A retrospective review of the prevalence and management of anaemia in children in at Red Cross War Memorial Children's Hospital

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Declaration

I, Martie Wege, hereby declare that the work on which this research project is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Signature: Signed by candidate

Date: 28/9/2015

List of Abbreviations

FBC: Full blood count

Hb: Haemoglobin

HIV: Human immunodeficiency virus

IDA: Iron deficiency anaemia

ID: Iron deficiency

IMCI: Integrated Management of Childhood Illness

MCV: Mean corpuscular volume

MEU: Medical Emergency Unit

MOPD: Medical outpatient department

NHLS: National Health Laboratory Services

PEM: Protein energy malnutrition

PICU: Paediatric intensive care unit

RBC: Red Blood Cells

RCH: Red Cross War Memorial Children's Hospital

RDW: Red cell distribution width

SAVACG: South African Vitamin A Consultation Group

SD: Standard deviation

SOPD: Surgical outpatient department

SSW: Short stay ward

UCT: University of Cape Town

UNICEF: United Nations Children's Fund

WHO: World Health Organization

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Chapter 1: Introduction and Literature review

1.1 Aim and objectives of the research project

1. To determine the prevalence of anaemia in children 6-36 months of age that visited the medical emergency unit (MEU) or medical outpatients department (MOPD) at RCH in Cape Town and a full blood count (FBC) was part of their special investigations.
2. To describe the morphology of anaemia in this group of patients.
3. To determine and describe the management of anaemia of the children in this study group.

1.2 Aim and objectives of literature review

The literature review focused on anaemia in children. The main objectives were to establish the reported prevalence of anaemia in the age group 6-36 month of age with a specific focus on children presenting to a hospital with acute medical problems excluding malaria, haemoglobinopathies, malignancies or aplastic anaemia.

1.3 Methodology of the literature review

A structured literature review was first performed on the 15th of February 2012 and repeated on the 13th of January 2015. The literature search was done in PUBMED (www.ncbi.nlm.nih.gov) using both text and MeSH terms.

The following terms and search filters were used

Search 1: Anaemia AND prevalence

("anaemia"[All Fields] OR "anemia"[MeSH Terms] OR "anemia"[All Fields]) AND ("epidemiology"[Subheading] OR "epidemiology"[All Fields] OR "prevalence"[All Fields] OR "prevalence"[MeSH Terms])

Search 2: Anaemia AND hospital AND children NOT malaria NOT sickle cell disease

("anaemia"[All Fields] OR "anemia"[MeSH Terms] OR "anemia"[All Fields]) AND ("hospitals"[MeSH Terms] OR "hospitals"[All Fields] OR "hospital"[All Fields]) AND ("child"[MeSH Terms] OR "child"[All Fields] OR "children"[All Fields]) NOT ("anemia, sickle cell"[MeSH Terms] OR ("anemia"[All Fields] AND "sickle"[All Fields] AND "cell"[All Fields]) OR "sickle cell anemia"[All Fields] OR ("sickle"[All Fields] AND "cell"[All Fields] AND "disease"[All Fields]) OR "sickle cell disease"[All Fields]) NOT ("malaria"[MeSH Terms])

Search 3: Anaemia AND South Africa

("anaemia"[All Fields] OR "anemia"[MeSH Terms] OR "anemia"[All Fields]) AND ("south africa"[MeSH Terms] OR ("south"[All Fields] AND "africa"[All Fields]) OR "south africa"[All Fields])

Search 4: Breastfeeding AND iron deficiency

("breast feeding"[MeSH Terms] OR ("breast"[All Fields] AND "feeding"[All Fields]) OR "breast feeding"[All Fields] OR "breastfeeding"[All Fields]) AND (("iron"[MeSH Terms] OR "iron"[All Fields]) AND ("deficiency"[Subheading] OR "deficiency"[All fields]

The results of the search were limited to human studies reported in the English language. Age limitations were customized for infants: 1-23 months and preschool children: 2-5years of age.

Abstracts from the articles were reviewed. Abstracts that focused only on anaemia caused specifically by malaria, haemoglobinopathies/haemolytic anaemia, aplastic anaemia, oncological diseases and chronic renal failure were excluded.

Studies that focused on anaemia in children with Human immunodeficiency virus (HIV) infection and abstracts that included studies on children more than three years old, were also excluded. All clinical trials and review articles were included in the search.

1.4 Results

The search yielded a total of 637 articles. Reference lists and related citations of identified articles were scanned for other relevant literature. By using these search strings, there were some overlap in the articles found but all the abstracts were reviewed and duplicates excluded. Two studies included patients with possible malaria but the aim of the study was not on malaria but on the accuracy of pallor in the diagnosis of anaemia. Another study done on children with high incidence of malaria looked at anaemia in African children presenting to hospital with severe febrile illness. As the focus of our study was children presenting to hospital with anaemia as well as the accuracy of clinical assessment of anaemia, these two studies were included. (See figure 1)

None of the articles reviewed totally mirrored the aim of our research project.

Paediatric textbooks were also consulted in the writing of this literature review.

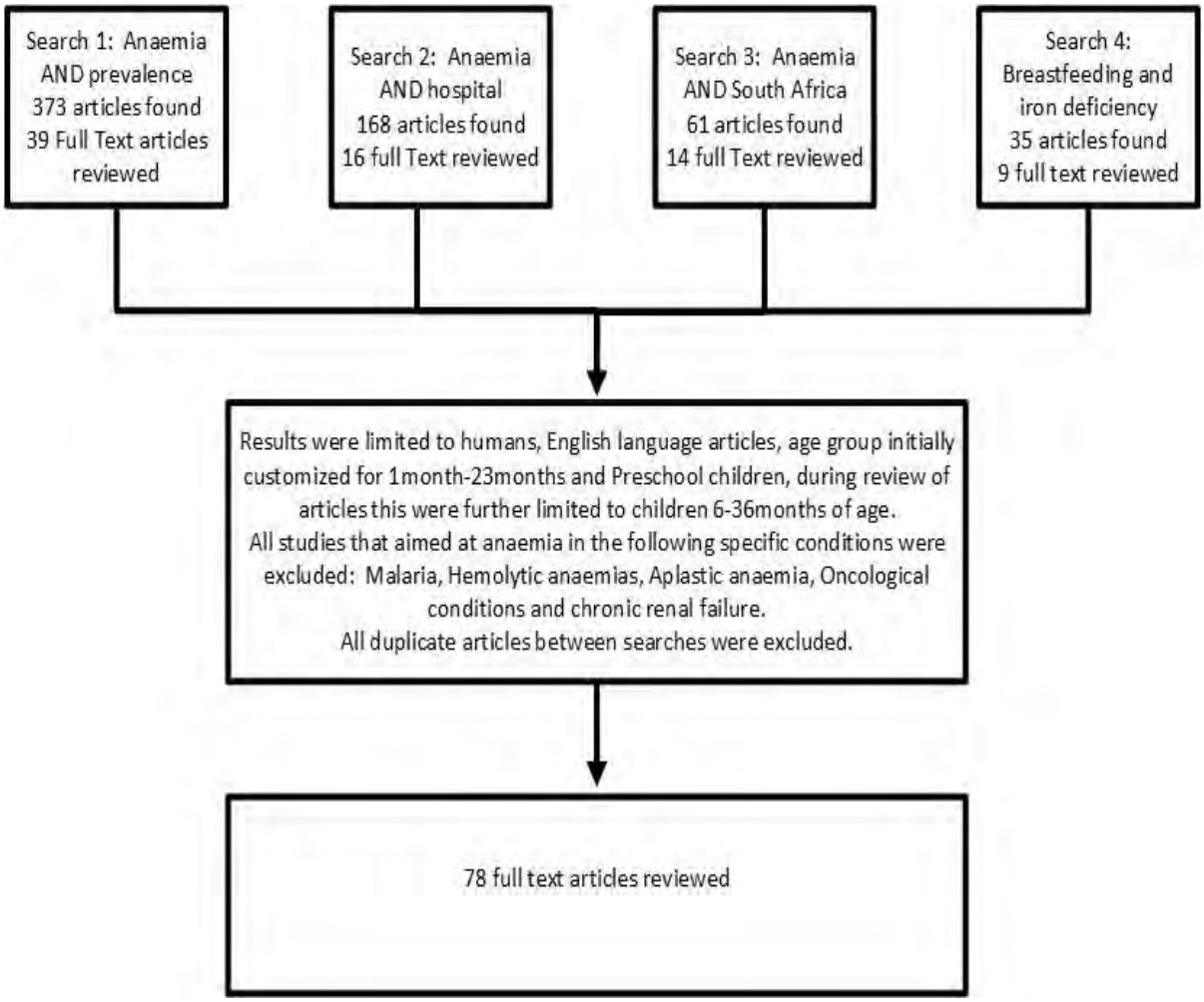

Figure 1: Summary of Pubmed search using different search strings

1.5 Backgrounds and discussion

Anaemia is a major public health problem in children although its prevalence in children from resource poor communities remains unclear.[1] Anaemia affects 1.62 billion (24.8%) people worldwide and pre-school children have the highest prevalence (47.4%).[1] Anaemia is a global public health problem in all age groups and plays a major role in the health of both developed and developing countries. The prevalence of anaemia in South

African pre-school children is 24.1%, however in the age group 6-11 month, the prevalence of anaemia is significantly higher at 48%.[2]

Anaemia is defined as a reduction in haemoglobin (Hb) concentration below the -2 standard deviation (SD) for age, it occurs through reduced production or increased loss of RBC. A low Hb will decrease the oxygen-carrying capacity of the blood.[3]

Anaemia is a condition that can be caused by various pathological processes. Anaemia warrants further investigation to establish a specific cause.[4] Nutritional anaemia is most common in developing countries with 50-60% of cases due to iron deficiency.[5] Many other disease processes, including malaria, hemoglobinopathies, malignancy, HIV and other chronic diseases can be associated with anaemia.[4] However iron deficiency anaemia (IDA) is still the most prevalent and preventable cause of anaemia in children and treatment is relatively cheap and very effective.[6]

Most children with anaemia are asymptomatic and are either diagnosed during routine screening or when investigated for other conditions. Some developed countries have routine screening programmes to look for anaemia in all infants 6-12 months of age.[7] Although anaemia has long been recognised as a public health problem, little progress has been made in reducing the global prevalence of anaemia. The World Health Organisation (WHO) and the United Nations Children's Fund (UNICEF) stress the importance of developing effective control programmes, that are both effective and sustainable based on local conditions.[8]

WHO gives a very useful classification to define anaemia as a public health problem.

(See Table 1)

According to the WHO, the prevalence of anaemia should be more than 40% in children between 6 and 24 months before implementing routine iron supplements.[6]

Table 1: Classification of anaemia as a public health problem: [2]

Prevalence of anaemia	CATEGORY OF PUBLIC HEALTH SIGNIFICANCE
<4.9%	No public health problem
5.0 – 19.9%	Mild public health problem
20.0 – 39.9%	Moderate public health problem
>40.0%	Severe public health problem

Definition of anaemia

The normal range for haemoglobin in children 6 month to 6 years is 10.5 – 14.0g/dL.

The normal range for mean corpus volume (MCV) is 70-74fl.

Anaemia is defined as Hb<10.5g/dl.

Microcytosis is defined as: MCV<70fl.

Macrocytosis for this study purpose MCV>100fl.[3]

The use of specific reference ranges to define anaemia

There are several different suggested ranges for normal haemoglobin levels in children depending on age.[2, 9, 10]

For this study the values suggested above were used.

There are great variability for the cutoff Hb level in children, according to age, gender and altitude. The most common criteria's used for childhood anaemia is the Classical Dallman Criteria and the WHO criteria.[2, 9] A lower Hb cut-off level of 10.5 for infants 4-6 months of age has been proposed.[11]

Celkan and colleagues showed that comparing the prevalence of anaemia in different countries is unreliable because diagnostic criteria used differ between countries. They also found that the prevalence of anaemia in their own study differ considerably dependant on which reference range was used.[10]

Classification of Anaemia

Anaemia is classified according to severity, morphology or aetiology. To establish the possible cause of anaemia, morphology is most useful. The MCV defines anaemia as normocytic, microcytic and macrocytic.[12, 13]
(See Table 2 and 3)

Anaemia may be mild (10-10.5g/dl), moderate (8-10g/dl), or severe (<8g/dl).[14]
Different reference ranges also exist to classify severity of anaemia. In areas with a high prevalence of malaria and sickle cell disease, severe anaemia will be classified as Hb of less than 5g/dl.[15]

Table 2: **Morphological classification for anaemia.[13]**

Microcytic	Normocytic	Macrocytic
•Iron deficiency •Thalassemia •Chronic systemic disease •Sideroblastic anaemia •Lead poisoning	•Infection •Recent blood loss •Haemolysis •Chronic inflammatory disease •Malignancy/marrow infiltration •Marrow aplasia •Transient erythroblastopenia of childhood	•Folate deficiency •Vitamin B12 deficiency •Hypothyroidism •Marrow failure •Liver disease

Table 3: Main Haematological parameters used to classify anaemia:[12]

Measurement	Indicator of	Advantages	Disadvantages
Hemoglobin	Anaemia	Simple to measure	Further investigations needed. Adjustment of thresholds is needed
Haematocrit (Hct) Or Packed cell volume (PCV)	Proportional volume of RBCs in whole blood	Simple to measure	As for Hemoglobin
Mean cell volume (MCV)	It is calculated form the RBC and Hct. It's the indicator of size of the RBC. Low is microcytic and high is macrocytic, normal is normocytic	Average size of RBCs can be characteristic of type of anaemia	More expensive to be reliable. Most common causes for microcytic anaemia is Thallasaemia and iron deficiency
Mean cell Haemoglobin (MCH)	Concentration of haemoglobin in an average RBC. If low, hypochromic; if normal normochromic	As for MCV	Usually slow to respond to iron deficiency
Red cell Distribution Width (RDW)	Abnormal range in size of RBC's. Normal = 11.5 – 14.5% RDW is the SD of MCV divided by the patients MCV and is calculated as a %.	Reflect the size of the RBC and can be characteristic of the type of anaemia. Usually high in iron deficiency and low in Thallasaemia and inflammation	Expensive machines required to be reliable. Not always that accurate in the diagnoses of iron deficiency anaemia

Anaemia in South African children

In South Africa many different socio-economical groups exist. Studies describe significant difference in the prevalence of anaemia in different communities. A study done in a community centre in Venda, Limpopo Province in 2007, found 75% of well children younger than 60 months of age to be anaemic.[16] A larger national study done by the South African Vitamin A Consultative Group (SAVACG) in 1994 found 20% of children younger than 72 months of age to be anaemic.[17, 18] In a study from 2002 conducted in two socially disadvantaged communities in the Western Cape, anaemia (Hb < 11) was present in 64% of infants of mixed ancestry and 83% of black African infants.[19] The Nutritional Intervention Research and Biostatistics Unit in South Africa in collaboration with UNICEF investigated the efficacy of micronutrient supplementations in South African infants; during this study they found that 40% of infants 6-12 months old were anaemic.[18] (See table 4)

In South Africa, screening for anaemia at primary health care (PHC) level is done as part of the Integrated Management of Childhood Illness (IMCI). These guidelines recommend screening for palmar pallor in sick children aged between two months and five years. When pallor is present, finger prick haemoglobin (Hb) should be done. According to these guidelines, children with Hb between 6 – 10g/dl should get appropriate deworming, receive a trial of iron therapy and their parents counselled about feeding practices. Children with poor response to iron or with Hb less than 6g/d should be referred for further management.[20] Clinical detection of anaemia is insensitive and many cases of anaemia are missed with the current IMCI screening guidelines.[21]

Table 4: Prevalence of anaemia in South Africa

Place where study was done	Venda, Limpopo Province	Peri-urban settlement in Cape Town	Disadvantaged communities in Western Cape	WHO: South Africa	Capricorn district, Limpopo Province, South Africa	South Africa	South Africa
Study title	Anaemia among clinically well under-fives attending a community health centre in Venda, Limpopo Province [16]	Full-term, peri-urban South African infants under 6 months of age are at risk for early onset anaemia [22]	Disadvantaged black and coloured infants in two urban communities in Western Cape, South Africa differ in micronutrient status [19]	WHO Global Database on Anaemia [2]	Prevalence of anaemia and its associated factors in African children at one and three years residing in the Capricorn District of Limpopo Province, South Africa [23]	Anthropometric, vitamin A, iron and immunisation coverage status in children aged 6-71 months in South Africa, 1994 [17]	The South African National Health and Nutrition examination survey (SANHANES-1): Nutritional status of children [24]
Study design	Cross-sectional observational study	Cross-sectional study	Cross-sectional study	Database	Prospective cohort study	National survey	Prospective cohort Cross-sectional survey
Year	2007-2008	2000	2000	1993-2005	2014	1994	2012
Age of children	<60 months	1-6 months	6-12 months	All age groups	1 year and followed until 3 years of age	6-71 months	<5 year old
Total amount of Patients included	2007: 92 2008: 74	113	120	-	219	18 219 households	Total 12 025 participants (includes adults)
Estimated prevalence of Anaemia	2007: 75% (39/92) 2008: 76% (54/74)	Hb below 11g/dl: 50% Hb below 10.5g/dl: 33% Hb below 9.5 g/dl: 12%	Coloured children: 64% Black infants: 83%	Preschool children: 24.1% 6-11 months: 48%	At 1 year of age: 52% At 3 years of age: 22%	Nationally, age 6-71 months: 21%	<5 year old: 10.7% Mild anaemia (Hb 10-10.9g/dl): 8.6% Moderate anaemia (Hb 7-9.9g/dl) : 2.1% Severe anaemia (Hb <7g/dl): 1.9%
Haemoglobin values used to define anaemia	10.4-11.8g/dl Adjusted for age and altitude	9.5 – 11 g/dl As specified above	<11g/dl	<11g/dl	<11g/dl	<11g/dl	<11g/dl

Anaemia in children presenting to hospital

All the above-mentioned studies focused on communities in South Africa, but the prevalence of anaemia in children that present to hospital is unknown. Anaemia is usually an incidental finding when patients present to the MEU with other problems or are ill enough to warrant routine blood tests to be done. Consequently, children who never have the occasional blood test done are likely to be missed. Although a FBC is a test frequently performed on children presenting to hospital, physicians frequently fail to act upon the abnormal results.[25] Few studies were identified where the researchers looked at paediatric inpatients that had a FBC during their admission to hospital and the response of the clinician to an abnormal Hb and MCV result.

Subramanian et al did a retrospective review on paediatric inpatients from Norfolk, United Kingdom in 2006. Only 25% (7/28) had the abnormal result documented while the remaining 75% (21/28) had no documentation of the abnormal result and did not receive any treatment or follow-up.[25]

In the MEU, a FBC is usually performed to look for evidence of infection, but sometimes physicians under-utilize the Hb, MCV and red cell distribution width (RDW) that were done without any additional costs. The study by Pusic et al published in 2005 looked at 935 children that had a FBC done at the Paediatric Emergency department over a four-month period and found an estimated anaemia prevalence of 19.7%, only 35% of the children that had an abnormal MCV or Hb, had documented treatment or follow-up investigation.[26]

An educational intervention was done to reinforce the use of the information available from the FBC; however there was no improvement in the rate of identifying children with suspected iron deficiency.[26]

Ballin and colleagues reported that children presenting to an Israeli paediatric emergency service with a suspected bacterial infection had an anaemia prevalence of 21.4%, compared to 14.1% in non-febrile children. The authors also reported that 60% of children that presented with fever and a positive blood culture had anaemia.[27]

There was a marked difference between the prevalence of anaemia in above studies when compared to a study done in Sudan and Eastern parts of Africa, where they found anaemia in 86% and 76% of the children presenting to hospital.[15, 28] The latter study was in a population with high prevalence of severe malaria, which makes it difficult to compare with a study of children presenting to hospitals in South Africa. (See Table 5)
A similar prevalence found in the previously described Cape Town study was in non-hospitalized children.[16,19]
The marked difference in the prevalence of anaemia in different population groups shows the importance to classify anaemia as a public health problem for specific groups. Children that present to hospital are more prone to anaemia and special care is needed in the evaluation and treatment of these individuals.

Table 5: **Prevalence of anaemia in children presenting to hospital**

Place where study was done	Montreal Children's Hospital, a 181-bed tertiary referral centre	Maccabi Health Services, Israel	Alkwaity Pediatric Hospital and Kassala Teaching Hospital, Sudan	Kenya: Kilifi District Hospital; Tanzania: Teule District Hospital Uganda: Mulago National Hospital, Kampala; St Marys Hospital, Lacor; Soroti and Mbale Regional Hospitals
Study title	Opportunistic screening for iron-deficiency in 6-36 month old children presenting to the PED [26]	Anaemia Associated with acute infection in children [27]	Iron deficiency anaemia among children under three years in Kassala, Eastern Sudan [28]	Anaemia and blood transfusion in African children presenting to hospital with severe febrile illness [15]
Study design	Retrospective study	Cross-sectional study	Cross sectional study	Randomized controlled trial
Year	1999-2000	2008	2011	2011-2013
Age of children	6-36 months of age	0-16 years of age	12-36 months of age	60 days and 12 years
Estimated Prevalence of anaemia	19.7%	Bacterial infection: 21.4% Control group: 14.3%	86%	76%
Haemoglobin level used to define anaemia	Hb <11g/dl	0-2 years: Hb <10.5g/dl 2-11years:Hb <11.5 Female 12-18 years: Hb <12g/dl Males 12-18 years: Hb <13	Hb <9g/dl	Hb <10g/dl

Iron deficiency anaemia in children

IDA manifesting as microcytic hypochromic anaemia is the most common nutritional disorder in the world.[29] Iron is an essential component for the formation of haemoglobin, the molecule responsible for carrying oxygen in red blood cells.[4, 12]

In developing countries, according to UNICEF, 90% of anaemia occurring in children 6-24 months and in pregnant women is due to iron deficiency (ID).[30]

According to WHO, the prevalence of anaemia for a specific population also estimates the prevalence of iron deficiency for that population. The WHO estimate that 50-60% of all anaemia is due to IDA and further more the incidence of ID without anaemia is 2.5 times more prevalent than IDA.[6, 31]

Published data from the 2005 National Food Consumption Survey found IDA to be present in 7.6% of South African children between one and nine years of age. (This was defined as a ferritin level less than 12mg/dL and a Hb level less that 11g/dL for children aged 1 – 5 years, or 11.5 for older children). Iron deficiency was more prevalent in children 1 – 3 years of age at 17%. The study also found that the iron status of children in South Africa had deteriorated since 1994. [17,19,32]

IDA also has consequences later in life manifesting as poor psychosocial development and behavioural problems. IDA has a major effect on a child's cognitive and motor development as well as physical growth. The prevalence of IDA in childhood peaks between 6 and 24 months of age, the period associated with rapid brain development and acquisition of

motor and cognitive skills.[33-35] There are studies that suggest that IDA alters immunity as evidenced by the high propensity for infection in children with IDA.[36]

Diagnosis of Iron deficiency anaemia

The diagnostic tools needed the diagnosis of ID and IDA in infants and toddlers are not universally agreed upon. The current gold standard for diagnosing IDA is a trial of iron therapy for a child with a history that suggests the likelihood of IDA and a low mean corpuscular volume (MCV). A repeat FBC that confirms a treatment response with an increase in Hb and MCV needs to be done.[4, 12]

The MCV indicates the size of the red blood cells (RBC). MCV reference values differ greatly with age. A MCV below SD (Standard deviation) -2 suggests a microcytic anaemia.[3] Another criteria used to diagnose IDA can include Hb<11g/dl and ferritin <12 mcg/l.[3, 14] During the first 18 months of life ferritin is a less useful marker for ID and IDA and should be used with more caution.[37]

Another useful laboratory investigations include the red cell distribution width (RDW) that increase with IDA.[38] The peripheral smear shows hypochromic, microcytic RBC, target cells or pencil cells. Biomarkers that can be used include serum iron and iron saturation levels that will be low with transferrin levels and Zinc protoporphyrin levels that will be elevated.[4, 12, 37] In a low-income setting with a high prevalence of IDA a careful history, FBC and trial of IDA remains sufficient for diagnosing IDA.[4, 39] (See figure 2)

Risk factors for anaemia

There are many risk factors for anaemia in infancy. The most common are insufficient maternal iron stores, low-birth-weight and insufficient intake.[4, 29]

Breastfeeding is the recommended feeding of choice in infants for various reasons particularly in low-income countries such as South Africa. In some developing countries with a high prevalence of anaemia, there is evidence to suggest that infants breastfed for more than six months have significantly lower Hb levels at nine months of age.[40] Although breast milk has a higher bioavailability and absorption of iron than formula milk, it has a lower iron concentration than most formula feeds.[41] The bioavailability and iron content in breast milk decrease over the course of lactation.[42]

Complementary foods are essential after six months of life for adequate supply of iron and other nutrients to promote development and growth in infants.[43]
Previous work by Eckard et al showed that all the breastfed infants that were studied and developed IDA before six months were born with decreased iron capacity. [44]
Previous research found that infants breastfed longer than four to six months of age need some form of iron supplementation to prevent IDA.[45]

The following groups have been identified as at risk of IDA: preterm infants, children with chronic diseases, infants of iron-deficient mothers, late or early weaning onto solids, the early and excessive use of cow's milk in young children, early gastro-intestinal infections and children with special health needs.[4, 29] Also at increased risk for anaemia are the younger

children and the children with acute infections (including malaria and bacterial infection).[2, 15, 27]

Prevention of Anaemia

IDA is the most common preventable form of anaemia. It is also easily treatable. Early treatment may prevent long-term consequences and poor cognitive function.[6] Factors that contribute to IDA include insufficient intake, increased iron requirements to meet the body's demands and losses through malabsorption or parasitic infections. Not all children that are iron deficient will be anaemic, but identifying them as a group at risk and providing early intervention may prevent anaemia.[29]

Current strategies to prevent anaemia go beyond the use of iron supplementation in infancy and include antenatal iron supplements, the implementation of delayed cord clamping at birth and the use of IMCI protocols, deworming, the promotion of animal source and iron fortified foods with micronutrient supplements. This also includes revised and updated national protocols and awareness among medical staff.[31]

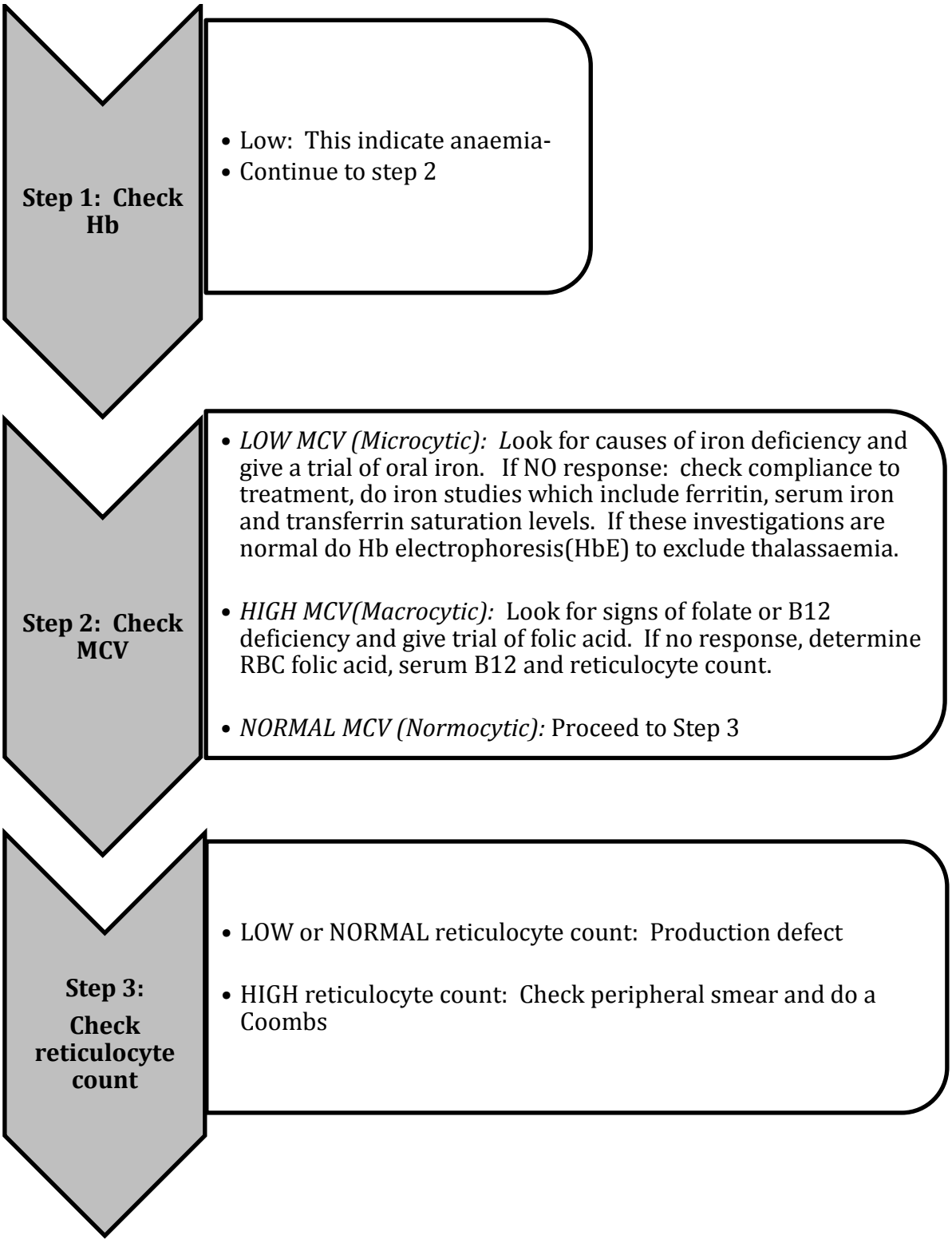

Figure 2: Diagnostic approach to children with anaemia in Cape Town, South Africa.[13]

1.6 References

1. Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al. Maternal and child undernutrition: global and regional exposures and health consequences. Lancet. 2008;371(9608):243-60.
2. World Health Organization (WHO). Worldwide prevalence of anaemia 1993-2005: WHO global database on Anemia. Geneva: WHO 2008.
3. Glader B. The Anemias. In: Kligman R, Behrman R, editors. Nelson Textbook of Pediatrics. Chapter 447. 18th edition. Philadelphia. Elsevier; 2007. p. 2003-2017
4. Janus J, Moerschel SK. Evaluation of anemia in children. American family physician. 2010;81(12):1462-71.
5. Charles CV, Summerlee AJ, Dewey CE. Anemia in Cambodia: prevalence, etiology and research needs. Asia Pacific journal of clinical nutrition. 2012;21(2):171-81.
6. United Nations Children's Fund, World Health Organization. Iron Deficiency Anaemia: Assessment, Prevention, and Control: A guide for programme managers 2001. [Accessed 2015 November 24] Available from: http://whqlibdoc.who.int/hq/2001/WHO_NHD_01.3.pdf.
7. Irwin JJ, Kirchner JT. Anemia in children. American family physician. 2001;64(8):1379-86.
8. World Health Organization. Focusing on anaemia: Towards an integrated approach for effective anaemia control. WHO/UNICEF/UNU. Geneva: WHO 2004.
9. Dallman PR, Siimes MA. Percentile curves for hemoglobin and red cell volume in infancy and childhood. The Journal of pediatrics. 1979;94(1):26-31.
10. Celkan T, Gur E, Can G, Yildiz I. Anemic or not? The Turkish journal of pediatrics. 2003;45(4):329-34.

11. Domellof M, Lonnerdal B, Abrams SA, Hernell O. Iron absorption in breast-fed infants: effects of age, iron status, iron supplements, and complementary foods. The American journal of clinical nutrition. 2002;76(1):198-204.

12. World Health Organization. Assessing the iron status of populations. Geneva, Switzerland: World Health Organization, 2004.

13. Hartley PS DF. Handbook of Paediatrics for developing countries: Blood disorders. Sixth ed. Harrison VC, editor: Oxford University Press, USA; 2005. p 253-257

14. Carley A. Anemia: when is it not iron deficiency? Pediatric nursing. 2003;29(3):205-11.

15. Kiguli S, Maitland K, George EC, Olupot-Olupot P, Opoka RO, Engoru C, et al. Anaemia and blood transfusion in African children presenting to hospital with severe febrile illness. BMC medicine. 2015;13:21.

16. Heckman J, Samie A, Bessong P, Ntsieni M, Hamandi H, Kohler M, et al. Anaemia among clinically well under-fives attending a community health centre in Venda, Limpopo Province. S Afr Med J. 2010;100(7):445-8.

17. South African Vitamin A Consultative Group. Anthropometric, vitamin A, iron and immunisation coverage status in children aged 6-71 months in South Africa, 1994. South African Vitamin A Consultative Group (SAVACG). S Afr Med J. 1996;86(4):354-7.

18. Smuts CM, Dhansay MA, Faber M, van Stuijvenberg ME, Swanevelder S, Gross R, et al. Efficacy of multiple micronutrient supplementation for improving anemia, micronutrient status, and growth in South African infants. The Journal of nutrition. 2005;135(3):653s-9s.

19. Oelofse A, Van Raaij JM, Benade AJ, Dhansay MA, Tolboom JJ, Hautvast JG. Disadvantaged black and coloured infants in two urban communities in the Western Cape, South Africa differ in micronutrient status. Public health nutrition. 2002;5(2):289-94.

20. World Health Organization, UNICEF. Integrated Management of Childhood Illness Handbook. Geneva 2005.p 157.

21. Chalco JP, Huicho L, Alamo C, Carreazo NY, Bada CA. Accuracy of clinical pallor in the diagnosis of anaemia in children: a meta-analysis. BMC pediatrics. 2005;5:46.

22. Sibeko LN, Dhansay MA, Charlton KE, Johns T, Van Stuijvenberg ME, Gray-Donald K. Full-term, peri-urban South African infants under 6 months of age are at risk for early-onset anaemia. Public health nutrition. 2004;7(6):813-20.

23. Mamabolo RL, Alberts M. Prevalence of anaemia and its associated factors in African children at one and three years residing in the Capricorn District of Limpopo Province, South Africa. Curationis. 2014;37(1):1-9.

24. Shisana O, Labadarios D, Rehle T, Simbayi L, et al. The South African National Health and Nutrition Examination Survey (SANHANES-1). Cape Town: HSRC Press. 2013.

25. Subramanian DN, Kitson S, Bhaniani A. Microcytosis and possible early iron deficiency in paediatric inpatients: a retrospective audit. BMC pediatrics. 2009;9:36.

26. Pusic MV, Dawyduk BJ, Mitchell D. Opportunistic screening for iron-deficiency in 6-36 month old children presenting to the Paediatric Emergency Department. BMC pediatrics. 2005;5:42.

27. Ballin A, Senecky Y, Rubinstein U, Schaefer E, Peri R, Amsel S, et al. Anemia associated with acute infection in children. The Israel Medical Association journal. 2012;14(8):484-7.

28. Mahmoud H MA, Osman S, AlKhawad MA, Awadia K. Iron Deficiency Anemia among Children under Three years in Kassala, Eastern Sudan. Sudanese Journal Of Public Health. 2014;9(1):33-7.

29. Jacobson D. Prevention and treatment of iron deficiency anaemia in children. Continuing Medical Education Journal. 2008;26(5):242-4.

30. Coutinho GG, Goloni-Bertollo EM, Bertelli EC. Iron deficiency anemia in children: a challenge for public health and for society. Sao Paulo medical journal. 2005;123(2):88-92.

31. Lutter CK. Iron deficiency in young children in low-income countries and new approaches for its prevention. The Journal of nutrition. 2008;138(12):2523-8.

32. Berry L HK, Hendricks M Nutrition - Iron deficiency in children [Accessed 2015 November 24]. Available from: http://www.childrencount.ci.org.za/indicator.php?id=4&indicator=37.

33. Peirano PD, Algarin CR, Chamorro R, Reyes S, Garrido MI, Duran S, et al. Sleep and neurofunctions throughout child development: lasting effects of early iron deficiency. Journal of pediatric gastroenterology and nutrition. 2009;48 Suppl 1:S8-15.

34. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. The American journal of clinical nutrition. 2007;85(4):931-45.

35. Aukett MA, Parks YA, Scott PH, Wharton BA. Treatment with iron increases weight gain and psychomotor development. Archives of disease in childhood. 1986;61(9):849-57.

36. Ekiz C, Agaoglu L, Karakas Z, Gurel N, Yalcin I. The effect of iron deficiency anemia on the function of the immune system. The hematology journal : the official journal of the European Haematology Association / EHA. 2005;5(7):579-83.

37. Sherriff A, Emond A, Hawkins N, Golding J. Haemoglobin and ferritin concentrations in children aged 12 and 18 months. ALSPAC Children in Focus Study Team. Archives of disease in childhood. 1999;80(2):153-7.

38. Sazawal S, Dhingra U, Dhingra P, Dutta A, Shabir H, Menon VP, et al. Efficiency of red cell distribution width in identification of children aged 1-3 years with iron deficiency anemia against traditional hematological markers. BMC pediatrics. 2014;14:8.

39. Short MW, Domagalski JE. Iron deficiency anemia: evaluation and management. American family physician. 2013;87(2):98-104.

40. Meinzen-Derr JK, Guerrero ML, Altaye M, Ortega-Gallegos H, Ruiz-Palacios GM, Morrow AL. Risk of infant anemia is associated with exclusive breast-feeding and maternal anemia in a Mexican cohort. The Journal of nutrition. 2006;136(2):452-8.

41. Dallman PR, Siimes MA, Stekel A. Iron deficiency in infancy and childhood. The American journal of clinical nutrition. 1980;33(1):86-118.

42. Siimes MA, Vuori E, Kuitunen P. Breast milk iron--a declining concentration during the course of lactation. Acta paediatrica Scandinavica. 1979;68(1):29-31.

43. Bortolini GA, Vitolo MR. The impact of systematic dietary counseling during the first year of life on prevalence rates of anemia and iron deficiency at 12-16 months. Jornal de pediatria. 2012;88(1):33-9.

44. Ziegler EE, Nelson SE, Jeter JM. Iron stores of breastfed infants during the first year of life. Nutrients. 2014;6(5):2023-34.

45. Oski FA. Iron deficiency in infancy and childhood. The New England journal of medicine. 1993;329(3):190-3.

Chapter 2: Publication-Ready Manuscript

PREVALENCE AND MANAGEMENT OF ANAEMIA IN CHILDREN PRESENTING TO THE MEDICAL EMERGENCY UNIT AND MEDICAL OUTPATIENT DEPARTMENT OF THE RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL

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2.1 Abstract

Introduction
Childhood anaemia is a major public health problem, iron deficiency being most common. WHO estimates anaemia to occur in 24.1% of pre-school South African children. Our study describes prevalence and management of anaemia in children aged 6-36 months presenting to a children's hospital.

Methods
In a retrospective crossectional study, laboratory data were used to estimate prevalence of anaemia in children aged 6-36 month presenting to medical emergency or ambulatory services of Red Cross Children's Hospital in 2012. A random sample of 50% of anaemic children was sampled for detailed review.

Results
2661 subjects were included. Anaemia (Hb < 10.5) was found in 40.8% (1088/2661. Children presenting to medical emergency had a higher prevalence of anaemia compared to those presenting to ambulatory services (42.7% vs. 34.9%; p=0.001).

Anaemia prevalence increased with decreasing age with RR 1.25 (95% CI 1.10-1.43) and RR 1.15 (95% CI 1.02-1.31) in children aged 6-11 months and 12-23 months respectively compared to children aged 24-36 months.

Microcytosis was found in 51.3% (558/1088) of anaemic children and in 19.3% (n=303/1573) of children without anaemia; p<0.001.

Folders were reviewed in 502 children with anaemia, 36.1% had mild anaemia (Hb 10 – 10.5g/dl), while moderate (Hb 8 – 10g/dl) and severe (Hb < 8g/dl) anaemia was found in 52.5% and 11.4% respectively.

Breastfeeding for longer than six months was associated with higher risk of microcytic anaemia [RR 1.26 (95%CI 1.08-1.47)].

Only 12.2% (31/254) of children with microcytic anaemia received adequate iron therapy, 50.0% (127/254) received no iron therapy.

Conclusions
Prevalence of anaemia in children presenting to hospital is higher than predicted for well children in South Africa. The risk is higher in younger and acutely sick children. Prolonged breasting is associated with increased risk of microcytosis. Most children with suspected iron deficiency anaemia did not receive appropriate treatment.

2.2 Introduction

Anaemia in children is a major public health problem. The actual prevalence of anaemia in children in most resource poor communities, including Cape Town, remains unclear. Globally the prevalence of anaemia in preschool children is estimated at 47%.[1] Nutritional anaemia is the most common cause of anaemia in developing countries with 50-60% of cases explained by iron deficiency.[2] The treatment of iron deficiency anaemia (IDA) is cheap and very effective. IDA has a very high morbidity and a major effect on a child's cognitive and motor development as well as physical growth. IDA also has further consequences later in life such as psychosocial development and behavioural problems.[3] Children with IDA are more prone to develop infections as IDA can also affect the immune system.[4] The most common manifestation of IDA is as a microcytic, hypochromic anaemia.[5]

In South Africa, screening for anaemia at primary health care (PHC) level is done as part of the Integrated Management of Childhood Illness (IMCI). These guidelines recommend screening for palmar pallor in sick children aged between two months and five years. When pallor is present, finger prick haemoglobin (Hb) should be done. According to these guidelines, children with Hb between 6 – 10g/dl should get appropriate deworming, receive a trial of iron therapy and their parents counselled about feeding practices. Children with poor response to iron or with Hb less than 6g/d should be referred for further management.[6]

Clinical detection of anaemia is insensitive and many cases of anaemia are missed with the current IMCI screening guidelines.[7]

Prevalence of Anaemia

According to the World Health Organization (WHO), the estimated prevalence of anaemia in South Africa's pre-school children is 24.1%, making this an important public health problem. (See Table 1)[8]

Table 1: Classification of anaemia as a Public Health problem

Anaemia prevalence	PUBLIC HEALTH SIGNIFICANCE
< 4.9%	No public health problem
5.0 – 19.9%	Mild public health problem
20.0 – 39.9%	Moderate public health problem
> 40.0%	Severe public health problem

South Africa has different socio-economical groups with significant differences in the prevalence of anaemia in its various communities. A study done in a community centre in Venda, Limpopo Province in 2007 found that 75% of clinically well children younger than 60 months were anaemic.[9] A larger national study done by the South African Vitamin A Consultative Group (SAVACG) in 1994 had earlier found anaemia in 20% of children younger than 72 months.[10] In a study done in 2002 conducted in two socially disadvantaged communities in the Western Cape, anaemia was present in 64% of infants of mixed ancestry and 83% of black African infants.[11]

Anaemia is usually discovered incidentally in children when a full blood count (FBC) is performed in the evaluation of other medical problems. Consequently, children who never have a routine blood test are likely to be missed. Even though a FBC is commonly performed on children [12], physicians frequently fail to act upon the results and under-utilize test results available to them.[13]

The prevalence of anaemia in hospitalized children in South Africa is currently still unknown. At the Red Cross War Memorial Children's Hospital (RCH), where this study was conducted general guidelines exist for management of anaemia.

2.3 Materials and methods

A retrospective review was done between August and September 2013. Data from the NHLS was used to identify children 6 - 36 months old seen at the Medical Emergency Unit (MEU) and ambulatory services of the Medical Outpatient Department (MOPD) of the RCH between the 1st of January 2012 and the 31st of December 2012. All children that had a FBC done were included. There were no other exclusion criteria.

The prevalence and morphology of anaemia was described. Stata was used to select a random sample of half of the children with anaemia, these folders was taken for a detailed folder review. Some of the children in this sample were transferred to other health facilities for further treatment. The management of anaemia for children who were not transferred was further analysed.

RCH is a dedicated referral children's hospital with 290 inpatient beds.[14] The hospital situated in Cape Town caters for about 18 500 inpatient and 260 000 outpatient visits each year. The patients come from various demographic and socio-economic backgrounds.[15] The Haematology Laboratory, a division of the National Health Laboratory Services (NHLS) at RCH performs around 38 000 FBC tests per year.

Ethics approval was obtained from the University of Cape Town's Human Research Ethics Committee.

The anthropometry of the children was classified using WHO weight for age Z-score (WAZ) growth charts. Children were categorized into the following groups:

- Normal: WAZ > -1 to +1
- Mild underweight: WAZ < -1 to -2
- Moderate underweight: WAZ <-2 to -3
- Severe underweight: WAZ < -3

Mild, moderate and severe malnutrition were used to correlate with the three WHO weight for age categories. Failure to thrive was defined as a plateau in weight gain.

HIV status was determined from the hospital record. A child was classified as HIV unexposed uninfected if the ELISA test was negative and the mother was HIV negative during pregnancy, HIV exposed uninfected if there was no confirmed infection but the mother was HIV positive during pregnancy. Finally HIV infection was diagnosed if the polymerase chain reaction (PCR) test was positive or two enzyme-linked immunosorbent assay (ELISA) tests were positive in the case of children more than 18 months of age.

Feeding choice was categorized into two groups; patients breastfed for more than six months AND patients not breastfed/breastfed for less than six months.

Case definition

Anaemia was defined as Hb less than 10.5 g/dl. MCV was classified as microcytosis if less than 70 fl, normal if 70 fl and less than 100fl. Above 100 fl was regarded as macrocytosis.[16]

Anaemia was further classified as mild (Hb 10.0 -10.5 g/dl), moderate (Hb 8.0 – 9.9 g/dl) or severe (Hb < 8 g/dl).[17]

Data analysis

All data were analysed using STATA version 13 (Statacorp, College Station, Texas, USA). Percentages were used to depict proportions of categorical data while medians with interquartile ranges (IQR) or mean and standard deviation (SD) summarise continuous data after testing for normality. The Chi-squared test was used to test strength of association between categorical variables.

An unadjusted general linear model was used to estimate the relative risk of anaemia by age categories using Poisson regression. The same model was used to estimate the independent effect of risk factors on microcytosis in a multivariable analysis.

A significance level was set at a two-tailed $p<0.05$ for all analysis.

2.4 Results

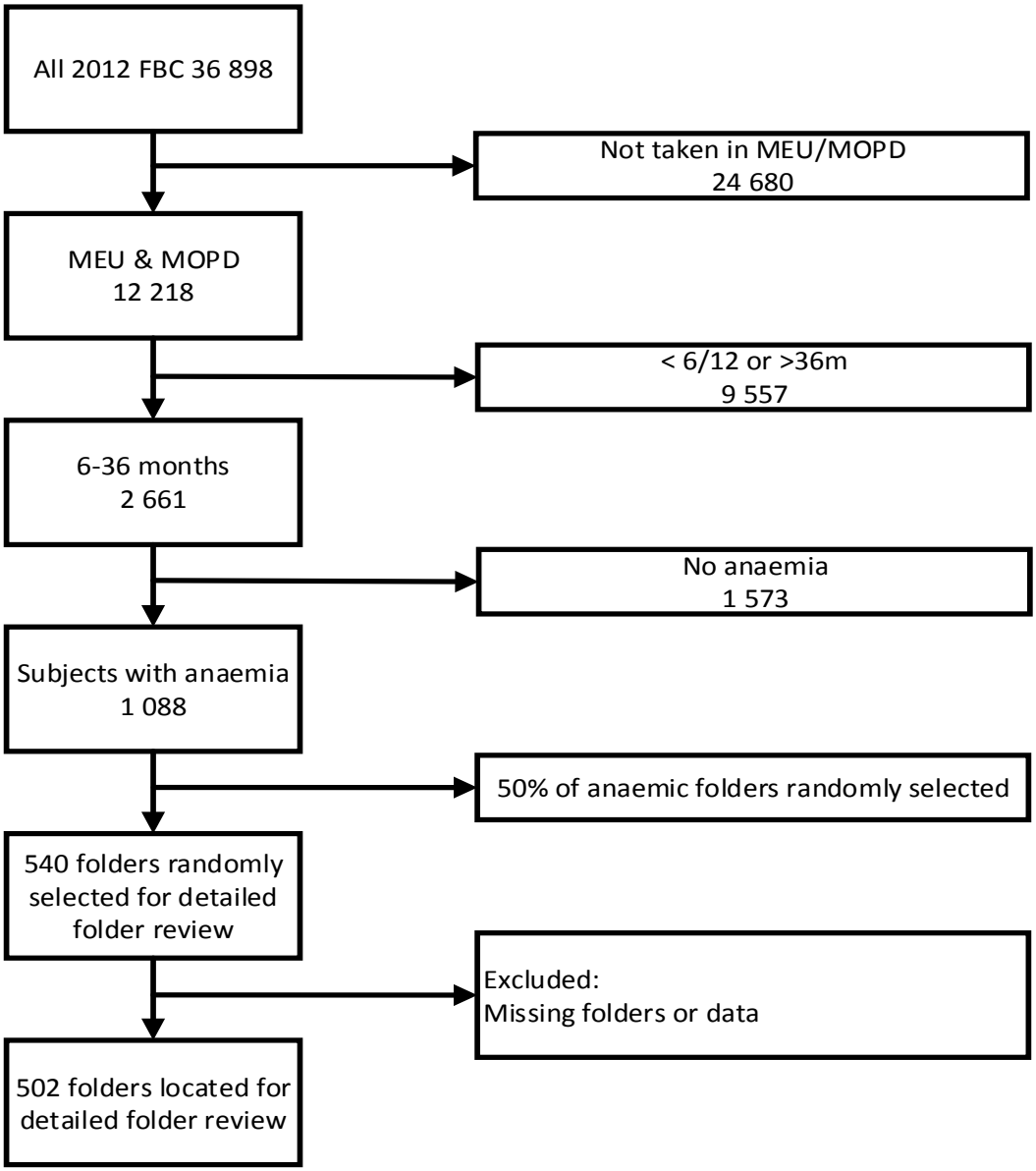

Figure 1: Selection and enrolment flow chart

There were 2661 subjects included. (See Figure 1). Boys made up 50.4% (n=1341) of the group. Most of the children presented to the MEU (76.7%; n=2042). The mean Hb of the group was 10.7g/dl (SD 1.5)

Anaemia was found in 40.8% (1088/2661) of the children. The prevalence was similar in boys, 42% (n=1118) and in girls 40% (n=1064); p=0.274. (See Figure 1)

The prevalence of anaemia in children with FBC done at the MEU was significantly higher than for those seen in MOPD 42.7% (872/2042), compared to 34.9% (216/619); p=0.001. The prevalence of anaemia was higher in the younger children, 44.7% (373/834) for those 6-12 months, 41.1% (n=482/1173) for 12-23 months old and 35.6% (n=1223/654) in children 24-36 months of age. This increased risk of anaemia in younger children was further confirmed when comparing the oldest stratum of 24-36 months old with 12-23 month (RR 1.15; 95% CI 1.02-1.31) and with 6-12 months old (RR 1.25; 95% CI 1.10-1.43).

(See Table 2)

In total 32.4% (861/2661) of children showed microcytosis on their FBC profile. Microcytosis was present in 19.3% (n=303/1573) of the children without anaemia and in 51.3% (558/1088) of anaemic children; p<0.001. Only one child had macrocytosis.

Table 2: Factors associated with anaemia in children attending hospital (N=2661)

Risk factor	Risk %(n/N)	Unadjusted RR	p-value
Age group			
6-11 months old	44.7 (373/834)	1	-
12-23 months old	41.1 (482/1173)	1.02 (0.88-1.18)	0.024
24-36 months old	35.6 (233/654)	1.00 (0.84-1.19)	<0.001
Gender			
Female	39.8 (526/1320)	1	-
Male	41.9 (562/1341)	0.95 (0.87-1.04)	0.274
Area of presentation			
Ambulatory services	34.9 (216/619)	1	-
Medical emergency unit	42.7 (872/2042)	1.22 (1.09-1.38)	0.001

RR = Relative risk; %(n/N) = per cent(stratum specific proportion)

Detailed review of children with anaemia

A detailed review of children with anaemia was done. A random sample of 50% (540) of the children with anaemia was taken for retrospective folder review. Folders of 502 of the children had sufficient data for inclusion. (See figure 1)

The sampled children had a median age of 15 (IQR 10-23) months. There were slightly more boys than girls in the group (55.6%, n=280). Five per cent (n=25) of the patients were from other African countries including Malawi, Zimbabwe, the Democratic Republic of the Congo and Burundi while the remainder were from South Africa.

Failure to thrive was found in 14.7% (n=74) of the children with 7.4% (n=37) having severe malnutrition. The two most common presenting symptoms were diarrhoea, 24.7% (124/502) and cough 21.7% (109/502). Only 1.4% (n=7) of the children were specifically referred for anaemia to the RCH. HIV infection was confirmed in 5.4% (n=27) of the children while 13.9% (70) were HIV exposed but not infected.

In 27.9% (140/502) there was no documentation of feeding choice. For the children with available data, the majority of the children 57.2% (n=207/362) were breastfed for more than six months. The other 42.8% (n=155) were either breastfed for less than six months or not breastfed at all.

Other baseline characteristics of the sampled children are shown in Table 3.

Table 3: Baseline characteristic of children with anaemia (N=502)

Baseline variable		Frequencies %(n)
Gender	Female	44.2(222)
	Male	55.8(280)
Vaccination status	Up to date	77.3(388)
	Not up to date	14.9(75)
	Not documented	7.8(39)
Nutritional status	Normal	61.5 (309)
	Mild UWFA	11.8(59)
	Moderate UWFA	19.3(97)
	Severe UWFA	7.4(37)
HIV status	Unexposed Uninfected	72.7(365)
	Exposed Uninfected	13.9(70)
	Exposed Infected	5.4(27)
	Not recorded	8.0(40)
Previous medical problem	None or unknown	69.1(347)
	Eczema	10.8(54)
	Prematurity	9.8(49)
	Congenital anomalies	3.2(16)
	Other	7.17 (36)
Presenting diagnosis	RTI	34.3(172)
	Gastro-enteritis	27.5(138)
	Neurological problems	12.7(64)
	Anaemia	1.8(9)
	Other	23.7(119)
Breast feeding	None or < 6 months	30.9(155)
	> 6 months	41.2(207)
	Unknown	27.9(140)

* UWFA = Underweight for age WHO weight for age Z-score, RTI = Respiratory tract infection

Microcytosis and severity of anaemia

Microcytosis was found in 65.5% (n=328) of the children, 34.6% (n=174) had a normocytic anaemia.

Mild anaemia was found in 36.1% (n=181), 52.6% (n=264) had a moderate anaemia and 11.4% (n=57) had severe anaemia. There was poor correlation between laboratory confirmed anaemia and clinical examination with only 18.1% (n=91) noted to be pale. The clinical detection however increased with severity of anaemia from 11.1% (20/181) in children with mild anaemia, 16.3% (43/264) in moderate and 49.1% (28) in the children with severe anaemia; p<0.001.

Anaemia was documented by clinicians as a problem in only 36.9% (n=185) of the 502 children with laboratory confirmation of anaemia.

Risk factors for microcytosis

The risk of microcytosis was higher in children who were breastfed for longer than six months with a prevalence of 73.9% (n=153/207) compared to 57.8% (n=91/155) in children breastfed for less than six months or not breastfed at all; adjusted RR 1.23 (1.04-1.45). There was no association between microcytosis and HIV status, gender or having a pre-existing medical condition. (See Table 4)

Table 4: Risk factors for microcytosis in children with anaemia

Risk factor	Risk %(n/N)	RR (95% Confidence Interval) Unadjusted	Adjusted*
Age			
6-11 months old	64.8 (118/182)	1	1
12-23 months old	66.0 (138/209)	1.02 (0.88-1.18)	1.02 (0.87-1.19)
24-36 months old	64.9 (72/111)	1.00 (0.84-1.19)	1.10 (0.89-1.35)
Nutritional status			
Normal	64.7 (200/309)	1	1
Mild under-nutrition	69.4 (41/59)	1.07 (0.89-1.30	1.10 (0.89-1.36)
Moderate under-nutrition	63.9 (62/97)	0.99 (0.83-1.17)	1.09 (0.92-1.32)

Severe under-nutrition	67.6 (25/37)	1.04 (0.82-1.32)	1.08 (0.83-1.41)
HIV status			
Unexposed uninfected	68.2 (249/365)	1	1
Exposed uninfected	60.0 (42/70)	0.88 (0.72-1.08)	0.88 (0.70-1.11)
Exposed infected	44.4 (12/27)	0.65 (0.42-1.00)	0.69 (0.44-1.08)
Breastfeeding			
Less than 6/12	58.7 (91/155)	1	1
More than 6/12	73.9 (153/207)	1.26 (1.08-1.47)	1.22 (1.04-1.44)
Pre-existing medical condition			
Absent	30.1 (47/156)	1	1
Present	69.87 (109/156)	1.12 (0.97-1.26)	1.09 (0.94-1.27)
Gender			
Female	58.1 (129/222)	1	1
Male	71.1 (199/280)	2.33 (1.7-1.40)	1.14 (0.98-1.32)

RR = Relative risk; %(n/N) = per cent(stratum specific proportion); * Multivariable model adjusted for age, sex, HIV status, breast-feeding , nutritional status and presence of pre-existing medical condition

Management of Anaemia (See Table 5)

Evidence of deworming could only be found for 47.4% (n=238) of the children and folic acid was prescribed for 37.5% (n=188) of them.

Only 3.2% (n=16) of the patients needed a blood transfusion. Thirteen of the 16 had Hb less than 8 and three had Hb between 8 and 10g/dl.

Iron studies were done for 5.4% (n=27) of the children. These included serum iron, ferritin, transferrin and transferrin saturation levels. Haemoglobin electrophoresis was performed on 3.6% (n=18). Two of the 18 were diagnosed with Beta Thalassaemia trait, two with sickle

cell disease (homozygous) and one with sickle cell trait (heterozygous). The remaining 13 had no haemoglobinopathy.

Twenty two per cent (n=111) of the children were transferred to other centres. Of the 391 children that were not transferred, 65.0% (254/391) had a microcytic anaemia. Of these only 12.2% (31/254) received iron therapy for more than three months and 50.0% (127/254) received no iron therapy at all. When therapy was given, most children received the correct dose of iron, with 83.5%(106/127) prescribed 3-6mg/kg/day of elemental iron.

Children were more likely to receive some form of therapy if they had severe anaemia. Of the children with microcytosis, 31.5% (23/73) of the children with mild anaemia received iron therapy, compared to 52.4% (77/147) of moderate and 79.4% (27/34) children with severe anaemia, $p<0.001$. Children with more severe anaemia were also more likely to be dewormed and their caregiver to receive dietary advice. (See table 5)

The dietician was consulted for 29.4% (115/391) of the children that continued to receive treatment at RCH. No record was available on whether the rest were given dietary advice or referred to a dietician.

Table 5: Management of children with microcytic anaemia (N=254)

Severity of anaemia	Iron therapy	Dietician referral	Deworming
	% (n)	% (n)	% (n)
Mild	31.5 (23/73)	26.0 (19/73)	37.0 (27/73)
Moderate	52.4 (77/147)	31.5 (46/147)	55.8 (82/147)
Severe	79.4 (27/34)	58.8 (20/34)	61.8 (21/34)

2.5 Discussion

This study shows a high prevalence of anaemia (40.8%) in children presenting to MEU and MOPD of a major children's hospital. This, according to WHO criteria, makes it a severe public health problem. Furthermore, the study shows anaemia is often poorly recognised and inadequately managed.

This is the first reported study of its sort for hospitalized children in South Africa. Most previous prevalence studies in hospitalized children in Africa were done in areas with a high incidence of malaria and sickle cell disease.[18, 19] The prevalence of anaemia in our study is much higher than that reported in high income countries. A study from Montreal Children's Hospital found anaemia (Hb <11g/dl) in 19.7% (184/934) of children 6-36months of age.[13] Authors reported that children presenting to an Israeli paediatric emergency service with a suspected bacterial infection had an anaemia prevalence of 21.4%, compared to 14.1% in non-febrile children. These authors also reported that 60% of children that presented with fever and a positive blood culture had anaemia.[20] The children included in these Israeli studies provided a similar cohort to our study group as they presented to the Paediatric Emergency Department (PED).

The increased prevalence of anaemia in younger children has been noted in other studies.[21-23]

A WHO study estimated the prevalence of anaemia to be 48% in the 6-11 month old age group of South African children.[10]

Children presenting with an acute severe illness in the emergency unit were more likely to be anaemic than those presenting to ambulatory care. Acute illness can cause a transient decrease in Hb without a change in the MCV. [24] The pathogenesis of anaemia in acute infection is generally not well understood. Children with bacterial infection have a much higher risk for anaemia compared to afebrile children and those with viral illnesses.[20]

Sixty per cent of children with anaemia have iron deficiency.[2] We identified a more than double prevalence of microcytosis (51.3% %) in anaemic children compared to non-anaemic ones (19.3%). This is much higher than the 8% reported in high-income countries.[12, 13] Although IDA is the most common cause of microcytosis, very few of the children in our study had iron studies performed.

Anaemia was clinically suspected in only 18% of laboratory confirmed cases. This study highlights that the use of clinical signs to screen for anaemia in children is unreliable. Even in children with severe anaemia, half of them were missed when screened by clinical signs. Guidelines such as the IMCI that use clinical suspicion to guide selection of laboratory confirmation should be reviewed, as this is likely to miss a majority of cases.[25]

It is also important to note that only a small proportion (5.2%) of the children had a documented Hb on their referral letter. This again shows that pallor is a poor screening tool for anaemia. It may also reflect the underutilization of fingerprick Hb at the PHC level. Screening for anaemia in acutely unwell children needs to be reinforced with the use of fingerprick Hb.

By WHO estimation, 12 % of healthy South African children <5year of age were found to be UWFA, 35.5% of children in our study were UWFA.[26] This was probably skewed by the fact that malnourished children are more likely to end up in hospital than their healthy counter parts.

Breastfeeding for more than six months was associated with microcytosis. Meinzen-Der and colleagues reported significantly lower Hb levels at nine months of age in infants exclusively breastfed for more than 6 months.[27] While bioavailability and absorption of iron is much better in breast milk compared to formula milk, iron content decreases significantly if breastfeeding is continued beyond 6 months of age.[28] Maternal iron deficiency is one of the greatest contributors to IDA in breast fed infants. Children breastfed for more than 6 months of age, should be considered for routine screening for IDA and appropriate iron supplementation.[29] As many of the patients in our study population came from low socio-economic circumstances, prolonged breastfeeding may have been associated with inadequate complementary feeds containing heme-iron in their diet.

The prevalence of iron deficiency is estimated at 60% in children with anaemia. Due to this high prevalence of iron deficiency, current guidelines recommend a trial of empirical iron therapy in children with low Hb.[2] This has been found to be more reliable than RBC morphology in diagnosing iron deficiency and less expensive than formal iron studies.[30]

Most of the children with suspected IDA in this group received inadequate treatment and follow up. Only 12% of the children with a microcytic anaemia received optimal iron therapy with adequate doses of elemental iron being continued for 4-8 weeks after Hb has normalised to replenish body iron stores. [31, 32] Fifty per cent of children with suspected

IDA received no therapy. This failure to diagnose and adequately treat children with IDA is a common worldwide phenomenon. An English study of paediatric inpatients found that only 12% patients received treatment for a low MCV.[12] Similarly, a Canadian study found that only just over a third (35%) of children received treatment or follow-up.[13]

IDA has negative effects on a child's cognitive, motor, social and emotional development.[33] Authors reported the benefits of preventing and treating IDA before it becomes chronic and severe. Children whose iron deficiency was corrected before 24 months of age had a better overall outcome in terms of behaviour, coordination and social or emotional development.[33] Opportunities were missed for the early treatment of children with suspected IDA in our study population.

Children included in this review were sick enough to require referral to hospital and as such may not reflect the haemoglobin status of healthy children. Incomplete documentation in the folders done by physicians resulting from the retrospective study design may have biased the results. This includes incomplete data on feeding choice as well as information on iron therapy for children transferred out of RCH. The averaged cut-off Hb used in our study for defining anaemia may overestimate the prevalence in younger children and underestimate the prevalence in older ones.[34]

2.6 Conclusion

Risk of anaemia is significantly higher in sick children presenting to hospital, however the management of microcytic anaemia for this group of children is suboptimal.

There is a need to increase the awareness of the high prevalence of anaemia in this population of children and to encourage clinicians to make better use of the routinely

performed FBC. Routine screening, measuring Hb and or FBC and iron supplementation should be considered for children at risk of anaemia, particularly in infancy as well as acutely unwell children and children breastfed for more than six months.

2.7 Acknowledgment

We would like to thank the National Health Laboratory Services for giving us access to the laboratory data on which this study is based.

2.8 References

1. Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al. Maternal and child undernutrition: global and regional exposures and health consequences. Lancet. 2008;371(9608):243-60.
2. Charles CV, Summerlee AJ, Dewey CE. Anemia in Cambodia: prevalence, etiology and research needs. Asia Pacific journal of clinical nutrition. 2012;21(2):171-81.
3. Aukett MA, Parks YA, Scott PH, Wharton BA. Treatment with iron increases weight gain and psychomotor development. Archives of disease in childhood. 1986;61(9):849-57.
4. Ekiz C, Agaoglu L, Karakas Z, Gurel N, Yalcin I. The effect of iron deficiency anemia on the function of the immune system. The hematology journal : the official journal of the European Haematology Association / EHA. 2005;5(7):579-83.
5. Kazal LA, Jr. Prevention of iron deficiency in infants and toddlers. American family physician. 2002;66(7):1217-24.
6. Phillips-Howard PA, Wannemuehler KA, ter Kuile FO, Hawley WA, Kolczak MS, Odhacha A, et al. Diagnostic and prescribing practices in peripheral health facilities in rural western Kenya. The American journal of tropical medicine and hygiene. 2003;68(4 Suppl):44-9.
7. Chalco JP, Huicho L, Alamo C, Carreazo NY, Bada CA. Accuracy of clinical pallor in the diagnosis of anaemia in children: a meta-analysis. BMC pediatrics. 2005;5:46.
8. World Health Organization (WHO). Worldwide prevalence of anaemia 1993-2005: WHO global database on Anemia. Geneva: WHO 2008.
9. Heckman J, Samie A, Bessong P, Ntsieni M, Hamandi H, Kohler M, et al. Anaemia among clinically well under-fives attending a community health centre in Venda, Limpopo Province. S Afr Med J. 2010;100(7):445-8.
10. South African Vitamin A Consultative Group. Anthropometric, vitamin A, iron and immunisation coverage status in children aged 6-71 months in South Africa, 1994. South African Vitamin A Consultative Group (SAVACG). S Afr Med J. 1996;86(4):354-7.

11. Oelofse A, Van Raaij JM, Benade AJ, Dhansay MA, Tolboom JJ, Hautvast JG. Disadvantaged black and coloured infants in two urban communities in the Western Cape, South Africa differ in micronutrient status. Public health nutrition. 2002;5(2):289-94.

12. Subramanian DN, Kitson S, Bhaniani A. Microcytosis and possible early iron deficiency in paediatric inpatients: a retrospective audit. BMC pediatrics. 2009;9:36.

13. Pusic MV, Dawyduk BJ, Mitchell D. Opportunistic screening for iron-deficiency in 6-36 month old children presenting to the Paediatric Emergency Department. BMC pediatrics. 2005;5:42.

14. Western Cape Government. Red Cross War Memorial Children's Hospital: Overview. Available from: http://www.westerncape.gov.za/redcrosshospital.

15. The children's hospital trust: Annual report 2011: Red Cross War Memorial Children's Hospital 2011. Available from: http://childrenshospitaltrust.org.za/wp-content/uploads/7234 CHT Anual Report.pdf.

16. Glader B. Nelson Textbook of Pediatrics: The Anemias. Chapter 447. 18th edition. Elsevier; 2007. p. 2003-2017

17. Carley A. Anemia: when is it not iron deficiency? Pediatric nursing. 2003;29(3):205-11.

18. Kiguli S, Maitland K, George EC, Olupot-Olupot P, Opoka RO, Engoru C, et al. Anaemia and blood transfusion in African children presenting to hospital with severe febrile illness. BMC medicine. 2015;13:21.

19. Luby SP, Kazembe PN, Redd SC, Ziba C, Nwanyanwu OC, Hightower AW, et al. Using clinical signs to diagnose anaemia in African children. Bulletin of the World Health Organization. 1995;73(4):477-82.

20. Ballin A, Senecky Y, Rubinstein U, Schaefer E, Peri R, Amsel S, et al. Anemia associated with acute infection in children. The Israel Medical Association journal. 2012;14(8):484-7.

21. Irwin JJ, Kirchner JT. Anemia in children. American family physician. 2001;64(8):1379-86.

22. Smuts CM, Dhansay MA, Faber M, van Stuijvenberg ME, Swanevelder S, Gross R, et al. Efficacy of multiple micronutrient supplementation for improving anemia, micronutrient status, and growth in South African infants. The Journal of nutrition. 2005;135(3):653s-9s.

23. Mamabolo RL, Alberts M. Prevalence of anaemia and its associated factors in African children at one and three years residing in the Capricorn District of Limpopo Province, South Africa. Curationis. 2014;37(1):1-9.

24. Reeves JD, Yip R, Kiley VA, Dallman PR. Iron deficiency in infants: the influence of mild antecedent infection. The Journal of pediatrics. 1984;105(6):874-9.

25. Janus J, Moerschel SK. Evaluation of anemia in children. American family physician. 2010;81(12):1462-71.

26. World Health Organization. WHO Global Database on Child Growth and Malnutrition. In: WHO, editor. Geneva 2012.

27. Meinzen-Derr JK, Guerrero ML, Altaye M, Ortega-Gallegos H, Ruiz-Palacios GM, Morrow AL. Risk of infant anemia is associated with exclusive breast-feeding and maternal anemia in a Mexican cohort. The Journal of nutrition. 2006;136(2):452-8.

28. Dewey KG, Cohen RJ, Brown KH. Exclusive breast-feeding for 6 months, with iron supplementation, maintains adequate micronutrient status among term, low-birthweight, breast-fed infants in Honduras. The Journal of nutrition. 2004;134(5):1091-8.

29. Kilbride J, Baker TG, Parapia LA, Khoury SA, Shuqaidef SW, Jerwood D. Anaemia during pregnancy as a risk factor for iron-deficiency anaemia in infancy: a case-control study in Jordan. International journal of epidemiology. 1999;28(3):461-8.

30. Wright CM, Kelly J, Trail A, Parkinson KN, Summerfield G. The diagnosis of borderline iron deficiency: results of a therapeutic trial. Archives of disease in childhood. 2004;89(11):1028-31.

31. Department of Health. The Standard Treatment Guidelines (STG) and Essential Medicine list (EML) for Hospital level Paediatrics. Chapter 3: Blood and bloodforming organs. Third

edition. Diale D MJ, editor: The National Department of Health, Pretoria, South Africa; 2013. 3.5-3.7 p.

32. Hartley PS DF. Handbook of Paediatrics for developing countries: Blood disorders. Sixth edition. Harrison VC, editor: Oxford University Press, USA; 2005. p 253-257

33. Chang S, Wang L, Wang Y, Brouwer ID, Kok FJ, Lozoff B, et al. Iron-deficiency anemia in infancy and social emotional development in preschool-aged Chinese children. Pediatrics. 2011;127(4):e927-33.

34. Sibeko LN, Dhansay MA, Charlton KE, Johns T, Van Stuijvenberg ME, Gray-Donald K. Full-term, peri-urban South African infants under 6 months of age are at risk for early-onset anaemia. Public health nutrition. 2004;7(6):813-20.

Appendices

Appendix 1: Anaemia at RCWMCH data sheet

Study number: __ __ __ __ __ __ Date of folder review__ __ / __ __ / 2 0 __ __

Patient information and background data		
1.	Patient name	
2.	Folder Number	
3.	Sex	☐ *Male* ☐ *Female*
4.	Date of birth	*___ ___ / ___ ___ / 2 0 ___ ___ (dd/mm/yyyy)*
5.	Suburb/Township of residence	
Previous History:		
6.	Immunization status	☐ *Up to date* ☐ *Not up to date* ☐ *Not recorded*
7.	Recent deworming: (within last 6 months)	☐ *Yes* ☐ *No* ☐ *Not documented*
8.	TB contact If YES, on INH prophylaxis	☐ *Yes* ☐ *No* ☐ *Unknown* ☐ *Yes* ☐ *No* ☐ *Unknown*
9.	Previous history of pulmonary TB If Yes, date of diagnosis (or month)	☐ *Yes* ☐ *No* ☐ *Not recorded* ☐ *On treatment* ☐ *Completed treatment* ☐ *Defaulted treatment*
10.	Smoking contact	☐ *Yes* ☐ *No* ☐ *Not recorded*
11.	HIV status of child	☐ *Negative* ☐ *Exposed Uninfected* ☐ *Infected* ☐ *Unknown*
12.	If HIV positive, is the child on HAART?	☐ *Yes* ☐ *No* ☐ *Unknown*
13.	HIV Stage of the child	☐ *1* ☐ *2* ☐ *3* ☐ *4* ☐ *Not Applicable*
14.	HIV treatment	☐ *1st line* ☐ *2nd line* ☐ *Not Applicable*
15.	Nevirapine prophylaxis	☐ *Yes* ☐ *No* ☐ *Not Applicable*
16.	Presence of pre-admission diagnosis (This refers to a preexisting diagnosis for all children that has never had a previous Full Blood count done at RCWMCH and fulfill inclusion criteria)	☐ *Not recorded* ☐ *Prematurity* ☐ *Neonatal Jaundice* ☐ *Hypoxic ischemic encephalopathy* ☐ *Congenital heart disease* ☐ *Malnutrition* ☐ *Previous Trauma history* ☐ *Other, specify*_____
17.	Birth history: Birthweight:	☐ *<1000g* ☐ *1000 – 2000g* ☐ *2000-2500g* ☐ *>2500g*
18.	Developmental milestones	☐ *Appropriate for age* ☐ *Delayed: Specify(if documented):*_____ ☐ *Not recorded*
19.	Dietary history: Breastfeeding for the first 6	☐ *Yes* ☐ *No* ☐ *Not documented*

	months of life Breastfeeding for more than 1year	☐ *Yes* ☐ *No* ☐ *Not documented*
20.	Dietary history: Age when solids were introduced to diet:	☐ *Before 4 months of age* ☐ *4-6months of age* ☐ *After 6 months of age* ☐ *Not documented*
21.	Dietary history: Seen by dietician: If yes, referred for NTP?	☐ *Yes* ☐ *No* ☐ *Yes* ☐ *No*
22.	Social history: Housing: Child social grant:	☐ *Formal* ☐ *Informal* ☐ *Yes* ☐ *No* ☐ *Not recorded*
23.	Previous contact with health care. If yes, location. Date of last contact or admission	☐ *Yes* ☐ *No* ☐ *Not recorded* ☐ *RXH* ☐ *Other, specify* _____ ___ ___ / ___ ___ / 2 0 ___ ___ *(dd/mm/yyyy)*
24.	Diagnosis at last admission or contact with health facility.	*Diagnosis:* _____ ☐ *Not Applicable*

Admission information:

25.	Date of admission	___ ___ / ___ ___ / 2 0 ___ ___ *(dd/mm/yyyy)*
26.	Time of admission	___ ___ *H* ___ ___ ☐ *Not recoded*
27.	Source of referral	☐ *Home* ☐ *Clinic/Day Hospital* ☐ *Children's Home* ☐ *Known to RXH* ☐ *Other, specify* _____
28.	Admission weight and height	*Wt:* ___ ___ . ___ ___ *kg* ☐ *Not recorded* *Ht:* ___ ___ ___ *cm* ☐ *Not recorded*
29.	Admission diagnosis (Only Primary diagnosis that brought child in to hospital)	☐ *AGE: (Specify hydration status on admission)* ☐ *Well hydrated* ☐ *5% or some dehydration* ☐ *10% or severe dehydration* ☐ *Shocked* ☐ *Malnutrition: (Specify according to WHO criteria)* ☐ *Severe* ☐ *PEM (with oedema)* ☐ *Moderate* ☐ *Pneumonia*

		☐ *Brongiolitis* ☐ *Asthma* ☐ *PTB* ☐ *UTI* ☐ *Sepsis* ☐ *Tonsilitis* ☐ *Otitis Media* ☐ *URTI* ☐ *Febrile seizures* ☐ *Other seizures: Specify: _____* ☐ *Anaemia: Specify:_____* ☐ *Bacterial meningitis* ☐ *Viral meningitis* ☐ *Other: Specify:_____*
30.	Temperature	__ __ . __ *°C*
31.	Heart rate	___ ___ ___ *beats / min* ☐ *Not recorded*
32.	Respiratory rate	___ ___ ___ *breaths / min* ☐ *Not recorded*
33.	Oxygen saturation	___ ___ ___ *%* ☐ *Room air* ___ ___ ___ *%* ☐ *On oxygen* ☐ *Not recorded*
34.	Pale:	☐ *Yes* ☐ *No* ☐ *Not recorded*
35.	Jaundiced:	☐ *Yes* ☐ *No* ☐ *Not recorded*
36.	Clubbing:	☐ *Yes* ☐ *No* ☐ *Not recorded*
37.	Oedema:	☐ *Yes* ☐ *No* ☐ *Not recorded*
38.	Supplemental Oxygen	☐ *Yes* ☐ *No* ☐ *Not recorded*
39.	Admission to ICU or High Care If *Yes*	☐ *ICU* ☐ *High Care* ☐ *Both* ☐ *Not recorded*
40.	Admission to ward	*Ward* *Date ((dd/mm/yyyy))* _____ __ __ to__ __ /__ __ / 2 0 __ __ _____ __ __ to__ __ /__ __ / 2 0 __ __ _____ __ __ to__ __ /__ __ / 2 0 __ __ _____ __ __ to__ __ /__ __ / 2 0 __ __
41.	Transferred to another Health facility:	☐ *Yes* ☐ *No* *Specify:* ☐ *GSH* ☐ *NSH* ☐ *KBH* ☐ *Victoria* ☐ *KDH* ☐ *Carnation* ☐ *Other*
42.	Assisted ventilation If *Yes*, Duration Type of assistance	☐ *Yes* ☐ *No* ☐ *Not recorded* _____ *days* ☐ *CPAP* ☐ *IPPV* ☐ *Other*_____

Special investigations:

	Laboratory number	
43.	Type of special investigations done: *Date of specimen collection*	☐ *FBC* ☐ *Smear* ☐ *Hb Electrophoresis* ☐ *Coombs*

		☐ *Reticulocyte count* ☐ *Iron studies (s-iron/ferritin)* ☐ *Bonemarrow aspiration* ☐ *Mantoux* ☐ *Other, specify* _____ _____ ___ ___ / ___ ___ / 2 0 ___ ___ *(dd/mm/yyyy)*
44.	Ward of FBC specimen collection Date of specimen collection	☐ *MOPD* ☐ *S12* ☐ *S11/A9/A7* __ __ / __ __ / 2 0 __ __ *(dd/mm/yyyy)* ☐ *Not recorded*
Treatment received at RCWMCH:		
45.	Bloodtransfusion	☐ *Yes* ☐ *No*
46.	Folate	☐ *Yes* ☐ *No* ☐ *Not recorded*
47.	Iron supplementation Correct dose?	☐ *Yes* ☐ *No* ☐ *Not recorded* ☐ *Yes* ☐ *No* ☐ *Not recorded*
48.	Vitamin C	☐ *Yes* ☐ *No* ☐ *Not recorded*
49.	Deworm	☐ *Yes* ☐ *No* ☐ *Not recorded*
50.	Any surgery during admission? If *Yes*, type of surgery Date of surgery	☐ *Yes* ☐ *No* ☐ *Not recorded* _____ ___ ___ / ___ ___ / 2 0 ___ ___ *(dd/mm/yyyy)*

Outcome and follow-up:

51.	Outcome of child If *Died*, cause of death (specify)	☐ *Discharged* ☐ *Transferred to another hospital* ☐ *Died* ☐ *Still at RXH* _____
52.	Anaemia: Morphology of anaemia:	☐ *Microcytic* ☐ *Normocytic* ☐ *Macrocytic*
53.	Cause for anaemia:	☐ *Iron deficiency* ☐ *Chronic Disease* ☐ *Haemolytic anaemia* ☐ *Malignancy* ☐ *Thalassaemia* ☐ *Chronic inflammatory disease* ☐ *Folate deficiency* ☐ *Cause not yet confirmed, await result/follow-up* ☐ *Specific cause not found* ☐ *Anaemia not investigated*
54.	Where Iron deficiency	☐ *Yes* ☐ *No*

	anaemia suspected or confirmed, was the child discharged with iron supplementation? Repeat prescription given? Total months treated?	 ☐ *Yes* ☐ *No* ☐ *Unknown* _____ *months*
55.	Date of discharge/ death/ transfer	___ ___ / ___ ___ / 2 0 ___ ___ *(dd/mm/yyyy)* ☐ *N/A*
56.	Discharge diagnosis *(mark all that apply)*	☐ *AGE* ☐ *CGE* ☐ *Malnutrition (specify according to WHO criteria)* ☐ *Severe UWFA* ☐ *PEM (with oedema)* ☐ *Moderate UWFA* ☐ *Mild UWFA* ☐ *Pneumonia* ☐ *Brongiolitis* ☐ *Asthma* ☐ *PTB* ☐ *UTI* ☐ *Sepsis* ☐ *Tonsilitis* ☐ *Otitis Media* ☐ *URTI* ☐ *Febrile seizures* ☐ *Other seizures: Specify:* _____ ☐ *Anaemia: Specify:*_____ ☐ *Bacterial meningitis* ☐ *Viral meningitis* ☐ *Other: Specify:*_____
57.	Any readmissions after discharge If *Yes*, specify diagnosis:	☐ *Yes* ☐ *No* *Specify:* _____ *Date:*___ ___ *to* ___ ___ / 2 0 ___ ___ *(dd/mm/yyyy)*
58.	Follow-up arrangements Where?	☐ *Yes* ☐ *No* ☐ *Not recorded* *Specify:*_____ *Date:*___ ___/20___ ___*(dd/mm/yyyy)*

Appendix 2: Ethics approval and annual progress report/renewal

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/research/humanethics/forms

26 July 2013

HREC REF: 422/2013

Dr M Wege
c/o Dr R Muloiwa
Paediatrics
Red Cross War Memorial Children's Hospital

Dear Dr Wege

PROJECT TITLE: A RETROSPECTIVE REVIEW OF THE PREVALENCE AND MANAGEMENT OF ANAEMIA IN CHILDREN AT RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL (RCWMCH)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Please note HREC approval is subject to approval from Red Cross War Memorial Children's Hospital to access patient records. Please provide the HREC with a copy of Institutional approval.

Approval is granted for one year till the 30th July 2014

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/research/humanethics/forms)

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical

s.thomas

Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

HREC office use only (FWA00001037; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.07.2015
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	13/11/2014

Principal Investigator to complete the following:

1. Protocol information

Date form submitted	13th November 2014		
HREC REF Number	422/2013	Current Ethics Approval was granted until	30th July 2014
Protocol title	A retrospective review of the prevalence and management of anaemia in children at Red Cross War Memorial Children's Hospital (RCWMCH)		
Principal Investigator	Martie Wege		
Department / Office Internal Mail Address	5th Floor ICH Building, Red Cross War Memorial Children's Hospital Rondebosch, 7700		
1.1 Does this protocol receive US Federal funding?		☐ Yes	☑ No

2. Protocol status (tick ✓)

☐	Research-related activities are ongoing
☑	Data collection is complete, data analysis only

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval	502	
Total number of records or specimens collected, reviewed or stored since last progress report	502	
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report	☑ Yes	☐ No

4. Signature

Signature of PI		Date	11th November 2014
Signature of Supervisor (if PI is a student)		Date	11th November 2014

Please complete the Closure form (FHS018) if the study is completed within the approval period

Appendix 3: Approval from Hospital research review committee

Dr TA Blake
Manager: Medical Services
Email: Thomas.Blake@pgwc.gov.za
Tel: +27 21 658 5788 fax: +27 21 658 5166
8 AUGUST 2013

DR M WEGE
DEPT PAEDIATRICS
RCWMCH

Dear Dr Wege,

Re: **RESEARCH AT RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL**

Approval to do research has been granted.

Kindly note that Folder reviews are done in the Medical Records Department. Should you wish to remove folders from the Department, no more than five (5) folders may be removed at any one time, and they must then be returned before the close of business the same day.

Yours faithfully,

DR T A BLAKE
CHAIRPERSON

HOSPITAL RESEARCH REVIEW COMMITTEE

RCWMCH

0216585788

0828225553

thomas.blake@westerncape.gov.za

Appendix 4: Manuscripts submission guidelines for PLOS one journal

Style and Format

File format

Length

Font

Headings

Layout

Page and line numbers

Footnotes

Language

Abbreviations

Reference style

Equations

Nomenclature

Manuscript Organization

Parts of a Submission

Additional Information Requested at Submission

Guidelines for Specific Study Types

Submission Guidelines

Style and Format

File format	Manuscript files can be in the following formats: DOC, DOCX, RTF, or PDF. Microsoft Word documents should not be locked or protected. LaTeX manuscripts must be submitted as PDFs. Read the LaTeX guidelines.
Length	Manuscripts can be any length. There are no restrictions on word count, number of figures, or amount of supporting information. We encourage you to present and discuss your findings concisely.
Font	Use any standard font and a standard font size.
Headings	Limit manuscript sections and sub-sections to 3 heading levels. Make sure heading levels are clearly indicated in the manuscript text.
Layout	Manuscript text should be double-spaced. Do not format text in multiple columns.
Page and line numbers	Include page numbers and line numbers in the manuscript file.
Footnotes	Footnotes are not permitted. If your manuscript contains footnotes, move the information into the main text or the reference list, depending on the content.
Language	Manuscripts must be submitted in English. You may submit translations of the manuscript or abstract as supporting information. Read the supporting information guidelines.
Abbreviations	Define abbreviations upon first appearance in the text. Do not use non-standard abbreviations unless they appear at least three times in the text. Keep abbreviations to a minimum.
Reference style	PLOS uses "Vancouver" style, as outlined in the ICMJE sample references. See reference formatting examples and additional instructions below.
Equations	We recommend using MathType for display and inline equations, as it will provide the most reliable outcome. If this is not possible, Equation Editor is acceptable. Avoid using MathType or Equation Editor to insert single variables (e.g., "$a^2 + b^2 = c^2$"), Greek or other symbols (e.g., β, Δ, or ′ [prime]), or mathematical operators (e.g., ×, ≥, or ±) in running text. Wherever possible, insert single symbols as normal text with the correct Unicode (hex) values. Do not use MathType or Equation Editor for only a portion of an equation. Rather, ensure that the entire equation is included. Avoid "hybrid" inline or display equations, in which part is text and part is MathType, or part is MathType and part is Equation Editor.
Nomenclature	Use correct and established nomenclature wherever possible.

Units of measurement	Use SI units. If you do not use these exclusively, provide the SI value in parentheses after each value. Read more about SI units.
Drugs	Provide the Recommended International Non-Proprietary Name (rINN).
Species names	Write in italics (e.g., *Homo sapiens*). Write out in full the genus and species, both in the title of the manuscript and at the first mention of an organism in a paper. After first mention, the first letter of the genus name followed by the full species name may be used (e.g., *H. sapiens*).
Genes, mutations, genotypes, and alleles	Write in italics. Use the recommended name by consulting the appropriate genetic nomenclature database (e.g., HUGO for human genes). It is sometimes advisable to indicate the synonyms for the gene the first time it appears in the text. Gene prefixes such as those used for oncogenes or cellular localization should be shown in roman typeface (e.g., v-fes, c-MYC).

Manuscript Organization

Manuscripts should be organized as follows. Instructions for each element appear below the list.

Beginning section	*The following elements are required, in order:* › Title page: List title, authors, and affiliations as first page of manuscript › Abstract › Introduction
Middle section	*The following elements can be renamed as needed and presented in any order:* › Materials and Methods › Results › Discussion › Conclusions (optional)
Ending section	*The following elements are required, in order:* › Acknowledgments › References › Supporting Information Captions (if applicable)
Other elements	› Figure captions are inserted immediately after the first paragraph in which the figure is cited. Figure files are uploaded separately. › Tables are inserted immediately after the first paragraph in which they are cited. › Supporting information files are uploaded separately.

Please refer to our downloadable sample files to make sure that your submission meets our formatting requirements:

- Download sample title, author list, and affiliations page (PDF)
- Download full manuscript sample (PDF)

Parts of a Submission

Title

Include a full title and a short title for the manuscript.

Title	Length	Guidelines	Examples
Full title	250 characters	Specific, descriptive, concise, and comprehensible to readers outside the field	Impact of Cigarette Smoke Exposure on Innate Immunity: A *Caenorhabditis elegans* Model Solar Drinking Water Disinfection (SODIS) to Reduce Childhood Diarrhoea in Rural Bolivia: A Cluster-Randomized, Controlled Trial
Short title	50 characters	State the topic of the study	Cigarette Smoke Exposure and Innate Immunity SODIS and Childhood Diarrhoea

Titles should be written in title case (all words capitalized except articles, prepositions, and conjunctions). Avoid specialist abbreviations if possible. For clinical trials, systematic reviews, or meta-analyses, the subtitle should include the study design.

Author list

Who belongs on the author list

All authors must meet the criteria for authorship as outlined in the authorship policy. Read the policy.

Those who contributed to the work but do not meet the criteria for authorship can be mentioned in the Acknowledgments. Read more about Acknowledgments.

Author names and affiliations

Enter author names on the title page of the manuscript and in the online submission system.

On the title page, write author names in the following order:

- First name (or initials, if used)
- Middle name (or initials, if used)
- Last name (surname, family name)

Each author on the list must have an affiliation. The affiliation includes department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country.

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- Abbreviations, if possible

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The introduction should:

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- Define the problem addressed and why it is important
- Include a brief review of the key literature
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Methods sections describing research using human or animal subjects and/or tissue or field sampling must include required ethics statements. See the reporting guidelines for human research, clinical trials, animal research, and observational and field studies for more information.

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Methods sections of manuscripts using data that should be deposited in a publicly available database should specify where the data have been deposited and provide the relevant accession numbers and version numbers, if appropriate. Accession numbers should be provided in parentheses after the entity on first use.

If the accession numbers have not yet been obtained at the time of submission, please state that they will be provided during review. They must be provided prior to publication.

A list of recommended repositories for different types of data can be found here.

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Methods sections describing research using cell lines must state the origin of the cell lines used. See the reporting guidelines for cell line research for more information.

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Methods sections of manuscripts adding new taxon names to the literature must follow the reporting guidelines below for a new zoological taxon, botanical taxon, or fungal taxon.

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These sections may all be separate, or may be combined to create a mixed Results/Discussion section (commonly labeled “Results and Discussion”) or a mixed Discussion/Conclusions section (commonly labeled “Discussion”). These sections may be further divided into subsections, each with a concise subheading, as appropriate. These sections have no word limit, but the language should be clear and concise.

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Acknowledgments

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Do not cite the following sources in the reference list:

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References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., "We used the techniques developed by our colleagues [19] to analyze the data"). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

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> Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of the references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE) also referred to as the "Vancouver" style. Example formats are listed below. Additional examples are in the ICMJE sample references.

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Source	Format
Published articles	Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (*Ailuropoda melanoleuca*). Genet Mol Res. 2011;10: 1576-1588. Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005 *Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers.*
Accepted, unpublished articles	Same as published articles, but substitute "In press" for page numbers or DOI.
Web sites or online articles	Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available: http://www.globalizationandhealth.com/content/1/1/14.

Books	Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.
Book chapters	Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.
Deposited articles (preprints, e-prints, or arXiv)	Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity; 1991. Preprint. Available: arXiv:1403.3301v1. Accessed 17 March 2014.
Published media (print or online newspapers and magazine articles)	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 29 Jan 2014. Available: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html. Accessed 17 March 2014.
New media (blogs, web sites, or other written works)	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.
Masters' theses or doctoral dissertations	Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available: http://cumincad.scix.net/cgi-bin/works/Show?2e09
Databases and repositories (Figshare, arXiv)	Roberts SB. QPX Genome Browser Feature Tracks; 2013. Database: figshare [Internet]. Accessed: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214.
Multimedia (videos, movies, or TV shows)	Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

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Authors can submit essential supporting files and multimedia files along with their manuscripts. All Supporting Information will be subject to peer review. All file types can be submitted, but files must be smaller than 10 MB in size.

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All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article.

Read our policy on data availability.

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See our list of recommended repositories.

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- Deposit data in the integrated repository of choice.
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If you have any questions, please email us.

Accession numbers

All appropriate datasets, images, and information should be deposited in public resources. Please provide the relevant accession numbers (and version numbers, if appropriate). Accession numbers should be provided in parentheses after the entity on first use.

Suggested databases include, but are not limited to:

- ArrayExpress
- BioModels Database
- Database of Interacting Proteins
- DNA Data Bank of Japan [DDBJ]
- DRYAD
- EMBL Nucleotide Sequence Database
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Manuscripts reporting a meta-analysis of genetic association studies must report results of value to the fiel and should be reported according to the guidelines presented in *Systematic Reviews of Genetic Association Studies* by Sagoo *et al.*

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Authors reporting research using cell lines should state when and where they obtained the cells, giving the date and the name of the researcher, cell line repository, or commercial source (company) who provided the cells, as appropriate.

Authors must also include the following information for each cell line:

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- Details of institutional review board or ethics committee approval; AND
- For human cells, confirmation of written informed consent from the donor, guardian, or next of kin

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- A reference to the published article that first described the cell line; AND/OR
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- The final antibody concentration or dilution.
- A reference to the validation study if the antibody was previously validated. If not, provide details of how the authors validate the antibody for the applications and species used.

We encourage authors to consider adding information on new validations to a publicly available database such as Antibodypedia or CiteAb.

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