Time</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for Social Sciences</td>
</tr>
<tr>
<td>TOF</td>
<td>Tetralogy of Fallot</td>
</tr>
<tr>
<td>ULN</td>
<td>Upper Limit of Normal</td>
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<tr>
<td>UON</td>
<td>University of Nairobi</td>
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ABSTRACT

Background: Cyanotic heart disease results in inadequate tissue oxygenation. This in turn triggers increased erythropoietin release from the kidneys in an effort to increase the red cell mass so as to increase oxygen delivery to the tissues. Polycythemia results and often iron deficiency develops as the iron stores are depleted as a result of the sustained erythropoiesis. Polycythemia and iron deficiency both lead to increased blood viscosity and a tendency to bleeding diathesis. The objective of this study was to examine the haematological profile of children with cyanotic heart disease in Kenyatta National Hospital and Mater Hospital, and to document the prevalence of abnormal coagulation and iron deficiency in these children.

Method: A cross-sectional descriptive study was carried out at Kenyatta National Hospital and Mater Hospital from August to December 2007. A total of 112 children meeting the eligibility criteria were recruited into the study. Haemoglobin level, Mean corpuscular volume, Mean corpuscular haemoglobin, Peripheral blood film report, serum ferritin levels, Prothrombin time, Activated partial thromboplastin time, Platelet counts and D-dimer levels were determined and recorded.

Results: The prevalence of iron deficiency was found to be 16.9% (95% CI 9.8-24.1%). Abnormalities in the coagulation tests were as follows: Prolonged APTT-
32.1% (95% CI 23.5-40.7%) of the study population, prolonged PT-3.6% (95% CI 1.4-8.8%), low platelets-7.1% (95% CI 3-11%), and raised D-dimer – 60% (95% CI 50.9-69.1%).

The sensitivity of Low MCV in detecting iron deficiency was 58.8% with specificity of 51.2%. For Low MCH the sensitivity was 52.9% with a specificity of 50.6%. Findings of microcytic hypochromic cells on peripheral blood film gave a sensitivity of 50% and specificity of 73.4%.

Conclusion: There is a high prevalence of iron deficiency among patients with congenital heart disease with cyanosis in Kenyatta National Hospital and Mater Hospital. Mild to moderate coagulation abnormalities were also noted.

Recommendation: Screening for iron deficiency using biochemical methods is recommended for patients with congenital cyanotic heart disease, and routine screening for coagulopathy is recommended prior invasive procedures.
INTRODUCTION AND LITERATURE REVIEW

In the United Kingdom there are about 4,600 babies born with congenital heart disease each year – one in every 145 births (1). At least three-quarters of babies with congenital heart disease are predicted to survive to adulthood as a result of improved medical and surgical care. Currently in there are approximately 150,000 people aged 16 and over living with congenital heart disease. Of these around 11,500 have the more complex forms of the disease, which require life-long care. It is estimated that the number of adults with congenital heart disease in the UK will grow by 25%, and the number with complex conditions by at least 50%, between 2000 and 2010 (1). Reports from India put the incidence of congenital heart disease at 4-10 per 1000 live births (2). In the United States the incidence of congenital heart disease is 8:1000 of all live births. This results in approximately 32,000 babies born each year with congenital heart disease (3). Palliative and corrective surgery is done commonly at a younger age and the survival of such patients has improved. Currently there are over 640,000 children with congenital heart disease surviving in the United States. In contrast, our patients present late, and even those diagnosed early will have delays in surgical correction mainly due to lack of finances or skilled personnel, or both. As a result, we have a bigger burden of more severely affected patients growing up with congenital heart defects.

Uncorrected congenital cyanotic heart lesions (and some acyanotic lesions which later have reversal of blood flow from right to left with the development of Eisenmenger’s complex) keep the body in a state of constant hypoxia. This hypoxia triggers a physiological increase in erythropoietin release leading to stimulation of the bone marrow
to produce more red cells in an effort to increase the body’s oxygen carrying capacity, and thus improve oxygen delivery to the tissues. With persisting right to left shunt, the arterial oxygen tensions remain perpetually low and so the production of more and more red cells goes unabated leading to polycythemia. This seemingly noble physiological response is the first step to the haematological derangements encountered in cyanotic cardiac patients.

With time, iron stores are depleted due to the sustained demand for haemoglobin formation. In these patients, the total haemoglobin is normal, high or slightly reduced compared to aged-matched normal individuals without cyanosis. However, the MCV, MCH and serum ferritin are usually comparatively lower than their peers as shown by Cemile B. et Al (4) - a phenomenon known as relative anaemia. Generally, the risk factors leading to iron deficiency in the general population also compound the risk for developing iron deficiency in this vulnerable group. These risk factors include

1. Lack of exclusive breastfeeding in the first six months as these patients usually tire easily on breastfeeding, making the mothers wean them early.
2. Use of cow’s milk-this is the next available option for most mothers in our setup who cannot breastfeed. Excessive use of cow’s milk before 1 year of age is associated with occult gastrointestinal bleeds predisposing to iron deficiency (5).
3. Dietary deficiency—The mainly plant based diets commonly consumed in the developing world contains non-heme iron which has a low bioavailability compared to animal based diet.

4. Consumption of diets high in inhibitors of iron absorption e.g. phytates and phosphates in cereals predisposes to iron deficiency.

Studies have been carried out in different parts of the world to examine the prevalence of iron deficiency in this group of patients. Drossos C. et Al from Italy in 1981 examining a total of 38 cyanotic heart disease children, found an iron deficiency prevalence of 37.5% and 12.5% in the 6 months to 5 year olds and those 6-12 years old respectively (6). Kaemmerer H et al in Germany found a prevalence of 37% among the 52 adults studied (7). In a study carried out by Gaiha et al in India, 6 out of the 33 cyanotic patients studied were found to be having iron deficiency, giving a prevalence of 18.2% (8).

Iron deficiency plays an important role in the propagation of hyperviscosity in these already polycythemic patients. Whole blood viscosity, is not only a function of haematocrit, but also of additional variables including deformability of erythrocytes, aggregation and dispersion of cellular elements, flow velocity (shear rate), temperature, vessel bore, endothelial integrity, and plasma viscosity of which fibrinogen concentration is an important determinant (9, 10, 11). Although blood viscosity rises remarkably as haematocrit levels increase through the range of 0.55 to 0.75, what may prove to be a relevant physiologic relationship is the effect of haematocrit level on vascular resistance rather than on viscosity. The effects of iron deficiency on viscosity have been the subject
Irrespective of cause, iron deficiency leads to a significant increase in whole blood viscosity in erythrocytotic patients, and the viscous effect rises with decreasing erythrocyte mean corpuscular haemoglobin (10, 11, 12, 13).

Patients with iron deficiency will present with symptoms of hyperviscosity e.g. symptoms of cyanotic spells, anorexia, exercise intolerance, poor appetite, poor weight gain and irritability. They are also at increased risk for cerebrovascular accidents (14). Treatment of iron deficiency has been shown to reverse all these symptoms. Abraham et al showed in their study that supplementing iron to CCHD patients with haematologic parameters consistent with iron deficiency significantly improved the patients' symptoms of hyperviscosity (15). Gaiha M et al showed that hyperviscosity symptoms occurred at a lower PCV level (0.52-0.58) among cyanotic patients with iron deficiency as compared to those who were iron sufficient where symptoms occurred at the mean PCV of 0.68. Among the iron deficient group, hyper cyanotic symptoms were relieved with iron supplements, and this symptomatic relief was accompanied by an average haemoglobin rise of 2.1g/dl (8). Similar effects were demonstrated by Perfloff J. K. et Al. (16).

Besides the above, it is known that iron deficiency is the commonest micronutrient deficiency in the world, with the developing world being hardest hit (17). Iron deficiency has been shown in various studies to have a detrimental effect in the growth of children. Deficiency has been associated with low IQ scores and poor psychomotor development (18, 19, 20). It has even been shown to affect the child's height (21).
ASSESSMENT OF IRON DEFICIENCY

A characteristic sequence of changes in the clinically useful indications of iron status occurs as body iron decreases from the iron-replete normal to the levels found in iron deficiency anemia. Initially, as a result of any of the causes of iron deficiency, iron requirements exceed the available supply of iron. Iron is mobilized from body stores, and iron absorption is increased. If the amounts of iron available from body reserves and absorption are inadequate, depletion of storage iron follows. At this stage, bone marrow examination shows absent, or nearly absent, hemosiderin iron. The serum ferritin falls, while the total iron-binding capacity rises. Exhaustion of iron reserves then results in an inadequate supply of iron to the developing erythroid cell, and iron-deficient erythropoiesis commences. Plasma transferrin receptor concentrations increase as the total body mass of tissue receptor expands. The plasma ferritin decreases to less than 12 μg/L reflecting the absence of storage iron, and the total iron-binding capacity continues to rise. The plasma iron declines, and in combination with the increase in total iron-binding capacity, the transferrin saturation falls to less than 16%. As a result, most iron is then derived from mono- rather than diferric transferrin, and most ferric transferrin-transferrin receptor complexes carry only one or two atoms of iron rather than as many as four with each intracellular delivery cycle. Marrow examination shows, in addition to the absence of hemosiderin iron, a decrease in the proportion of sideroblasts, because too little iron is available to support siderotic granule formation. The erythrocyte zinc protoporphyrin progressively
increases with reduction of the amount of iron available for heme formation. Measurement of reticulocyte cellular indices, such as the reticulocyte hemoglobin content, seems to provide an early means of detecting iron-restricted erythropoiesis both in uncomplicated iron deficiency and in the "functional" lack of iron in patients receiving recombinant erythropoietin.

The use of serum ferritin for the assessment of iron stores has become well established (24). During the first months of life, mean serum ferritin concentrations change considerably, reflecting changes in storage iron concentration. Concentrations are lower in children (less than 15 years) than in adults, and this varies with age (see appendix 1). Iron overload causes high concentrations of serum ferritin, but these may also be found in patients in liver disease, infection, inflammation or malignant disease. Careful consideration of the clinical evidence is required before concluding that a high serum ferritin concentration is primarily the result of iron overload and not a result of tissue damage or enhanced synthesis of ferritin. A normal ferritin concentration provides good evidence against iron overload but does not exclude genetic haemochromatosis. This is because haemochromatosis is a late-onset condition and iron stores may remain within normal range for many years.

In patients with acute or chronic disease, interpretation of serum ferritin is less straightforward and patients may have serum ferritin concentrations of up to 100μg/l despite absence of stainable iron in the bone marrow. Ferritin synthesis is
enhanced by interleukin 1 – the primary mediator of the acute phase response. In patients with chronic disease, the following approach should be adopted: low serum ferritin concentrations indicate absent iron stores, values within the normal range indicate either low or normal levels, and high values indicate either normal or high levels.

COAGULATION

Several case reports have been published documenting increased bleeding tendencies among patients with cyanotic heart disease. Dore et al, reporting on 307 consecutive cardiac surgeries from 1991-1994 reported a significant increase in bleeding among the cyanotic heart disease patients compared to the acyanotic group (25).

Bleeding in cyanotic heart disease is thought to be multifactorial (figure 2). It may be caused by a decrease in the coagulation factors synthesized in the liver, i.e., vitamin-K-dependent factors II, VII, IX and X. This can be explained by deficient synthesis resulting from hypoxic damage to the liver and sluggishness of microcirculation caused by high blood viscosity (26). In patients with CCHD, platelets have both qualitative and quantitative abnormalities. Platelet count and haematocrit are inversely related, that is, an increased haematocrit is associated with thrombocytopenia (26,27). Deficiency of the high molecular weight forms of the Von Willebrand factor in plasma, has also been reported (27, 28, 29). Increased fibrinolysis has been observed in some patients. Chan and others (30) observed an increase in thrombin generation and consumptive
coagulopathy as indicated by high levels of thrombin–antithrombin complexes in cyanotic and acyanotic heart disease patients.
Figure 1: Schematic representation of the pathophysiology of coagulation abnormalities in CCHD
Haemostatic tests in a large series (235 consecutive patients with congenital heart disease) were performed by Colon-Otero et al in 1987 (31). Abnormal results were obtained in 45 (19%) patients; 29 patients (12%) had abnormal results of only 1 test, and 16 patients (7%) had abnormalities in 2 or more tests. The abnormal tests included prolongation of prothrombin time (PT), partial thromboplastin time (PTT), activated partial thromboplastin time (APTT), thrombin time, thrombocytopenia, bleeding time, and euglobulin lysis time. Among these, prolonged values for the PT or the PTT or APTT were seen most frequently.

The authors also found that patients with poor cardiac performance, high haematocrit values, and clinically evident cyanosis had the highest incidence of abnormalities. The PT and APTT were significantly longer and the platelet counts were significantly lower in patients with haematocrit values >60%. A positive correlation was seen between arterial oxygen saturation and platelet counts. This study provided the evidence that the abnormalities in platelet counts and PT are a consequence of hypoxia. It also suggested that DIC may be a contributing factor to thrombocytopenia, at least in some patients. Decreased levels of factors VII or IX in association with abnormalities of PT, PTT, or APTT provide evidence that impaired vitamin K–dependent carboxylation is an important causative factor.

Goel et al in a recent study confirmed that haemostatic abnormalities are commonly present in CCHD (26). The authors studied platelet count, bleeding time, PT, APTT, assay of fibrinogen, D-dimer, factors VII and VIII, and antithrombin III. They found that
coexisting abnormalities of >1 test were seen in a significantly larger number of cyanotic children as compared to the acyanotic (16 of 25 vs 5 of 25). An elevated level of D-dimers was observed in a large proportion of cyanotic children, indicating a state of DIC. Derangement of other coagulation tests or thrombocytopenia was present in many of these patients. The authors postulate that chronic subclinical compensated DIC, reduced synthesis of coagulation factors, or deranged platelet aggregation accounts for the haemostatic abnormalities in CCHD. In another recent study, pre-existing platelet activation or the activation of the coagulation or fibrinolytic system was studied in patients with congenital heart disease (32). The authors performed platelet activation tests and measured thrombin-antithrombin complex, prothrombin fragment F1.2, tissue plasminogen activator, plasminogen activator inhibitor type 1, plasminogen, and fibrin D-dimers. It was shown that raised levels of fibrin D-dimers and plasminogen activator inhibitor type 1 were present in patients with CCHD, signifying a state of accelerated fibrinolysis.

Platelet counts and haematocrit tend to be inversely related. Mildly reduced platelet counts of 100,000/mm³ to 150,000/mm³ have been commonly reported, and occasionally, severe thrombocytopenia with platelet counts of 50,000/mm³ has been described in association with polycythemia (10). Ekert and Gilchrist (33) reported that of 17 cyanotic heart disease children studied, thrombocytopenia was noted in 13 (platelet counts of <150,000/mm³). No correlation was noted however, between platelet count and the bleeding time, suggesting that abnormal platelet function may be present in these patients. Several authors have reported
that platelet function abnormalities are present in children with CCHD and that these are improved by corrective surgery for the congenital heart defect (34).

Lill M et al in a recent publication (2006) tried to explore the pathogenesis of thrombocytopenia in cyanotic heart disease patients. They evaluated 105 adult cyanotic patients. They excluded patients with impaired platelet activation and those with DIC, thus: Normal thrombopoietin levels implied normal megakaryocyte mass. Normal prothrombin time, activated partial thromboplastin time, and D-dimers excluded disseminated intravascular coagulation. Normal platelet factor 4 and β thromboglobulin indicated absent or minimal platelet activation. Twenty-five percent (25%) of the patients with CCHD were thrombocytopenic because platelet production was decreased despite normal megakaryocyte mass. They hypothesized that right-to-left shunts deliver whole megakaryocytes into the system arterial circulation, bypassing the lungs where megakaryocytic cytoplasm is fragmented into platelets, thus reducing platelet production. In conclusion, platelet counts in CCHD appear to represent a continuum beginning with low normal counts and ending with thrombocytopenia.
OTHER COMPLICATIONS OF CONGENITAL CYANOTIC HEART DISEASE

The polycythemia resulting from compensatory erythrocytosis stimulated by low arterial oxygen tension, is the major contributor to the morbidity and complications encountered in patients with CCHD. Secondary gout could result from elevated uric acid observed in the CCHD resulting from continuous breakdown of large numbers of red blood cells.

Cerebro-Vascular Accidents:
Thrombotic episodes are common in children less than 2 year old. Polycythemia leads to increased blood viscosity and slugging of blood predisposing to thrombotic episodes. The risk is increased significantly in children with iron deficiency anaemia, through reduced red cell deformability of the iron deficient rbc’s which increases clogging of the microvasculature. Hemorrhagic cardiovascular accidents are also encountered as a result of reduced clotting factors in this group of patients.

Cerebral abscesses:
Commonly seen in children more than 2 years of age. Right to left shunting seen in many CCHD compromise the filtration of bacteria from the venous blood that usually happens in the lungs, resulting in arterial blood being bacteremic, hence lodging of bacteria in the brain with subsequent formation of brain abscesses.
**Bacterial endocarditis:**

This frequent complication among adults with cyanotic heart disease, is infrequent below the age of 2 years. Multiple pulmonary arterial thrombi may occur when there is a prolonged decrease in pulmonary arterial flow. Such thrombi occlude precapillary arterioles. As their number increases, they proportionately diminish the cross-sectional area of the pulmonary arterial system, leading to increase in pulmonary arteriolar resistance and a corresponding increase in right to left shunting of blood.

**Myocardial infarction/fibrosis:**

This results from repeated microthrombi occluding the cardiovascular circulation with resultant infactions.

**Hemoptysis:**

Results less commonly from severe pulmonary hypertensive disease, associated with plexiform, angiomatoid, or panarteritic lesions of the lung. In the absence of pulmonary hypertension, hemoptysis may be secondary to rapture of extensive bronchial collateral vessels, as a result of infection, trauma and thrombosis.

**Joint pains and polyarthropathy:**

This results from clogging of the microcirculature in the bones by red cells leading to impaired circulation and infarction.
STUDY JUSTIFICATION

This study aims at determining the prevalence of iron deficiency and coagulation derangements among our local population of cyanotic heart disease patients. Results of this study will shed light on the extent of these problems in our set up and perhaps form a basis for improved management of these patients.
RESEARCH QUESTIONS AND OBJECTIVES

RESEARCH QUESTION

What are the haematological manifestations of cyanotic heart disease in children at Kenyatta National Hospital and Mater Hospital?

OBJECTIVES

BROAD OBJECTIVE

To determine the haematological abnormalities seen in children with cyanotic heart disease in Kenyatta National Hospital and Mater Hospital

A. PRIMARY AIMS

a. To determine the prevalence of Iron deficiency among the paediatric cyanotic heart disease patients in Kenyatta National Hospital and Mater Hospital

b. To determine the prevalence of abnormal coagulation among the paediatric cyanotic heart disease patients seen in Kenyatta National Hospital and Mater Hospital

B. SECONDARY AIM

a. To evaluate the sensitivity and specificity of red cell indices and peripheral blood film appearance in diagnosing iron deficiency in cyanotic heart disease patients seen in Kenyatta National Hospital and Mater Hospital
STUDY POPULATION

The study population included children less than 18 years of age, with cyanotic heart disease confirmed on ECHO presenting at the paediatric cardiac clinic of Kenyatta and Mater Hospitals or admitted in the wards at Kenyatta National Hospital. These were patients who had not undergone surgical correction. Both Mater Hospital and Kenyatta National Hospital have comprehensive cardiac programs. Most of the patients seen in these clinics are referrals from within and outside Nairobi. The patients seen at the Mater Hospital’s cardiac clinic are derived from the KNH out patient pool and are referred there for the purpose of sponsored surgery. The Mater cardiac program is sponsored by the Mater Heart Trust Fund, Terres de Hommes, the Rotary and Lions club of Kenya.

INCLUSION CRITERIA:

1. Patients who were below 18 years of age
2. Cyanotic heart lesion confirmed on echocardiography, with the ECHO report available to the investigator for perusal.
3. Guardian had to have given written consent.

EXCLUSION CRITERIA

1. When consent was not given the patient was not included
2. Patients with cyanosis secondary to pulmonary disease, for example those with Pneumocistis Jiroveci Pneumonia.
3. Patients with congenital cyanotic heart disease in the setting of other complex congenital malformations e.g. spina bifida, polycystic kidney disease, bladder extrophy, e.t.c.

4. Those on warfarin, aspirin or hematinsics

**SAMPLE SIZE**

The sample size was determined as follows

\[
N = \frac{Z^2(p \cdot q)}{d^2}
\]

Where

- \( N = \) sample size
- \( Z = \) Z score corresponding 95% of confidence level = 1.96
- \( p = \) presumed prevalence iron deficiency among cyanotic heart disease patients (6)
- \( q = 1 - p \) (1 - 0.375 = 0.625)
- \( d = 10\% \) sampling error =0.1

\[
1.96^2(0.375 \times 0.625) = 90
\]

\[
(0.1)^2
\]

**SAMPLING**

The investigator visited the two hospitals between 8am and 3pm during the study duration. Patients who meet the eligibility criteria were recruited consecutively as they presented until the desired sample size was attained.
STUDY AREA

This study was carried out at Kenyatta National Hospital and Mater Hospital. In Mater hospital, patients were recruited from the paediatric cardiac clinic which runs every Monday. In Kenyatta National Hospital, patients were recruited from the paediatric cardiac clinic, the cardiothoracic surgery ward, paediatric wards and medical wards. Patients who met the eligibility criteria and gave written consent were recruited to the study as they presented till the desired sample size was attained.

STUDY DURATION

This study was carried out over 5 months, that is, from August 2007 to December 2007.

STUDY DESIGN

This was a cross-sectional descriptive study.

PROCEDURES

CLINICAL PROCEDURES

Once a patient was identified, the investigator explained to the guardian the purpose of the study, what it entailed and the potential risks and benefits expected before proceeding to seek consent. Those who gave consent were recruited.
Height and length were measured in centimeters using a stadiometer. For those who could not stand, length was taken with the patient lying supine on the stadiometer with knees extended. Weight was taken using a step-on weighing scale, which was calibrated daily. For infants, an infant weighing scale was used. Under arm temperature was taken using a mercury thermometer placed under the arm for 5 minutes. All these were recorded onto the questionnaire. The same machines were used throughout the study.

Date of birth, sex and residence was inquired and recorded on the questionnaire. A short medical history was obtained from the guardian to assess the patient’s state of health and any current concerns particularly those suggesting a current infectious illness or an inflammatory process. A complete physical examination was then carried out to confirm or rule out these concerns. The investigator then noted those he diagnosed as having an infection or an obvious inflammatory process on their questionnaire. Further history was taken to ascertain whether the patient was using warfarin, aspirin or any haematinics. This was also noted on the questionnaire. Use of warfarin, aspirin or haematinics was an exclusion criterion. The cardiac lesion diagnosed and noted on the patient’s echocardiogram was recorded on the questionnaire.

**SPECIMEN COLLECTION, TRANSPORT AND STORAGE**

After administration of the questionnaire, a finger prick sample was collected into a microcapillary tube following aseptic techniques. The tube was then span in a
microcentrifuge for 7 minutes and the PCV determined from the microcapillary tube on the Hawksley Micro-haematocrit reader. This was done by the bed side.

For patients with haematocrit of 55% and below, sodium citrate and blood were mixed at a ratio of 1:9. For those with haematocrit values of above 55%, a correction factor for the decreased serum was employed, in a bid to have an appropriate ratio between the blood and the anticoagulant. This was done following a standard formula as described on appendix 2.

**Drawing of blood**

Blood was drawn from a venous access after cleaning of the puncture site with methylated spirit, without application of a pressure cuff where possible. If need arose, pressure cuff was used but effort was made to limit its application to not more than a minute. About 5 to 6ml of venous blood was drawn using a 10cc syringe and an appropriately sized needle. 1ml was put into a plain bottle and another 1ml into the EDTA bottle. The rest was collected in a bottle with sodium citrate at appropriate ratios between blood and the anticoagulant, and this was used for the coagulation studies. The samples were stored in a cooler with ice packs and transported to the University of Nairobi Haematology laboratory for analysis.

At the laboratory the serum was separated from the sample in the plain bottle and stored at -60 degrees centigrade awaiting batch analysis for the serum ferritin levels.

The coagulation sample was span, plasma separated, and this was used for D-dimer levels, the APTT and PT test as outlined below.
LABORATORY METHODS

Complete Blood Count

The complete blood count was done at the UON Haematology laboratory using the electronic cell counter – Cell-Dyn 1300, calibrated using commercially availed material and controlled through the tri-level controls. This was an automated process. This laboratory has internal and external quality controls.

Ferritin Levels

These were done through a semi automated process at the UON clinical chemistry laboratory using Humalyzer 2000.

Test principle:

Ferritin in a sample or standard cause agglutination of latex particles coated with anti-ferritin antibodies. The agglutination is proportional to the ferritin concentration in sample or standard and can be measured by turbidimetry.

A known quality control sample was run with the tests to confirm validity of the tests.

APTT test

This was done in manually using the Pathromtin SL kit. The test was done in duplicate and the average of the two taken.
Test principle:
The incubation of plasma with the optimal quantity of phospholipids and a surface activator leads to activation of factors of the intrinsic coagulation system. The addition of calcium ions triggers the coagulation process; the time to formation of a fibrin clot is measured.

Procedure:
Citrated plasma 100μl plus an equal amount (100μl) of Pathromtin SL reagent, both pre-warmed to +37°C were pipetted into a test tube and incubated for 2 minutes at +37°C. Thereafter 100μl of calcium chloride solution pre-warmed to +37°C, was added and at this time the timer was started, and the time to clot formation recorded.
A known quality control sample was run together with the test to ascertain validity of the tests.

PT test
This was done manually using the Thromborel S kit. The test was done in duplicate and the average of the two taken.

Test principle:
The coagulation process is triggered by incubation of plasma with the optimal amount of thromboplastin and calcium. The time to clot formation is then measured.
Procedure:
Citrated plasma 100μl was pipetted into a test tube pre-warmed to 37°C and incubated for 1 minute. Pre-warmed 200μl of Thromborel S was added, at the same time the stop watch started to measure the time to clot formation. A known quality control sample was run together with the test to ascertain validity of the tests.

D-dimer levels
These were determined using the d-dimer kit commercially available from International Laboratories®, the NycoCard D-Dimer Single Test. It was an automated process and the results were read from the manufacturer-supplied machine.

Test principle:
This test is based upon an immunometric flow-through principle. The plasma sample is applied to the test well of the device. When the sample has soaked into the device, D-dimer molecules are trapped on a membrane carrying D-dimer specific monoclonal antibodies conjugated with ultra-small gold particles. The D-dimer on the membrane will bind the gold-antibody conjugate in a sandwich-type reaction. The excess conjugate is removed from the membrane by the washing solution. In the absence of D-dimer levels above 0.1mg/l in the sample, the membrane appears reddish with colour intensity proportional to the D-dimer concentration. The colour intensity is evaluated using NycoCard reader II.
Procedure:
Fifty microlitres (50μL) of washing solution was placed into the test device and allowed to soak. Fifty microlitres of the sample was added, then 50μL of conjugate was added and allowed to soak. Fifty microlitres of washing solution is finally added, and then the values read from the machine in mg/l. A control sample with known values was run with every test batch to ascertain validity of the results obtained.

QUALITY ASSURANCE

1. All equipment and machines were calibrated following standard procedure as recommended by the manufacturers
2. Quality reagents that were not expired were used.
3. Standard operating procedures were followed in carrying out the laboratory investigations.
4. Coagulation tests were carried out in duplicate
5. A qualified laboratory technologist was contracted to carry out the laboratory investigations.
6. Qualified haematologist was available to evaluate results
STUDY DEFINITIONS

1. A case of cyanotic heart disease: Any child below 18 years of age who has been diagnosed to be having a cyanotic heart disease as evidenced by availability of an echocardiogram report from a cardiologist.

2. Iron deficiency: Depletion of the body's iron stores as evidenced by low ferritin levels that are below the lower limit of the age specific reference range. In this study, ferritin was the gold standard for measuring iron stores.

3. Grading of degree of low platelets and degree of prolongation of the APTT and PT was done as outlined in the Division of AIDS table for grading paediatric and adult adverse events (see appendix 1).

4. Low MCV and low MCH refer to values of MCV and MCH that are below the lower limit of the age specific reference ranges as given in appendix 1.

5. The lower bound of the reference values for ferritin, MCV, MCH was -2SD of the mean of the reference population (33,34).
SCHEME OF THE STUDY

Figure 2: Flow diagram showing how the study was conducted
DATA ANALYSIS

The study population was described in terms of their demographic and clinical characteristics using means, medians and proportions. Prevalence of low ferritin, and abnormal coagulopathy was calculated as proportion of children with values below -2SD of the reference mean. Children with iron deficiency were compared to those without to determine associated factors. Chi-square tests and t-test were used to determine factors with significant associations. Pearson correlations and multiple regression analysis was carried out to characterize the relationship between ferritin, red blood cell indices (MCV, MCH, HCT), and coagulation factors (APTT, PT, platelets and D-dimers).

ETHICAL CONSIDERATIONS

1. Informed consent was sought before recruitment (see appendix 4)
2. The information acquired was treated with utmost confidentiality handled by using study codes to identify participants.
3. Permission was sought from the two hospitals’ ethical committees before the study was carried out.
4. The participants had the investigations done at no extra cost to them.
5. The results of the investigations were available in the patients’ files to assist in their management.
RESULTS

The study was carried out between the months of August to December 2007 at Mater hospital and Kenyatta National Hospital. We recruited 112 children with cyanotic heart disease who met the inclusion criteria.

DEMOGRAPHIC FACTORS

The recruited patients' age ranged from 1 month to 17 years, with a median age of 4 years and five months. The mean age was 5 years 10 months. Males were 59 while 53 were female, giving a male to female ratio of 1:1. The commonest heart lesion encountered was Tetralogy of Fallot (54%).

Fifty nine out of 112 patients (52.6%) were below 5 years of age, while 53 out of 112 (47.3%) were older than 5 years. The nutritional status was analyzed based on the CDC 2000 guidelines. Twenty (17.9%) were classified as moderately stunted, while 25 (22.3%) were severely stunted. Moderate wasting was seen in 17 (15.2%) of the study population while 23 (20.5%) were severely wasted as shown on table 1 below.
Table 1: Summary Of The Characteristics Of The Study Population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Category</th>
<th>Number (N=112)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt; 1 year</td>
<td>20</td>
<td>17.8%</td>
</tr>
<tr>
<td></td>
<td>&gt;1 year – 5 years</td>
<td>39</td>
<td>34.8%</td>
</tr>
<tr>
<td></td>
<td>&gt;5 years – 12 years</td>
<td>35</td>
<td>31.3%</td>
</tr>
<tr>
<td></td>
<td>&gt; 12 years</td>
<td>18</td>
<td>16.1%</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>59</td>
<td>52%</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>53</td>
<td>48%</td>
</tr>
<tr>
<td>Nutritional status</td>
<td>Normal &amp; mild</td>
<td>67</td>
<td>59.8%</td>
</tr>
<tr>
<td></td>
<td>stunting</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderately stunted</td>
<td>20</td>
<td>17.9%</td>
</tr>
<tr>
<td></td>
<td>Severely stunted</td>
<td>25</td>
<td>22.3%</td>
</tr>
<tr>
<td></td>
<td>Normal &amp; mild</td>
<td>72</td>
<td>64.3%</td>
</tr>
<tr>
<td></td>
<td>wasting</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate wasting</td>
<td>17</td>
<td>15.2%</td>
</tr>
<tr>
<td></td>
<td>Severe wasting</td>
<td>23</td>
<td>20.5%</td>
</tr>
</tbody>
</table>
Heart lesions were divided into those with predominantly left to right shunt and those with predominantly right to left shunt. Complex lesions seen in this study included dextrocardia and a host of other complicated cardiac anatomy lesions. The most frequently encountered heart lesion was Tetralogy of Fallot followed by tricuspid atresia, as shown on table 2 below.

**Table 2: Frequency of encountered heart lesions**

<table>
<thead>
<tr>
<th>LESION</th>
<th>NUMBER</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PREDOMINANTLY RIGHT TO LEFT SHUNT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TETRALOGY OF FALLOT</td>
<td>61</td>
<td>54</td>
</tr>
<tr>
<td>TRICUSPID ATRESIA</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>PULMONARY ATRESIA WITH VSD</td>
<td>6</td>
<td>5.3</td>
</tr>
<tr>
<td>DOUBLE OUTLET RIGHT VENTRICLE</td>
<td>4</td>
<td>3.5</td>
</tr>
<tr>
<td>CRITICAL PULMONARY STENOSIS</td>
<td>3</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>PREDOMINANTLY LEFT TO RIGHT SHUNT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRUNCUS ARTERIOSUS</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>TRANSPOSITION OF GREAT ARTERIES</td>
<td>5</td>
<td>4.4</td>
</tr>
<tr>
<td>EISENMENGERS AND OTHER COMPLEX</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td><strong>HEART LESIONS</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Eighteen out of 106 patients had ferritin levels below the lower limit of their age specific reference ranges giving an iron deficiency prevalence of 16.9% (CI 9.8-24.1%). Half the study population had MCV and MCH values below the age specific reference ranges (50.5% for low MCV and 50% for low MCH). Findings of microcytic hypochromic cells were seen in 33 out of 106 patients giving a prevalence of 31.1%. APTT was prolonged in 36 out of 103 patients giving a prevalence of 34.9% (CI 23.5-40.7%). These derangements were however mostly mild to moderate (grade 1 and 2) in severity, that is, 28 of 103 (27.1%). Grade 3 and 4 derangement was seen in only 8 out of 103 (7.7%). Prolongation of PT was seen in 4 out of 103 patients, giving a prevalence of 3.6% (CI 1.4-8.8%). Seventeen patients out of 103 had platelet levels below the lower limit of their age specific reference ranges, giving a prevalence of 16.5% (CI 9.2-23.4%). On analyzing the severity of this derangement using the DAIDS table (see appendix 1), we note that only 8 out of 103 (7.1%) had a significant derangement. Out of these, 6 (5.8%) had mild to moderate derangement (grade 1 and 2), while 2 (1.9%) had grade 3 and 4 derangement. D-dimers were raised in 60 out of 100 patients, giving a prevalence of 60% (CI 50.9-69.1%).

A summary of these findings have been presented in table 3 below. Normal and acceptable values denote values within the normal reference ranges as shown in page appendix 1 and values above the given upper limits of these reference ranges, apart from the values for APTT, PT and D-dimers where values above the upper limit of the reference ranges denote derangement. For the peripheral blood film (PBF) findings, our derangement of interest is findings of microcytic hypochromic cells.
### Table 3: Summary of the haematological parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (95% CI)</th>
<th>Median</th>
<th>Inter-Quartile range</th>
<th>Normal and acceptable values (%)</th>
<th>Derangements of interest (%)</th>
<th>Grading of the derangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Ferritin</td>
<td>48.9 (40.5-57.3)</td>
<td>39</td>
<td>61.25</td>
<td>88/106 (83.1%)</td>
<td>18/106 (16.9%)</td>
<td>N/A</td>
</tr>
<tr>
<td>MCV</td>
<td>76.3 (74.2-78.4)</td>
<td>77</td>
<td>17</td>
<td>51/103 (49.5%)</td>
<td>52/103 (50.5%)</td>
<td>N/A</td>
</tr>
<tr>
<td>MCH</td>
<td>25.8 (24.9-26.8)</td>
<td>25.45</td>
<td>7.17</td>
<td>51/102 (50%)</td>
<td>51/102 (50%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Hypochromic microcytic cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>APTT</td>
<td>26.6 (21.7-31.6)</td>
<td>22.6</td>
<td>25.5</td>
<td>67/103 (67%)</td>
<td>36/103 (34.9%)</td>
<td>28/103 (27.1%)</td>
</tr>
<tr>
<td>PT</td>
<td>5.3 (1.8-8.8)</td>
<td>1.7</td>
<td>3</td>
<td>99/103 (96.2%)</td>
<td>4/103 (3.8%)</td>
<td>1/103 (0.9%)</td>
</tr>
<tr>
<td>Platelets</td>
<td>336 (306-366)</td>
<td>317</td>
<td>224</td>
<td>95/103 (92.3%)</td>
<td>8/103 (7.7%)</td>
<td>6/103 (5.8%)</td>
</tr>
<tr>
<td>D-dimer</td>
<td>2.9 (1.8-3.9)</td>
<td>0.9</td>
<td>2.9</td>
<td>40/100 (40%)</td>
<td>60/100 (60%)</td>
<td>N/A</td>
</tr>
</tbody>
</table>
There was no statistically significant difference in the occurrence of any of the haematological derangements between the predominantly left to right shunting lesions and the predominantly right to left shunting lesions as shown on table 4 below.

### Table 4: Influence of the type of heart lesion on the haematological derangements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CCHD predominantly Left to right shunt</th>
<th>CCHD predominantly Right to left shunt</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Ferritin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron Deficiency</td>
<td>12/70 (14.6%)</td>
<td>2/5 (28.6%)</td>
<td>0.43 (0.06-3.63)</td>
<td>0.302</td>
</tr>
<tr>
<td>Rbc indicators</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOW MCV</td>
<td>36/82 (43.9%)</td>
<td>2/7 (28.6%)</td>
<td>1.96 (0.31-15.57)</td>
<td>0.690</td>
</tr>
<tr>
<td>LOW MCH</td>
<td>22/82 (26.8%)</td>
<td>1/7 (14.3%)</td>
<td>2.20 (0.24-51.26)</td>
<td>0.690</td>
</tr>
<tr>
<td>PBF-Microcytic hypochromic cells</td>
<td>28/82 (43.1%)</td>
<td>1/7 (14.3%)</td>
<td>3.11 (0.34-71.99)</td>
<td>0.420</td>
</tr>
<tr>
<td>Coagulation tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prolonged APTT</td>
<td>46/75 (61.3%)</td>
<td>2/6 (33%)</td>
<td>3.17 (0.46-26.91)</td>
<td>0.179</td>
</tr>
<tr>
<td>Prolonged PT</td>
<td>13/75 (17.3%)</td>
<td>3/6 (50%)</td>
<td>0.21 (0.03-1.50)</td>
<td>0.088</td>
</tr>
<tr>
<td>Low Platelets</td>
<td>3/75 (4%)</td>
<td>0/5 (0%)</td>
<td>-</td>
<td>0.649</td>
</tr>
<tr>
<td>Raised D-dimers</td>
<td>14/75 (18.7%)</td>
<td>2/5 (40%)</td>
<td>0.34 (0.04-3.30)</td>
<td>0.260</td>
</tr>
</tbody>
</table>

In this study all iron deficient patients fell in the age group of less than 5 years of age. This was statistically significant as denoted by the p value of 0.000. however, the odds ratio could not be computed because of the zero entry. The other significant factor associated with iron deficiency was stunting, specifically, severe stunting. There was a 5
fold increased likelihood of having iron deficiency with the presence of severe stunting (odds ratio 4.88 [95% CI 1.48-16.2], p = 0.005). No statistically significant relationship was noted between iron deficiency and sex (p=0.301) and between iron deficiency and wasting (p=0.673) as shown in table 5 below.

Table 5: Non-cardiac correlates of Iron deficiency

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Iron Deficiency N (%)</th>
<th>No Iron Deficiency N (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60 months</td>
<td>18/18 (100%)</td>
<td>41/94 (43.6%)</td>
<td>-</td>
<td>-</td>
<td>0.000</td>
</tr>
<tr>
<td>≥60 months</td>
<td>0/18 (0%)</td>
<td>53/94 (56.4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11/18 (61.1%)</td>
<td>48/94 (51%)</td>
<td>1.5</td>
<td>0.49-4.76</td>
<td>0.301</td>
</tr>
<tr>
<td>Female</td>
<td>7/18 (38.9%)</td>
<td>46/94 (49%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrition Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stunting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>9/18 (50%)</td>
<td>16/94 (17%)</td>
<td>4.88</td>
<td>1.48-16.2</td>
<td>0.005</td>
</tr>
<tr>
<td>Moderate</td>
<td>1/18 (5.5%)</td>
<td>19/94 (20.2%)</td>
<td>0.22</td>
<td>0.01-1.85</td>
<td>0.19</td>
</tr>
<tr>
<td>Mild to Normal</td>
<td>8/18 (44.5%)</td>
<td>59/94 (62.8%)</td>
<td>0.47</td>
<td>0.15-1.46</td>
<td>0.23</td>
</tr>
<tr>
<td>Wasting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>5/18 (27.8%)</td>
<td>32/93 (34.4%)</td>
<td>0.73</td>
<td>0.21-2.48</td>
<td>0.78</td>
</tr>
<tr>
<td>Moderate</td>
<td>6/18 (33.3%)</td>
<td>22/93 (23.6%)</td>
<td>1.61</td>
<td>0.47-5.38</td>
<td>0.386</td>
</tr>
<tr>
<td>Mild to normal</td>
<td>7/18 (38.9%)</td>
<td>39/93 (42.0%)</td>
<td>0.88</td>
<td>0.28-2.75</td>
<td>0.98</td>
</tr>
</tbody>
</table>
The Spearman Rho correlation was used to examine the relationship between haematocrit and ferritin, MCV, MCH, platelets, PT, APTT and D-dimers. There was a significant inverse correlation between Haematocrit levels and ferritin ($r = -0.197, p = 0.048$), platelets ($r = -0.374, p = 0.000$) and D-dimers ($r = -0.272, p = 0.010$). The inverse correlation with APTT was not significant. In addition there was a positive correlation between the haematocrit and MCV ($r = 0.228, p = 0.021$) and a non significant positive correlation with MCH as shown in table 6 below.

### Table 6: Relationship between Haematocrit and other Haematological parameters

<table>
<thead>
<tr>
<th></th>
<th>Ferritin</th>
<th>MCH</th>
<th>MCV</th>
<th>PLT</th>
<th>APTT</th>
<th>PT</th>
<th>D-dimer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient</td>
<td>-.197</td>
<td>.152</td>
<td>.228</td>
<td>-.374</td>
<td>-0.021</td>
<td>0.115</td>
<td>-.272</td>
</tr>
<tr>
<td>P value</td>
<td>0.048</td>
<td>0.127</td>
<td>0.021</td>
<td>0.000</td>
<td>0.841</td>
<td>0.264</td>
<td>0.010</td>
</tr>
<tr>
<td>Number</td>
<td>102</td>
<td>103</td>
<td>103</td>
<td>103</td>
<td>96</td>
<td>96</td>
<td>96</td>
</tr>
</tbody>
</table>

Subsequently, a regression analysis with the dependent variable being HCT was carried out to examine the relationship between the correlating variables. There was a statistically significant inverse correlation between HCT and ferritin ($p = 0.001$), and between HCT and platelets ($p = 0.016$). The inverse correlation with d-dimer was not significant ($p = 0.539$). There was also a significant positive correlation between HCT and MCV ($p = 0.046$). The adjusted R square for this model is 0.208. See table 7 below.
Table 7: Regression analysis examining the relationship between HCT and Ferritin, MCV, platelet and D-dimer

Model summary:

R = .494 \quad R^2 = .244 \quad \text{Adjusted } R^2 = .208 \quad p = 0.000

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(Constant)</td>
<td>43.011</td>
<td>12.679</td>
<td>3.392</td>
</tr>
<tr>
<td></td>
<td>FERRITIN</td>
<td>-0.113</td>
<td>0.031</td>
<td>-3.610</td>
</tr>
<tr>
<td></td>
<td>MCV</td>
<td>0.291</td>
<td>0.144</td>
<td>2.023</td>
</tr>
<tr>
<td></td>
<td>PLT</td>
<td>-0.023</td>
<td>0.009</td>
<td>-2.465</td>
</tr>
<tr>
<td></td>
<td>D-DIMER</td>
<td>-0.170</td>
<td>0.276</td>
<td>-0.617</td>
</tr>
</tbody>
</table>

Dependent Variable: HCT

Correlation between platelets and the other haematological variables showed that there is a significant inverse relationship between platelets and MCH (r=-.350, p<0.000), MCV (r=-.405, p=0.000) and HCT (r=-.374, p=0.000). The inverse relationship between platelets and APTT, PT and D-dimer are not significant. These findings have been summarized in table 8 below.
Table 8: Relationship between Platelets and other haematological parameters

<table>
<thead>
<tr>
<th></th>
<th>Ferritin</th>
<th>MCH</th>
<th>MCV</th>
<th>PT</th>
<th>APTT</th>
<th>HCT</th>
<th>D-dimer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td>-.070</td>
<td>-.350</td>
<td>-.405</td>
<td>-.106</td>
<td>-.083</td>
<td>-.374</td>
<td>-.008</td>
</tr>
<tr>
<td>coefficient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.487</td>
<td>0.000</td>
<td>0.000</td>
<td>0.304</td>
<td>0.422</td>
<td>0.000</td>
<td>0.941</td>
</tr>
<tr>
<td>Number</td>
<td>102</td>
<td>102</td>
<td>103</td>
<td>96</td>
<td>96</td>
<td>102</td>
<td>96</td>
</tr>
</tbody>
</table>

A regression analysis of the above correlating variables with platelet as the dependent variable shows a significant inverse correlation between platelets and HCT (p=0.001). The inverse correlations between platelets and MCV and MCH in this case are not significant. The adjusted R square for this model is 0.248. See table 9 below.

Table 9: Regression analysis examining the relationship between Platelets and MCV, MCH and HCT

Model summary:

\[ R = .540 \quad R^2 = .270 \quad \text{Adjusted } R^2 = .248 \quad p = 0.000 \]

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>95% Confidence Interval for B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Constan t)</td>
<td>560.751</td>
<td>99.822</td>
<td>000</td>
</tr>
<tr>
<td>MCV</td>
<td>-1.905</td>
<td>2.633</td>
<td>-.136</td>
</tr>
<tr>
<td>HCT</td>
<td>-3.547</td>
<td>1.008</td>
<td>-.317</td>
</tr>
<tr>
<td>MCH</td>
<td>-7.597</td>
<td>5.808</td>
<td>-.241</td>
</tr>
</tbody>
</table>

* Dependent Variable: PLT
A correlation analysis between ferritin and the other haematological parameters showed that there is a significant inverse correlation between ferritin and HCT ($r = -0.197$, $p=0.048$), and a significant positive correlation between ferritin and MCH ($r = 0.427$, $p=0.000$), and MCV ($r = 0.367$, $p=0.000$). The positive correlation between ferritin and D-dimer and between ferritin and PT are not significant. Similarly, the inverse correlation between ferritin and platelets and ferritin and APTT are not significant.

Table 10: Relationship between Ferritin and MCV, MCH, haematocrit, platelets, APTT, PT and D-dimers

<table>
<thead>
<tr>
<th></th>
<th>HCT</th>
<th>MCH</th>
<th>MCV</th>
<th>PLT</th>
<th>APTT</th>
<th>PT</th>
<th>D-dimer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin Correlation coefficient</td>
<td>-0.197</td>
<td>0.427</td>
<td>0.367</td>
<td>-0.707</td>
<td>-0.077</td>
<td>0.142</td>
<td>0.015</td>
</tr>
<tr>
<td>P value</td>
<td>0.048</td>
<td>0.000</td>
<td>0.000</td>
<td>0.487</td>
<td>0.447</td>
<td>0.160</td>
<td>0.884</td>
</tr>
<tr>
<td>Number</td>
<td>102</td>
<td>102</td>
<td>102</td>
<td>102</td>
<td>99</td>
<td>99</td>
<td>96</td>
</tr>
</tbody>
</table>

The subsequent regression analysis with ferritin as the dependant variable shows that the positive correlation between ferritin and MCV and between ferritin and MCH are not statistically significant ($p=0.738$ and $p=0.280$ respectively). The adjusted $r$-square for this model is 0.097. These findings have been summerized in table 11 below.
Table 11: Regression analysis examining the relationship between ferritin, MCV and MCH

Model summary:

$$R = .340 \quad R^2 = .115 \quad \text{Adjusted } R^2 = .097 \quad p = 0.002$$

Table 11: Regression analysis examining the relationship between ferritin, MCV and MCH

Model summary:

$$R = .340 \quad R^2 = .115 \quad \text{Adjusted } R^2 = .097 \quad p = 0.002$$

<table>
<thead>
<tr>
<th>Coefficients*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1 (Constant)</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>1</td>
</tr>
</tbody>
</table>

a. Dependent Variable: FERRITIN

SENSITIVITY AND SPECIFICITY OF RED CELL INDICES IN DIAGNOSING IRON DEFICIENCY

Table 12: Sensitivity and specificity of red cell indices in diagnosing iron deficiency

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW MCV</td>
<td>58.8% (10/17)</td>
<td>51.2% (42/86)</td>
<td>19.2% (10/52)</td>
<td>86.3% (44/51)</td>
</tr>
<tr>
<td>LOW MCH</td>
<td>52.9% (9/17)</td>
<td>50.6% (43/85)</td>
<td>17.6% (9/51)</td>
<td>84.3% (43/51)</td>
</tr>
<tr>
<td>MICROCYTIC HYPOCHROMIC</td>
<td>50% (9/18)</td>
<td>73.4% (69/94)</td>
<td>26.5% (9/34)</td>
<td>88.5% (69/78)</td>
</tr>
</tbody>
</table>

From table 12 above we see that the sensitivity and specificity of low MCV in diagnosing iron deficiency is low (58.8% and 51.2% respectively). Similarly, low MCH has a low
sensitivity and specificity in diagnosing iron deficiency (52.9% and 50.9% respectively).

Findings of microcytic hypochromic cells on the peripheral blood film will give a sensitivity of 50% and a specificity of 73.4%.
DISCUSSION

DEMOGRAPHIC FACTORS

The study population comprised 59 males and 53 females. The age ranged from 1 month to 17 years, with a median age of 4 years and five months. The mean age was 5 years 10 months. The commonest heart lesion encountered was Tetralogy of Fallot, which is generally the commonest cyanotic heart lesion. This study’s findings are in keeping with what was expected. The study locations of Kenyatta National Hospital and Mater hospital have comprehensive paediatric cardiology programs with a large number of patients on follow up and as such were ideal recruitment centres.

From this study, we found that 40.1% (45 out of 112) children were stunted. This is much higher than the national figure of 30% reported in the Kenya demographic and health survey 2003. Out of these, 22.3% were severely stunted while 17.9% were classified as moderately stunted. Wasting was seen in 35.7% (40 out of 112) of our study population, a figure much higher than the national figure of 6% reported in the Kenya demographic and health survey 2003. Severe wasting was seen in 20.5% of the study population while moderate wasting was seen in 15.2%. This was however an expected finding given the burden of the heart disease on its own is expected to interfere with growth and general well being and food intake.

Examining the relationship between the nutritional status and iron deficiency, severe wasting gave a 5 fold risk of having iron deficiency, an observation that was statistically significant. Besides the heart disease itself, iron deficiency is known to cause anorexia
and has a direct negative effect on growth, specifically height. Malnutrition is known to involve also micronutrient deficiencies, of importance here is iron and vitamin c.

HAEMATOLOGICAL PARAMETERS

The overall prevalence of iron deficiency in this study was 16.9%, which is almost similar to what was found in the study done in India by Gaiha et al where they reported a prevalence of 18.2% (10). Murila et al in Kenya found an iron deficiency prevalence of 7% in a generally healthy population of children less than 5 years of age (35).

Interestingly, in our study all the 18 patients found to be iron deficient were below 5 years of age. In contrast, Drossos C. et Al from Italy in 1981 examining a total of 38 cyanotic heart disease children, found an iron deficiency prevalence of 12.5% in children 6 to 12 year old. In the same study however, children less than 5 years still contributed the bulk of the iron deficiency group with a prevalence of 37.5%.

Ferritin measurements as indicator of iron status are prone to giving false negative results as they are affected by many factors causing inflammation including infection. This leads to a falsely elevated ferritin level, thus the false negatives. In our local set up, with high prevalence of infectious diseases, it is possible that our ferritin assays could have underestimated the prevalence of iron depletion.

The baseline coagulation screening tests done in this study were APTT, PT and platelet levels. Prolongation in APTT was seen in 32.1%, prolongation in PT in 3.6% of the patients while significant reduction in platelets was seen in 7.1% of the study population.
This is a low prevalence compared to the findings by Colon-Otero et al in 1987 (29) where they reported abnormal test results in 19% of their patients.

In this study, grading of the degree of coagulation tests’ derangement was based on the DAIDS (Division of AIDS) Table for grading severity of paediatric adverse experiences (see appendix 1), where significant derangements are in the grades 3 and 4. With this categorization, grade 3 and 4 abnormalities were seen in 7.7%, 2.9% and 1.9% for APTT, PT and platelets respectively.

An isolated prolongation in the APTT is indicative of a decrease in factors VIII, IX, XII, prekallikrein, High molecular weight kininogen, Von Willebrand’s disease or circulating anticoagulants e.g. Lupus. It could also be raised in mild factor II, V or X deficiency. An isolated prolongation in PT is usually indicative of factor VII deficiency, early oral anticoagulant and Lupus anticoagulant. In some cases, mild factor II, V or X deficiency could also lead to an isolated prolongation in APTT. When both APTT and PT are raised, we suspect lack of vitamin K or administration of oral anticoagulants (in both cases the PT is usually relatively more prolonged than the APTT). Another cause will be liver disease leading to deficiencies in multiple clotting factors. In this study, we also noted raised D-Dimers in 60% of our study subjects. This was however mild elevation above our reference cut off 0.3mg/l. This is indicative of a low grade DIC going on in these patients.
The correlation and regression analysis done in this study showed that haematocrit values had a significant inverse relation with ferritin (and thus iron deficiency) and with platelets (predisposing to coagulopathy). These observations affirm the hypothesis that the raise in haematocrit seen in congenital cyanotic heart disease is key to the subsequent iron deficiency and coagulopathy observed in these patients.

SENSITIVITY AND SPECIFICITY OF THE RED CELL INDICES IN DIAGNOSING IRON DEFICIENCY

It is worthwhile to note that this study was not powered to answer this question. However, from the figures in table 12, we see that red cell indices have a low sensitivity and specificity in diagnosing iron deficiency in this group of patients. If one uses values of MCV that are below the age specific reference range as a screening tool for iron deficiency in this group of patients, only 58% will be picked by this screening test. Low MCH will on the other hand only pick 52.9% of the true iron deficient patients. Findings of microcytic hypochromic cells on a peripheral blood film will only pick 50% of the true iron deficient patients. Similarly, the specificity of these tests is also low-about 50% for low MCV and low MCH, and 73.4% for microcytic hypochromic cells. These tests however have high negative predictive values, ranging from 84% to 88.5%.

A regression analysis done in this study examining the correlation between ferritin levels and MCV and MCH did not show a significant correlation. This could be explained by the low number of subjects analyzed in this study.
STRENGTHS OF THE STUDY

1. Compared to study previously published on this subject matter, this study had a significantly larger patient population giving strength to the observed outcomes.

2. Consistency was maintained throughout the study in that the principle investigator recruited all the patients, and a single laboratory technologist was used for each test and a single consultant pathologist reported all the peripheral blood films.

STUDY LIMITATIONS

1. This study did not have a control group which could have helped in strengthening the study observations.

2. Other factors like liver dysfunction that could have interfered with some of our results in the coagulation screen were not isolated in our data collection and analysis.

3. There was no absolute way of ruling out inflammatory processes during data collection. These are known to interfere with ferritin levels.
CONCLUSION

1. The prevalence of iron deficiency in children with cyanotic heart disease attending Kenyatta National Hospital and Mater Hospital is 16.9%.

2. The prevalence of severe derangements in coagulation tests in congenital cyanotic heart disease patients in Kenyatta National Hospital and Mater Hospital is low, at 7.7% for prolonged APTT, 2.9% for prolonged PT and 1.9% for low platelets. The derangements are mostly of the mild and moderate grade.

3. The sensitivities of low MCV, low MCH and findings of hypochromic microcytic cells on peripheral blood film in detecting iron deficiency this group of patients are low. The sensitivity and specificity of low MCV was 58.8% and 51.2% respectively, and that of low MCH was 52.9% and 50.6% respectively. Findings of microcytic hypochromic cells on the peripheral blood film gave a sensitivity of 50% and specificity of 73.4%.
RECOMMENDATIONS

1. Children with cyanotic heart disease should routinely be screened for iron deficiency using biochemical methods like ferritin levels, and those found deficient should be treated accordingly.

2. Red cell indices and red blood cell morphology have a low sensitivity in detecting iron deficiency, and cannot be relied upon as good screening tools for iron deficiency in our local population of cyanotic heart disease patients. We recommend the use of ferritin assays in screening children suspected to be iron deficient.

3. Abnormalities in coagulation tests do occur in cyanotic heart disease patients, mostly mild and moderate in severity, we recommend coagulation test screening before major surgery and invasive procedures.

4. Further studies should be carried out to determine the specific abnormalities leading to prolongation of the PT and APTT in children with cyanotic heart disease.
REFERENCES


30. Levin E, Wu J, Devine DV. Hemostatic parameters and platelet activation marker expression in cyanotic and acyanotic pediatric patients undergoing cardiac surgery in the presence of tranexamic acid. Thrombosis and Haemostasis. 2000;83:54-59


# APPENDIX 1

## REFERENCE INTERVALS USED

### FERRITIN

<table>
<thead>
<tr>
<th>AGE</th>
<th>LOWER LIMIT</th>
<th>UPPER LIMIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td>200 µg/l</td>
<td>600 µg/l</td>
</tr>
<tr>
<td>2 - 5 months</td>
<td>50 µg/l</td>
<td>200 µg/l</td>
</tr>
<tr>
<td>6 months - 15 years</td>
<td>7 µg/l</td>
<td>140 µg/l</td>
</tr>
<tr>
<td>&gt; 15 years - male</td>
<td>15 µg/l</td>
<td>200 µg/l</td>
</tr>
<tr>
<td>- female</td>
<td>12 µg/l</td>
<td>150 µg/l</td>
</tr>
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</table>

### MCV

<table>
<thead>
<tr>
<th>AGE</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td>92 fl</td>
<td>114 fl</td>
</tr>
<tr>
<td>2 months</td>
<td>87 fl</td>
<td>103 fl</td>
</tr>
<tr>
<td>3-6 months</td>
<td>68 fl</td>
<td>84 fl</td>
</tr>
<tr>
<td>6 months-2years</td>
<td>77 fl</td>
<td>95 fl</td>
</tr>
<tr>
<td>2-6 years</td>
<td>75 fl</td>
<td>87 fl</td>
</tr>
<tr>
<td>6-12 years</td>
<td>77 fl</td>
<td>95 fl</td>
</tr>
<tr>
<td>&gt;12 years</td>
<td>83 fl</td>
<td>101 fl</td>
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</table>

### MCH

<table>
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</thead>
<tbody>
<tr>
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<td>36 pg</td>
</tr>
<tr>
<td>2 months</td>
<td>27 pg</td>
<td>33 pg</td>
</tr>
<tr>
<td>3-6 months</td>
<td>24 pg</td>
<td>30 pg</td>
</tr>
<tr>
<td>6 months-2years</td>
<td>25 pg</td>
<td>29 pg</td>
</tr>
<tr>
<td>HCT</td>
<td>AGE</td>
<td>LOWER LIMIT</td>
</tr>
<tr>
<td>-----</td>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td></td>
<td>1 month</td>
<td>33%</td>
</tr>
<tr>
<td></td>
<td>2 months</td>
<td>28%</td>
</tr>
<tr>
<td></td>
<td>3-6 months</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>6 months-2 years</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>2-6 years</td>
<td>34%</td>
</tr>
<tr>
<td></td>
<td>6-12 years</td>
<td>35%</td>
</tr>
<tr>
<td></td>
<td>&gt;12 years – male</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>-female</td>
<td>36%</td>
</tr>
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<table>
<thead>
<tr>
<th>HB</th>
<th>AGE</th>
<th>LOWER LIMIT</th>
<th>UPPER LIMIT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 month</td>
<td>11.5g/dl</td>
<td>16.5g/dl</td>
</tr>
<tr>
<td></td>
<td>2 months</td>
<td>9.4g/dl</td>
<td>13g/dl</td>
</tr>
<tr>
<td></td>
<td>3-6 months</td>
<td>11.1g/dl</td>
<td>14.1g/dl</td>
</tr>
<tr>
<td></td>
<td>6 months-2 years</td>
<td>11.1g/dl</td>
<td>14.1g/dl</td>
</tr>
<tr>
<td></td>
<td>2-6 years</td>
<td>11.0g/dl</td>
<td>14.0g/dl</td>
</tr>
<tr>
<td></td>
<td>6-12 years</td>
<td>11.5g/dl</td>
<td>15.5g/dl</td>
</tr>
<tr>
<td></td>
<td>&gt;12 years – male</td>
<td>13.0g/dl</td>
<td>17.0g/dl</td>
</tr>
<tr>
<td></td>
<td>-female</td>
<td>12.0g/dl</td>
<td>15.0g/dl</td>
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</tbody>
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<table>
<thead>
<tr>
<th>PLATELETS</th>
<th>LOWER LIMIT</th>
<th>UPPER LIMIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td>200 X 10^9/l</td>
<td>400 X 10^9/l</td>
</tr>
<tr>
<td>Age Group</td>
<td>Grade 1 (Mild)</td>
<td>Grade 2 (Moderate)</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>2 months</td>
<td>210 X 10^9/l</td>
<td>200 X 10^9/l</td>
</tr>
<tr>
<td>3-6 months</td>
<td>210 X 10^9/l</td>
<td>200 X 10^9/l</td>
</tr>
<tr>
<td>6 months-2 years</td>
<td>200 X 10^9/l</td>
<td>200 X 10^9/l</td>
</tr>
<tr>
<td>2-6 years</td>
<td>200 X 10^9/l</td>
<td>200 X 10^9/l</td>
</tr>
<tr>
<td>6-12 years</td>
<td>170 X 10^9/l</td>
<td>170 X 10^9/l</td>
</tr>
<tr>
<td>&gt;12 years</td>
<td>150 X 10^9/l</td>
<td>150 X 10^9/l</td>
</tr>
</tbody>
</table>

Grading Low Platelets-
- Grade 1 (Mild) - 100,000 - 124,999/mm³
- Grade 2 (Moderate) - 50,000 - 99,999/mm³
- Grade 3 (Severe) - 25,000 - 49,999/mm³
- Grade 4 (Life threatening) - <25,000/mm³

Prolonged APTT - test time - control time > 15 seconds
- Grade 1 (Mild) - 1.1 - 1.66 x ULN
- Grade 2 (Moderate) - 1.67 - 2.33 x ULN
- Grade 3 (Severe) - 2.34 - 3.00 x ULN
- Grade 4 (Life threatening) - > 3 x ULN

Prolonged PT - test time - control time > 3 seconds
- Grade 1 (Mild) - 1.1 - 1.25 x ULN
- Grade 2 (Moderate) - 1.26 - 1.5 x ULN
- Grade 3 (Severe) - 1.51 - 3.00 x ULN
- Grade 4 (Life threatening) - > 3 x ULN

Raised D-Dimer - Levels >0.3mg/l

Abnormal coagulation- prolongation in PT, APTT, low platelets or raised D-dimer

Stunting - Mild: >-2SD Height for age on the CDC 2000 growth reference chart
- Moderate: -2SD - < -3SD Height for age on the CDC 2000 growth reference chart
  - Severe: < -3SD Height for age on the CDC 2000 growth reference chart

- Wasting
  - Mild: > -2SD weight for height on the CDC 2000 growth reference chart
  - Moderate: -2SD - < -3SD weight for height on the CDC 2000 growth reference chart
  - Severe: < -3SD weight for height on the CDC 2000 growth reference chart
### DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS

#### DIVISION OF AIDS (DAIDS)
**TABLE FOR GRADING SEVERITY OF PEDIATRIC (>3 MONTHS OF AGE) ADVERSE EXPERIENCES**

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>GRADE 1</th>
<th>GRADE 2</th>
<th>GRADE 3</th>
<th>GRADE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HEMATOLOGY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin &gt;3 mo to &lt;2 y.o.</td>
<td>9.0-9.9</td>
<td>7.0-8.9</td>
<td>&lt;7.0</td>
<td>Cardiac Failure Secondary to anemia</td>
</tr>
<tr>
<td>Hemoglobin ≥2 y.o.</td>
<td>10-10.9</td>
<td>7.0-9.9</td>
<td>&lt;7.0</td>
<td>Cardiac Failure Secondary to anemia</td>
</tr>
<tr>
<td>Abs Neutrophil Ct</td>
<td>750-1200</td>
<td>400-749</td>
<td>250-399</td>
<td>&lt;250</td>
</tr>
<tr>
<td>Platelets</td>
<td>50,000-75,000</td>
<td>25,000-49,999</td>
<td>&lt;25,000 or bleeding</td>
<td>&lt;250,000 or bleeding</td>
</tr>
<tr>
<td><strong>GASTROINTESTINAL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin</td>
<td>1 1-1.9xN</td>
<td>2.0-2.9xN</td>
<td>3.0-7.5xN</td>
<td>&gt;7.5xN</td>
</tr>
<tr>
<td>AST (SGOT)</td>
<td>1 1-4 9xN</td>
<td>5.0-9 9xN</td>
<td>10.0-15 0xN</td>
<td>&gt;15 0xN</td>
</tr>
<tr>
<td>ALT (SGPT)</td>
<td>1 1-4 9xN</td>
<td>5.0-9 9xN</td>
<td>10.0-15 0xN</td>
<td>&gt;15 0xN</td>
</tr>
<tr>
<td>GGT</td>
<td>1 1-4 9xN</td>
<td>5.0-9 9xN</td>
<td>10.0-15 0xN</td>
<td>&gt;15 0xN</td>
</tr>
<tr>
<td>Pancreatic Amylase</td>
<td>1 1-1 4xN</td>
<td>1.5-1 9xN</td>
<td>2.0-3 0xN</td>
<td>&gt;3 0xN</td>
</tr>
<tr>
<td>Total Amylase + Lipase*</td>
<td>1 1-1 4xN</td>
<td>1.5-2 4xN</td>
<td>2.5-5 0xN</td>
<td>&gt;5 0xN</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>7 5-9 9</td>
<td>10-12 4</td>
<td>12-15 0</td>
<td>&gt;15 0 or Gout</td>
</tr>
<tr>
<td><strong>CPK</strong></td>
<td></td>
<td></td>
<td></td>
<td>Severe- Hospital and Rx</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>Mild</td>
<td>Moderate- Needed</td>
<td>No Rx</td>
<td>Moderate Rx Needed</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Soft stools</td>
<td>Liquid stools</td>
<td>Liquid Stools and Mild Dehydration Bloody stools</td>
<td>Dehydration requiring IV therapy or Hypotensive Shock</td>
</tr>
<tr>
<td>Constipation</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
<td>Distention and Vomiting</td>
</tr>
<tr>
<td>Nausea</td>
<td>Mild</td>
<td>Moderate-Decreased intake</td>
<td>Severe-Little intake</td>
<td>Unable to ingest food or fluid for &gt;24 hours</td>
</tr>
<tr>
<td>Vomiting</td>
<td>&lt;1 episode/day</td>
<td>1-3 episodes/day or duration&gt;3d</td>
<td>&gt;3 episodes/day or duration&gt;7d</td>
<td>Intractable Vomiting</td>
</tr>
</tbody>
</table>

*Both amylase and lipase must be elevated to the same grade or higher (i.e., if total amylase is Grade 4, but lipase is only Grade 1, the Toxicity Grade is 1). In pediatric HIV patients, the most common source of serum amylase is the salivary glands. Salivary amylase elevations are generally not clinically significant. When amylase is released from damaged pancreatic cells, it can be a marker of pancreatitis. In most cases of clinical pancreatitis, lipase will also be elevated. However, lipase is also a non-specific marker. Combined elevation of amylase and lipase (each >5 x normal) often indicates pancreatic disease and requires evaluation. However, in the absence of pancreatic disease, drug can be resumed even at Grade 3 and 4 toxicities.*
APPENDIX 2

CORRECTION FORMULA FOR CALCULATING THE AMOUNT OF SODIUM CITRATE AND BLOOD MIXING VOLUMES FOR POLYCYTHAEMIC PATIENTS

Principle

For accurate coagulation testing, the ideal concentration of the blood-anticoagulation mixture is 10.9-12.9 mmol of sodium citrate per litre. The standard 9:1 blood-anticoagulation mixture maintains this ideal concentration for haematocrit up to 55%. This standard ratio however, produces altered test results in patients whose haematocrit are >55%. In a patient whose Hct is >55% the results may be prolonged due to a decrease in plasma volume and excess anticoagulation. Consequently, the blood to anticoagulant ratio will be adjusted for such Hct values to insure accurate results, using the formula:

\[ C = 1.85 \times 10^{-3} (100 - h) \times V \]

Where:

- \( C \) = volume of 3.2% sodium citrate in ml
- \( h \) = Hct in percent
- \( V \) = volume of whole blood in ml

Example:

Patient with Hct of 57%, where \( C = 0.1 \text{ml} \) sodium citrate, we will add 1.3mls whole blood to make a total of 1.4ml.
0.1 = 85 \times 10^{-3} (100-57) \times V \\
0.1 = 0.00185 \times 43 \times \\
0.1 = 0.0795 \times V \\
V = 1.3 \text{ml} \\

The amounts are then doubled or tripled to ensure recovery of the desired plasma.
APPENDIX 3

QUESTIONNAIRE

Study Title

Haematological manifestations of cyanotic heart diseases at Kenyatta National Hospital and Mater Hospital, Nairobi.

Identification

Identification number  
Initials:  

Age

Date of birth  (dd/mm/year)

Sex

a) Male  
b) Female  

Residence

Physical Examination

Weight (Kg)  Height (cm)  Temp °C  

Signs of systemic infection or inflammation e.g. Pneumonia, septicaemia, malaria etc

a) Present  
b) Absent
Family history

Family history of a bleeding disorder

a) Yes

b) No

Drug history

Current use of warfarin, aspirin or haematinics

a) Yes

b) No

Outcome Variables

- Cardiac lesion
  
a) Tetralogy of Fallot
  
b) Pulmonary atresia with VSD
  
c) Critical Pulmonary Stenosis
  
d) Tricuspid atresia
  
e) Double-outlet right ventricle with pulmonary stenosis
  
f) Double-outlet right ventricle without pulmonary stenosis
  
g) Transposition of the great arteries
  
h) Ebstein anomaly of the tricuspid valve
  
i) Total anomalous pulmonary venous return
  
j) Truncus arteriosus
  
k) Single ventricle
  
l) Hypoplastic left heart syndrome
  
m) others (specify)
Haematological Variables

1. Coagulation screening tests:
   a) Prothrombin time
      Test (sec) □□□□ Control(sec) □□□□
   b) Activated partial thromboplastin time
      Test (sec) □□□□ Control(sec) □□□□

2. Full Heamogram
   a) Haemoglobin level □□□□
   b) Mean corpuscular volume □□□□
   c) Mean corpuscular haemoglobin □□□□
   d) Platelet count □□□□

3. D-dimers □□□□

4. Peripheral blood film (Tick as appropriate)
   a) Normocytic Normochromic □□□□
   b) Microcytic Normochromic □□□□
   c) Microcytic Hypochromic □□□□
   d) Macrocytic □□□□
   e) Others □□□□

5. Ferritin Levels □□□□
APPENDIX 4
CONSENT FORM

Introduction
My name is Dr. Moses Lang’o, a postgraduate student at the School of Medicine, college of Health Sciences at the University of Nairobi. In partial fulfillment for the masters degree of Medicine in Pediatrics and child health, I am carrying out a study to examine the problems in the blood associated with bleeding tendencies and anaemia, that are commonly encountered in children with heart disease.

These changes as mentioned are common, and the tests carried out to assess these changes are routine. Your child is a potential candidate and I would like to have him/her in this study. However, it is imperative that you understand what the study entails, what is expected of you, and all the benefits and disadvantages your child may have by enrolling into this study.

The study: Patients with cyanotic heart disease like your child commonly have a different hematological response from those without cyanosis secondary to the strain of reduced oxygenation inherent with the heart lesion. As such, they commonly have bleeding problems and are predisposed to iron deficiency anaemia.
I would like to examine a number of our patients suffering from cyanotic heart disease who are attending our hospitals and establish the extent of this problem in our set up.

Procedure:
If you agree to join the study, I shall ask you some questions regarding the child like age, his/her past medical history, etc. I shall document these findings together with the heart findings as reported on your ECHO report and I shall proceed to withdraw a small amount of blood from your child through a finger prick (about 2-3 drops) which I shall examine and document the haematocrit. Thereafter I shall withdraw about 5-7mls of blood which I shall take to the laboratory and examine for the haematological derangements earlier explained. These finding will be done free of charge and you will have them in your child’s file to assist in your child’s management. I shall then request you to bring your child for a second visit where I shall draw a second blood sample to assess your child for any bleeding disorder. The child’s health comes first and at any one time the study shall not take precedence over giving your child any medical attention he or she may require. If abnormalities are encountered, I shall recommend the appropriate treatment for your child. All information obtained will be treated with utmost confidentiality.
Benefits: Your child will benefit by having a free blood evaluation that will assist the doctors in managing him/her better. The information we get from this study will give information that may assist other children with similar conditions like your child.

Risks: The child will experience pain and discomfort during withdrawal of the blood. Drawing blood also predisposes to a risk of excessive bleeding from the puncture site, which can be contained by application of firm pressure. There is a slight risk of infection at the puncture site.

Child’s Rights: The participation of your child in this study is voluntary and he or she is free to decline to participate or withdraw without suffering any loss or his/her treatment being affected in any way.

My contacts are Dr. Moses Lang’o
P. O. Box 15143-00100 Nrb
Tel: 0722,428092
I can be contacted 24 hrs a day in case of any queries or concerns. You can also get in touch with the chairperson of the ethics and research committee if Kenyatta National Hospital. Prof Bhatt
on tel no. 726300-9 or P. O. Box 20723 Nairobi, in case of any ethics concerns.

Consent

I ........................................................ Parent/Guardian to
........................................................ , having been explained to about this study,
by........................................................ give consent for my child to participate. My rights have been explained to me and assured.

Signed ........................................ Date ........................................

Dr Moses Lang’o

Signed ..................... Date ..........................
APPENDIX 5

KNH ETHICS AND RESEARCH COMMITTEE APPROVAL LETTER

Ref. KNH-ERC/ 01/ 4638

Dr. Lango Moses
Dept. of Paediatrics & Child Health
School of Medicine
University of Nairobi

Dear Dr. Lango

REVISED RESEARCH PROPOSAL “HAEMATOLOGICAL MANIFESTATIONS IN CYANOTIC HEART DISEASES AT K.N.H AND MATER HOSPITAL, NAIROBI”

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and approved your revised research proposal for the period 1st August 2007 - 9th August 2008.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH-ERC

KENYATTA NATIONAL HOSPITAL
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Tel: 7263009
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Email: KNH-finan@Kon-Healton.org

10th August 2007

cc: Prof. K.M. Bhatt, Chairperson KNH-ERC
      The Deputy Director CS, KNH
      The Dean, School of Medicine, UON
      The Chairman, Dept. of Paediatrics & Child Health, UON
      Supervisor: Dr. J.N. Githanga, Dept. of Human Pathology, UON
                 Dr. C.A. Yuko Jowi, Dept. of Paediatrics & Child Health, UON