BONE MARROW MORPHOLOGY IN PEADIATRIC MORTALITIES ASSOCIATED WITH SEVERE ACUTE RESPIRATORY ILLNESS AT KENYATTA NATIONAL HOSPITAL

BY

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2017
DECLARATION

I, DR LILIAN KERUBO BOSIRE declare that this dissertation is my original work under the guidance of the supervisors indicated below and has not been submitted to the University of Nairobi or any institute of higher learning.

Signature ___________________ Date ___________________

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DEDICATION

I dedicate my work to my husband Ezekiel Mwabili for his unconditional support and understanding he has given me during the study period.
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3. The entire pediatric respiratory surveillance (PRESS) team for teamwork and information required to perform my study.

4. Lecturers, staff members and my fellow postgraduate colleagues in the department of Human Pathology for their support and encouragement.
**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BMB</td>
<td>Bone Marrow Biopsy</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control</td>
</tr>
<tr>
<td>CMV</td>
<td>Cytomegalovirus</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated Intravascular Coagulation</td>
</tr>
<tr>
<td>EBV</td>
<td>Ebstein Barr Virus</td>
</tr>
<tr>
<td>ERC</td>
<td>Ethical Regulatory Committee</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and Eosin</td>
</tr>
<tr>
<td>HHH</td>
<td>Histiocytic Hyperplasia Haemophagocytosis</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodefiency Virus</td>
</tr>
<tr>
<td>HEP A</td>
<td>Hepatitis A</td>
</tr>
<tr>
<td>HLH</td>
<td>Hemophagocytic Lymphohistiocytosis</td>
</tr>
<tr>
<td>HPS</td>
<td>Hemophagocytic Syndrome</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin-1</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile Range</td>
</tr>
<tr>
<td>KDHS</td>
<td>Kenya Demographic Health Survey</td>
</tr>
<tr>
<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
</tr>
<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
</tr>
<tr>
<td>PAS</td>
<td>Periodic Acid Schiff</td>
</tr>
<tr>
<td>PH</td>
<td>Power of Hydrogen</td>
</tr>
<tr>
<td>PRESS</td>
<td>Pediatric Respiratory Surveillance Study</td>
</tr>
<tr>
<td>RCPA</td>
<td>Royal College of Pathologists of Australia</td>
</tr>
<tr>
<td>SARI</td>
<td>Severe Acute Respiratory illness</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>TB</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>VAHS</td>
<td>Viral Associated Hemophagocytic Syndrome</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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ABSTRACT

Background
In Kenya, severe acute respiratory tract infections (SARI) are the leading cause of death among children. The predominant mechanism of death is related to respiratory disease. These children frequently have hematologic abnormalities such as anemia, leukocytosis, thrombocytopenia and thrombocytosis. There is no African series that has examined the bone marrow morphological features in SARI mortalities. Asian and Eastern European series have identified haemophagocytic syndrome as a prominent feature in SARI mortalities. In Kenya, the contribution of the haemophagocytic syndromes and the diagnostic potential of bone marrow biopsies in SARI patients are unknown. In addition, the contribution of specific infectious disease morbidity to bone marrow pathology is also unclear.

Objectives: The broad objective was to describe the morphological changes in bone marrow obtained from autopsies of SARI related mortalities in children under 5 years. The specific objectives were to describe the hematopoietic and histiocytic characteristics of bone marrow in pediatric SARI mortalities and to correlate the bone marrow morphological findings to SARI etiology.

Study Design: Cross- Sectional Descriptive Study.

Study Site: Anatomic Pathology core research and Unit of Haematology and Blood Transfusion laboratory, Department of Human Pathology, University of Nairobi.

Study Population: These were SARI mortalities at Kenyatta National Hospital among infants and children aged 1-59 months that occurred between August, 2014 and December, 2015. The autopsies were performed on sixty four eligible SARI mortalities during that period as part of a larger study, titled ‘Pediatric Respiratory Epidemiology Surveillance Study (PRESS)’.

Study Procedures: Fifty nine archived formalin fixed paraffin embedded (FFPE) bone marrow tissue specimens from the PRESS study were retrieved, sectioned and stained with Hematoxylin and Eosin (H/E), reticulin, modified Grunwald Giemsa (MGG), Periodic Acid Schiff (PAS) and Perl’s Prussian Blue. The specimens were examined using light microscopy by the principal investigator, hematopathologist and anatomic pathologist. These were examined using a standardized Royal College of Pathologists of Australia (RCPA) 2014 protocol approach. Variables were bone marrow cellularity, and nature of erythropoiesis, nature of myelopoiesis, megakaryocytes, lymphocytes, plasma cells, histiocytes and hemophagocytosis. Data was coded, cleaned and analyzed using statistics and data version13. Numeric variables were summarized using means (standard deviations) or medians (interquartile range) that were reported in histograms and dot plots. Categorical variables were summarized using frequencies and proportions that were reported in tables. Chi-square test of homogeneity was done to compare the distribution of morphological findings by type of infection. The chi-square statistic and corresponding p-values were reported.
Results:
Fifty two bone marrow biopsies were satisfactory for evaluation. The age range of the study population was 1 to 48 months with a median of 8 months. Females were 32 (54.2%) cases. The total blood count showed a mean hemoglobin level of 9.42 and standard deviation of 1.80, white blood cell count(WBC) had a median of 13 and Interquartile range(IQR) of 8.1.

Of the 52 bone marrow biopsies evaluated, H&E stained well in 47 (90.4%) cases. Normal cellularity was observed in 35 (67%) cases. Erythropoiesis was normal in 25 (48%) cases) increased in 16 (30.8%) cases and reduced in 11 (21%) cases. Granulopoeisis was increased in 31 (59.6%) cases and reduced in 7 (13.5%) cases. Left shift granulopoeisis was reported in 38 (73.1%) cases. Megakaryopoeisis was well represented in 40 (76%) cases. Plasma cells were increased in 5 (9.6%) cases and lymphocytes increased in 15(28%) cases. Histiocytosis was seen in 14 (26.9%) cases and Histiocytic Hyperplastic Hemophagocytosis (HHH) was present in 13 (25%) cases: mild HHH in 6 (11.4%) cases, moderate HHH in 2 (3.9%) cases and severe HHH in 2(3.9%) cases. Bone marrow hypoplasia with HHH was reported in 3 (5.8%) cases. Evidence of infection seen in the bone marrow were tuberculosis (n=1), malarial pigment (n=2) and parvovirus related changes (n=1). The finding of HHH was not significantly associated with any particular pattern of infection. There was no significant association between type of infection and presence of HHH. There was no association between bone marrow morphology and the infectious etiological agent.

Conclusion: The main changes observed in bone marrow were granulocytic hyperplasia (59.6%), erythroid hyperplasia (30%) and histiocytosis (26.9%). Dyplasias involving the erythroid (44%) and myeloid lineages (14%) were common features. Hemophagocytosis (25%) was a significant finding in Kenyan SARI mortalities.
1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Hematologic complications of severe acute respiratory infections (SARI) contribute to mortality in approximately 60% of cases. Clinical intervention focused on these complications can alter the cause of disease and reduce the mortality rate of SARI, which in a recent survey was 22% (unpublished observations, Henry Njuguna) (1). Studies that focus on these cases in Africa are limited. Autopsy studies would provide insight into these complications and their likely impact on mortality.

Studies in Japan, Italy and India show that there are significant hematologic features among children with SARI. These changes include hemophagocytosis, anemia, thrombocytopenia and leukopenia. Furthermore, leukocytosis is common and inhibits red blood cell and platelet production. In developing African countries there are limited studies on hematological features among children with SARI. In Zambia, Chintu et al did not present features on the bone marrow present in their autopsy cohort. The magnitude and spectrum of hematologic complications is unknown in our setting (2).

Autopsy studies would provide deep insights into hematologic disorders in fatal SARI cases. A complete diagnostic autopsy that includes histologic examination of bone marrow tissue would provide information about direct bone marrow infections, qualitative assessment of hematopoietic activity and presence of abnormal function such as hemophagocytosis. Bone marrow histology maybe stained by routine morphological techniques and special stains such as those required for assessment of iron. These tissues can also be examined using advanced pathology techniques such as immunohistochemistry and molecular techniques.
The aim of this study was to identify the hematological complications which occur in acute respiratory illness especially those associated with sepsis which is common and frequently fatal. In the United States of America alone 800,000 cases of severe sepsis occur each year and each organ dysfunction increases the mortality rate of severe sepsis by 20%. The study was aimed at providing information on hematological complications to critical care specialists to manage these complications (3).

1.2 Disease Classification of Acute Respiratory Illness

Respiratory illness can be classified as upper respiratory tract illness and lower respiratory illness. Upper respiratory illness includes sinusitis, rhinitis, pharyngitis, epiglottitis and laryngitis. Lower respiratory illness includes pneumonia and bronchiolitis (4).

1.3 Epidemiology of Acute Respiratory Illness

Acute respiratory illnesses cause an estimated 20% of deaths in children less than five years of age. Most of these infections are treatable and preventable. The World Health Organization (WHO) estimates that 46% of the world under five years mortality occurs in Sub-Saharan Africa. The Kenya Demographic Health Survey 2014 survey estimated the infant mortality is 39 per 1000 and the under-five mortality is 52 per 1000. Respiratory diseases cause hospitalization and deaths within the children, an estimated 35 million cases and one million cases of deaths annually (5).

1.4 Hematological Complications of Childhood Severe Acute Respiratory Illness

The main hematological complications of acute respiratory infection are anemia, thrombocytopenia, alterations of white cell count and function, immunocytopenia, and alterations in hemostasis (6). Studies done in Italy showed the main hematological
manifestations seen in patients with adenovirus and rhinovirus were neutrophilia and monocytosis respectively (7).

The main pathogenic mechanism of anemia is mediated by cytokines such as Tumor Necrosis Factor alpha (TNF) and Interleukin 1 (IL1). The mechanisms are shortened red cell survival, impaired bone marrow erythropoietin response and impaired iron mobilization and utilization. Alteration of white cell count is due to direct damage of hematopoietic precursors, splenic sequestration and mobilization of mature neutrophils faster than proliferation rate (6).

Studies done in India demonstrated that the most hematological manifestations are autoimmune hemolytic anemia, autoimmune thrombocytopenia, disseminated intravascular coagulation and hemophagocytic syndrome (8).

Raymond S. M. et-al performed a study on hematological manifestations of severe acute respiratory disease and the main hematological variables were lymphopenia and thrombocytopenia. Postmortem findings showed no features of bone marrow failure or Hemophagocytic syndrome. Dysmorphic megakaryocytes containing inclusion bodies, vacuoles, degenerated nuclei were seen in infected bone marrow. The postmortem findings of patients with thrombocytopenia with active bone marrow favored immune thrombocytopenia (9).

Dysfunction of the hematological organ system may contribute to multiple organ dysfunction and death. The knowledge of these hematological changes and pathophysiological changes is essential to improve outcomes in patients with SARI (3).
1.5 Assessment of Hematopoietic Abnormalities in Pediatric Autopsies

1.5.1 Bone Marrow Morphology in Bacterial Infections

Bone marrow abnormalities associated with respiratory infections include: quantitative changes in hematopoietic tissue, interstitial edema, megakaryocytes with or without dysplastic changes, necrosis, bone marrow hypoplasia, granulomas and hemophagocytic syndrome (10).

Severe bacterial infection causes an increase in bone marrow cellularity due increased granulocyte hyperplasia; there is an increase in immature granulocytes in relation to mature neutrophils. Megakaryopoiesis is increased and erythropoiesis is reduced. In chronic infection there is plasma cell satellitosis, with occasional cells containing Russell bodies. The macrophages may be seen ingesting granulocytes (10).

Mycobacterial infections may show granulocyte hyperplasia, perivascular plasmacytosis, caseating granulomas and or histiocytic aggregates and extensive necrosis. Acid fast stain reveals single, linear bacilli that stains red in color (10).

A study done in China on autopsies of three patients who died due to acute respiratory disease demonstrated extensive lung lesions with inflammatory infiltrate, mononuclear cells and desquamation of the epithelial cells. The bone marrow had decreased hematopoietic tissue, with a relative reduction in granulocyte and megakaryocyte lineages. There were also localized polychromatophilic erythroblasts (11).

1.5.2 Bone Marrow Morphology in Viral Infection

Viral infections increase bone marrow lymphocytes, plasma cells, and macrophages with or without hemophagocytosis. Morphological changes in viral infection are plasmacytoid lymphocytes and intranuclear inclusions (12). The myeloid erythroid ratio is increased. The
bone marrow features are giant pronormoblasts, which have a blue cytoplasm with an irregular cytoplasmic border, a nucleus with fine chromatin and large nucleoli.

The lymphocytic nuclei appear homogeneous with a central area and condensation of chromatin at the periphery of nuclei (lantern cells). The bone marrow is hypercellular with bizarre megakaryocytes and isolated histocytes (10).

Epstein Bar Virus (EBV) is a lymphotropic herpes virus that causes lymphocytosis and the bone marrow shows atypical lymphocytes with abundant azurophilic granules in the cytoplasm and eosinophilic viral inclusion bodies (10).

The hallmark of cytomegalovirus in the bone marrow is viral inclusions in the nuclei and cytoplasm of the infected cells. The nuclear inclusions are single, round to oval in shape, have a halo around them with margination and condensation of the nuclear chromatin. They appear dark blue or amphophilic with routine staining and react with PAS. Cytoplasmic inclusions are multiple, irregular in shape and basophilic with routine staining.

The bone marrow findings of HIV are hypercellular with granulocytic and erythroid hyperplasia. Megaloblastic erythroid changes, nuclear fragmentation, ring sideroblasts and decreased or absent iron stores. Micro or macro megakaryocytes, hypo segmented and hyperlobulated forms, large megakaryocytes with hyperchromatic nuclei and bizarre nuclei shaped are also seen. There are also increased plasma cells, macrophages as well hemophagocytosis (10).
1.5.3 Bone Marrow Morphology in Fungal Infections

Fungi have no specific hematological manifestations. The bone marrow reaction to fungal infection is formation of granuloma and necrosis. The fungi are found within the histiocytes or admixed within necrotic debri (10).

Candida albicans has non–branching pseudo hyphae and small budding yeast forms. Filaments have periodic constrictions seen. Neutrophilic inflammations with small lymphocytes and macrophages, fibrin, coagulative necrosis are features also seen (13). Cryptococcus is spherical to oval measuring 5-10 micrometers in diameter with thick capsule with narrow based unequal buds. There may be an inflammatory reaction surrounding it.

Histoplasma capsulatum is oval with narrow based buds and on H&E the basophilic cytoplasm is separated from the surrounding tissue by clear zone corresponding to the cell wall. Clustering of yeasts in histiocytic cells is a diagnostic feature. Scattered small granulomas with small yeasts in the parenchyma are also seen. Fungi are seen with PAS, GMS and mucicarmine stain (13).

1.6 Histiocytic Abnormalities in Childhood Severe Acute Respiratory Illness

The Histiocytic Hyperplastic Hemophagocytic Syndrome (HHPS) is a group of disorders that is found in children with severe acute respiratory illness and they cause proliferation of macrophages and dendritic cells. Classification is macrophage related, dendritic-related and Langerhans cell histiocytosis (most common). It is an inflammatory condition that is characterized by fever, cytopenia, hepatosplenomegaly, adenopathy and coagulopathy. The other findings are increased serum transaminases, low albumin, and increased serum ferritin levels (14).
The pathophysiology is dysregulation of T lymphocytes and production of cytokines. The diagnostic criteria for HHPS is high fever for more than one week, unexplained progressive cytopenia affecting two cell lineages - bone marrow showing mature histiocytes->3%, prominent hemophagocytosis, and or hemophagocytes in liver, spleen or lymph nodes. Infective organisms causing HS are *EBV*, *histoplasmosis*, *candida*, *Cryptococcus* and *pneumocystitis* (15) (6).

Ali et al described hemophagocytic lymphohistiocytosis (HLH) as a life threatening disease characterized by lymphocytic and hemophagocytic infiltration of vital organs and activation of the T lymphocytes and macrophages. HLH is classified as primary or secondary, however distinction is made through molecular techniques. Secondary HLH is caused through intense immunological activation of the immune system especially in the immunocompromised patients with viral infections (14).

Studies done on twenty four children with respiratory infections demonstrated bone marrow hyperplasia in 17 cases. Hemophagocytic histocytes were seen in fourteen cases, with increased plasma cells in three cases and dyserythropoietic changes in eight cases (16).

Studies were also done in Japan where seventy autopsies were performed to investigate the pathogenesis of HHH. The outcome was to determine the risk factors for HHH. It was found that HHH was associated with excessive inflammation especially in sepsis and hematological diseases. The bone marrow sample of these diseased patients was categorized in terms of activity of haemaphagocytosis through microscopic examination (17).

Mild HHH – >4 high power fields to view haemaphagocytosis.

Moderate HHH – 1-3 haemophagocytic cells per HPF.
Severe HHH-more than 3 haemophagocytic cells per HPF

Hypoplastic HHH - severe HHH with hypocellular bone marrow.

The complications of HHH are bone marrow failure and organ dysfunction which are fatal. This study will aim to diagnose HHH using hematoxylin and eosin staining in children dying due to SARI.

1.7 Association of Respiratory Infectious Agent to Bone Marrow Morphology

Studies done in South East Asia (India) and Africa (Nigeria) pointed to the main causative agents for SARI as being *Streptococcus pneumoniae* and *Haemophilus Influenza* type b; others included *Staphylococcus aureus, Klebsiella pneumonia*, and *Mycobacterium tuberculosis* (18).

The viral agents found were *Respiratory Syncytial virus* (15-40%), *Influenza A and B, par influenza, Human metapneumovirus* and *Adenovirus. Pneumocystis carini* is common in HIV patients. Other organisms causing pneumonia were *Mycoplasma pneumoniae, Chlamydia pneumoniae, Pseudomonas* species, *Escherichia coli, influenza, Histoplasma* and *Toxoplasma* (18).

The bone marrow features depend on the infectious agent. Viral infections cause reduced megakaryopoesis, left shift granulopoeisis and maturation arrest, lymphocytosis and a hypercellular marrow. Bacterial infections cause granulocytic hyperplasia, increased megakaryopoesis, reduced erythopoiesis and a hypercellular bone marrow. Fungal infections cause granuloma formation, inflammatory reaction and a hypercellular bone marrow (10).

The respiratory infectious agents were compared to the bone marrow morphology to correlate if there were any associations between the respiratory infectious agent and bone marrow infectious agent.
2.0 JUSTIFICATION

Respiratory infections are a major cause of mortality in Africa and account for 46% of all under five mortalities. In Kenya the mortality of severe acute respiratory illness has reduced from 75% to 46% but it has not been sufficient to reach Millennium Developmental Goals (5). Clinical management of respiratory tract infections and hematological comorbidities appear neglected yet important. The aim of this study was to provide information to medical personnel on the anticipated hematological complications that would arise from severe acute respiratory illness. Provision of relevant interventions may reduce the mortalities and morbidities that may arise in children with severe acute respiratory illness (5).

HHH is a syndrome that may be fatal and yet it is underdiagnosed. It is usually associated with severe acute respiratory illness therefore examination of histiocytic abnormalities in children dying due to severe acute respiratory illness would provide insight on the management of hematological complications such as pancytopenia and hypofibrinogenemia.

Postmortem examination of the hematopoietic abnormalities seen in children dying due to acute respiratory infections was to provide pathological co-relation to hematological findings this would provide a better understanding of the pathophysiology of this illness and reduce multiple organ dysfunction associated with SARI.

Severe acute respiratory illness occurs due to different infectious agents. This study aimed to correlate the respiratory infectious agent to the bone marrow morphology. Morbidities such as multiple organ failure, bleeding and sepsis are higher in patients with viral associated HHH as opposed to other infections this information would help in the anticipation of any complications that may arise from the existing infection.
3.0 RESEARCH QUESTION

What are the morphological bone marrow features in children dying due to severe acute respiratory illness at Kenyatta National Hospital?

3.1 BROAD OBJECTIVE

To describe the morphology of bone marrow in children dying due to severe acute respiratory illness at Kenyatta National Hospital.

3.2 SPECIFIC OBJECTIVES.

1) To describe the hematopoetic characteristics of bone marrow in children dying due to acute respiratory illness.

2) To describe histiocytic characteristics of bone marrow in children dying due to acute respiratory illness.

3) To correlate the bone marrow morphological findings in children dying due to acute respiratory illness with the infectious agent.
4.0 METHODOLOGY

4.1 Study Design

A cross sectional descriptive study design was adopted to investigate the morphology of bone marrow in children dying due to acute respiratory illness in Kenyatta National Hospital that occurred in August 2014 and December 2015.

4.2 Study Site

This study was conducted in Kenyatta National Hospital (KNH) which is the national referral and primary teaching hospital for the University of Nairobi. It contains four pediatric wards with approximately 60 beds per ward. The hospital has over 14,000 pediatric admissions annually. It also has a mortuary which has a capacity of storing up to one hundred and thirty six bodies.

4.3 Study Duration

The study was conducted from August 2014- December 2015.

4.4 Study Population

The study population comprised of patients aged 1-59 months who died due to SARI at Kenyatta National Hospital between August, 2014 and December, 2015.

4.4.1 Case Definition

SARI case was defined as a patient with:

- History of fever >38.0°C
- History of cough
– Onset of symptoms within 14 days

4.4.2 Inclusion Criteria

Archived bone marrow Formalin Fixed Paraffin Embedded specimens obtained from SARI autopsies

4.4.3 Exclusion Criteria

Specimens unsatisfactory for evaluation

4.5 Sample Size Calculation

Sample size was calculated using the Daniel’s formula (1999) for finite population

\[ n \geq \frac{NZ^2_{\alpha/2}P(1 - P)}{d^2(N - 1) + Z^2_{\alpha/2}P(1 - P)} \]

Where:

n= minimum sample size required

N=Total estimated accessible population (N=100)

\[ Z_{\alpha/2} = \text{Critical value for standard normal distribution at } \alpha\text{-level of significance (} \alpha=0.05, \ Z_{\alpha/2}=1.96 \]

P=Estimated prevalence (proportion pediatric bone marrow abnormalities among children under 5 with SARI in Kenya P=0.5

\[ d=\text{Margin of error (} d=0.08 \]

\[ n = \frac{100 \times 1.96^2 \times 0.39 \times (1 - 0.39)}{0.08^2 (100 - 1) + 1.96^2 \times 0.39 \times (1 - 0.39)} = 59.06, \text{rounded up to } 60 \text{ samples} \]

The minimum required sample size was 59 (19)
**Sampling Method**

The ideal sampling method would be simple random sampling but due to the few numbers all the available number of bone biopsies of children who died due to SARI at the KNH between August 2014 and December 2015 were done.

**4.6 Methodology**

Clinical data was abstracted from the files for all pediatric autopsies who met the SARI case definition obtained from August 2014 - December 2015 in KNH. The bodies were transported from the ward and taken to the mortuary for preservation. Bone biopsies and open lung tissue biopsies were collected. Trucut lung biopsies were tested using Real time PCR for respiratory pathogens (*Influenza A, Influenza B, adenovirus, RSV, parainfluenza1-3, human metapneumovirus, Streptococcus.pneumoniae and tuberculosis*). The bone biopsy was collected from the second rib at the level of costocondral junction where the whole rib was taken and cut into smaller sections and fixed in 10% neutral formalin. Decalcification was not done. Formalin fixed paraffin embedded wax blocks was retrieved. Sections were cut not more than 3-4 micrometers thick. Six sections were cut at three levels 25%, 50% and 75% into the cross-sectional diameter of the core and serial sections mounted stepwise onto glass slides (refer Appendix V). The slides were stained with hematoxylin and eosin, MGG for staining hematopoetic cells, Perl’s’ Prussian for iron stores, PAS for granulocytic series and ZN stain for acid fast bacilli refer to Appendix VI, VII, VIII, IX and X). The following features were reported based on the slides assessment:

- Adequacy of bone trabecular and overall cellularity
- Status of erythropoiesis
- Activity and pattern of granulopoiesis
- Status of megakaryocytes and activity
- Plasma cells, lymphocytes, histiocytes, and presence of malignant cells
- Presence of infection
- Presence of hemophagocytosis that was graded as mild, moderate and severe
- A concluding diagnosis or statement was made.

Microscopy and reporting was co-related with ante mortem blood picture (refer to Appendix XI)

**STUDY PROCEDURE**

- **IDENTIFICATION OF SARI CASES**
- **RETRIVAL OF CLINICAL AND DEMOGRAPHIC DATA**
- **RETRIVAL OF SPECIMEN BLOCKS**
- **MICROSCOPIC ANALYSIS**
- **SPECIMEN BLOCK PROCESSING**
- **DATA ENTRY INTO PROFORMA**
- **STORE RECORDS**

Figure 1: Methodology Flow Chart
4.7 Quality Assurance

4.7.1 Pre-Analytical Stage

Pathology reports and specimen blocks were checked to confirm whether they corresponded to each other. Blocks and slides were properly labeled.

4.7.2 Analytical Stage

The slides were examined initially by the PI and findings confirmed by two consultant pathologists.

4.7.3 Post-Analytical Stage

Data was reported and entered into the data collection form.

4.8 Data Collection Instrument

The data was collected using a predesigned proforma.

4.9 Data Management and Analysis

Clinical and demographic Data was abstracted from patients’ files and recorded in the bone marrow biopsy data collection tool. Data was stored in Microsoft Access database. Data coding, cleaning and analysis were done using statistics and data version 13

Descriptive statistics was done to explore and summarize the variables; for categorical variables, frequencies and proportions were reported in tables. For numeric (continuous/discrete) variables such as age and total blood count histograms and dot plots were plotted to shows distributions, means (standard deviations) or medians (interquartile range) were computed and presented in tables.
Bivariate analysis was used to determine associations (chi square tests) or correlation (Spearman correlation) between variables at α level of significance 0.05. The test statistics and corresponding p-values were reported. Microphotographs were used to show some of the histomorphological findings.

4.10 Ethical Consideration

Prior to performing the study the Principal Investigator from the main PRESS study had sought approval from Kenya Medical Research Institute Ethical Review Committee (see Appendix II). The principal investigator of the PRESS study obtained an informed consent from the guardian for the larger study which covered this particular study; therefore there was no need for obtaining consent for this aspect of the study. (Appendix I)

Permission to conduct this study was sought from Kenyatta National Hospital and University of Nairobi Ethical Research committee (KNH/UON-ERC) and principal investigator of PRESS (Appendix III) The study was undertaken after formal approval. (Appendix IV)

Strict confidentiality was observed throughout the study period held in trust by the participating investigators, research staff and study institutions.
5.0 STUDY RESULTS

5.1 Demographic Characteristics of SARI Cases

Bone marrow tissues were obtained from a total of 59 children who died due to severe acute respiratory illness. The children were aged between 1 and 48 months. The distribution showed a right skew with a median of 8 months, IQR of 4-12 months.

The females were (54%) n=32 and males were 45.8% (n=27). The female to male ratio was 1.18.

Figure 2: Children’s Age in Months
5.2 Comorbidities of Children with SARI

The main comorbidities in children with SARI were meningitis (10) cases, gastroenteritis (7) cases, malnutrition (7) cases and sepsis (7) cases.

Table 1: Comorbidities in Children with SARI

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Frequency (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Meningitis</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Gastroenteritis</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Malnutrition</td>
<td>7</td>
</tr>
<tr>
<td>Comorbidities</td>
<td>Sepsis</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Rickets</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Pneumocystis carinii</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Meningoencephalitis</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Down’s syndrome</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Pulmonary Hypertension</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Dehydration</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2: Etiological Agents in SARI

The main etiological agents were viral and bacterial seen in 32 and 18 patients respectively.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Frequency (x)</th>
<th>Proportion %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etiological agents</td>
<td>Viral</td>
<td>32</td>
<td>54.24</td>
</tr>
<tr>
<td>(n=59)</td>
<td>Bacterial</td>
<td>18</td>
<td>30.51</td>
</tr>
<tr>
<td></td>
<td>Fungal</td>
<td>9</td>
<td>15.25</td>
</tr>
<tr>
<td></td>
<td>Parasitic</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Protozoan</td>
<td>1</td>
<td>0.02</td>
</tr>
</tbody>
</table>

NB: Details of specific infectious agents are indicated in table 10

Duration of deaths of SARI cases

More than half (75.4%; n=43) of the children died within 4 days of hospital admission, with majority of the deaths occurring between day 0 (19.3%; n=11) and 1 (26.3%; n=15).
5.3 Total Blood Counts

Fifty four children had missing blood counts parameters. The most commonly reported parameter was the WBC counts and HB. Platelet counts and other parameters such as white cell differential counts and MCV were often not reported in the files.

Table 3: Summary Findings in Total Blood Count of Children with SARI

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median</th>
<th>IQR</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (n=42)</td>
<td>13.00</td>
<td>8.10</td>
<td>0.50</td>
<td>71.80</td>
</tr>
<tr>
<td>MCV (n=14)</td>
<td>76.10</td>
<td>17.9</td>
<td>50.1</td>
<td>89.30</td>
</tr>
<tr>
<td>Neutrophils (n=23)</td>
<td>7.00</td>
<td>25.50</td>
<td>0.35</td>
<td>88.00</td>
</tr>
<tr>
<td>Lymphocytes (n=23)</td>
<td>6.00</td>
<td>6.00</td>
<td>0.00</td>
<td>49.00</td>
</tr>
<tr>
<td>Monocytes (n=10)</td>
<td>1.41</td>
<td>1.78</td>
<td>0.08</td>
<td>37.00</td>
</tr>
<tr>
<td>Eosinophil (n=11)</td>
<td>0.02</td>
<td>0.11</td>
<td>0.00</td>
<td>1.21</td>
</tr>
<tr>
<td>Basophils (n=11)</td>
<td>0.19</td>
<td>0.35</td>
<td>0.01</td>
<td>1.24</td>
</tr>
<tr>
<td>Platelets (n=15)</td>
<td>222.00</td>
<td>183.00</td>
<td>48.00</td>
<td>613</td>
</tr>
</tbody>
</table>
### Hemoglobin (n=42)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>9.42</td>
<td>1.80</td>
<td>5.40</td>
<td>12.70</td>
</tr>
</tbody>
</table>

5.3.1 Dot Plots for HB

The total blood count in 42 patients showed a mean haemoglobin level of 9.42 and a standard deviation of 1.80. The lowest and highest levels were 5.4g/dl and 12.7g/dl.

![Figure 4: Hemoglobin dot plot](image-url)
5.3.2 Dot Plots for MCV

The lowest and highest MCV was 50.2L and 90FL respectively with median count of 76.10.

Figure 5: MCV dot plot
The distribution of white blood cell count was spread. Majority had a blood cell count below 30x10⁹/l. The Highest count was 71.80x10⁹/l, lowest was 0.50x10⁹/l and median of 13.

Figure 6: White Blood Cell Count Dot Plot
The distribution of Eosinophils and Monocytes was concentrated around the median whereas Basophils, Neutrophils and lymphocyte count was spread out.

Figure 7: Summary of White Blood Cell Count Parameters Dot Plot

5.4 Bone Marrow Morphology Findings

Fifty two (88.1%) cases were satisfactory for evaluation and 47 (90.4%) cases were well stained using H&E. Normal trabecular architecture was observed in 40 (76 %) cases and normal marrow architecture in 37 (71.2%) cases. Normal cellularity was observed in 35 (67.3%) cases. Hypercellularity was reported in 7 (13.5%) cases and hypocellularity in 10 (19.2%) cases.
Table 4: Summary of the Overall Bone Marrow Morphology Findings

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Frequency</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequacy (n=59)</td>
<td>Adequate</td>
<td>52</td>
<td>88.1</td>
</tr>
<tr>
<td></td>
<td>Inadequate</td>
<td>7</td>
<td>11.9</td>
</tr>
<tr>
<td>Staining quality (n=52)</td>
<td>Good</td>
<td>47</td>
<td>90.4</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>5</td>
<td>9.6</td>
</tr>
<tr>
<td>Trabecular bone Architecture (n=52)</td>
<td>Normal</td>
<td>40</td>
<td>76.7</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>12</td>
<td>23.3</td>
</tr>
<tr>
<td>Marrow architecture (n=52)</td>
<td>Normal</td>
<td>37</td>
<td>71.2</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>15</td>
<td>28.9</td>
</tr>
<tr>
<td>Overall cellularity (n=52)</td>
<td>Normocellularity</td>
<td>35</td>
<td>67.3</td>
</tr>
<tr>
<td></td>
<td>Hypercellularity</td>
<td>7</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>Hypocellularity</td>
<td>10</td>
<td>19.2</td>
</tr>
</tbody>
</table>

5.4.1 Hematopoietic Characteristics

*Erythropoiesis*

Erythropoiesis was increased in 16 cases (30%), preponderance of immature forms was present in 13 cases (25%) and dysplasia in 23 cases (44%). Dysplastic forms such as binucleate forms, trinucleate forms, mitotic forms, irregular nuclear membrane and fragmented forms were also seen.
Table 5: Summary of the Bone Marrow Erythropoiesis Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Frequency</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity</td>
<td>Normal</td>
<td>25</td>
<td>48.0</td>
</tr>
<tr>
<td>(n=52)</td>
<td>Increased</td>
<td>16</td>
<td>30.8</td>
</tr>
<tr>
<td></td>
<td>Reduced</td>
<td>11</td>
<td>21.2</td>
</tr>
<tr>
<td>Maturity</td>
<td>Normal</td>
<td>39</td>
<td>75.0</td>
</tr>
<tr>
<td>(n=52)</td>
<td>Preponderance to immature forms</td>
<td>13</td>
<td>25.0</td>
</tr>
<tr>
<td>Morphology</td>
<td>Normal</td>
<td>29</td>
<td>55.8</td>
</tr>
<tr>
<td>(n=52)</td>
<td>Abnormal</td>
<td>23</td>
<td>44.2</td>
</tr>
</tbody>
</table>

Granulopoeisis

Granulopoeisis was increased in 31/52 (59.6%) cases. Dysplastic features were observed in 14/52 (28.6%) cases. Left shifted granulopoeisis was reported in 38/52 (73.1%) cases.

Table 6: Summary of the Bone Marrow Granulopoeisis Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Frequency</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity</td>
<td>Normal</td>
<td>14</td>
<td>26.9</td>
</tr>
<tr>
<td>(n=52)</td>
<td>Increased</td>
<td>31</td>
<td>59.6</td>
</tr>
<tr>
<td></td>
<td>Reduced</td>
<td>7</td>
<td>13.5</td>
</tr>
<tr>
<td>Maturity</td>
<td>Normal</td>
<td>14</td>
<td>26.9</td>
</tr>
<tr>
<td>(n=52)</td>
<td>Left shift</td>
<td>38</td>
<td>73.1</td>
</tr>
<tr>
<td>Morphology</td>
<td>Normal</td>
<td>35</td>
<td>71.4</td>
</tr>
<tr>
<td>(n=49)</td>
<td>Abnormal</td>
<td>14</td>
<td>28.6</td>
</tr>
</tbody>
</table>

Megakaryopoeosis

Megakaryocytes were well represented in 40(76.9%) cases and normal morphology in 46(92.0%) cases. Dysplastic features such as hypolobulation (n=2) and juvenile forms (n=2) were seen in 4/52 (7.7%) biopsies.
Table 7: Summary of the Bone Marrow Megakaryopoiesis Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Frequency</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells</td>
<td>Normal</td>
<td>40</td>
<td>76.9</td>
</tr>
<tr>
<td>(n=52)</td>
<td>Increased</td>
<td>4</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>Reduced</td>
<td>8</td>
<td>15.4</td>
</tr>
<tr>
<td>Location</td>
<td>Interstitial</td>
<td>51</td>
<td>98.1</td>
</tr>
<tr>
<td>(n=52)</td>
<td>Paratrabecular</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>Morphology</td>
<td>Normal</td>
<td>48</td>
<td>92.3</td>
</tr>
<tr>
<td>(n=52)</td>
<td>Abnormal</td>
<td>4</td>
<td>7.7</td>
</tr>
</tbody>
</table>

**Lymphocytes**

Increased numbers were observed in 15/52 (28.9%) cases. Lymphoid aggregates were present in only 2/52 (3.9%) biopsies.

Table 8: Summary of the Bone Marrow Lymphocyte Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Frequency</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells</td>
<td>Normal</td>
<td>37</td>
<td>71.1</td>
</tr>
<tr>
<td>(n=52)</td>
<td>Increased</td>
<td>15</td>
<td>28.9</td>
</tr>
<tr>
<td>Distribution</td>
<td>Interstitial</td>
<td>49</td>
<td>94.2</td>
</tr>
<tr>
<td>(n=52)</td>
<td>Diffuse</td>
<td>3</td>
<td>5.8</td>
</tr>
<tr>
<td>Morphology</td>
<td>Normal</td>
<td>52</td>
<td>100</td>
</tr>
<tr>
<td>(n=52)</td>
<td>Lyphoid aggregate</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>51</td>
<td>98.1</td>
</tr>
</tbody>
</table>

**Plasma cells**

All the plasma cells had normal morphology. Increased plasma cells were reported in 5/52 (9.6%) biopsies.
5.4.2 Histiocytic Characteristics

Histiocytosis was observed in 14 (26.9%) cases and HHH present in 13 (25%) cases. Mild HHH was present in (6/52; 11.4%), moderate HHH in (2/52:3.9%) and severe HHH in (2/52 3.9%) cases. Bone marrow hypoplasia with HHH was seen in 3/52(5.8%) cases. Evidence of infection seen in the bone marrow were tuberculosis (n=1), malaria pigment (n=2) and parvovirus related changes (n=1). Fisher’s exact test was done to evaluate the relationship between type of infection and presence of HHH; the test showed that there was no significant association between type of infection and presence of HHH (P-value=0.274). None of the biopsies had foreign cells.

The Iron stores were absent in all the cases as expected. The other special stains that were done were MGG, reticulin and PAS and ZN stain. The MGG was done in all the cases but was not useful as it was not properly done. The reticulin stain showed an increase in two cases. The two cases were case no 27 and 44. The PAS stain was not evaluable in 42 cases because of poor processing.

Table 9: Summary of the Bone Marrow Histiocytic Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Frequency</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells</td>
<td>Normal</td>
<td>38</td>
<td>73.1</td>
</tr>
<tr>
<td>(n=52)</td>
<td>Increased</td>
<td>14</td>
<td>26.9</td>
</tr>
<tr>
<td>Grading (n=52)</td>
<td>Absent</td>
<td>39</td>
<td>75.0</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>6</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>2</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Hypo</td>
<td>3</td>
<td>5.8</td>
</tr>
<tr>
<td>Infection (n=48)</td>
<td>Absent</td>
<td>44</td>
<td>91.7</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>4</td>
<td>8.3</td>
</tr>
</tbody>
</table>
**Respiratory Etiological Agents**

The PRESS (Paediatric respiratory surveillance study) identified the most common viral agent was respiratory syncytial virus and influenza while the most common bacteria was Strep pneumonia and Klebsiella. (Unpublished data)

**Table 10: Summary of Viruses and Bacteria Causing Severe Acute Respiratory Illness (n=44)**

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Frequency</th>
<th>Bacteria</th>
<th>Viral (21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>9</td>
<td><em>Strep pneumoniae</em></td>
<td>5</td>
</tr>
<tr>
<td>Influenza</td>
<td>4</td>
<td><em>Klebsiella pneumonia</em></td>
<td>3</td>
</tr>
<tr>
<td>HMPV</td>
<td>4</td>
<td><em>E. coli</em></td>
<td>4</td>
</tr>
<tr>
<td>HIV</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>2</td>
<td>MTB</td>
<td>2</td>
</tr>
<tr>
<td>Para-influenza virus</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>2</td>
<td><em>Moraxella</em></td>
<td>1</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIAT</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV B19</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.4.3 Associated Bone Marrow Features to Respiratory Infections

There was no significant (p-values>0.05) difference in the distribution of hematopoetic and Histiocytic abnormalities among biopsies with history of bacterial and viral infections.

Table 11: Distribution of Hematopoietic and Histiocytic Characteristics by type of Infection

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Bacterial (n=13)</th>
<th>Viral (21)</th>
<th>Chi-square statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythropoiesis</td>
<td>Abnormal</td>
<td>10</td>
<td>15</td>
<td>0.125</td>
<td>0.724</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>3</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granulopoiesis</td>
<td>Abnormal</td>
<td>13</td>
<td>20</td>
<td>0.638</td>
<td>0.425</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Megakaryopoiesis</td>
<td>Abnormal</td>
<td>2</td>
<td>6</td>
<td>0.776</td>
<td>0.378</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>11</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Abnormal</td>
<td>6</td>
<td>7</td>
<td>0.559</td>
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</table>
6.0 HISTOMORPHOLOGICAL MICROPHOTOGRAPHS

6.1 Hematopoetic Morphology in Severe Acute Respiratory Illness

Figure 8: BMB section, arrows showing binucleate erythroid forms, and nuclear budding paraffin-embedded H&E X40
Figure 9: BMB section, arrow shows left shift in myeloid series, paraffin-embedded H&E X100
6.2 Histiocytic Morphology in Severe Acute Respiratory Illness
Figure 12: BMB section, arrows showing histiocytic cells with severe HHH, paraffin-embedded H&E X40

6.3 Infections Seen in Severe Acute Respiratory Illness

Figure 13: BMB section, figure a) shows Langhan giant cell and figure b) shows features of Langhan giant cell and granuloma, paraffin-embedded H&E X40, X10
Figure 14: BMB section, arrows showing histiocytes with malaria pigment, paraffin-embedded H&E X40

Figure 15: BMB section, arrow showing a giant cell, paraffin-embedded H&E X40
Figure 16: BMB section, arrow showing erythroid hypoplasia in parvovirus infection, paraffin-embedded H&E X40
7.0 DISCUSSION

This study was performed to describe the morphological changes in SARI. The specific objectives were to describe the hematological and histiocytic characteristics of the bone marrow and correlate the bone marrow morphology to the etiological infectious agent.

Fifty four children out of fifty nine had some missing blood count parameters. The total blood count showed a mean haemoglobin level of 9.42 and a standard deviation of 1.80. The white blood cell count had a median of 13.0 and IQR of 8.1. Parameters reported in the total blood count were neutrophils (n=23), eosinophils (n=11,) basophils (n=11) monocytes (n=10) and lymphocytes (n=23). There was no correlation between antemortem hematological findings and postmortem bone marrow findings. The possibility was due to release of neutrophils from the storage pool causing a decrease in the marrow neutrophils during acute infections.

Fifty two cases were satisfactory for evaluation while 7 cases were unsatisfactory. This can be attributed to processing. The fixative used to preserve the bone biopsy specimens was 10% neutral formalin. However, the use of Zenker’s solution would have enhanced the tinctorial effect of the giemsa and H&E stain. Fragmentation of bone was seen in forty cases. The reason for this was due to using blunt knives during bone biopsy collection. This would have been improved by use of tungsten knife blades. The benefits include durability, cost efficiency, provision of clean cuts and ability to section hard specimens like bone. The marrow cellularity was normal in 37 cases, while in seven cases the marrow was hypercellular. This can be attributed to infection.

Erythropoiesis was increased in 16 (30.8%) cases which was similar to a study by Costa et al that showed 22/77 (30%) neonatal patients had erythroid hyperplasia (20).
The main confounding factor affecting dyserthropoiesis in this study was degenerative postmortem changes. The postmortem interval time for this study was 1-7 days. Studies by Shatish showed bone marrow erythroid dysplasia in autopsies performed 16 hours after death. Mitigation by prompt fixation, prompt preservation at 1-6 degrees Celsius and performance of autopsies in less than 7 hours after death is essential for obtaining efficient results (21).

In this study erythroid dysplasia was seen in 23 (44%) cases. Merissa demonstrated 13/20 (66%) cases with erythroid dysplasia which was due to delayed fixation and post mortem artifact changes (22). Heerema reported 46/77 (59%) cases with erythroid dysplasia due to fetal hypoxia and degenerative postmortem changes (23). Merissa and Heerema showed that erythroid dysplasia was due to postmortem changes which was similar to this study. Studies by Wang showed dyserthropoiesis in bone biopsies stored at room temperature for up to 72 hours (24). Similarly, in this study storage temperature for up to 6hrs may have been a contributing factor to dyserthropoiesis observed in the marrow. The bodies remained at ambient room temperatures prior to collection from the wards, during transportation to the morgue and delays while awaiting storage.

There was granulocytic hyperplasia in 31 (59%) cases with a predominant left shift granulopoeisis which was similar to Schid et al which showed (34%) a hyperplastic granulopoeisis with a left shift granulopoeisis (25). The morphology of the granulocytic lineage was predominantly normal except in 14 cases where there were dysplastic forms seen in adenovirus, PJP, HIV and strep pneumonia. Megakaryopoesis was suppressed in four (15%) cases which was due to infection similar to studies by Edward, where 4/14 (17%) cases demonstrated reduced megakaryopoesis in neonatal autopsies with infection (26).
Lymphocytosis was seen in 15 (28%) cases both present in bacterial and viral infection this was similar to studies done by Korppi et al that showed a marked lymphocytosis in both mixed infections rather than viral infections alone (27). Histiocytes were increased in 14/52 (26.9%) comparable to studies done by Nilsson which showed that bone marrow haemaphagocytosis was seen in 33% of children (28). Studies by Janka also showed that a third of patients with severe acute respiratory illness showed increased hemophagocytosis in the bone marrow (29).

In this study, 13 (25%) cases had HHH studies performed at two different hospitals in India identified HHH in 35 of 107 deceased patients (32.7 %) and 102 of 230 autopsies (44.3 %) respectively (30) (31). This difference may be due to bacterial sepsis and recent blood transfusion which contributed significantly in developing HHH in these two hospitals. However, from this study there was no significant association between infection and HHH. In this study 25% of cases had HHH which was an important finding that has often been ignored or even unnoticed by hematopathologist. Even though bone marrow examination is not indicated in SARI cases, severe pancytopenia is a clinical feature of HHH. Other clinical features include: fever, lymphadenopathy and hepatosplenomegaly which if diagnosed and treated early may prevent bone marrow failure, multiple organ dysfunction and death.

Infections associated with HHH in this study were malaria (n=2) cases and tuberculosis (n=2) which was similar to Chandra et al where the infections associated with HHH were viral, tuberculosis, malaria (32). Although two patients had tuberculosis, the evidence of TB was seen in the bone marrow in one case while the other was seen in the lung. Evidence of other infections seen in the bone marrow were parvovirus related changes and malaria pigment.

Bain et al demonstrated that viral infections cause reduced megakaryopoiesis, left shift granulopoiesis, lymphocytosis and a hypercellular marrow. In addition, bacterial infections cause granulocytic hyperplasia, increased megakaryopoiesis, reduced erythropoiesis and a
hyper cellular bone marrow (10). In this study there was no correlation of bone marrow morphological findings to the etiological infectious agent.

In this study the morphology was well demonstrated by hematoxylin and eosin stain in 47 cases. All the cases had been poorly stained with MGG and appeared too dark. The reason of poor staining could have been due to poor quality stain, poor quality methanol, unfiltered giemsa stock and incorrectly stored giemsa stock. The reason for the dark staining was probably due to long duration of staining. These pitfalls seen in MGG staining made evaluation of certain infections such as fungal elements and examination of hematopoietic cells difficult. Giemsa staining of bone marrow trephine biopsies in the institution has not been optimum therefore standardization is necessary to improve the quality of staining. Technical aspects in preparation that should be looked at include: measuring accurate volumes of giemsa powder, the use of high grade methanol or glycerol, filtering the small quantities of giemsa stock before making the working solution and storage of giemsa solution in a dark amber bottle away from sunlight. Decreasing the duration time of staining and determination of the optimal staining for each new batch of giemsa stock is also required. Staining using automated methods remains standard for reproducibility (33).

All 59 cases had been stained with PAS however only ten cases were evaluable. This was due to poor staining of autopsy bone marrow specimens making it difficult to evaluate fungal elements and granulopoetic series in this study. Suggestions of improvement in staining of PAS involve developing an institutional standardized operating procedure (SOP). The technical aspects include: use of a shorter decalcification time with Gooding and Stewart’s media and the choice of Zenker’s fluid as a fixative which provides excellent tissue morphology in paraffin embedded blocks compared to neutral formalin (34).
8.0 STUDY LIMITATIONS

Many of the bone marrow biopsies were poorly processed, largely due to lack of an SOP in sample management. Hematological changes are time bound which was also a limiting factor in analysis of the bone marrow changes.

Identification of microorganisms was difficult since MGG and PAS were poorly stained. The use of immunohistochemistry would have enhanced identification of microorganisms but this was not done due to cost considerations. Clinical data on total blood count parameters were often missing from the files and were not documented, making it difficult to correlate the antemortem hematological findings to the post mortem bone marrow morphology.

9.0 CONCLUSIONS

The main changes observed in bone marrow were granulocytic hyperplasia (59.6%), erythroid hyperplasia (30%) and histiocytosis (26.9%). Dyplasias involving the erythroid (44%) and myeloid lineages (14%) were common features. Histiocytic hyperplastic hemophagocytosis (25%) was a significant finding in Kenyan SARI mortalities. There was no correlation of the bone marrow morphological findings to the infectious disease etiology.

10.0 RECOMMENDATIONS

Histiocytic hyperplastic Hemophagocytosis is a significant finding in acute respiratory illness and its clinical significance to infectious agent should be analyzed using immunohistochemical means of detection for infectious agents. Micro-organisms that are difficult to identify using routine or special stains can be identified using monoclonal antibodies. Improvement on MGG and PAS staining in autopsy bone marrow material should be a useful method for detection of micro-organisms and hematopoetic cells because it is cost effective in our setup.
Standardization of autopsy bone marrow trephine techniques needs to be performed such as timing of decalcification and use of tungsten blades. Children with acute respiratory illness should be well investigated for infection. Documentation in children with SARI should be well maintained, complete and easily accessible.
11.0 BIBLIOGRAPHY


APPENDICES

APPENDIX I: Consent Form Postmortem Study
Flesch-Kincaid readability level – 8.1

Consent form for postmortem specimens collection

<table>
<thead>
<tr>
<th>Today’s date</th>
<th>Decedents unique identification number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Name of decedent: 

Name next of kin: 

Filenum---*:

Receive our condolences on the death of (name). (Name) had an illness affecting his/her lungs which may have led to his/her death. Lung illnesses are one of the leading causes of sickness and death in our country. We would like to find out the cause of the lung illness in (name) that may have led to his/her death. This will enable us to know the leading germs that cause lung illnesses. This knowledge will then help us choose vaccines and other prevention and treatment options that might help us to avoid similar deaths in future.

What we will do

We will take some lung specimen using a needle and syringe. As is normally done in an autopsy we will open the chest and take samples of the lungs and other organs in the chest that may be damaged or have disease causing germs. These specimens will be sent to laboratories in KNH and KEMRI/CDC in Nairobi, and to CDC Laboratories in Atlanta, Georgia, USA for analysis so as to identify germs that may have led to your child’s illness and death. For each specimen, we will place a number that uniquely identifies the samples. This number is similar to the one on questionnaires that collected clinical data when the child was in the ward. In addition, we will collect some blood and test it for HIV and influenza.

We will let you know the results of tests done once they are ready and the cause of death.
**Benefit from being in this study:**

The study will provide no direct benefit to you. In general, the study will help us to learn more about causes of lung illnesses and bone marrow illness that lead to death in our country so that we can provide better care to prevent similar deaths in the future. If you would like we will call you and tell you what we think was the cause of death for your child. It may take up to six months for us to determine the final cause of death.

**Risks from being in this study:**

The body may have cuts on the chest and abdomen which will be sewed together after the post mortem.

**How will the deceased information be protected?**

No names shall appear on samples collected. Instead, numbers will be used to identify the samples. Most of the samples collected will be tested here in the hospital and at KEMRI/CDC laboratory. However, for some tests that cannot be done here, part of the samples may be sent to special laboratories in CDC in the United States.

All the study records will be kept secretly and securely. There will be people involved in the study who will need to see the deceased’s health records. These people may include members of the study team, the study monitors, and members of the ethics committees that oversee the study. In addition, the information collected about the deceased will be shared with our data team, who are located at KEMRI/CDC offices in Nairobi. The information shared with the data team will not contain names or any other personal identifying information. Reports and publications from this study will not contain decedent’s name or any other personal identifying information.

**What happens if I do not want a post mortem for the deceased?**

You can choose not to have the deceased participate in this study. The body will still receive the usual care at the mortuary.
Will it cost anything?

It will not cost you anything to have the deceased participate in this study. We will meet the post-mortem costs, costs of any laboratory tests done and mortuary fees for a period of up to 5 days since death.

Who do I call if I have questions or problems?

- If you have questions or complaints about this study, you can call the principal investigator, Henry Njuguna, at phone#0724256803
- If you have questions about rights as a study participant, call the Ethical Review Committee for Research in Human Subjects. You should contact the ethics committee if you feel you have not been treated fairly or if you have other concerns. The ethics committee contact information is: 0722205981

What does your signature (or initials/mark) on this consent form mean?

Your signature (or initials/mark) on this form means:

- You have been informed about this study’s purpose, procedures, possible benefits and risks.
- You have been given the chance to ask questions before you sign.
- You have voluntarily agreed to have the deceased body to be used in this study.

Please indicate Yes or No:

I agree to have the deceased body be used in this study

☐ Yes  ☐ No

I agree to allow the deceased medical records to be reviewed by study staff, ethics committee members, and legal authorities:

☐ Yes  ☐ No

I agree to allow specimens from the deceased to be stored and possibly used after this study is over to test for things related to respiratory disease:
☐ Yes  ☐ No

I agree to specimens and data being sent outside the country for research:

☐ Yes  ☐ No

Consent Agreement:

I have read or had the consent form read to me and had a chance to ask questions. I agree to have the deceased body to take part in the study. I understand that I am free to choose not to have his/her body take part in this study and that saying “NO” will have no effect on post-mortem care.

<table>
<thead>
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<th>Next of kin</th>
<th>Name:</th>
<th>Signature:</th>
<th>Date__/<strong>/</strong></th>
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<tr>
<td>Witness*</td>
<td>Name:</td>
<td>Signature:</td>
<td>Date__/<strong>/</strong></td>
</tr>
</tbody>
</table>

*Next of kin can sign or make a mark and have his/her consent confirmed by the signature of a witness
APPENDIX II: Approval Letter from KEMRI CDC ERC

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 64840-0030, NAIROBI, Kenya
Tel (254) 0202-272244, 271339, 0722-268539, 0722-269031; Fax (254) 0202-272239
E-mail: director@kemri.org | info@kemri.org | Website: www.kemri.org

KEMRI/RES/7/3/1

January 24, 2014

TO: DR. HENRY NJENGA NJUGUNA (PRINCIPAL INVESTIGATOR)

THROUGH: DR. STEPHEN MUNGA,
ACTING DIRECTOR, CGHR,
KISUMU

Dear Sir,

RE: SSC PROTOCOL NO. 2692 (RESUBMISSION): ETIOLOGY OF PEDIATRIC RESPIRATORY MORTALITY AT KENYATTA NATIONAL HOSPITAL, NAIROBI, KENYA

Reference is made to your letter dated 16th January, 2014. The ERC Secretariat acknowledges receipt of the revised document on 22nd January, 2014.

This is to inform you that the Ethics Review Committee (ERC) reviewed the document submitted, and is satisfied that the issues raised at the 22nd meeting of 26th November 2013, have been adequately addressed.

This study is granted approval implementation effective this January 24, 2014. Please note that authorization to conduct this study will automatically expire on January 23, 2015. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the ERC secretariat by December 12, 2014.

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the SSC and ERC for review prior to initiation.

You may embark on the study.

Yours faithfully,

DR. ELIZABETH BUKUSSI,
ACTING SECRETARY,
KEMRI/ETHICS REVIEW COMMITTEE

In Search of Better Health
Hello Lilian,

Sorry for the delayed response. I am out of country with intermittent access to email.

Once you've made the revisions, you can go ahead and submit it for ethical clearance. You can print this email as an acknowledgement that you can use data collected as part of the PRESS study for your project.

Regards,

Henry
APPENDIX IV: Approval from ERC-UON

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19576 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44455

Ref: KNH-ERC/A388

Dr. Lilian Kerubo Bosire
Reg.No.H58/69955/2013
Dept. of Human Pathology
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Bosire

REVISED RESEARCH PROPOSAL - BONE MARROW MORPHOLOGY IN PEDIATRIC MORTALITIES ASSOCIATED WITH SEVERE ACUTE RESPIRATORY ILLNESS AT KENYATTA NATIONAL HOSPITAL (P/599/01/2016)

This is to inform you that the KNH-UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and approved your above revised proposal. The approval period is from 3rd October 2016 – 2nd October 2017.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.

b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.

c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.

d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH-UoN ERC within 72 hours.

e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period.

(f) Clearance for export of biological specimens must be obtained from KNH-UoN ERC for each batch of shipment.

g) Submission of an executive summary report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH-UoN ERC website http://www.erc.uonbi.ac.ke

Scanned by CamScanner
APPENDIX V: Sectioning of Blocks

1. Release the brake and rotate the hand wheel until the handle is at 1 o’clock position and re-apply the brake.
2. Push the quick release lever of the cassette clamp backward, insert the cassette clamp backward, insert the cassette, release the lever and check that the cassette is firmly clamped.
3. Use the vertical and horizontal tilt controls to orientate the specimen correctly with the knife edge and lock the orientation head.
4. Release the brake and turn the coarse advance knob clockwise and anticlockwise to bring the tissue block closer or away from the cutting edge.
5. Trim the block using the coarse advance knob until the full face is attained.
6. Set the section thickness with thickness control knob.
7. Turn the hand wheel to cut the sections.
8. Pick the sections and float in warm water to remove the creases.
9. Fish the sections and mount on clean microscope slides. Label the slides with a lead pencil or diamond pencil.
10. Put the slides in a hot air oven at 56c for 1hour. Remove the slides and stain.
APPENDIX VI: Haematoxylin and Eosin Staining Preparation

Reagents

1. Eosin 1% aqueous solution

   Eosin 10g distilled water- 1litres

2. Harris-haematoxylin solution

   Haematoxylin-5g
   Ethyl alcohol-50ml
   Ammonium alum-100g
   Distilled water-1 litre
   Mercuric oxide red 2.5g

3. Scotts tap water

   Na hydrogen carbonate-3.5g
   MgSo4 -20g
   Distilled water-1 litre
   Acid alcohol
   -0.5%Hcl in 70% alcohol

Procedure for staining

1. Dissolve the ammonium aluminium in distilled water heat, stirring frequently.
2. Dissolve the haematoxylin in the alcohol and add to aluminium solution.
3. Bring to the boil while stirring.
4. Mix and allow cooling.
5. Filter into a glass stain bottle and the solution is ready for use.
6. De-wax sections with two changes of xylene.
7. Re-hydrate sections with two changes of absolute alcohol and wash in running tap water.
8. Stain with haematoxylin sol for up to 5 minutes.
9. Wash in running tap water.
10. Differentiate in acid alcohol for approximately 5 minutes.
11. Wash in running tap water.
12. Blue in Scotts tap water for few seconds.
13. Wash in running tap water.
14. Stain with eosin for approximately for 5 minutes.
15. Wash in running tap water.
16. Dehydrate, clear and mount section.
APPENDIX VII: May Grunwald Giemsa

Reagents

1. May Grunwald stain
2. Giemsa stain
3. Phosphate buffer ph. 6.8
4. Tap water

Procedure for preparing stain

Prepare working stain solutions by: Mixing equal parts of the 0.3% MGG by dissolving 0.3g of MGG in 100mls of absolute ethanol.

Warm in water bath at 50 degrees Celsius for 30 minutes then 20 degrees Celsius for 30 minutes with equal parts of PH of 6.8

1. Mix one part commercially available Giemsa with nine parts of P.H 6.8 buffered water in the second staining jar.
2. Label the slide.
3. Fix with Methanol for 10 minutes.
4. Transfer it into a jar of MGG working solution for 15 minutes.
5. Transfer into a jar of Giemsa stain working solution for 15 minutes.
6. Wash in three successive buffered water changes for 5 dips.
7. Allow to stand for three minutes.
8. Dry slide and examine under the microscope.

Quality control

- Changing of the working staining solution and the buffer washes every start of a shift.
- Ensure that water isn’t mixed with ethanol.
APPENDIX VIII: Zien Neelsens Stain Preparation

Reagents

Carbolfuschin 1L

Sulphuric acid 1L

Malachite green 1L

Procedure for preparing stain

1. Bring section to water.
2. Cover the section with a piece of filter paper.
3. Flood the section with filtered carbolfuschin for 15 minutes.
4. Remove the filter paper and wash well in tap water.
5. Decolorize with 20% sulphuric acid for 3 minutes and rinse in water.
6. See if the sections are pale pink.
7. Repeat step 5 and 6 until the desired picture is obtained.
8. Counter stain with methylene blue or malachite green for 2-3 minutes
9. Wash in water.
10. Dehydrate in 3 changes of Ethanol.
11. Clear in 3 changes of xylene.
12. Mount in DPX.

Results:

Nuclei-blue

AAFB- bright red

RBC-pale pink
APPENDIX IX: Periodic Acid Schiff’s Stain Preparation

Reagents

Periodic acid 1L

Schiff’s 1L

Procedure for preparing stain

1. Bring section to water.
2. Oxidize for 10 minutes in 1% periodic acid.
3. Wash in running tap water for 5 minutes.
4. Rinse in distilled water.
5. Place in Schiff’s reagent for 15 minutes or until section turns magenta colour.
6. Rinse in three changes of freshly prepared 0.5% sodium metabisulphite.
7. Wash in running tap water for 10 minutes.
8. Stain in alum- haematoxylin for 6-7 minutes.
9. Differentiate in 1% acid alcohol-3 dips.
10. Blue in running tap water for 10 minutes or Scott’s tap water for 1 minute.
11. Counter stain in 0.3% tartrazin in cellosolve for 3 minutes.
12. Dehydrate in absolute ethanol, clear in xylene and mount in DPX.

Results:

Nuclei-blue

Cytoplasm-yellow

Positive controls –red or magenta
APPENDIX X: Iron Stain Preparation

Reagents

Xylene

Absolute alcohol

Neutral red

4% potassium Ferocynide

4% HCL

Procedure for preparing stain

1. Bring section to water

2. Flood section with a mixture of equal parts of 4% potassium Ferocynide and 4% HCL for 15 minutes

3. Rinse in distilled water

4. Counter stain with 0.5% neutral red for 30 seconds

5. Rinse in distilled water

6. Dehydrate in 3 changes of absolute alcohol

7. Clear in 3 changes of xylene and mount in DPX

Results

Nuclei – red

Ferric iron- blue

Positive control – splenic autopsy
APPENDIX XI: Bone Biopsy Proforma

SECTION A

1. Age (months) .............................................................................................................................................

2. Sex: ............................................................................................................................................................

3. Clinical history: .............................................................................................................................................

4. Date of Death: ................................................................................................................................................

5. Date of Total Blood Count: ...........................................................................................................................

6. Results of Total Blood Count:

<table>
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<tr>
<th>Results of TBC</th>
<th>HB- in g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td></td>
<td>MCV in FL</td>
</tr>
<tr>
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<td>NEUTROPHILS-in x10^9/L</td>
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<tr>
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<td>LYMPHOCYTES-in x10^9/L</td>
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<tr>
<td></td>
<td>EOSINOPHILS-in x10^9/L</td>
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<tr>
<td></td>
<td>BASOPHILS-in x10^9/L</td>
</tr>
<tr>
<td></td>
<td>PLATELETS-in x10^9/L</td>
</tr>
</tbody>
</table>

7. Date of Specimen Collection: ................................................................................................................................................

8. Block Number: ..................................................................................................................................................

9. Slide Number: ..................................................................................................................................................

SECTION B

1. Adequacy:

   Adequate □

   Inadequate □
2. Staining quality

- Good
- Poor

3. Trabecular Bone Architecture:

- Osteoblastic activity
- Osteoclastic activity
- Osteonecrosis
- Osteopenia
- New lamellar bone
- Normal

5. Marrow architecture

- Normal
- Abnormal (if present describe)

5. Overall Cellularity:

- Hypercellular
- Normocellular
SECTION C

1. Erythropoiesis

   a) Cellularity
      - Please Tick One
      - Increased
      - Normal
      - Reduced
      - Absent

   b) Maturity
      - Normal
      - Left shift (if present describe)

   c) Morphology
      - Normal
      - Abnormal (if present describe)

2. Granulopoiesis

   a) Cellularity
      - Reduced
      - Normal
      - Increased
      - Absent

   b) Maturity
Normal □
Left shift □
Maturation arrest □
c) Morphology
Normal □
Abnormal (if present describe) □

3. Megakaryopoiesis
a) Number
Increased □
Normal □
Reduced □
Absent □
b) Morphology
Normal □
Abnormal (if present describe) □
c) Location
Interstitial □
Paratrabecular □
d) Distribution
Clustering □
Discrete □
4. Lymphocytes

a) Number

- Increased
- Normal
- Reduced
- Absent

b) Distribution

- Interstitial
- Paratrabecular
- Nodular
- Diffuse

c) Lymphoid aggregate

- Present
- Absent

d) Morphology

- Normal
- Abnormal (if present describe)

5. Plasma cells

a) Number

- Increased
- Normal
- Decreased

b) Morphology
### SECTION D

1. Histocytes
   a) Number
      - Increased
      - Normal
      - Reduced
      - Absent
   b) Grading (HHH)
      - Mild
      - Moderate
      - Severe
      - Hypo
      - Absent
   c) Infections
      - Absent
      - Present (if present describe)
   d) Foreign cells
e) Iron stores

- Reduced
- Absent
- Normal
- Increased

f) Special stains

- Present (if present describe)
- Not applicable

Concluding statement:

Signature of Consultant /Student:

Date of Report:
## APPENDIX XII: Dummy Tables of Analysis

### Descriptive Analysis Table

<table>
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<tr>
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<tr>
<td><strong>Total blood count parameters</strong></td>
<td><strong>Mean (sd) or median (iqr)</strong></td>
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<td>Adequacy</td>
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<td>Frequency (%)</td>
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</table>

**Morphology**
- Normal
- Abnormal

**Histiocytic Characteristics**
- Number
- Increased
- Normal
- Reduced
- Absent

**Grading (HHH)**
- Mild
- Moderate
- Severe
- Hypo
- Absent

**Foreign cells**
- Present
- Absent

**Iron stores**
- Reduced
- Absent
- Normal
- Increased

**Bivariate Analysis Table**

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<td>Freq (%)</td>
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APPENDIX XIII: Plagiarism Report

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### PRIMARY SOURCES

1. www.fmshk.com.hk
   - Internet Source
   - 1%

   - Internet Source
   - 1%

3. Submitted to Aga Khan University
   - Student Paper
   - 1%

   - Publication
   - <1%

   - Publication
   - <1%

   - <1%