An evaluation of microcytic red blood cell disorders in patients reporting to a tertiary care centre in the Cook Islands

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Abstract

Objective:

Anaemia is a medical condition characterised by reduced levels of red blood cells (RBCs) and haemoglobin (Hb) in the blood. It is a relatively common condition which appears in many population health reports. The main objective of this study was to obtain an evaluation of microcytic RBC disorders in the Cook Islands population. This evaluation was assessed from the results of patient's laboratory full blood count (FBC) tests at the Rarotonga Hospital Laboratory (RHL) (the national laboratory for the Cook Islands), using their standardised patient reference ranges. This study is of interest due to the lack of existing published studies based on microcytic anaemias in the local Cook Islands population. It is suspected that the cause of anaemia may be overlooked or misdiagnosed in this group, especially in the case of thalassaemia/haemoglobinopathies and iron deficiency anaemia (IDA) It is important to evaluate the prevalence of microcytic RBC disorders in the population to enable appropriate health care interventions and the provision of a suitable diagnostic service to support these interventions.

Methods:

An audit was performed on 874 FBC results with low MCV (mean cell volume) and/or low MCH (mean cell haemoglobin) values. Mentzer Index (MI) and a web-based tool were applied to differentiate between IDA and thalassaemia as possible causes of microcytic anaemia. Where available, serum ferritin, CRP and alpha thalassaemia results were included in the evaluation of individual cases.

Results:

Of the 874 patient FBC results, the MI suggested IDA in 646 (74%) individuals and thalassaemia in 225 (26%) individuals. Of the IDA estimates, 485 (80.6%) were females and 142 (59.2%) males. Thalassaemia estimates were more common among males, (40.4%) compared to females (19.1%). Part of the results includes the observation that most cases with microcytosis and/or hypochromia were not investigated according to best practice guidelines of my study.

Conclusion:

This study has provided a preliminary evaluation of microcytic red cell disorders in the Cook Islands population presenting to RHL. It raises the likelihood that a significant number of cases of thalassaemia/haemoglobinopathy may be present in the population. A clear guideline for the investigation of microcytosis and hypochromia should be implemented to avoid missing or misdiagnosing patients with these FBC results. In addition, RHL should implement investigative protocols for thalassaemia/haemoglobinopathy. Additionally, thalassaemia/haemoglobinopathy prenatal screening should be included as part of the antenatal screening of pregnant mothers.

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor used artificial intelligence tools or generative artificial intelligence tools (unless it is clearly stated, and referenced, along with the purpose of use), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Victoria Wuatai
Signature

18th April 2025
Date

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Ethics Approval

This research, ethics application number 23/107, was granted full ethics approval by Auckland University of Technology Ethics Committee (AUTEC) on July 3, 2023, for three years, expiring on July 3, 2026. In addition, the Cook Islands Research Committee (CIRC) also granted approval for this research on July 17, 2023, for the period from May 2023 to May 2024, followed by a permit granted to undertake research in the Cook Islands by the chairperson of the CIRC. The permit number for this research is 17/23.

Chapter 1 Introduction

Anaemia is a medical condition characterised by a decreased number of red blood cells (RBCs) and haemoglobin (Hb) concentration in the blood (Kundu et al., 2023; Williams et al., 2023). It is a relatively common condition which appears in many population health reports. In this context, two main categories of anaemia are identified. First, there are non-nutritional anaemias caused by infection, inflammation, blood loss or genetic disorders. Secondly, there are nutritional anaemias caused by deficiencies of iron, vitamin A, vitamin B_{12}, folate or riboflavin (Williams et al., 2023). In other words, anaemia is a multifactorial condition with numerous causal factors including both internal (i.e., non-nutritional) and external (nutritional) micro-environments (Loechl et al., 2023).

The main objective of this study was to obtain an evaluation of microcytic anaemias in the Cook Islands population. This evaluation was assessed from the results of patient laboratory full blood count (FBC) tests at the Rarotonga Hospital Laboratory (RHL) which is the National Laboratory for the Cook Islands, using their standardised patient reference ranges. This study is of interest due to the lack of existing published studies based on microcytic anaemias in the local Cook Islands population. Moreover, it is suspected that the cause of anaemias may be overlooked or misdiagnosed in this group, especially in the case of thalassaemia/haemoglobinopathies and iron deficiency anaemia (IDA). If this is so, appropriate treatment and management may not be provided, which can cause further health complications for patients and their families. Furthermore, the increase in the number of migrant workers from other countries has resulted in increased rates of inter-marriage amongst migrants and natives. This may be of additional interest, as inherited anaemias could contribute to the prevalence of this condition in the Cook Islands population. It is important to evaluate the prevalence of microcytic anaemias in the population to enable appropriate health care interventions and consideration of the provision of a suitable diagnostic service to support these interventions.

Moreover, adapting these healthcare interventions in the Cook Islands will invest in a quality health system that meets the needs of the people; and stands for the Health Ministry's mission "To provide accessible, affordable healthcare and equitable health services of the highest quality, by and for all, in order to improve the health status of people living in the Cook Islands" (Te Marae Ora Cook Islands Ministry of Health, n.d.)

Anaemia is the primary term and health condition that is further split into different disease categories, indicating their types and severity. It is defined as low Hb concentration (Kundu et al., 2023; Seu et al., 2019) in the circulating blood of a human body. To be able to indicate there is a low Hb concentration, it is compared and aligned to the standardised Diagnostic Medical Laboratory patient reference ranges that were adjusted to age, sex, physiological condition, and height above sea level. The types and severity of anaemia can be described using these following terms: microcytosis, normocytosis, macrocytosis, hypochromia, and normochromia.

Hb concentration in the blood stream indicates the iron (i.e. haem) concentration. Iron is one of the main components in the RBCs that are involved in the transportation of oxygen (O_2) (which is picked up from the air we breathe) and delivered to anywhere in the body, hence the colour of the RBCs. Therefore, Hb is the O_2 transport protein that is essential for the body's metabolic processes. These metabolic processes include cellular proliferation, DNA synthesis, neurotransmitter synthesis and energy production (Kang et al., 2021). When a patient has a low Hb concentration they are considered anaemic.

There are numerous studies in the literature stating the global prevalence of anaemia. Loechl et al. (2023) discussed the prevalence of anaemia as, critical worldwide economic and health issue that needs an emphasis on public health relevance interventions. These authors also provided a consideration of the clinical context of the disease. Anaemia contributes to increased morbidity and mortality, particularly in women and young school children (Chaparro & Suchdev, 2019; Raiten et al., 2021) with a globally reported maternal mortality rate with severe anaemia of 2.36% (Daru et al., 2018) and with 41.8% of women globally being anaemic during their pregnancy (McLean et al., 2009). Additionally, for young children aged 6 to 59 months, a global figure of 40% were found to be anaemic in 2019 compared to 48% in 2000 (Stevens et al., 2022). The World Health Assembly (WHA) has introduced a global nutritional target for women between the ages of 15 and 49 years to reduce the anaemia rate to half of its current prevalence by 2025. However, it is not a reasonable target due to the diverse specific causes of anaemia either caused by non-nutritional or nutritional factors (Williams et al., 2023). However, there is a huge need for the quality implementation of effective multi-sectoral approaches and actions to address these causes.

This perspective raises a variety of questions requiring consideration. For example, what are the possible causes and predisposing factors of anaemia, and whether these are

related to age, sex, environmental or other influences. The approach to reducing the prevalence of anaemia in a population requires a systematic diagnostic approach to classify it at least into broad categories with similar aetiology and it's approach to treatment (Williams et al., 2023). This approach might then be expected to alleviate the problem and likely result in a reduction of anaemia prevalent in a region.

Iron deficiency (ID) is prevalent globally, affecting an estimated 2.5 billion people worldwide (Camaschella, 2019). ID is the most prominent nutrient deficiency in developing countries seen in pre-school children and pregnant women (Özdemir, 2015). In developed countries, where anaemia is used as a secondary indicator of ID, 30-40% of children and pregnant women are observed to have ID (World Health Organisation, 2001). Özdemir (2015) states that the terms ID and IDA are frequently used interchangeably because anaemia is a sign of ID, but it is important to note that ID can also be present without anaemia. A review by Al-Naseem et al. (2021) has discussed that ID without anaemia (e.g. having Hb concentration below the normal reference range of a particular age/gender group) is present in patients with anaemic symptoms of fatigue but is poorly recognised by clinicians. However, a combination of tests is required for the confirmation or exclusion of iron deficiency (Al-Naseem et al., 2021).

Insufficient nutritional requirements in the body lead to the occurrence of IDA status. Different stages of the human body's development impose different nutritional demands that are crucial for body development and quality of life. Infant nutritional requirements in their early stage of life are unique. They need the greatest amount of nutrition for body growth and the development of major organs in the body, in particular the brain. For an infant to obtain the essential nutrients for growth, they must have adequate and appropriate food sources to avoid the causes of nutrient deficiency. Iron is the key important nutrient of interest in this study because of its deficiency state (i.e., ID) has caused significant morbidity and mortality in young children. Thus, ID is an ongoing global public health issue (Chaparro & Suchdev, 2019; Raiten et al., 2021; Williams et al., 2023).

Activity and Nutrition Aotearoa (ANA) is an organisation that has a vision that everyone in Aotearoa can eat well and lead an active life (Activity and Nutrition Aotearoa [ANA], 2020). This organisation reported on a survey that was carried out in 2008/09 on iron intake amongst the New Zealand population. They found a 5.6% overall prevalence of low iron intake (i.e. below the recommended daily dietary

amount). It was found males had a low prevalence iron intake of 1.2% as compared to 9.7% for women. However, Māori and Pacific women had higher prevalence of low iron intake of 18.4% and 19.9% respectively, compared to 9.3% of New Zealand European and other women. However, females between the ages of 15 and 18 years had the highest rate of inadequate iron intake at 34.2%, where Māori in this age group has a prevalence of 49% while 40% of Pacific women in this age group had inadequate iron intake, potentially leading to IDA. Another survey undertaken subsequently, in 2014/15, found no significant differences in IDA prevalence of 6.9% (as compared to 5.6% from the first survey) amongst adults or any population sub-groups after performing biochemical analyses (ANA, 2020). Therefore, the first survey shows higher prevalence of insufficient iron intake amongst Māori and Pacific Islanders versus New Zealand Europeans. But the second survey showing biochemical evidence of ID showed no such discrepancies. This is indicating possible increased risk of IDA amongst Māori and Pacific Islanders versus New Zealand Europeans in New Zealand, but more studies need to be done. Although these two surveys have discussed two different means of assessing iron status (i.e., iron intake through self-reported food consumption versus biochemical analysis of iron status), however they are not comparable with each other, and authors were able to demonstrate possible ethnic differences in the prevalence of IDA within the NZ population. The authors emphasise that biochemical testing is more reliable than dietary data when evaluating individual functional iron levels in individuals. Future surveys should use the same measurement method for assessing IDA prevalence to enable adequate comparison between two study timeframes. Overall, these surveys indicated that Pacific women have a higher prevalence rate of IDA than those women who are non-Pacific (ANA, 2020; Ministry of Health New Zealand, 2011). We can conclude from these studies that Pacific women's iron intake is inadequate, due to the foods they consume, compared to the higher level of iron in the diet consumed by non-Pacific women. Additionally, dietary intake surveys based on iron intake and other micronutrients will likely contribute to the reduction of the IDA prevalence rate and these surveys need to be performed.

There may not be any published specific figures on ID or anaemia for the Cook Islands population or ethnicity, but the above figures for Pacific women are inclusive of the Cook Islands ethnicity. This should alarm the health authorities in the Cook Islands and encourage them to perform some studies or surveys to determine the prevalence of ID in

the local Cook Islands population. These studies must be published to enable other researchers to compare the findings with other populations or ethnicities studies.

Anaemia is sub-classified into disease states indicating their type and severity. Table 1 shows the combination and brief RBC descriptions of these sub-classifications according to their size and colour, and with the most common sub-classifications marked as *. These sub-classifications of anaemia are dependent on haematological RBC indices such as mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), Hb concentration, haematocrit (Hct), RBC count and RBC distribution width (RDW) (Keohane et al., 2016), which all correlate to the medical laboratory standardised reference ranges. MCV and MCH are the two RBC indices first in line to describe the types of anaemia: MCV is designated for RBC size and MCH is for the average amount of Hb in each RBC (Keohane et al., 2016). There are numerous studies that have utilised these RBC indices for their sample collection, data analysis and for the evaluation and investigation of the prevalence and likely cause of RBC disorders. For example, a study conducted by Kandhro et al. (2017) utilised these RBC indices to identify the most reliable bioinformatics-based discrimination formulas for differentiating thalassaemia traits (TT) from IDA. The formulae included Mentzer Index (MI), England-Fraser formula, Green-King formula, Shine-Lal formula, Ehsani formula; Srivastava formula; Palestinian population; RDW Index; Ricerca;and Huber-Herklotz Index. Additionally, these RBC indices were also used to identify and to confirm the best logarithm-based formula for differentiating TT from IDA in pregnancy during the first trimester (Shuang et al., 2023). This is the logarithm-based formula XS-1 model that has been established as a web-based tool formula (see Figure 12).

Table 1

Sub-classifications of anaemia according to RBC size and colour

Haematological disease consequences (combinations)	RBC size	RBC colour
Microcytic Hypochromic*	Small	Less
Microcytic Normochromic*	Small	Normal
Microcytic Hyperchromic	Small	More
Normocytic Hypochromic*	Normal	Less
Normocytic Normochromic*	Normal	Normal
Normocytic Hyperchromic	Normal	More
Macrocytic Hypochromic	Large	Less
Macrocytic Normochromic*	Large	Normal
Macrocytic Hyperchromic	Large	More

Note. * indicates the most common combinations in haematology.

Furthermore, these RBC indices cannot be individually used for evaluation of erythrocytes (Balcázar-Villarroel et al., 2024). They must be collected to produce a reliable and precise study. However, of these RBC indices, MCV is the most important. MCV indicates the type of anaemia present. Studies have also indicated a few haematological RBC formulas shown in Table 2 that are associated with these RBC indices, hence explaining why MCV is popular. Narchi and Basak (2010) stated that the Green-King Index was the best discriminatory index for IDA versus alpha thalassaemia detection, whereas Sherali et al. (2023) described the Mentzer Index as a valuable tool for the detection of IDA in children.

Table 2

Haematological RBC indices and associated RBC formulas

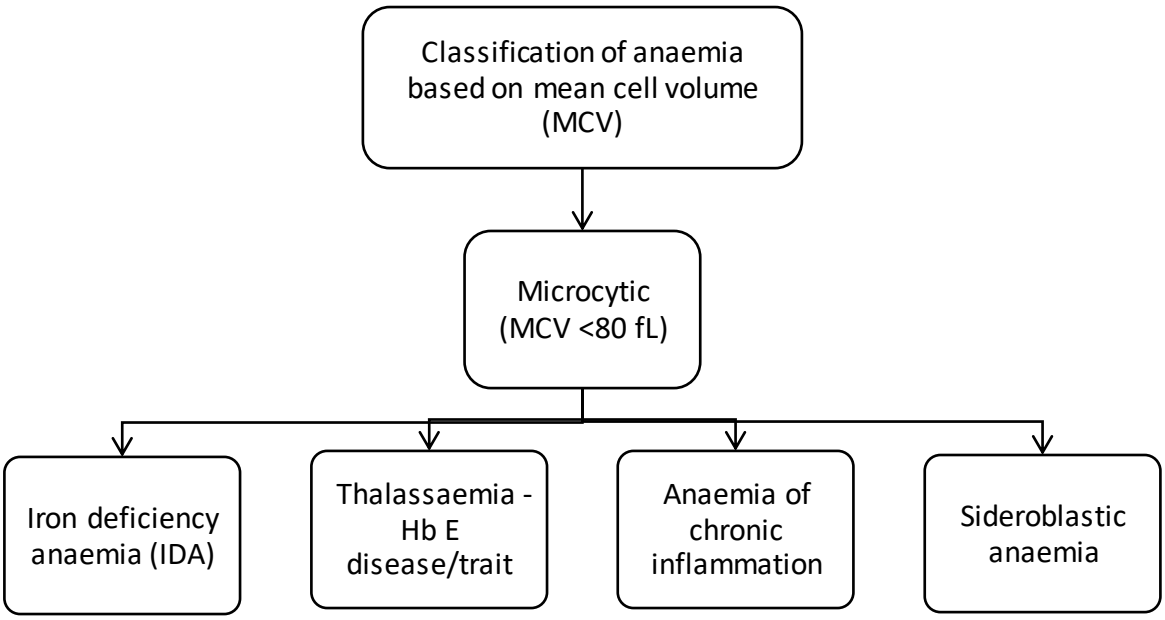
Index	Calculation	Cut-off value
Mentzer index	MCV/RBC	13
Green-King formula	MCV^2 x RDW/(Hb x 100)	65
England-Fraser formula	MCV – (5 x Hb) + RBC + 3.4	0
Shine-Lal formula	MCV^2 x MCH/100	1530
Srivastava formula	MCH/RBC	4.4
RDW index	MCV x RDW/RBC	220

Note. *RBC in this instance refers to RBC ***count*** and *Hb refers to Hb concentration. These are found in a patient FBC results performed on an automated blood cell count analyser in a haematology department. The information on RBC indices formulas was adapted from Narchi and Basak (2010).

MCV is the most useful RBC index when diagnosing the type of anaemia. This research study aimed to give an estimate of the possible microcytic anaemia in the local Cook Islands population with inclusive background knowledge of anaemias and their causes. Figure 1 shows an algorithm of RBC diseases being classified as microcytic anaemic where MCV is <80 fL. This is the MCV value that was used in this which corresponds to the lower cut-off in the reference range utilised by the RHL. Although Figure 1 indicates four types of microcytic anaemia, IDA and thalassaemia are the two microcytic anaemias that will be investigated in this thesis based on the study population and data collected. The selection of patients with values of low MCH (< 27 pg) on its own was also considered as part of this study due to its close correspondence with MCV, which is an initial point for microcytic anaemia classification.

Figure 1

Classification algorithm of anaemia by MCV



Note. Information adapted from Keohane et al. (2016, p. 292).

1.1 Iron Physiology

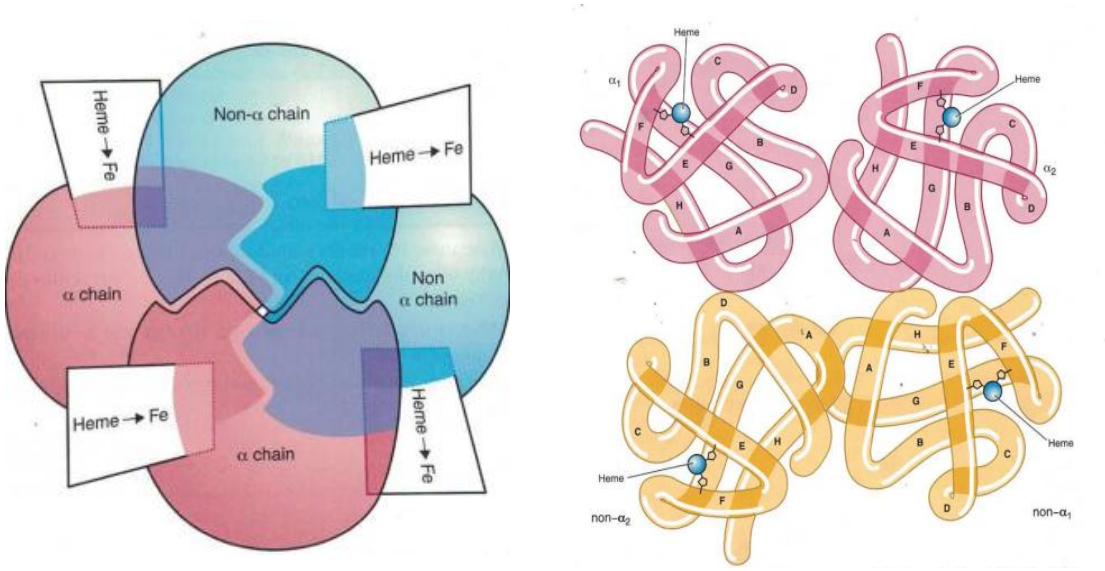
There are numerous reviews that emphasise the importance of iron in the body. For instance, a review by Lanser et al. (2021) discussed iron being crucially involved in multiple important biochemical pathways such as mitochondrial respiration which is used for energy production/conversion. Similar points were stated by Kang et al. (2021) in a review on "Ethnic Differences in Iron Status." At this stage, it is clearly understood that iron is essentially important for the body; however, it must be at an adequate level

for it not to become a problem for the body, which is also discussed by numerous studies and reviews based on iron mechanisms (Ganz & Nemeth, 2015).

In a single RBC, a haemoglobin molecule (which also relates to Hb concentration) is a globular tetramer consisting of four globin proteins, comprising of one haem subunit in each of those four globins, with an iron atom in the middle (see Figure 2). In a normal human adult, the completion of mature RBC synthesis in the bone marrow creates at least two million RBCs per second. There are about 280 million Hb molecules in a RBC. The iron atoms in the middle of haemoglobin molecules are the oxygen binding sites for RBCs. These iron atoms provide more than a billion oxygen binding sites (Drakesmith et al., 2015).

Figure 2

Haemoglobin molecule



Note. Pictures adapted from Rodak (2007, pp. 106,108). The picture on the left shows the globin tetramer of the haemoglobin molecule. The right picture shows the four globin chains of a complete haemoglobin molecule where pink represents alpha (α) 1 (left) and α 2 (right). The yellow coloured represents the non-α 2 (left) and non-α 1 (right). More information relating to this figure is discussed later in relation to RBC disorders such as thalassaemia.

According to Lanser et al. (2021), iron is best known for its essential role as a functional component of the haemoglobin (consisting of the most iron). The myoglobin protein enables O_2 binding and O_2 transportation throughout the body. Myoglobin is another protein component found in muscle cells which is used for O_2 storage (Shaffer, 2023). In terms of iron storage, myoglobin stores a lesser amount of iron in the body than the iron stored in the haemoglobin molecule (Bain et al., 2012; Conway & Henderson,

2019). Table 3 shows an example of the distribution of iron content in various proteins in a 70kg man. The distribution of iron is dependent on a person's body weight.

Table 3

Distribution of iron content in a 70kg man's body

Protein	Location	Iron Content (mg)
Haemoglobin	RBCs	3000
Myoglobin	Muscle	400
Cytochromes and iron sulphur proteins	All tissues	50
Transferrin	Plasma and extracellular fluid	5
Ferritin and haemosiderin	Liver, spleen and bone marrow	100–1000

Note. Information adapted from Bain et al. (2012, p. 176).

1.2 Iron Sources, Absorption and Iron Metabolism

Iron is a strong irritant and a toxic element in its free state in the body (Shaffer, 2023). Due to this problem, the body has a system of organic molecules that bind to free iron atoms to prevent them from being transferred and stored, thus causing oxidative damage (Conway & Henderson, 2019). These free iron atoms are capable of causing cellular damage from the formation of reactive oxygen species (i.e., free radicals) such as hydroxyl radicals, superoxide anion radicals, free nitrogen radicals and singlet oxygen radicals (Jakubczyk et al., 2020).

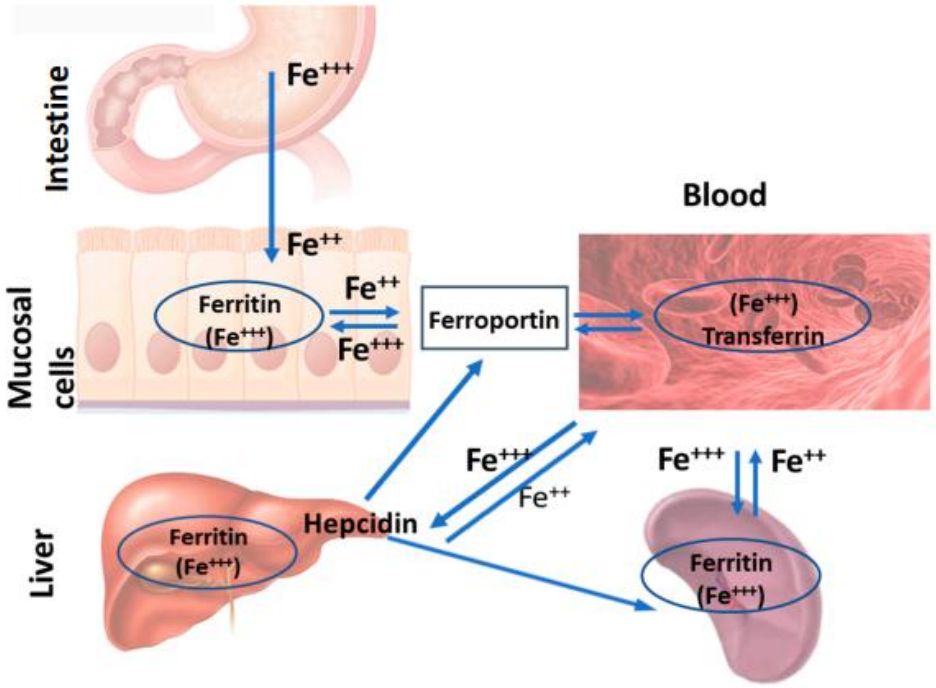
Iron is not synthesised in the body; however, it is acquired from the absorption of two dietary sources, namely, haem iron and non-haem (elemental) iron. Haem iron is the form contained in the haemoglobin and myoglobin is obtained from animal-based foods. This haem form is much more freely absorbed as compared to the non-haem iron which is gained from animal and plant-based foods (Conway & Henderson, 2019; Kang et al., 2021). The haem iron has less susceptibility to absorption inhibitors such as phytates and tannins (Bailey et al., 2015). The absorption of haem iron has its own unique proteins and pathway in the absorption process; however, this is not clearly understood (Anderson & Frazer, 2017). On the other hand, the absorption mechanism of the non-haem iron in the body is well-understood (Anderson & Frazer, 2017). The body's iron demand must be met and firmly regulated to prevent the occurrence of iron-related medical consequences due to insufficient iron availability (Camaschella et al., 2020). Dietary non-haem iron exists in two valency states known as ferrous, Fe^{2+} (reduced), and ferric Fe^{3+} (oxidised) ions. Inorganic iron in the ferric state must first be

reduced to Fe^{2+} iron by the enzyme ferrireductase, specifically duodenal cytochrome b (DCYTB), which is located on the apical membrane of enterocytes in the duodenum. After reduction, the Fe^{2+} iron is transported into the enterocytes through the divalent metal transporter 1 (DMT1). (Conway & Henderson, 2019; Kang et al., 2021). When the Fe^{2+} ion is absorbed into the cytoplasm of the enterocyte, it can be utilised in three ways. Firstly, it can be utilised within the mitochondria for various enzyme reactions. Secondly, iron can be stored as ferritin within the enterocyte for intracellular functions. Lastly, it can be exported out of the enterocytes by a non-haem iron export protein, called ferroportin (FPN). FPN is located on the basolateral membrane and is transported via transferrin to the bone marrow and other distant body sites (Conway & Henderson, 2019). The FPN export protein is not only present in the duodenal enterocytes where it is responsible for cellular iron export into the blood, but it is also responsible for iron mobilisation from liver cells (hepatocytes) and the iron recycling macrophages of the reticuloendothelial system (RES) (i.e., spleen, liver and bone marrow). These cells recover iron from senescent and damaged RBCs for re-use in new RBCs (Drakesmith et al., 2015; Theurl et al., 2016; Yiannikourides & Latunde-Dada, 2019).

Figure 3 illustrates the body tissues involved in the regulation of systemic iron homeostasis. Once a Fe^{2+} ion is exported, it must be first oxidised by ferroxidase hephaestin to Fe^{3+} ion before binding to transferrin (TF) molecules in the bloodstream and then transported to other body sites (Anderson & Frazer, 2017; Conway & Henderson, 2019; Nishito & Kambe, 2018).

Figure 3

Tissues involved in the regulation of systemic iron homeostasis



Note. Adapted from Yiannikourides and Latunde-Dada (2019).

TF is the result of apotransferrin glycoprotein binding to free iron in the plasma. TF molecules have essential roles in the circulation that carry circulating iron to erythroblasts in the bone marrow for erythropoiesis. TF also provide iron tissues requiring growth and reparative processes, and into iron-storage molecules such as ferritin and haemosiderin (Conway & Henderson, 2019). Table 3 provides an illustration of iron distribution in the body. The bone marrow is one of the body sites that has a high requirement for iron for erythropoiesis (i.e., erythroid marrow proliferative activity) (Anderson & Frazer, 2017; Keohane et al., 2016). Erythropoiesis involves bone marrow functionality to produce the daily requirement of normal RBCs and replace the approximately 1% loss of RBCs from circulation due to RBC senescence in a normal healthy individual (Keohane et al., 2016).

1.3 Iron Homeostasis

Insufficient and excess iron in the body are both problematic. The human body processes consists of an equilibrium mechanism to maintain a stable state in the body, to reduce the effect of an internal health problem for an individual. Iron homeostasis involves the mechanism of balancing iron in the body from the moment when iron is

absorbed by ingestion in the diet, to cellular metabolism and utilisation, then finally to iron excretion or loss (Conway & Henderson, 2019; Fleming & Ponka, 2012).

Systemic iron homeostasis as shown in Figures 3 and 4 depends on the amount of iron absorption in the diet. The human body has a daily requirement of 20–30 mg iron for metabolic processes (Lanser et al., 2021) and, out of this, about 1–2 mg of absorbed iron is lost daily by the removal of cells from intestinal mucosa, the shedding of skin cells, and sweat and urine formation (Conway & Henderson, 2019); thus, these losses are not regulated. The RBCs have a life-span of 120 days in the circulation of a normal healthy human being (Keohane et al., 2016). Erythropoiesis demands the highest amount of iron, 20–25 mg iron per day, for adequate productivity (Kang et al., 2021).

Table 4

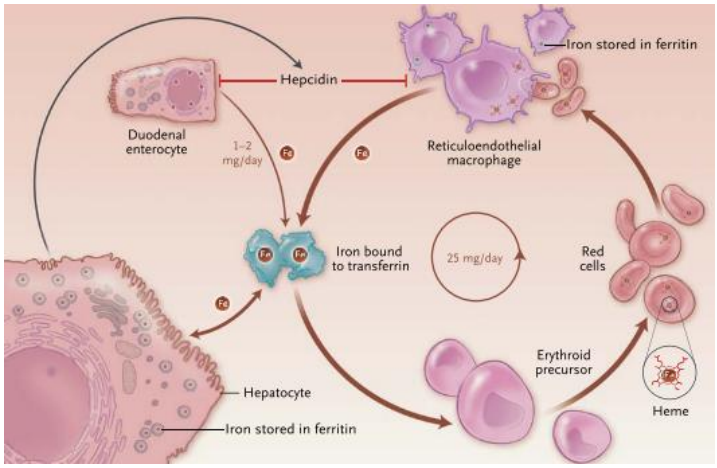
Recommended daily dietary allowance for iron

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	0.27 mg*	0.27 mg*		
7 – 12 months	11 mg	11 mg		
1 – 3 years	7 mg	7 mg		
4 – 8 years	10 mg	10 mg		
9 – 13 years	8 mg	8 mg		
14 – 18 years	11 mg	15 mg	27 mg	10 mg
19 – 50 years	8 mg	18 mg	27 mg	9 mg
51+ years	8 mg	8 mg		

Note. * indicates adequate intake for this age group. Information adapted from National Institutes of Health (2023).

Figure 4

Iron cycle showing the regulation of systemic iron homeostasis



Note. Adapted from Fleming and Ponka (2012).

Erythropoietin (EPO) and two other hormones, hepcidin and erythroferrone, are involved in the regulation of systemic iron homeostasis (Coffey & Ganz, 2018; Kang et al., 2021). Erythropoiesis is described as the proliferative activity of erythroid cells in the bone marrow (Keohane et al., 2016). Of these three hormones mentioned above, hepcidin is the main regulator of body iron balance that is produced in the liver (Lanser et al., 2021). It is described as a systemic iron-regulator hormone (Ganz & Nemeth, 2012), which acts as a negative regulator of iron release from enterocytes and macrophages in response to increased iron level, thus, modulating and controlling the levels of iron that are stored in the body (Rishi et al., 2015). Hepcidin is a small peptide molecule, and its release is triggered by adequate or high iron stores as detected by the liver itself from circulating iron levels (Fleming & Ponka, 2012). Additionally, the expression of hepcidin is reduced by increased erythropoiesis, ID and tissue hypoxia ,enabling more iron to be released for erythropoiesis (Conway and Henderson (2019). EPO is a glycoprotein hormone produced within the peritubular cells located in the renal cortex of the kidney. This hormone plays a crucial role in maintaining adequate oxygen levels in the blood. When partial pressure of oxygen (pO_2) is low, it stimulates the bone marrow to produce more RBCs (Krantz, 1991). Erythroferrone is a hormone primarily produced by erythroblasts, and it plays a crucial role in regulating hepcidin, the central hormone that governs the levels of iron in the plasma and the overall iron content within the body (Srole & Ganz, 2021). To regulate the iron lost by the various excretion processes mentioned above, a balance of the total iron stores must be maintained in the body, which is controlled by dietary intake, iron released from hepatocytes and iron recycling macrophages (Dev & Babitt, 2017). Table 4 shows a recommended daily intake for iron and Figure 4 illustrates the iron cycle. When there is no balanced iron regulation in the body, associated iron-related diseases will occur in an individual.

1.4 Iron Status

Sources of haem iron include lean red meat, liver, eggs, seafood and spinach, whereas non-haem iron-containing foods include nuts, fruits, vegetables, beans and grains (Conway & Henderson, 2019). The iron status of an individual depends on their daily iron intake and the iron cycle as depicted above Figure 4. Consequently, when an individual has a normal iron status, erythropoiesis is not constrained by iron availability or limited iron stores in the body (Bain et al., 2012). Under this condition an individual will be able to cope with daily physiological needs and, in surviving an urgent

physiological condition such as acute blood loss from injury, they will have adequate reserves. Defining the limits of normality is challenging, and there is debate about whether physiological normality means having just a minimal amount of storage iron. In extreme conditions such as IDA and haemochromatosis, however, these limits are clear says Bain et al. (2012).

Not only is there the question of increased or decreased iron in the body, but the maldistribution of iron is also a possibility in anaemia associated with inflammation or infections, where erythropoiesis is partially affected and the iron released from recycling macrophages of the RES results in accumulation of iron in the body (Bain et al. (2012). Therefore, the determination of iron status of an individual can be evaluated by the measurement of Hb concentration (the initial indication of an anaemia state) along with multiple biomarkers that are currently used for anaemia diagnosis and differentiation. These multiple biomarkers include serum iron, ferritin, TF and soluble transferrin receptor (sTfR) (Kang et al., 2021).

Figure 1 (on page 7) shows the different profiles of microcytic anaemias. When managing a patient with these anaemias, the process begins with a comprehensive history-taking and a physical examination (Keohane et al., 2016). If someone experiences sudden fatigue and shortness of breath, it may indicate a rapid decrease in Hb levels. Conversely, if there are minimal or no symptoms, it could indicate a chronic condition where the body has adapted to the lower Hb levels. A comprehensive history and physical examination can provide valuable insights to suggest a likely cause of the microcytic anaemia. This will enable a more rational and cost-effective approach when ordering the necessary diagnostic tests (Keohane et al., 2016).

The initial laboratory diagnosis of anaemia involves accurately measuring Hb, Hct, MCV, and RBC count from a FBC analysis, and comparing them to the reference ranges of healthy individuals that are of the same age, sex, race, and environment (Keohane et al., 2016). An abnormal condition may be indicated by knowledge of the previous haematological values of an individual. Assessing anaemia effectively requires consideration of the multiple causes and test results of Hb, Hct, MCV and RBC count from a FBC analysis with further investigation of the utilisation of biomarkers (such as iron, ferritin, sTfR), which enable a specific diagnosis as seen in Figure 10 of Thalassaemia syndrome.

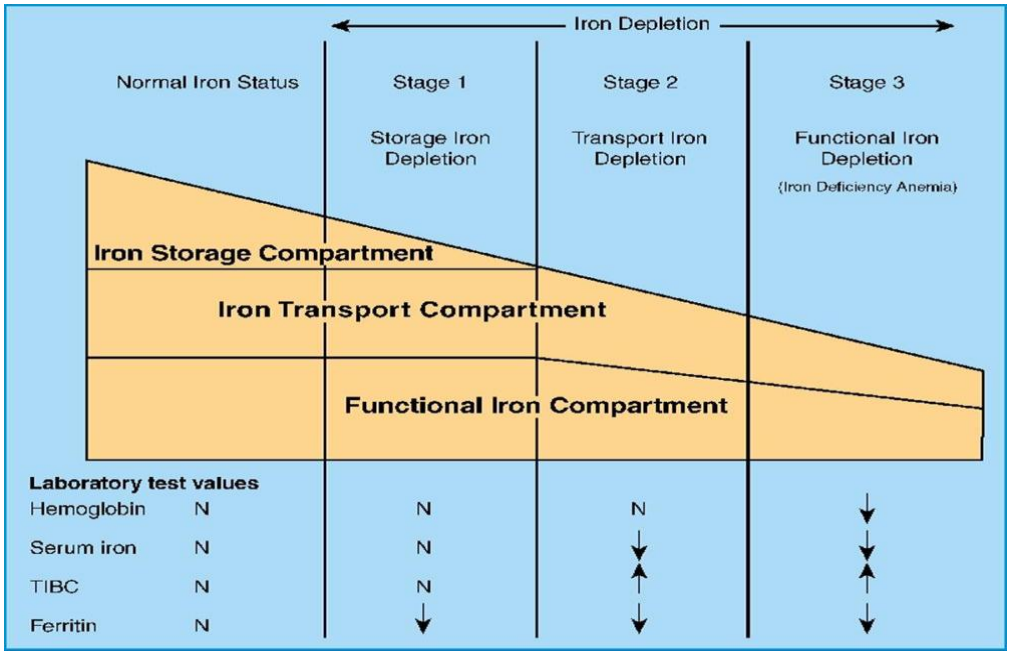
1.4.1 Iron Deficiency Anaemia

Due to anaemia being the primary indicator of ID, the terms ID and IDA are often used interchangeably (Özdemir, 2015). However, it is important to note that ID can occur without anaemia, as indicated by the Hb level, but it can still have an impact on body tissues.

The manifestation of ID occurs in various stages (stage 1, 2 and 3) (Keohane et al., 2016). Insufficient intake of iron supplements or iron sources primarily leads to a reduction in iron stores. It is important to note that the absence of anaemia does not rule out ID, as Hb levels can remain normal for a while despite reduced iron stores (see stage 2, Figure 5). Initially, plasma ferritin and plasma transferrin saturation are the only markers that show a decrease. Once iron stores have been depleted, a decrease in haemoglobin levels indicates a negative iron balance. In conclusion, the term ID is used to describe low body iron levels, while IDA refers specifically to the progression to anaemia. Figure 5 illustrates the development of IDA through three stages also known as three compartments. IDA is generally defined as an anaemia with inadequate iron stores (Keohane et al., 2016)

Figure 5

Development of IDA



Note. Figure adapted from Keohane et al. (2016, p. 299).

Ferritin, the stored form of iron, is found in the macrophages of the bone marrow and liver cells during stage 1 (storage compartment). Serum transferrin is involved in stage

2 (transport compartment), whereas stage 3 (functional compartment) focuses on iron within Hb, myoglobin, and cytochromes. Hb, intracellular ferritin and haemosiderin make up almost 90% of the total iron distribution in a normal human (Keohane et al., 2016) (see Table 5). Table 5 summarises how iron is distributed in the human body, typically totalling about 3.5 grams.

Table 5

Iron compartments in a normal human

Compartment	Form and Anatomical Site	Total Iron Percentage	Typical Iron Content (g)
Functional	Hb iron in the blood	~ 68	2.400
	Myoglobin iron in muscles	~ 10	0.360
	Peroxidase, catalase, cytochromes, riboflavin enzymes in all cells	~ 3	0.120
Storage	Ferritin and haemosiderin mostly in macrophages and hepatocytes, small amounts in all cells except mature RBCs	~18	0.667
Transport	Transferrin in plasma	< 1	0.001

Note. Information adapted from Keohane et al. (2016, p. 138).

In the United States, researchers have estimated that stage 1 ID affects 14% of 1 to 2-year-olds, 3.7% of 3 to 5-year-olds, 9.3% of females aged 12 to 19, and 9.2% of females aged 20 to 49 (Cogswell et al., 2009). This stage is often called the latent or subclinical ID, where the iron stores are insufficient but the haemoglobin level remains within the normal range, making it difficult to detect the deficiency (Keohane et al., 2016). In the Cook Islands, there are no published data available on iron status or IDA in the population like there are for the United States.

Causes/aetiology

IDA occurs when the intake of iron is insufficient to meet the body's demand. This can happen when the need for iron increases but is not compensated for by an increase in intake. Additionally, the impaired absorption of iron or the chronic loss of Hb from the body can also lead to the development of IDA. Table 6 shows various body state conditions that cause IDA. Howard and Hamilton (2013) noted that ID is commonly caused by long-term blood loss. This blood loss usually occurs from the gastrointestinal tract or the uterine area but can sometimes happen in the urinary tract or other parts of the body. ID in older patients may indicate gastrointestinal cancer. Numerous research articles based on IDA screening have stated that the most common cause of IDA is

nutritional, especially in children (Kumar et al., 2022). A research article by Sherali et al. (2023) on screening for IDA in Pakistan discussed how ID is responsible for approximately half of the anaemia cases worldwide in children under five years old, where according to the World Health Organization (WHO) the estimate is 42%. Hence, the prevalence of IDA among children in Pakistan is acknowledged as a significant public health problem, mirroring the worldwide recognition of IDA as a common cause of anaemia and a global health problem (Loechl et al., 2023; Özdemir, 2015; Speckert et al., 2023). Furthermore, Abdulraheem et al. (2023) emphasised that IDA is a significant health issue specifically impacting women in their reproductive years.

Table 6

Causes of IDA

Body state conditions
• Bleeding from the gastrointestinal tract (e.g., benign ulcer, malignancy, hookworm) • Menorrhagia • Pregnancy • Malabsorption (e.g., coeliac disease, Helicobacter pylori gastritis) • Malnutrition • Bleeding from urinary tract • Pulmonary haemosiderosis (ongoing bleeding into lungs causing shortness of breath and haemoptysis).

Note. Information adapted from Howard and Hamilton (2013, p. 24).

Types of iron deficiency anaemia

Brittenham et al. (2023) discussed two types of ID. The first type is absolute ID, which occurs when there is insufficient iron stored in the body. The second type is functional ID, which happens when iron mobilisation is not enough, even if iron stores are adequate. Therefore, the decreased availability of iron for erythropoiesis is caused by absolute ID, functional ID, or a combination of the two.

Absolute ID

Both physiological and pathological factors influence the development of absolute ID. These factors can include insufficient dietary iron intake, poor iron absorption, and situations where the demand for iron exceeds its supply. Such circumstances commonly arise during growth, pregnancy, menstruation, postpartum haemorrhage, haemolysis, or when individuals are undergoing treatment with erythropoiesis-stimulating agents (Pasricha et al., 2021). During pregnancy, iron absorption increases over nine times to meet the demands of a 30% expansion in Hb mass (Bothwell, 2000). In terms of iron

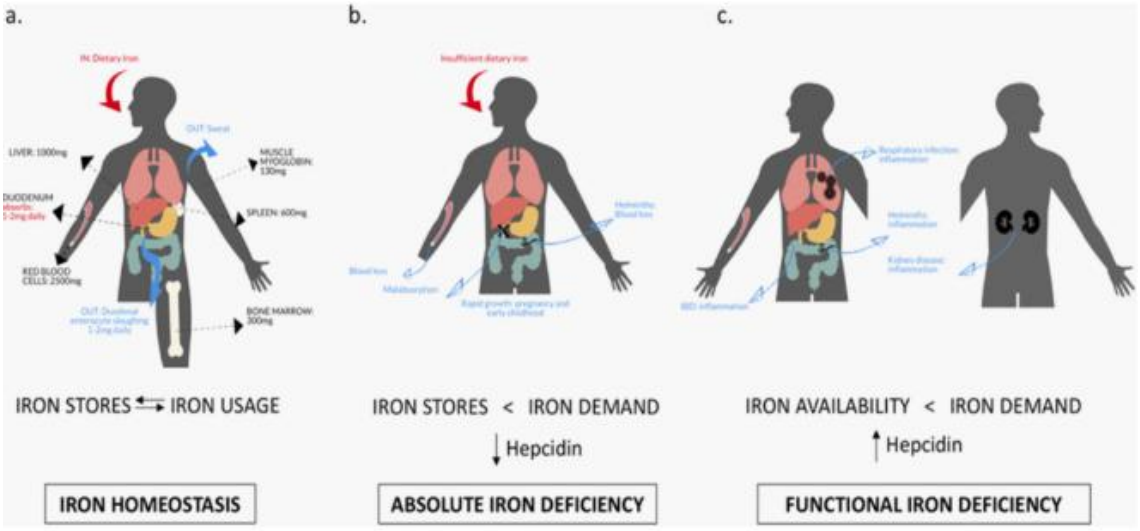
demand, pregnancy requires the highest intake at 27 mg per day, followed by premenopausal women at 18 mg, infants at 11 mg, and men with the lowest requirement at 8 mg (Brittenham et al., 2023; National Institutes of Health, 2023) (refer to Table 4 on page 12). It has been estimated that absolute ID contributes to 50% of all cases of anaemia in pregnancy in low- and middle-income countries (Geller et al., 2006).

Functional ID

Despite having enough iron stores, the presence of systemic inflammation can lead to the development of functional ID (Brittenham et al., 2023), therefore the mechanism of functional ID occurs when an inflammation is causing the effect of becoming iron deficient. Chronic medical conditions such as anaemia of chronic inflammation is a type of functional ID that concurrently suppresses iron absorption, reducing iron stores, thus limiting iron availability for erythropoiesis (Lanser et al., 2021). Additionally, functional ID frequently occurs in populations living in areas with high infection rates, individuals with complex and chronic medical and surgical conditions, and patients undergoing treatment with erythropoiesis-stimulating agents (Pasricha et al., 2021). In low- and middle-income countries, infection is a significant cause of systemic inflammation. This inflammation limits the absorption and mobilisation of iron, leading to functional ID. It also contributes to absolute ID. Inflammation's effects are significant when dietary iron is limited and may worsen iron depletion (Hurrell, 2010). Refer to Figure 6 for illustration of absolute and functional ID indicating the hepcidin activity through the process of erythropoiesis.

Figure 6

Illustration of absolute and functional ID between iron stores, usage and hepcidin levels



Note. Figure adapted from Brittenham et al. (2023). Figure 6a shows an overview of the circulation of iron in the body from where it is stored and its loss. Figure 6b shows absolute ID where iron stores are inadequate to meet the demand, thus hepcidin is downregulated. Figure 6c shows when iron stores are replete but hepcidin expression is upregulated due to the presence of inflammation, compromising the iron supply. Therefore, both of these ID situations (6b and 6c) may occur at the same time.

Clinical presentation

The clinical presentation of an individual considered to have IDA after long-standing ID include, skin pallor, concave nails (koilonychia), sore tongue and papillary atrophy, angular stomatitis, dysphagia, pica and hair thinning (Howard & Hamilton, 2013; Mehta & Hoffbrand, 2014; Özdemir, 2015).

The most frequently emphasised finding in IDA is its impact on the neurocognitive system. Many meticulously designed prospective studies have provided evidence of motor and cognitive retardation, as well as mood disorders, in children with ID (Akman et al., 2004; Oski et al., 1983). Lozoff et al. (1998) demonstrated that children with ID exhibited signs of fatigue more quickly, engaged in less play, and displayed greater hesitancy than their fully healthy counterparts. Even more significant, is that these effects continued to exist a decade after treatment (Lozoff et al., 2000). The progression from ID to IDA can have detrimental effects on mental and motor functions, which may unfortunately be irreversible. There is still much to learn about the mechanism through which ID contributes to the development of neurocognitive disorders. According to several studies, ID was associated with reduced expression of dopamine receptors, impaired myelinisation, and dysfunction of enzymes crucial for nerve tissue (Beard, 2001; Erikson et al., 2001; Ortiz et al., 2004). Another significant but contentious

clinical impact of ID is its influence on the immune system (Joynson et al., 1972; Lanser et al., 2021). Furthermore, recent studies and a meta-analysis conducted in 2010 have demonstrated a strong association between IDA and febrile convulsions (Idro et al., 2010; King & King, 2014).

Laboratory diagnosis

The initial way to diagnose IDA in a laboratory is by conducting an FBC using an automated cell counter analyser on a patient's blood sample. The FBC analysis generates various RBC indices, like MCV, MCH, Hb, Hct, etc. These indices are crucial in the initial diagnosis of IDA, particularly when their values are low below the baseline. Additionally, medical laboratory staff will also perform a microscopic examination of a peripheral blood smear (Özdemir, 2015).

However, current knowledge emphasises the importance of iron studies as the mainstay of confirming the diagnosis of IDA (Keohane et al., 2016). These studies involve conducting tests for serum iron, total iron-binding capacity (TIBC), soluble transferrin receptor (sTfR) saturation, and serum ferritin levels.

At the RHL, the tests for diagnosing IDA include the FBC, serum iron, serum ferritin, and examination of a peripheral blood film.

1.4.2 Thalassaemias

According to the World Health Organisation Southeast Asia (2021), thalassaemias are the most common monogenetic inherited disorders and they have become a global concern because of high population migration. Keohane et al. (2016) explained that Whipple and Bradford discovered in 1936 a significant number of children's autopsies from Mediterranean ethnicities that confirmed the diagnosis and the presence of these disorders.

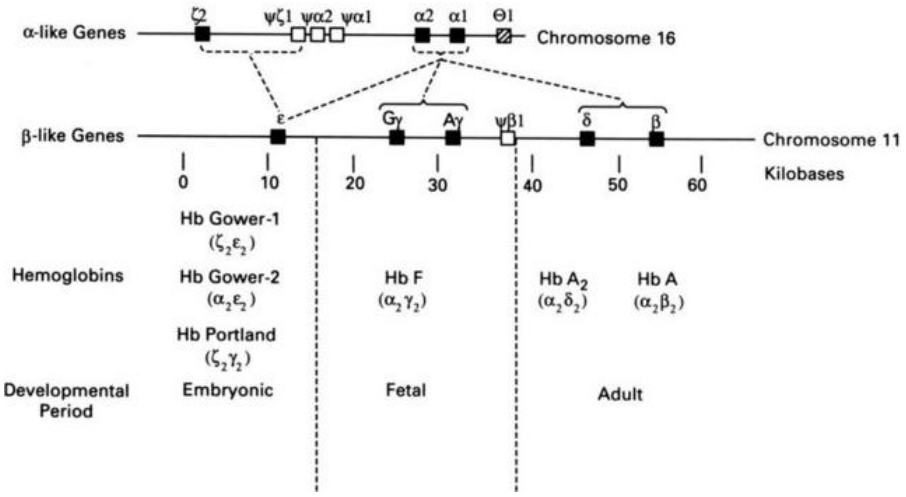
The global burden of thalassaemia is clear in the approximately 56,000 conception cases reported across various countries (Abu-Shaheen et al., 2020). The authors propose the increased adoption of premarital screening and genetic counselling (PMSGC) in high-risk regions of the Gulf Cooperation Council Countries. They believed this would effectively reduce global instances of thalassaemia.

Thalassaemias result from genetic defects that modify the globin chain of the haemoglobin tetramer. The haemoglobin tetramer is composed of two alpha (α)-

subunits (α1 and α2) and two beta (β)-subunits (β1 and β2), which are structurally similar and of similar size (Traeger-Synodinos & Harteveld, 2014) (refer to Figure 2 on page 8). Genetic expressions of these are found on chromosome 16, which contains the α-like globin chains and its cluster consisting of the functional embryonic globin gene zeta (ζ_2) with two functional adult genes (α1 and α2). On chromosome 11, are the β-like globin genes and its cluster which consists of five functional genes: the epsilon (ε), Ggamma (Gγ), Agamma (Aγ), delta (δ) and β. Refer to Figure 7 for an illustration of these gene expressions according to stage of life (Keohane et al., 2016).

Figure 7

Globin gene expression at different stages of normal human development.



Note. The black boxes indicate functional globin genes and white boxes are pseudogenes (non-functional segment of DNA, not capable of coding for a protein). Figure adapted from Keohane et al. (2016, p. 457).

The most clinically significant defects are those that affect the α- or β-globin genes. These globin genes make up haemoglobin A (Hb A as $\alpha_2\beta_2$), which is the main adult Hb and accounts for 95% to 100% of normal Hb in adults. Haemoglobin A_2 (HbA_2 as $\alpha_2\delta_2$) makes up to 3.5% and haemoglobin F (Hb F as $\alpha_2\gamma_2$) accounts for up to 2% in normal adults (Keohane et al., 2016; McKenzie et al., 2015). The classification of thalassaemias is based on the affected globin chain defects, resulting in two disease groups: alpha (α) and beta (β) thalassaemia. Listed below are the findings from Keohane et al. (2016) describing the clinical significance of α and β thalassaemias.

Alpha thalassaemia

The classification of α-thalassaemia into four significant clinical definitions depends on the number of functional/non-functional α-genes. According to McKenzie et al. (2015), α-thalassaemias comprise α silent carrier, α-trait (heterozygous or homozygous

inheritance), Hb H disease, and Hb Bart's disease (also known as Hydrops fetalis). Table 7 outlines more characteristics of these α-thalassaemias.

- Alpha thalassaemia silent carrier - (-α/αα) or (α+/α)

The inheritance of one or two deleted or mutated α-globin genes is often associated with either an absence of noticeable symptoms or only a mild form of microcytic anaemia in affected individuals.

- Alpha thalassaemia trait - (-α/-α or α+/α+) homozygous or (--/αα or α^0 /α) heterozygous

Unlike the silent carrier, which is less significant, this form is the result of a deletion or mutation affecting a pair of the α-globin genes, making it more significant. It is possible for individuals to present with anaemia symptoms that are either mild or moderate in nature.

- Haemgoblobin H disease - (--/-α) compound heterozygous

This particular phenotype is a direct consequence of the genetic alteration involving the deletion or mutation of three crucial α-globin genes, significantly impacting the overall expression of the gene. This condition is characterised by the presence of chronic haemolytic anaemia, an enlarged spleen (splenomegaly), and jaundice, often necessitating regular blood transfusions for effective management.

- Alpha thalassaemia major (Hydrops fetalis) - (--/--)/(α^0/α^0)

This represents the most severe form of the disease, a consequence of the complete absence of all four α-globin genes. This condition is almost always lethal, causing death either before birth or very soon after birth.

Beta thalassaemias

When both parents pass on one beta gene each, this can be abbreviated by the genotypic representation of β/β, which is the typical inheritance of β-genes or β-allele. Inheritance of β-allele can either be normal (β) or abnormal genes of either β^+ and β^0. Table 8 provides more description on the clinical significance of beta thalassaemias, with β^+ showing reduced functionality and β^0 showing complete absence of β chain synthesis, respectively (McKenzie et al., 2015).

- Thalassaemia minor (carrier) - $\beta^0\beta$ or $\beta^+\beta$ (heterozygous)

People who possess one typically functioning β-globin gene alongside a mutated β-globin gene, categorised as either β^+ or β^0, usually experience no symptoms or only a mild form of anaemia characterised by small RBCs.

- Thalassaemia intermedia - $\beta^+\beta^+$ (homozygous) or $\beta^0\beta^+$ (double/ compound heterozygous)

This particular phenotype is a direct result of inheriting either homozygous or compound heterozygous mutations in the β-globin gene (β^+ or β^0) genes. This condition is characterised by a moderate level of microcytic anaemia, a condition that may necessitate periodic blood transfusions and may also manifest with symptoms such as an enlarged spleen and excessive iron buildup in the body.

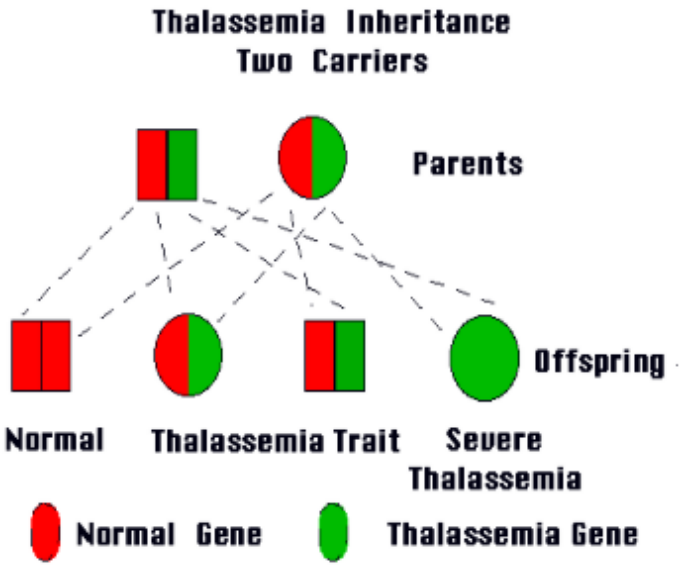
- Thalassaemia major - β^0/β^0 (homozygous)

This most severe form of the disease is a result of homozygous inheritance of the β^0 mutation. Those afflicted with thalassemia major commonly experience severe anaemia, an enlarged spleen, and skeletal malformations, necessitating regular blood transfusions and iron chelation therapy for survival.

Thalassaemia occurring in an individual is inherited in an autosomal recessive manner. The likelihood of an affected offspring is illustrated in Figure 8.

Figure 8

Example of an autosomal recessive inheritance of thalassaemia



Note. The combination of the inheritance from both parents will have 25% offspring free from or not carriers of the condition, 50% will only be carriers but will not express the condition, and 25% will express the condition. Figure adapted from, Sickle cell and thalassemic disorders - how do people get thalassemia? (https://sickle.bwh.harvard.edu/tha1_inheritance.html)

Typically, patients with mild genetic defects such as a silent carrier and thalassaemia minor show no symptoms (see Table 7 and Table 8). However, in cases of more severe genetic defects, patients display symptoms resulting from decreased normal haemoglobin production, synthesis of abnormal haemoglobins, imbalanced α- and β-globin chain synthesis, and ineffective erythropoiesis (McKenzie et al., 2015).

Around 5% of the world's population are carriers of a clinically significant genetic defect for thalassaemia. Thus, there are approximately 56,000 infants being born annually with the most severe form, thalassaemia major (Abu-Shaheen et al., 2020; Modell & Darlison, 2008). According to a 2021 regional review by WHO Southeast Asia, α-thalassaemia is more common in this region than in other parts of the world. World Health Organisation Southeast Asia (2021) has stated that up to 40% of people in this region could be carriers of the thalassaemia trait.

Abnormal globin chain production because of genetic defects leads to the abnormal maturation of RBCs, resulting in the microcytic RBCs as seen in thalassaemia patients. Furthermore, either a mutation or the absence of the genes results in the absence of chains resulting in the absence or reduction of a particular type of haemoglobin produced. The most frequent occurrence of deleted genes characterises α-thalassaemia,

while β-thalassaemia is more commonly associated with a reduction in globin chain production from non-deletional mutations (Traeger-Synodinos & Harteveld, 2014). Sabath (2017) found that chain deletion is responsible for 95% of α-thalassaemia cases, with the remaining cases attributed to point mutations. In contrast, point mutations cause 95% of β-thalassaemia cases. The type of mutation encountered determines the degree of reduction in globin chain production, which directly corresponds to the severity of the clinical disorder.

Thalassaemias are also called thalassaemia syndromes as in the literature by Karakaş et al. (2015) and Keohane et al. (2016). These disorders are classified into different categories based on which globin genes are affected on chromosomes 16 and 11 — including α, β, δ, γ, or combinations such as β/δ/γ. (Weatherall & Clegg, 2001). Depending on the specific chain and the level of globin chain production, the genetic defect can have different consequences. Sabath (2017) explained that the most common defects in thalassaemia are deletional, especially in α-thalassaemia cases. Thus, a scarcity of the affected globin chains reduces the production of Hb A molecules, which affects the oxygen-carrying capacity of circulating RBCs.

Table 7

Alpha thalassaemia characteristics

Genotype	Phenotype	Severity	Haemoglobin types present
(-α/αα) (α^{+}/α)	Silent carrier – 3 functioning alpha genes	Asymptomatic	Normal Hb A is 97–98% Normal HbA2 and HbF
(-α/-α or α^{+}/α^{+}) homozygous or (--/αα or (α^{0}/α) heterozygous	α-thalassaemia trait/minor – affected individuals have one set of functioning α genes inherited from one parent or one absent and one functioning gene from each parent	Mild to moderate anaemia	Birth = Hb Bart's is 5–10% Adult = normal, 90–95% Hb A present elaborate (normal A2 and F) A mild decrease in haemoglobin level but RBCs are microcytic hypochromic in adults
(--/-α) (α^{0}/α^{+}) compound heterozygous	Haemoglobin H disease (HbH)	Chronic, moderately severe haemolytic anaemia. Secondary complications include hepatospleno-megaly, folic acid supplementation with some transfusion dependence episodes	Birth = Hb Bart's – 25–40% Adult = HbH – 2–40% Hb A markedly decreased. Decreased A2 and F Microcytic hypochromic anaemia
(--/--) (α^{0}/α^{0})	Hydrops fetalis (no functional alpha genes inherited)	Fatal due to excess fluid build-up in the foetus prior to birth. Haemoglobin production is severely reduced resulting in severe anaemia, growth abnormalities, enlarged liver and spleen. Babies are stillborn or die shortly after birth.	Hb Bart's (80–90%), Hb Portland (10–20%) No Hb A, A2 Severely anaemic

Note. Information adapted from McKenzie et al. (2015) and Keohane et al. (2016).

Table 8

Beta thalassaemia characteristics

Genotype and Zygosity	Phenotype	Severity	Haemoglobin present
β^0/β^0 (homozygous)	Major Inheritance of two affected genes from each parent.	Severe Severe haemolytic anaemia, hepatosplenomegaly due to increased red cell turnover, ineffective erythropoiesis leading to the administration of regular blood transfusions	No Hb A produced, variable Hb A_2 with normal to increased Hb F. Hb F is the foetal haemoglobin composing of $\alpha_2\gamma_2$
$\beta^0\beta^+$ (double/ compound heterozygous)	Intermedia Inheritance of one severe affected gene from one parent (β^0) and a reduced functionality gene (β^+) from the other parent	Moderate to severe A milder form of haemolytic anaemia as compared to homozygous inheritance above which occurs later in life. Blood transfusions are required for poor nutrient diets or low oxygen environment	Markedly decreased Hb A, variable Hb A_2 and mildly increased Hb F
$\beta^+\beta^+$ (homozygous)	Intermedia Inheritance of one reduced functionality β-gene from each parent	Moderate to severe Milder form of anaemia and less transfusion dependent.	Moderate decrease of Hb A, variable Hb A_2 and mild increase of Hb F
$\beta^0\beta$ or $\beta^+\beta$ (heterozygous)	Minor Inheritance of one affected and a normal β-gene	Mild to moderate Mild haemolytic anaemia, mild microcytic hypochromic anaemia. Patients are usually asymptomatic	Mild reduced production of Hb A and mild increased production of Hb A_2

Note. Information adapted from McKenzie et al. (2015) and (Keohane et al., 2016).

Other clinically significant microcytic red cell disorders related to thalassaemia

There are other forms of thalassaemia either associated with defects in the β cluster genes or with structural haemoglobin variants (Keohane et al., 2016) and these are discussed below.

β cluster gene defects

Examples of thalassaemias associated with defects in the β cluster genes are the hereditary persistence of foetal haemoglobin (HPFH) and δβ-thalassaemia. They are typically characterised by the continuous synthesis of increased levels of haemoglobin F in adult life (Keohane et al., 2016). These two conditions are quite similar; however, they are differentiated by the amount of haemoglobin F produced and the distribution of haemoglobin F in the RBCs. Individuals with these defects are usually asymptomatic and of little concern; however, their interactions with other forms of thalassaemias or structural haemoglobin variants indicate a significant situation (Marengo-Rowe, 2017).

The difference between these two conditions is based on their clinical presentation. Individuals with inherited heterozygous HPFH have significant variation, with normal RBC size and Hb F levels are between 15% to 30%. In contrast, individuals with heterozygous δβ-thalassaemia have microcytic hypochromic RBCs with Hb F levels between 5% to 20%. Homozygous inheritance for HPFH is asymptomatic with slightly microcytic hypochromic RBCs and Hb F levels of 100%. These individuals have 100% Hb F level but express a mild β-thalassaemia intermedia phenotype (Marengo-Rowe, 2017).

Structural haemoglobin variants

Compound heterozygous conditions with structural haemoglobin variants such as haemoglobin S (Hb S)-thalassaemia, haemoglobin C (Hb C)-thalassaemia and haemoglobin E (Hb E)-thalassaemia are of clinical significance due to their inheritance with other forms of thalassaemias. For example, Hb S and Hb C are mostly common in Africa, Hb E in Southeast Asia) and their co-inheritance with other thalassaemia forms lead to severe clinical consequences. Their inheritance patterns can either be heterozygous, homozygous and compound heterozygous (Keohane et al., 2016).

Hb S-thalassaemia results from the inheritance of a β-thalassaemia gene from one parent while the Hb S gene is inherited from the other parent. The interaction of β^{+}-thalassaemia or β^{0}-thalassaemia with Hb S produces only a minimal amount of β chain, showing a similar clinical syndrome to sickle cell anaemia (Keohane et al., 2016)

presented with microcytosis and splenomegaly distinguishable from sickle cell anaemia. The interaction of β^0-thalassaemia with Hb S is present with severe pain crises, high incidence of stroke and shorter life expectancy (Quinn et al., 2004) as well as microcytosis and increased Hb A_2 (Keohane et al., 2016). Haemoglobin electrophoresis shows mostly Hb S with moderately elevated Hb A_2, and variable amounts of Hb F and Hb A. This depends on the specific abnormal β gene inherited from the parents (Keohane et al., 2016).

Hb C co-inheritance with β-thalassaemia is presented with a moderate to severe degree of haemolysis, splenomegaly, microcytosis hypochromic and numerous target RBCs. This occurs when there are high concentrations of Hb C and minimal or no production of β-globin chains in an individual (Marengo-Rowe, 2017).

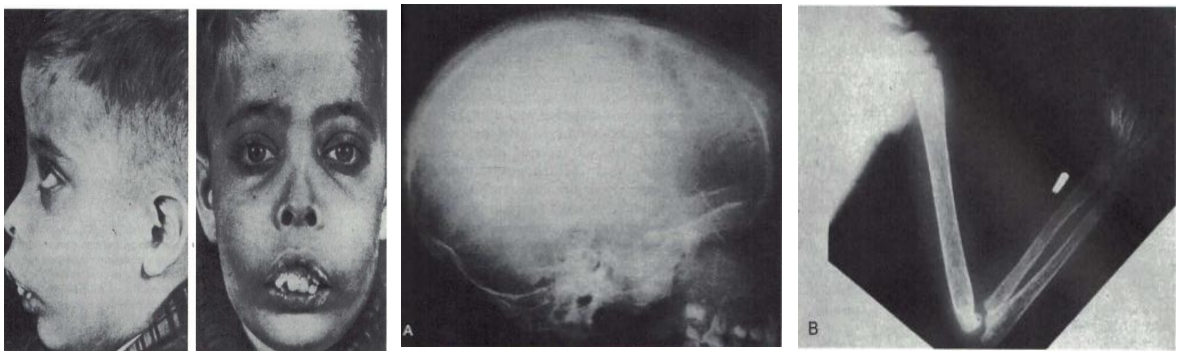
Homozygous Hb E clinically appears similar to a mild β-thalassaemia due to a reduced rate of Hb E synthesis. However, when the mutation is co-inherited, with thalassaemia, it results in a marked reduction of β chain production. The clinical consequences will range from intermediate to major in terms of the degree of transfusion-dependence (Marengo-Rowe, 2017). This condition is a significant concern in the Southeast Asian region where it has a high prevalence of 50% to 60% Hb E (Fucharoen & Winichagoon, 2011).

Clinical presentation

Thalassaemia was first described in 1925 by Cooley and Lee in four children with anaemia, splenomegaly, mild hepatomegaly, and "mongoloid" faces which can be confirmed on x-ray images (see Figure 9) (Keohane et al., 2016).

Figure 9

X-rays images of bone changes in an individual diagnosed with β-thalassaemia major



Note. Images were adapted from Keohane et al. (2016). The left image shows a typical facial appearance of a child with an untreated homozygous β-thalassaemia major. Middle and right images show a skull, forearm and hand radiographs of bone changes.

In Figure 9, the X-ray images provide examples that illustrate the usual bone changes observed in a patient who has β-thalassaemia major, which is a particularly severe form of thalassaemia. The left image shows the characteristic facial appearance of a child with untreated homozygous β-thalassaemia major, often referred to as “chipmunk facies.” This results from expansion of the facial bones due to increased bone marrow a” The middle and right images display radiographs of the skull, forearm, and hand, revealing further skeletal abnormalities. The classic "hair-on-end" appearance within the skull, thinning of the cortical bone, expansion of the medullary cavities, and bone development that is delayed are all included in these conditions. The direct result of marrow hyperplasia, which is characterized by excessive bone marrow activity, is these changes, and it's brought on by ineffective erythropoiesis, a defining feature of β-thalassaemia major.

Additional clinical observations include poor growth, concurrent infection, pale appearance and slight yellowing of the skin (Mehta & Hoffbrand, 2014).

Regular blood transfusions and increased iron absorption may lead to signs of melanin pigmentation (Mehta & Hoffbrand, 2014) and iron haemosiderin (Anderson et al., 2000). Iron overload can cause growth and endocrine defects, including thyroid issues, heart failure, liver abnormalities, and diabetes mellitus (Mehta & Hoffbrand, 2014). To prevent complications in the body caused by iron overload, it is necessary for the patient to undergo iron chelation therapy to maintain optimum ferritin levels (Bain, 2020).

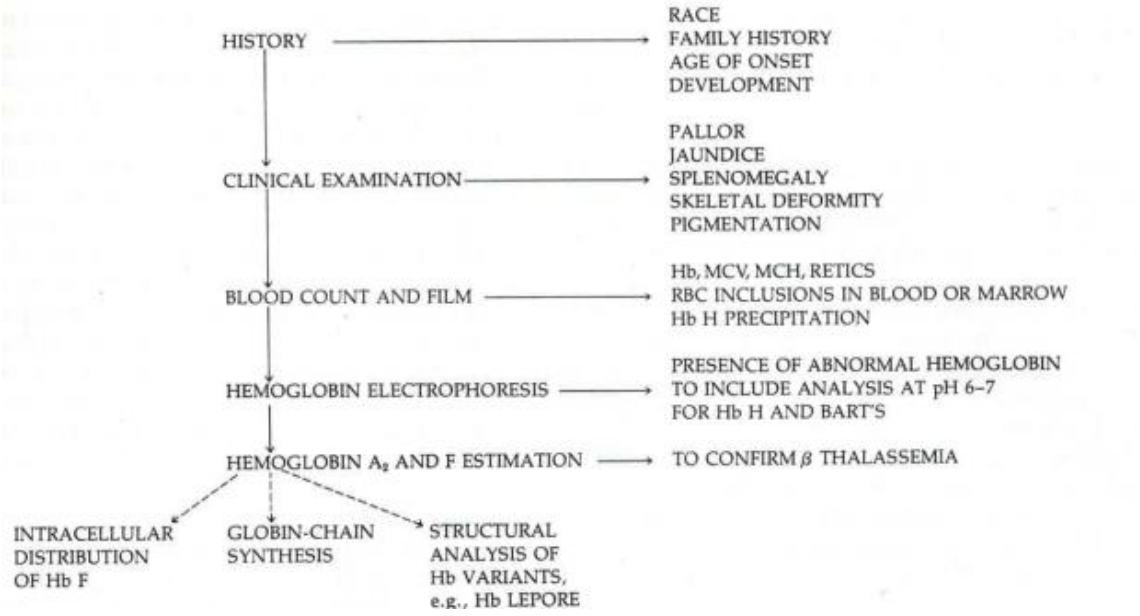
Laboratory diagnosis

Anaemia in thalassaemias is caused by chronic haemolysis and ineffective erythropoiesis because of reduced synthesis of Hb A (which is the normal type of haemoglobin produced in a normal adult) (Keohane et al., 2016).The severity of the anaemia depends on the specific gene defects and the number of genes involved. The presence of abnormal haemoglobins with high oxygen affinity (such as HbH and Hb Bart’s) lead to hypoxia in the presence of anaemia (McKenzie et al., 2015).

The physical characteristics and histories of an individual are paramount in thalassaemia diagnosis. Figure 10 illustrates a typical flow chart approach for diagnosing thalassaemia syndromes.

Figure 10

A flow chart approach for diagnosis of thalassaemia syndromes



Note. The above has been adapted from Keohane et al. (2016).

A FBC analysis can reveal the first clues to a disease. Laboratory diagnosis of thalassaemias follows a similar approach to IDA, using MCV, MCH, and usually MCHC to confirm microcytosis and hypochromia. Reticulocyte counts and iron studies such as iron, ferritin, and TIBC are also used to exclude IDA. The examination of a Wright-stained blood smear may show distinctive features of thalassaemia blood film such as microcytosis hypochromia, target cells, elliptocytes, basophilic stippling, nucleated RBCs, polychromasia with extreme poikilocytosis in homozygous and double heterozygous β-thalassaemia. In heterozygous α^0-thalassaemia, there is mild microcytic hypochromia but less poikilocytosis. In heterozygous β-thalassaemia and Hb H disease, there is a slight to moderate poikilocytosis and microcytosis (Keohane et al., 2016). The typical appearance of thalassaemia-stained blood smears will show microcytosis and hypochromia, except in the silent carrier phenotype (Keohane et al., 2016).

Many studies have highlighted the strong contribution of first-line tests, or screening tests, in detecting thalassaemias. Ghosh et al. (2020) emphasised the importance of not underestimating the value of routine haematological analysis, specifically FBC, morphology, and RBC indices, in the work-up of RBC disorders. The combination of

RBC indices, RBC morphology, and high-performance liquid chromatography (HPLC) data aids in detecting cases where HPLC alone would have been insufficient (Bain et al., 2012; Traeger-Synodinos et al., 2015).

Supravital staining for α-thalassaemia Hb H inclusions and electrophoresis are among the additional diagnostic options that should be considered. In the present day, molecular genetic tests are proposed and are ideal for accurately diagnosing thalassaemia, as they can identify complex mutations in the α- and β-globin genes (Keohane et al., 2016).

In the Cook Islands, the RHL currently is the first site for the detection of possible thalassaemias. If the diagnosis is suspected and/or inconclusive, the RHL will send further blood samples to advanced laboratories in New Zealand for additional testing such as haemoglobin electrophoresis and a conclusive diagnosis by, for example, molecular testing for the identification of gene mutations or defects.

1.4.3 Anaemia of Chronic Inflammation

Anaemia of chronic inflammation (ACI) is the name given to the anaemia that accompanies chronic infection, inflammatory disorders, trauma, organ failure, or neoplastic disorders. According to McKenzie et al. (2015), these health conditions do not have any connection to bleeding, haemolysis, or marrow-related problems. ACI is the leading cause of anaemia in hospitalised patients and is considered the second most common type of anaemia after IDA, accounting for around 40% of all anaemias globally (Kassebaum et al., 2014).

Patients with anaemia of chronic inflammation (ACI) experience disturbances in iron metabolism, erythropoietin (EPO) dysfunction, suppressed erythropoiesis, and reduced RBC survival. EPO, a glycoprotein hormone produced by the kidneys, normally stimulates RBC production (Keohane et al., 2016). In ACI, immune activation from inflammation or infection disrupts these processes (Weiss et al., 2019). In the short term, hepcidin increases during inflammation without harmful effects. However, prolonged elevation reduces intestinal iron absorption and limits iron release from macrophages. As a result, iron becomes unavailable for developing RBCs, leading to reduced RBC production and survival (Keohane et al., 2016). Despite adequate total body iron, it becomes trapped in macrophages and hepatocytes. This iron sequestration,

driven by chronically high hepcidin levels, contributes to anaemia in ACI as seen in Figure 6(c) (Keohane et al., 2016).

Refer to Figure 6 illustrating the mechanism of functional ID in relation to ACI. Microcytic anaemia usually accompanies an inflammatory state (DeLoughery, 2014), but occasionally patients with ACI may have mild to moderate normocytic normochromic anaemia (Weiss & Goodnough, 2005).

Causes/aetiology

ACI occurs due to a two-step mechanism. Firstly, inflammatory cytokines suppress renal production of EPO, leading to reduced RBC production (Keohane et al., 2016). According to Weiss and Goodnough (2005), the evidence suggests that microcytosis may occur due to limited iron availability for erythropoiesis. In ACI, hepcidin, an acute-phase reactant, is responsible for the decreased absorption and release of iron, leading to ID. The protein FPN regulates cellular iron transport. The binding of hepcidin to FPN, blocks the absorption of iron by enterocytes and the release of iron from body stores to developing RBCs (Ganz & Nemeth, 2011). Refer to Figure 4 on page 12.

Types of anaemia of chronic inflammation

Table 9 lists conditions that are related to ACI. These diseases trigger long-term immune activation, which disrupts iron metabolism and suppresses RBC production, leading to ACI (Howard & Hamilton, 2013).

Table 9

Medical conditions that are related to ACI

Chronic infections	• Osteomyelitis • Bacterial endocarditis • Tuberculosis • Chronic abscesses • Bronchiectasis • Chronic urinary tract infections • HIV • AIDS • Malaria
Other chronic inflammatory disorders	• Rheumatoid arthritis • Polymyalgia rheumatica • Systemic lupus erythematosus • Scleroderma • Inflammatory bowel disease • Thrombophlebitis
Malignant diseases	• Metastatic or associated with infections or carcinoma • Lymphoma
Others	• Congestive heart failure

Note. Information adapted from Howard and Hamilton (2013).

Clinical presentation

ACI usually presents with clinical symptoms that align with the underlying disorder. The severity of the anaemia is generally mild and is related to the activity of the underlying disease (McKenzie et al., 2015). Table 9 give examples of underlying disorders.

Laboratory diagnosis

The FBC analysis is still the initial test used to screen for ACI conditions, along with blood film examination. In the initial phase of ACI, it may present as normocytic normochromic before transitioning to microcytic hypochromic in chronic cases (McKenzie et al., 2015). Blood film examination is also carried out to test for microcytic features and to consider reactive changes that may occur because of the underlying disorder, such as neutrophilia, leucocytosis, thrombocytosis, and rouleaux formation (Uprichard & Uprichard, 2013).

Further studies on biomarkers like serum iron, ferritin, and TIBC contribute to the diagnosis of ACI, wherein the serum ferritin is usually elevated. C-reactive protein

(CRP) and erythrocyte sedimentation rate (ESR) are inflammatory markers that are typically raised in inflammatory diseases/conditions, providing valuable diagnostic information (Howard & Hamilton, 2013).

When a patient presents with microcytosis, the extent to which the MCV is decreased can serve as an indication of aetiological factors. For example, an MCV value below 70fL is rarely seen in patients with anaemia of inflammation (DeLoughery, 2014). Distinguishing IDA and ACI can be achieved by evaluating serum ferritin and transferrin. In IDA, ferritin is decreased, while in ACI, it can be either normal or increased (Keohane et al., 2016).

In the RHL, the tests available for diagnosis are: FBC, serum iron, serum ferritin, blood film examination, and selected inflammatory markers like CRP and ESR. However, knowledge of the patient's medical history can help diagnose the cause of the anaemia.

1.4.4 Sideroblastic Anaemias

Sideroblastic anaemias (SAs) encompass various diseases, both hereditary and acquired, which are caused by abnormal incorporation of iron into haem (Keohane et al., 2016). Research on SA patients has revealed disruptions in the enzymes that regulate haem synthesis (Abu-Zeinah & DeSancho, 2020). There are two forms of enzymes called aminolevulinic acid synthase (ALAS): nonerythroid or hepatic (ALAS1) and erythroid (ALAS2) (McKenzie et al., 2015). Both ALAS1 and ALAS2 catalyse the first and rate-limiting step in haem biosynthesis - an essential process for producing haemoglobin, cytochromes, and other haem-containing proteins (McKenzie et al., 2015).

Causes/aetiology

The synthesis of haem occurs in the bone marrow, in the mitochondria and the cytoplasm of the RBC precursors such as the pronormoblasts/proerythroblast to reticulocyte (Keohane et al., 2016).

Mutations affecting the first enzymatic step in haem synthesis lead to SAs, which can be either hereditary or acquired (McKenzie et al., 2015). The X-linked and autosomal types are forms of hereditary SA, with the defective X-linked recessive gene being the most common cause (Koc & Harris, 1998). The classification of acquired SA is determined by whether the anaemia is of unknown cause (idiopathic) or is caused by an underlying disease or toxin. According to Keohane et al. (2016), disorders included in acquired SA are refractory anaemia with ring sideroblasts (RARS), a type of Myelodysplastic

Neoplasm, and these are classified as primary acquired SA. Secondary acquired disorders in SA are those caused by tuberculosis drugs, chloramphenicol, alcohol, lead, and chemotherapeutic agents.

Lead and alcohol are the main culprits behind acquired SA, with lead poisoning being a long-standing problem. There have been instances where children have accidentally consumed peeled paint containing lead. In industrial settings, adults are mainly exposed to lead compounds through inhalation, leading to lead poisoning (McKenzie et al., 2015).

Clinical presentation

The symptoms of the underlying disorder are more prominent in patients with drug-induced and malignant secondary acquired SA. Generally, patients diagnosed with hereditary or refractory anaemia with ring sideroblasts display primary anaemia symptoms. Additionally, patients with hereditary SA often show symptoms related to iron overload such as liver and spleen enlargement, or diabetes; where later on, cardiac complications will develop (McKenzie et al., 2015).

Laboratory diagnosis

Similar to IDA, the anaemia can present as microcytic hypochromic. In contrast to IDA, iron is abundant in the bone marrow in SA, where it awaits incorporation into the RBCs' haem component. In this case, applying the Prussian blue stain to the bone marrow smear reveals normoblasts (immature RBCs containing Hb and a nucleus) with iron deposits in the mitochondria arranged in a circle around the nucleus (Keohane et al., 2016). The presence of ring sideroblasts is a key indicator of a diagnosis of SA. Furthermore, total body iron concentration will be elevated (McKenzie et al., 2015). Iron deposits (Pappenheimer bodies) in the cytoplasm of RBCs as well as a dimorphic RBC population are observed in the peripheral blood film.

Female carriers of the X-linked type of SA seldom exhibit symptoms of anaemia. In contrast, males show the typical clinical manifestations of SA (McKenzie et al., 2015)

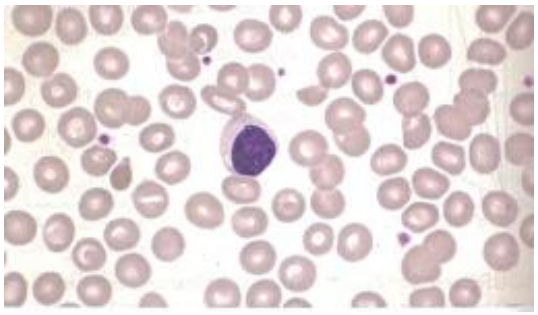
1.4.5 Summary

This review highlights the different types of microcytic anaemias. A lack of globin in thalassaemia; restricted iron delivery to the haem group of Hb in ACI; a lack of iron delivery to the haem group in IDA; and defects in the haem group in SAs (DeLoughery, 2014). Figure 11 illustrates these characteristics in light microscope

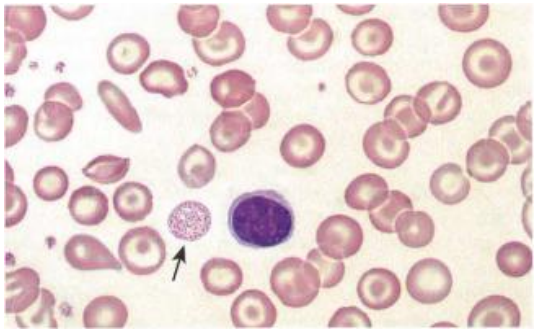
images of these microcytic anaemias. It highlights the characteristic RBC changes seen in various microcytic anaemias. In α-thalassaemia trait, mild microcytosis and target cells are observed, while β-thalassaemia minor shows more pronounced microcytosis, hypochromia, poikilocytosis, target cells, and basophilic stippling. IDA features severe microcytosis, hypochromia, and marked variation in RBC size and shape. In contrast, ACI typically shows mild microcytosis with less prominent morphological changes. Hb H disease presents with microcytic hypochromic RBCs, target cells, and Hb H inclusions. Lastly, SA, including the form caused by lead poisoning, displays basophilic stippling and, in bone marrow samples, ring sideroblasts—indicative of defective haemoglobin synthesis. These morphological features are valuable in differentiating between the various types of microcytic anaemia.

Figure 11

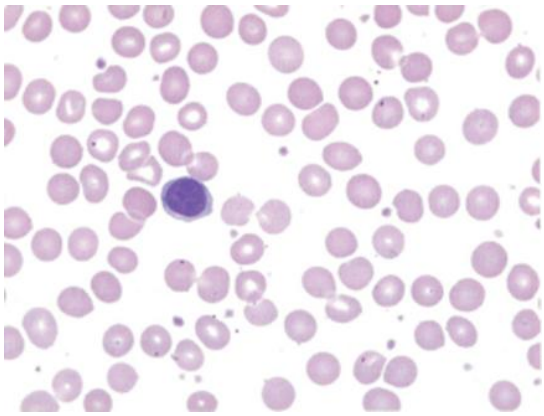
Some microscopic images of the microcytic anaemias



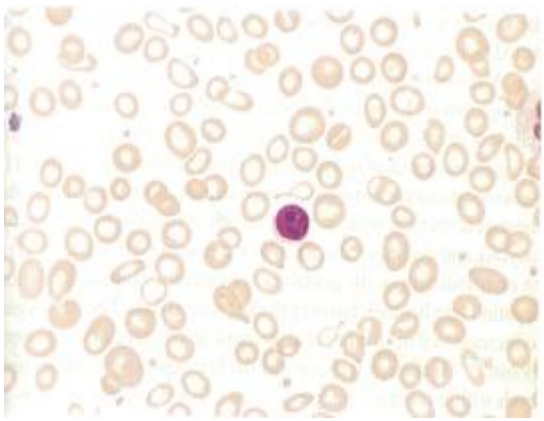
α-thalassaemia trait/minor showing mild microcytic hypochromic anaemia and target RBCs.



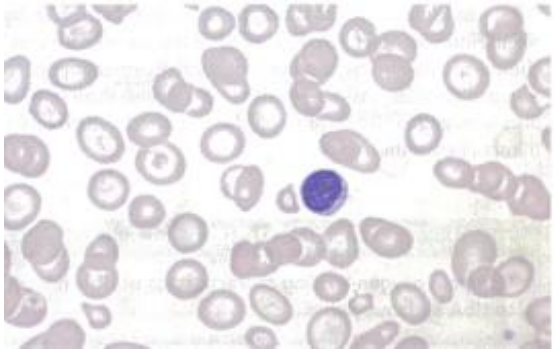
β-thalassaemia minor showing microcytic hypochromic RBCs, poikilocytosis, target RBCs and a stippled RBC indicated by the arrow.

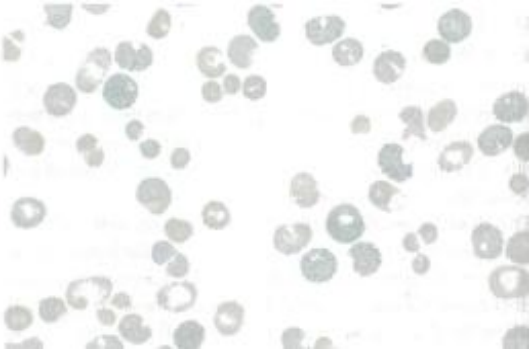


Anaemia of chronic inflammation

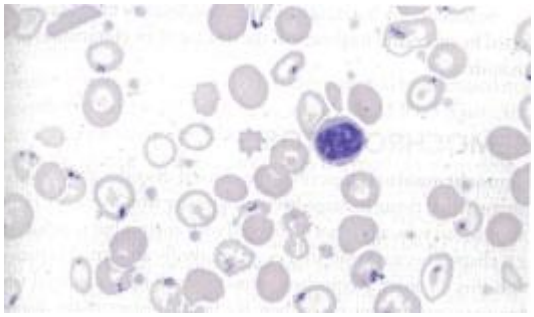


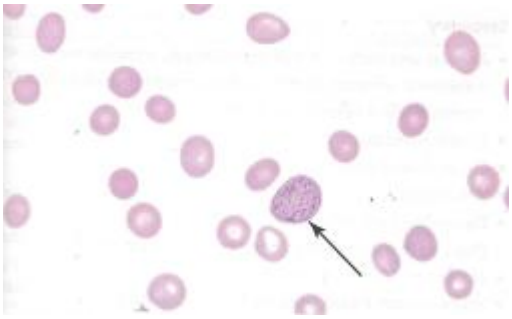
Iron deficiency anaemia





Hb H disease showing microcytic hypochromic RBCs, target RBCs and Hb H inclusions (right) in the RBCs.





Sideroblastic anaemia (left) and a picture of lead poisoning in acquired sideroblastic anaemia (right)

Note. The above pictures have been adapted from McKenzie et al. (2015) and Keohane et al. (2016).

Blood film examination is an essential component of the evaluation of any microcytic anaemia. This requires the necessary skill and in-depth morphology training that is crucial for reporting with confidence and ensuring.

Now that all the microcytic anaemias stated in this thesis have been discussed, the focus of this thesis is to evaluate and estimate the possible types of microcytic anaemias present within the Cook Islands population. Potentially, the evaluation made in this

thesis will give an insight for Te Marae Ora Cook Islands Ministry of Health to reassess or review the current diagnostic protocols for classifying microcytic anaemias. One essential diagnostic issue is to avoid administering incorrect diagnostic management plans to patients.

If the findings from this research are significant, then a reassessment or review of the current diagnosis practice of microcytic anaemias must be looked at to include new recommendations to be integrated into these protocols. This will ensure the improvement of the diagnosis and management plans of patients found or suspected to have these medical conditions in the Cook Islands.

The RHL diagnosis of these disorders can be complex, although performing an FBC analysis, iron studies, inflammation markers analysis and blood film examinations will improve the diagnosis of these RBC disorders within the Cook Islands health system, both by clinicians and those using the RHL platforms. By implementing some good medical guidelines for these conditions will ensure instant and correct diagnosis and management within the Cook Islands population, instead of waiting for a prolonged period for a diagnostic plan, or especially when blood samples have been referred to a reference laboratory in New Zealand for further analysis. By sending samples to a diagnostic laboratory, it will become more costly and time consuming as compared to when these extra laboratory tests are accessible and to be performed by the RHL.

Chapter 2 Research Design and Materials/Methods

2.1 Research Study Design

This study was conducted in the RHL, which is the national medical laboratory for the Cook Islands. It is a population-based, cross-sectional, exploratory study. The data collection for this study began after receiving ethical approval from Auckland University of Technology Ethics Committee (AUTEC) and the Cook Islands Research Ethics Committee (CIREC) (see Appendix A part a, b and c). In addition, permission to undertake research in the Cook Islands was also obtained from the Cook Islands Research Committee before research commencement.

An audit of 1,000 patient FBC results which satisfied the proposed selection criteria of the research was undertaken. This number was considered to be of adequate size; to be a reliable reflection of the population. However, the initial master copy dataset collected for this study was 1,463 results. To ensure a correct evaluation of the microcytic anaemias in the population, the exclusion of repeated patients (i.e., duplicate FBC results from the same patient in the 1,463-result dataset) was carried out for an accurate evaluation of the microcytic anaemias in the dataset. After exclusions, this study had a total of 874 de-identified results which is was considered an adequate size for a reliable reflection of the population. Section 2.1.1 and section 2.1.2 for describe detail of the data collection steps for this study. The final dataset which is called 'Study Data', consists of 874 results and encompasses both laboratory data from the study period and archived laboratory data prior to this period.

There were no interactions with participants, as these participants had already given consent for their blood samples to be taken for laboratory investigation as requested by their doctors. In addition, confidentiality and privacy has been applied to this research as per policy of Te Marae Ora Cook Islands Ministry of Health, hence the participants are treated anonymously throughout.

2.1.1 Steps of Data Collection

It is standard practice for authorised health staff to collect patient blood samples from various phlebotomy locations across Rarotonga and other parts of the Cook Islands. The RHL has its own phlebotomy outpatients' section within the laboratory, where patient samples collected at this site by laboratory staff reached the sample processing

laboratory quickly. Patient blood samples collected in other phlebotomy locations are referred to the RHL for laboratory analysis, adhering to strict protocols for transportation and storage. This ensures maintenance of sample integrity prior to laboratory analysis.

The initial data collection point of the Study Data was from an EDTA sample which underwent FBC analysis. Additional tests may have been requested by the referring practitioner. Where relevant to this study, the results of these were also collected. Examples of these are biomarkers (ferritin and iron), inflammatory markers (CRP and ESR) and other related research RBC markers such as, absolute reticulocytes count obtained from an FBC analysis.

The patient data available for this study was restricted to the original blood tests requested by doctors. No additional laboratory tests were performed on these patient samples unless requested by doctors for further laboratory testing (based on initial results), and/or already requested in the original laboratory blood test requisition form. Patient consent was waived for this study on the assurance that patient identity would be anonymised, and patient privacy and confidentiality maintained at all times.

The steps of data collection were as follows (See Table 10 for a flow chart summary).

1. Patient data was selected and retrieved with RBC parameters of low MCV and/or low MCH from automated blood cell counter analyser, Sysmex XN1000 and archived storage compartment in the haematology department. Table 11 describes the selection criteria selection for low MCV and low MCH for the different age groups, according to the normal reference ranges of the haematology analytes. The selected patient data included all FBC indices, including measurement of red cells, white cells and platelets (Seo & Lee, 2022). “Low” in this study refers to values below the relevant RHL reference ranges.

2. The patient data was carefully analysed to identify any recurring elements through an exclusion process, to accurately estimate the disorder. Patients who had duplicate FBC results in the collected dataset (i.e., those having other FBC results from other dates) were identified, and the duplicate FBC results excluded to ensure accurate evaluation of the microcytic anaemias. Therefore, the study dataset does not include duplicate of patients FBC results.

3. Once the final patient data was selected, it was cross-checked with the Rarotonga Hospital healthcare system (i.e., Medtech Evolution (ME)) for any additional information such as clinical conditions and demographic details.

4. Further relevant laboratory information for each patient during the study period was obtained from designated laboratory departments (e.g., the Biochemistry Department) or the ME system. This included tests such as serum iron, serum ferritin, and inflammatory markers (from the Biochemistry Department), as well as blood film examinations (from the Haematology Section). These tests, available in the RHL, were requested by health staff to evaluate suspected RBC disorders.

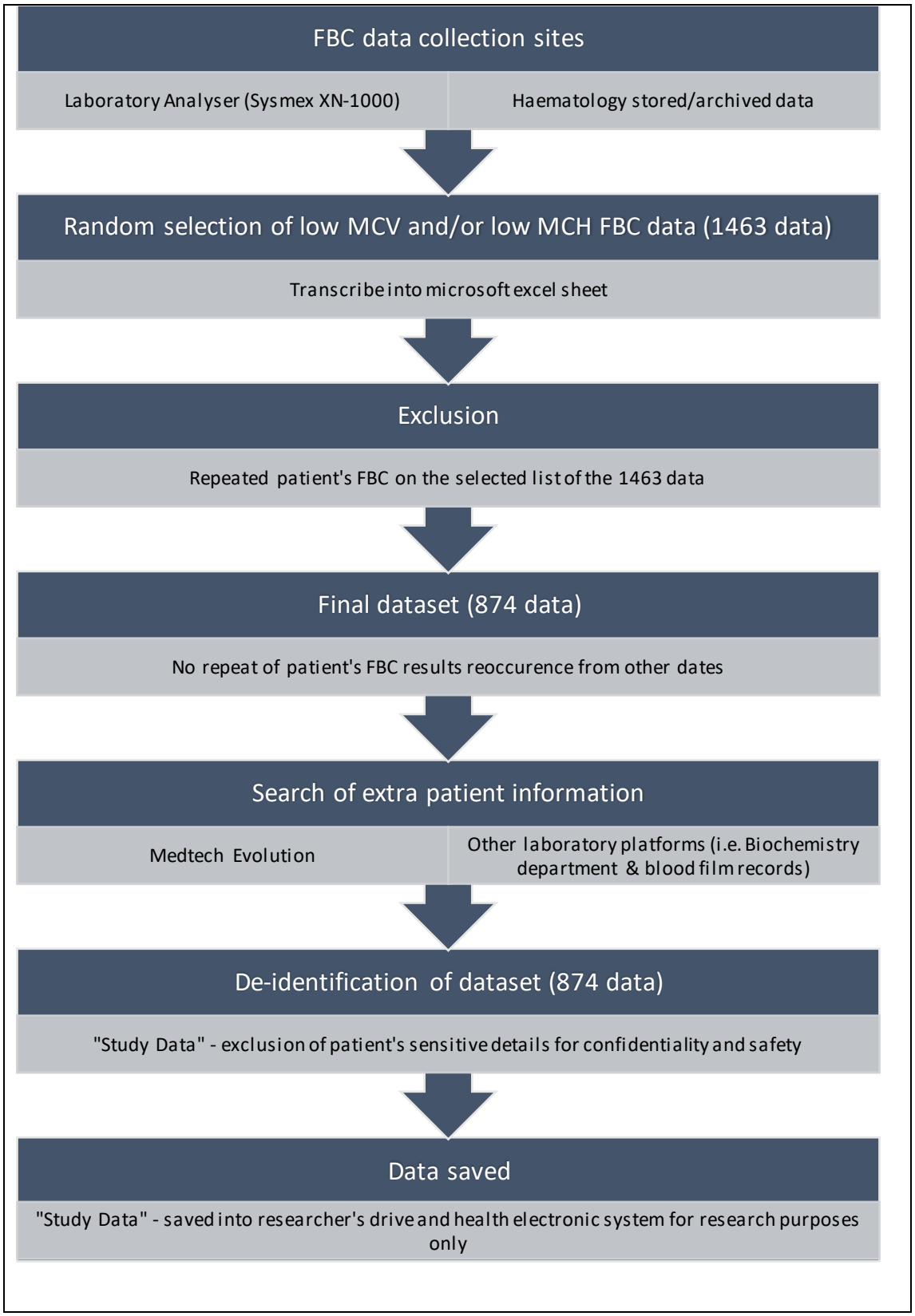
5. Personal details (i.e., names and other sensitive information) were removed from the data to protect patient safety and confidentiality during the study (i.e., de-identified data was used).

6. The data was de-identified and assigned a numeric code for patient/data identification. This data, known as the 'Study Data', was approved to be shared with Auckland University of Technology (AUT) by Te Marae Ora Cook Islands Ministry of Health. The purpose of sharing this data was for research only.

2.1.2 Summary of Data Collection Steps

Table 10

A flow chart illustrating the data collection steps



RHL references ranges applied in the selection of the 'Study Data'

The Study Data selection focused on MCV and/or MCH. The reference ranges vary. Table 11 and Table 12 displays the normal reference ranges among different groups and genders for the tests used in the evaluation of microcytic anaemia at the RHL. The RBC parameters (Hb, RBC, Hct, MCV, MCH, MCHC) illustrated in Table 11 and blood film examinations are the tests used for screening of microcytic anaemias such as IDA and thalassaemia in RHL haematology department. Biomarker analytes such as serum ferritin and serum iron in Table 12 are available biochemical test used in RHL biochemistry department for microcytic anaemia evaluation. Any test result below or above these reference ranges is recognised as abnormal.

Table 11

RHL normal reference ranges for haematology RBC parameters relevant for microcytic anaemia evaluation

	Laboratory RBC parameters					
	Hb (g/L)	**RBC (x 10^{12}/L)**	**Hct (ratio)**	**MCV (fL)**	**MCH (pg)**	**MCHC (g/L)**
Female (≥ 16 years	115 – 155	3.6 – 5.6	0.35 – 0.46	80 – 99	27 – 33	320 – 360
Male (≥ 16 years)	130 – 175	4.3 – 6.0	0.40 – 0.52	80 – 99	27 – 33	320 – 360
Pregnant	100 – 145	3.4 – 5.0	0.30 – 0.44	80 – 99	27 – 33	320 – 360
Female (< 16 years)	115 – 150	4.0 – 5.35	0.35 – 0.44	78 – 93	25 – 31	320 – 360
Male (< 16 years)	125 – 160	4.40 – 5.70	0.37 – 0.47	78 – 93	25 – 31	318 – 353
< 13 years	115 – 145	4.2 – 5.6	0.35 – 0.43	75 – 90	24 – 30	315 – 350
< 8 years	113 – 145	4.1 – 5.4	0.33 – 0.42	74 – 87	24 – 29	319 – 353
< 4 years	105 – 140	4.0 – 5.4	0.32 – 0.41	70 – 86	23 – 29	317 – 353
< 1 year	105 – 136	4.0 – 5.3	0.31 – 0.40	69 – 84	22 – 29	315 – 353
≤ 120 days	97 – 130	3.3 – 4.8	0.29 – 0.38	72 – 91	24 – 32	323 – 361
≤ 60 days	93 – 158	2.9 – 4.8	0.27 – 0.46	89 – 100	28 – 34	324 – 364
≤ 30 days	100 – 180	3.5 – 5.0	0.31 – 0.55	85 – 123	28 – 37	324 – 364
≤ 14 days	125 – 205	3.5 – 6.0	0.39 – 0.63	86 – 124	28 – 37	324 – 364
≤ 7 days	135 – 215	3.5 – 6.0	0.42 – 0.66	88 – 126	28 – 37	324 – 364
≤ 24 hours	145 – 225	3.5 – 6.0	0.45 – 0.67	95 – 121	31 – 37	295 – 335
Cord blood	124 – 192	3.53 – 5.52	0.37 – 0.56	99 – 119	32 – 39	305 – 345

Note. Normal references ranges obtained from RHL.

Table 12

RHL normal reference ranges for biomarker analytes relevant for microcytic anaemia evaluation

Gender and Age	Serum ferritin (ug/L)	Gender and Age	Serum iron (umol/L
All (< 4 years)	15.0 – 400.0	All (< 2 months)	10.0 – 31.0
All (< 11 years)	15.0 – 80.0	All (< 1 year)	4.0 – 27.0
All (< 14 years)	15.0 – 150.0	All (< 3 years)	5.0 – 23.0
Male (< 30 years)	20.0 – 320.0	All (< 10 years)	6.0 – 25.0
Female (< 30 years)	20.0 – 170.0	All (< 18 years)	8.0 – 32.0
Male (< 40 years)	20.0 – 400.0	All (≥ 18 years)	10.0 – 30.0
Female (< 40 years)	20.0 – 190.0		
Male (≥ 40 years)	20.0 – 450.0		
Female (≥ 40 years)	20.0 – 380.0		

Note. Normal reference ranges obtained from RHL.

2.1.3 Study Data Exclusion/Inclusion Criteria

Excluded from the study are patient FBC results without low MCV and/or low MCH. For every FBC result analysed with a low MCV and/or low MCH value during the study period of July 2023 to February 2024 along with archived data from June 2021 to June 2023 were chosen for inclusion in the study without considering age or gender.

The second exclusion criteria pertains to the duplication of patients' FBC data in the initial inclusion list. Whereby, each result was from a different patient, enabling an evaluation of frequency of microcytic anaemias in the study population.

All data were collected consecutively. Thus, it represents an unbiased dataset as it does not discriminate based on gender, age, ethnicity, or physiological condition such as pregnancy.

2.1.4 Study Data Management

Participants whose data were used in this research were anonymous. There was no cost to the participants; however, the laboratory data collected for this research will provide a better understanding of RBC disorders; and eventually contribute positively to the future health of Cook Islanders. The Te Marae Ora Cook Islands Ministry of Health policy for patient confidentiality and privacy was strictly applied to this study.

Data management plan

The IT personnel have created a secure electronic folder to store the collected patient data (Study Data) for this research. Appendix B part d and e display letters correspondence and approval from the various organisation regarding data management. This folder is strictly secured in that only the primary researcher and IT personnel Level 3 (i.e., the IT manager and deputy) can access this stored data. The involvement of the IT personnel is necessary when there are any technical issues that need to be resolved. However, permission to access this folder must be obtained from the primary researcher first.

AUT's data storage policy has been approved by Te Marae Ora Cook Islands Ministry of Health. Following AUTEC's recommendation of a 10-year data storage period, the primary researcher or IT personnel can completely delete this secure folder from the electronic system at Te Marae Ora Cook Islands Ministry of Health. The primary researcher must be consulted for permission before the folder can be removed by IT personnel.

For the purpose and completion of this research, Te Marae Ora Cook Islands Ministry of Health has approved the sharing of de-identified data with AUT.

2.1.5 Data Analysis Plan

Before statistical analysis, the initial analysis was done during the data collection stage, where data were sorted carefully to meet research criteria of including patient FBC data having low MCV and /or low MCH and excluding recurring FBC results from the same patients on different dates within the Study Data collection timeframe. The data was entered into a Microsoft Excel spreadsheet where they were subjected to statistical analysis. The final dataset included FBC results from 874 participants.

A biostatistician was consulted for statistical analysis advice and assistance to ensure suitable statistical analysis methods were used for the research dataset. Hypothesis testing using a linear regression model (i.e., univariate or multivariate) was used to compare differences between groups against the numerical response variables of MCV and MCH. This enabled detection of significance and correlation amongst these numerical and categorical variables. Descriptive statistical analysis was also applied to different groups (e.g., age, gender, and ethnicity) for enhanced evaluation. Statistical analysis was performed using Microsoft Excel and RStudio software version 2024.04.0.

Re-categorising the ethnicity into fewer groups enabled an effective comparison among the different groups. This was to reduce the effect of considerably different numbers in different groups. The re-categorising of the ethnicities below was matched to the ethnicity's breakdown provided in Central Intelligence Agency (CIA) (n.d.). CIA is the principal civilian foreign intelligence services of the United States which collects information on foreign countries to help understand global issues and make informed decisions. This re-categorising of ethnicities and these figures are agreeable as these are the main ethnic groups present in the local Cook Islands population after Cook Islands Maori.

Re-categorising of ethnicities in the 874-results dataset gives six ethnic groups:

- Cook Islands Maori
- Fijian
- New Zealand/European
- Filipino
- Other Pacific islands

- Others

Additionally, the Mentzer Index (MI) was applied to further evaluate and apply other RBC indices. The MI is used to assist in the differentiation of IDA and thalassaemia without further testing (Sherali et al., 2023; Vehapoglu et al., 2014). This MI calculation was applied to the whole dataset of 874 results. Another approach was to use the XS-1 model calculation on the web-based tool as used by Shuang et al. (2023) (refer to Figure 12). This model calculation was applied to differentiate thalassaemia trait (TT) from IDA among identified pregnant mothers in the 874-results dataset. An illustration of the web-based tool is seen in Figure 12 below. The breakdown of the statistical analysis methods performed on the datasets is presented below.

Dataset statistical analysis:

1) Plotting the dataset using both Microsoft Excel and RStudio software version 2024.04.0.

   Creating descriptive analysis by using tables and plots to identify the distribution of response variables against different characteristics.
   - Tables and graphs presentation of the genders, ethnicities and age groups
   - Graphs on MCV and MCH spread among different group categories such as genders and ethnicities
2) Using parametric statistical methods on RStudio software version 2024.04.0.

   A linear regression model was applied to the dataset of 874 results and filtered to identify the significance and correlation relationships between numerical response variables of MCV, MCH and RBC count among different categorical variables according to age, gender, and ethnicity. The two different analysis modes applied were the univariate and multivariate analysis approaches.

3) Additional dataset analysis was conducted using different analysis tools below:

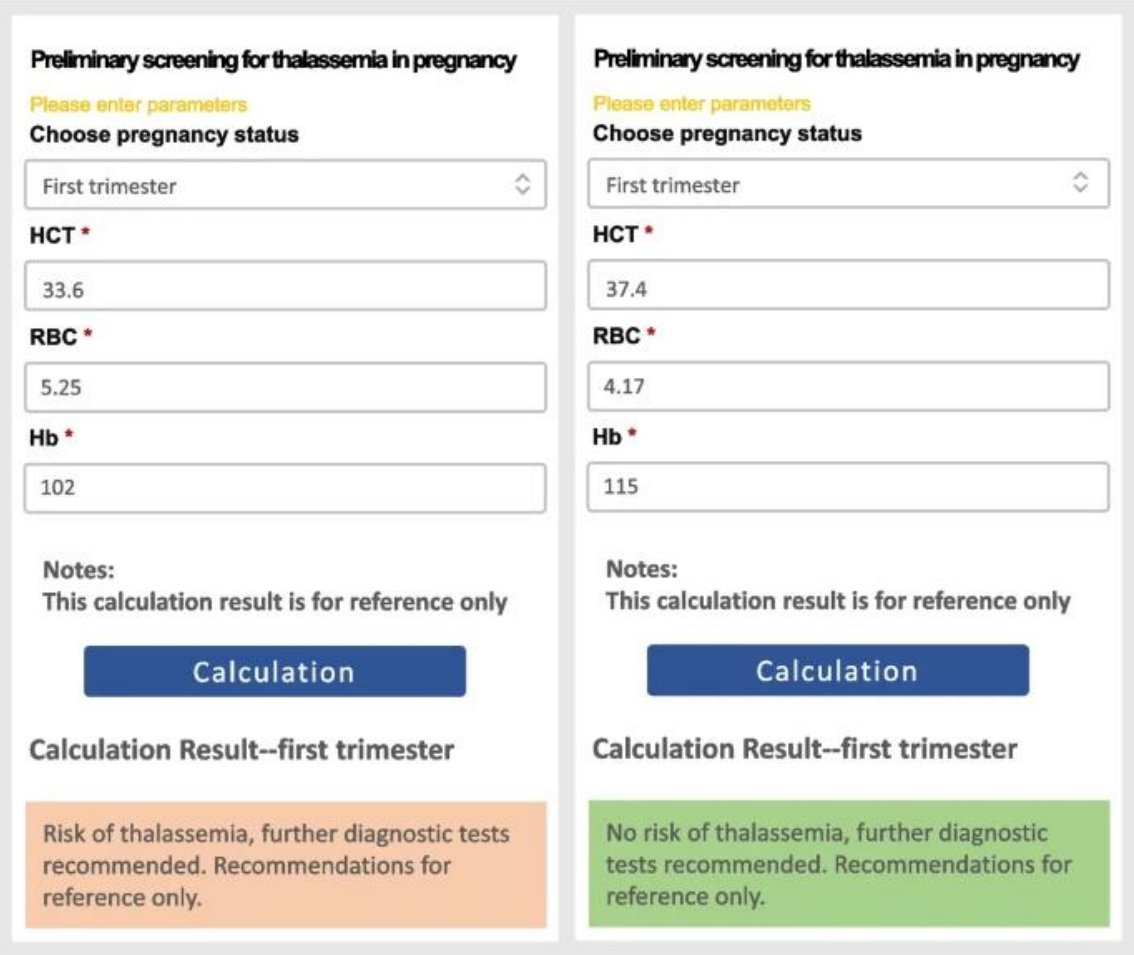
- Mentzer Index (MI)

  MCV/RBC is the formula for this discriminate formula and the cut-off value for IDA versus TT, where MI ≤ 13 is TT and MI > 13 is IDA. Descriptive analysis on MI calculation is performed to illustrate a clear indication of possible TT versus IDA by ethnicity.
- Web-based tool

This web-based tool was established with the XS-1 model which is the combination of log(e) from 3 different RBC indices such as Hct, RBC and Hb values. The thalassemia cut-off value for this calculation is <4 and results 4 and above are treated for IDA.

Figure 12

Web-based tool for determining characteristics of thalassaemia in pregnancy



Note. The above shows two scenario examples of using this web-based tool for thalassaemia screening in pregnancy. This calculation web-based tool is accessible from Shuang et al. (2023).

2.1.6 Ethics

Before the commencement of this research, various consultation processes criteria needed to be achieved. There were various ethics-related aspects required to support the AUTEC application and for approval of the research study to proceed. This includes obtaining support evidence from the various areas within the Cook Islands communities and Te Marae Ora Cook Islands Ministry of Health. Appendix B part a, b and c display the supporting letters from the various communities residing within the Cook Islands.

The involvement of these consultation processes was to ensure that this research is not discriminating against any ethnicity groups residing in the Cook Islands.

Additionally, it was advised to obtain a permit to undertake research in the Cook Islands from the Cook Islands National Research Committee (CINRC), as it is now a requirement under the National Research Policy of the Cook Islands (National Research Council, n.d.) for all research carried out in the Cook Islands to be granted a research permit before commencement, as this research involved human participants. Similar to the requirement of the AUTEC, Cook Islands Research Ethics Committee (CIREC) approval was also required. Therefore, there were two ethics bodies involved in this study, AUTEC and CIREC.

This research project was granted permission by the Cook Islands National Research Committee, with full approval from AUTEC and CIREC.

Since patient data were used for this study, the key ethical point to consider before Study Data analysis began was for data to be appropriately used, by ensuring confidentiality and privacy of the patients was maintained throughout the study process.

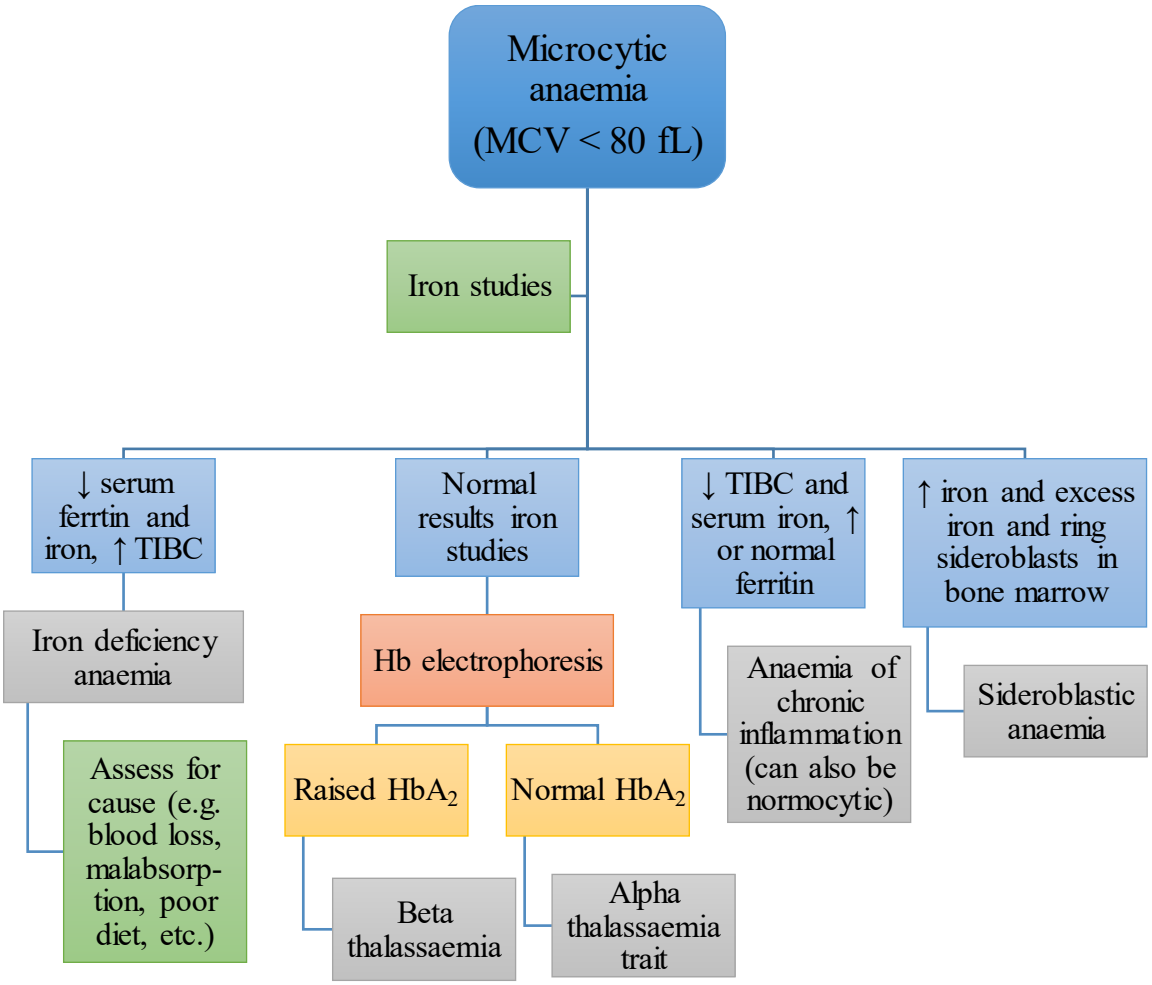
The Study Data for this research were de-identified once the dataset was finalised and before the analysis began.

2.1.7 Microcytic Anaemias Testing Algorithm Summary

An algorithm provides appropriate and effective guidance information when differentiating and diagnosing disorders. Therefore, to assess microcytic anaemias effectively, a microcytic anaemia testing algorithm applicable to this study has been provided (see Figure 13 below). This algorithm is a gold standard laboratory test, used for microcytic anaemia diagnosis. However, in the RHL, the current tests available for differentiating and diagnosing microcytic anaemia are the FBC, serum iron, ferritin and blood film examination. Only when a sample requires further testing for diagnosis confirmation will it be referred to New Zealand (NZ) laboratories for further analysis, as the required tests are not available at the RHL.

Figure 13

Microcytic anaemia testing algorithm



Note. Information in the figure adapted from Gram project microcytic anaemia (https://gramproject.com/diagram/microcytic-anaemia/). Data selected for this study has been respectively followed according to different age groups and genders reference ranges provided in Table 11.

2.2 Materials/Methods

The collaboration of various laboratory platforms in the RHL played a crucial role in generating data for this research. Therefore, descriptions and discussions of the RHL's platforms involved in the generation of data for this research are provided below. The alignment of these methods facilitated the evaluation of microcytic anaemias in the chosen population, which is the primary focus of this study's dataset.

2.2.1 Rarotonga Hospital Information Technology System

Medtech Evolution (ME) is the current hospital information technology system that all departments in the Rarotonga Hospital utilise for entering, sharing and storing patients'

healthcare-related information. This system provides easy accessibility to patient information across approved Rarotonga Hospital departments. These include the department of general practices, specialists, allied health professionals (e.g., RHL services), hospital outpatient clinics, accident and emergency clinics, and corporate health service providers.

This technology system stands out as an excellent healthcare management system for the Rarotonga Hospital, and it has been recognised as one of the most advanced in existence today. Its simple characteristics possesses a remarkable strength and adaptability to meet the growing needs of a healthcare system (Sayer et al., 2023). With its process and workflow engine, the ME application suite brings together a collection of integrated applications designed to enhance patient care in the Rarotonga Hospital. Therefore, the implementation of this health information management system has fostered an essential electronic connection between healthcare providers and individuals seeking medical care.

2.2.2 Haematology Department Platform

Automated blood cell counter

The Sysmex XN-1000 of the XN series is an automated haematology analyser used in clinical laboratories for in-vitro diagnostic purposes. It is designed to analyse human blood, human body fluids, or control blood. Using it for any other purpose is considered non-specified (Sysmex Corporation, 2012). The Sysmex XN1000 analyser in the RHL has not been set up or specified for human body fluid analysis. The Sysmex XN 1000 is a powerful tool that has a small footprint. It has a built-in automated re-run/reflex ability that reduces the need for manual interventions. Additionally, it has several excellent diagnostic features.

This instrument is used to perform blood count analysis in clinical laboratories, specifically for screening patient populations. It can provide quantitative, identification, and existence ratio analysis of different components of blood and body fluid, such as RBCs, white blood cells (WBCs), platelets (PLTs), and other cells (Sysmex Corporation, 2012). This analysis is done using electrical impedance, laser light scattering, and dye bonding. The Information Processing Unit (IPU) screen shows the analysis results. The XN series is composed of various components and options that can be used together in different combinations, which include the analyser, sampler section, IPU, pneumatic unit (controls the flow of samples and reagents in the instrument), SP-

10 (a fully automated slide maker and stainer) and additional components. These components and options can also be purchased separately.

In the Haematology Department of the RHL, the available XN-1000 analyser lacks an SP-10 unit. Figure 14 shows a picture of the XN-1000 and IPU unit similar to that at RHL. This analyser has the capacity to analyse 36 analysis parameters in three different types of analysis mode: the whole blood, low WBC and pre-dilution. The analyser generates an interpretive program (IP) message, which serves as a 'flag' to identify individuals at risk of specific haematological disorders based on their FBC results. With Sysmex XN-1000 IP common messages encountered include the identification of RBC agglutination (clumping), indications of ID, and abnormalities related to haemoglobin defects (Sysmex Corporation, 2012).

Figure 14

External view of the XN1000 analyser with an IPU unit

Note. Figure adapted from Sysmex XN-1000 (https://www.sysmex-europe.com/products/products-detail/xn-1000/). The pneumatic system is not included in this image.

Haematology Department smear staining machine

The Hematek® 3000 System is the available staining machine in RHL (see Figure 15). This was purchased due to its semi-automated slide staining capabilities, with the capacity to stain up to 60 slides per hour (Siemens Healthineers, 2024). The system enables RHL staff to define their own settings and achieve precise control, resulting in increased productivity and superior quality outcomes, and thus, more importantly, reduced time spent on staining a blood smear.

Therefore, this machine has been designed for optimised daily workflow in smaller laboratories like RHL with an average preparation of 10 slides per day made for suspected haematological conditions in the Haematology Department. Its all-in-one stain pack offers an easy load-and-go operation, providing enough stain for up to 900 slides.

The manufacturer's reagents used for staining are the Hematek Modified Wright's Stain and the Hematek Wright-Giemsa. The designers have designed the Hematek® 3000 system cellular applications to examine peripheral blood and bone marrow smears, providing detailed analysis.

Smear manual staining is an available option but not preferred by the haematology staff due to its time-consuming nature.

Figure 15

RHL Hematek 3000 System staining machine

Note. Figure adapted from Siemens Healthineers (2024).

2.2.3 Biochemistry Department Platform

The RHL currently utilises biochemistry analysers, specifically the cobas c311 and cobas e411, for routine biochemical testing. Descriptions and explanations of these two analysers, used in the Biochemistry Department, are provided below.

Roche cobas c311

The cobas c311 analyser (see Figure 16) is a stand-alone system that simplifies testing for healthcare professionals. It offers a diverse menu of clinical chemistry applications. The analyser can perform ion-selective electrode (ISE) measurements for sodium,

potassium, and chloride in serum, plasma, and urine. Additionally, it can measure HbA1c levels in whole blood, making it a versatile option (Roche Diagnostics, 2024).

The specifications of this analyser are:

- Has the ability to perform clinical chemistry tests, such as ISE analysis for sodium (Na), potassium (K), and chloride (Cl), as well as HbA1c measurement using whole blood.
- The sample throughput for photometry tests alone can reach up to 300 tests per hour, while for ISE tests alone, it can reach 450 tests per hour.
- The system allows for the utilisation of a range of sample types, including serum, plasma, whole blood, urine, cerebrospinal fluid (CSF), and supernatant (e.g., haemolysate).
- The system includes an automated sample rerun and dilution feature, allowing for continuous operation even while loading additional samples.
- This device incorporates a non-contact ultrasonic mixer and can measure three serum indices (lipaemic, haemolytic, and icteric), as well as detect sample clotting.
- Features a cassette reagent compartment, this system can store up to 45 reagents, with 42 dedicated to chemistry and 3 to ISE. The readable barcodes on the reagent packs make tracking and identification easy.

This platform analyses serum iron using a colorimetric assay, which quantitatively measures iron levels in the patient through photometric testing (Roche Diagnostics, 2023b).

Roche cobas e411

The cobas e 411 analyser (see Figure 17), with its patented electrochemiluminescence (ECL) technology, is a fully automated system that excels in performing immunoassay analyses (Axonia Medical, 2021). It is versatile for a multitude of applications, including quantitative and qualitative in-vitro assays for anaemia markers, bone, cardiac, and tumour markers, critical care, fertility/hormones, pregnancy care, and infectious diseases. The analyser is available in either a rack system or a disk system, allowing for flexibility in installation. The RHL has the disk system installed. What sets the cobas e411 apart is its extensive test menu, offering a wide range of options. With Roche's clinical chemistry and immunology test menu, the RHL has access to the most

comprehensive range of tests available. This allows both routine and specialised parameters to be run on a single analytical platform. With its efficient processes, the laboratory can conduct both routine and specialised tests with precision and promptness. Designed to prioritise patient needs, this instrument ensures continuous access and delivers robust performance, while also offering simplicity and reliable results.

Serum ferritin is analysed on this instrument. This assay measures ferritin levels in human serum and plasma, assisting the diagnosis of IDs and iron overload (IO) (Roche Diagnostics, 2023a). When conducting ferritin analysis, the testing principle utilised is the sandwich principle, also referred to ECL enzyme immunoassay (ECLIA, which is the same as ECL). The ferritin assay on cobas e411 takes a total of 18 minutes to measure (Roche Diagnostics, 2023a).

Figure 16

Roche cobas C311 chemistry analyser

Note. Figure adapted from Roche diagnostics cobas® 4000 analyzer series (https://diagnostics.roche.com/global/en/products/systems/cobas-4000-analyzer-series-sys-93.html)

Figure 17

Roche cobas e411 chemistry analyser

Note. Figure adapted from Roche diagnostics cobas® e411 analyzer (https://diagnostics.roche.com/global/en/products/instruments/cobas-e-411-ins-502.html)

2.2.4 Quality Measures in the Rarotonga Hospital Laboratory

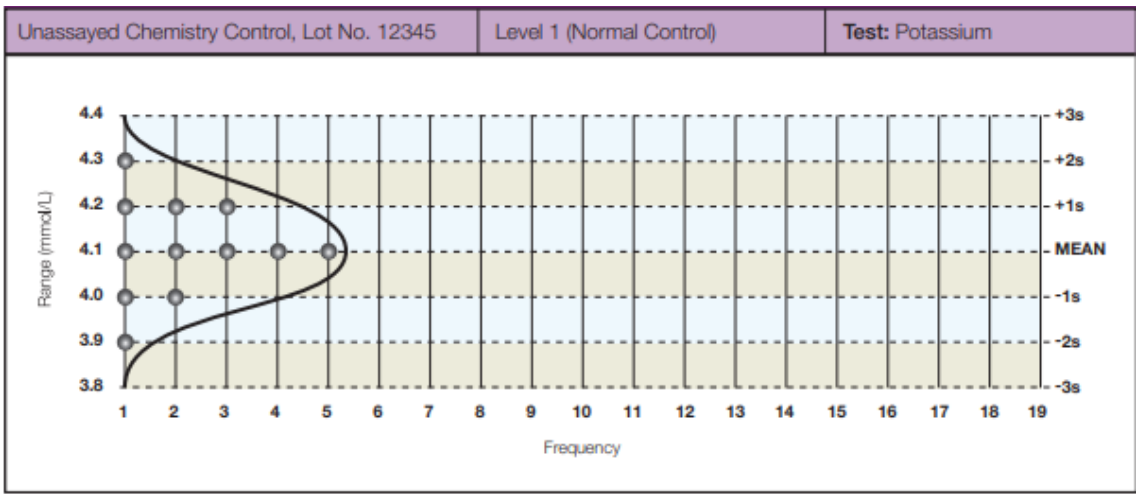
To fulfil its requirements, the RHL needs to perform regular maintenance on analysers and conduct daily quality control (QC) analysis. The International Standard Organisation ISO 15189 (a laboratory standard) requires the RHL to meet these essential requirements for measuring and ensuring quality and competence in diagnostic medical laboratory services (International Accreditation New Zealand [IANZ], n.d.). IANZ, an accreditation body, utilises this ISO laboratory standard for ensuring

laboratory services meet the laboratory regulatory standards. Their service provides effective quality processes for assessing professional assurance in laboratories by measuring laboratory expertise and technical competence (Spectrum Lab, n.d.).

On a daily basis, designated RHL staff maintain the analysers, and then carry out QC analysis. These tasks are the first steps to take before beginning the routine laboratory workload. The RHL uses commercially made QCs, running them in the morning and twice in the afternoon to validate patient results and analyser performance. The laboratory compares the QCs performed on each analyser against the manufacturers' reference ranges. The QC results validate the instrument, ensuring it meets the specified requirements (Cooper, 2008). This guarantees the reliability of patient test results. After validating the test system, the healthcare professionals can then utilise patient results for diagnosis, prognosis, or treatment planning. In addition, these QC results are plotted on a Levey-Jennings Chart (LJC) automatically by the analyser as another way of identifying that QC tests are reproducing reliable and precise values, so as to guarantee reliability and correct patient results (Cooper, 2008). Figure 18 shows an example of a relative distribution of QC values when the analytical process is within control.

Figure 18

Relative distribution of QC values



Note. Figure adapted from Cooper (2008). Within controlled analytical processes, around 68% of all QC values usually fall within ±1 standard deviation (1s). Similarly, the vast majority of QC values, about 95.5%, cluster within a range of two standard deviations (2s) from the mean. The analytical process, when in control, will cause around 4.5% of data exceeding the ±2s limits. Typically, most QC values, about 99.7%, fall within ±3 standard deviations (3s) of the mean. The ±3s limits only have 3 out of 1000 points falling outside, which is a mere 0.3%. It is advisable to avoid reporting patient results if any value falls beyond this range, as it is considered a significant error.

Laboratory analyser quality controls

Sysmex XN1000

The Sysmex XN-1000 QCs include three levels of blood concentration: XN Check™ assay Level 1, Level 2, and Level 3 (see Figure 19). The provider of these QC samples is Roche Diagnostics New Zealand Limited. XN Check comes in three varying concentrations to meet different needs. XN-Check level 2 covers the normal range, while XN-Check level 1 is used for abnormally low readings. To monitor abnormally high values, XN-Check level 3 is used. XN Check offers assay data for various diagnostic parameters. These include FBC parameters, WBC differential count, and additional information on immature myeloid cells, nucleated RBCs, and reticulocytes.

Figure 19

Sysmex XN1000 QC materials used by RHL

Note. The picture was adapted from Sysmex XN (https://www.sysmex-europe.com/products/products-detail/xn-check/)

cobas c311

PreciControl ClinChem 1 and 2 are the QCs used on the cobas c311 analyser. They are intended to be used for monitoring accuracy and precision for quantitative methods specified for the analyser. These are lyophilised human serum controls, and concentrations and activities are usually within the pathological range of a test assay.

cobas e411

PreciControl Universal (PCU) is the QC used for immunoassay analysers such as the cobas e411 in the RHL. This is a lyophilised control serum made from human serum, and it comes in two different concentrations known as PCU1 and PCU2. To ensure accuracy and precision, these controls are employed for monitoring immunoassays.

Hematek® 3000 System

QC of the staining machine involves preparing and staining a blood smear from a typical FBC sample. The Haematology Department performs this weekly to ensure quality staining material and machine functionality.

2.2.5 Rarotonga Hospital Laboratory Information System

Laboratory data make up around 70% of the information found in a patient's medical record (McCudden et al., 2020). To ensure the integrity of this information, a comprehensive framework of technical and regulatory requirements, intricate system architecture, and stringent security controls are in place.

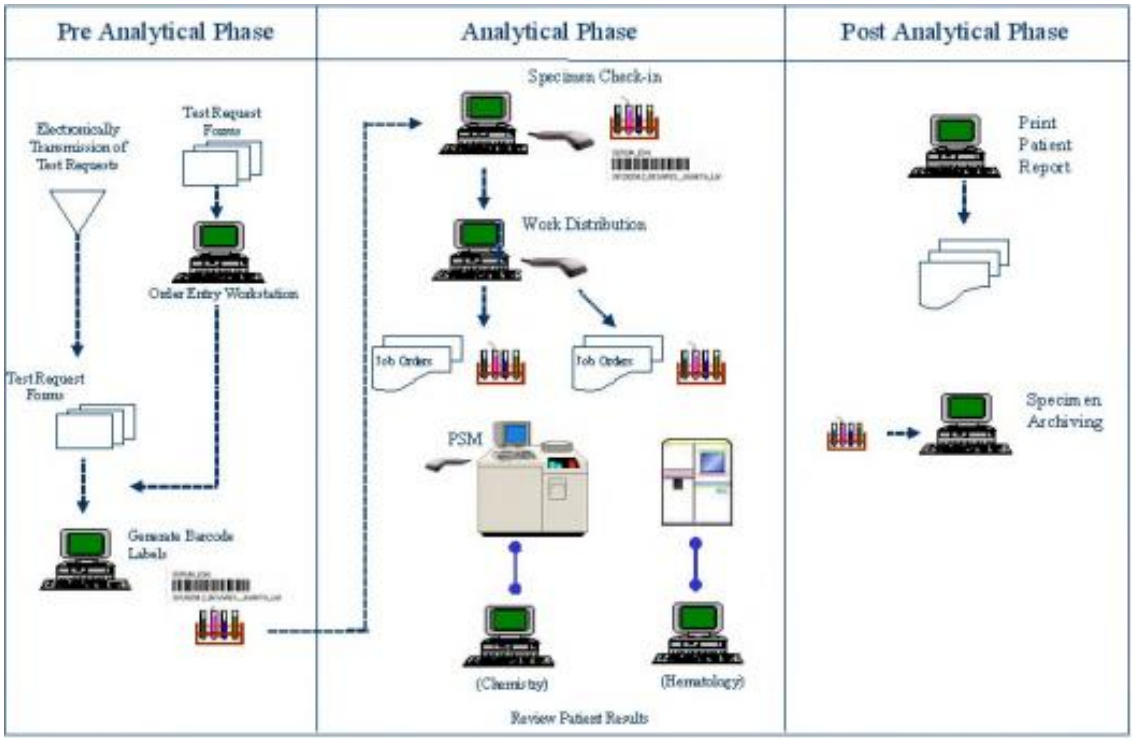
Sysmex HCLAB is the Laboratory Information System (LIS) available in the RHL. This LIS was installed in late November 2022, initially in the department of haematology and biochemistry. Installation processes for other RHL departments (i.e., Blood Transfusion, Immunology and Microbiology) were in process at the time of data collection and writing (April 2024) for this study.

LISs are essential for managing data in laboratories. They collect test requests and patient information from admission, discharge, and transfer systems, then send that information to the analytical instruments. The instruments return the results to the LIS, which transmits them to clinical viewers for healthcare providers to make decisions. Figure 20 below illustrates the process of an LIS, starting at pre-analytical phase, and progressing through the analytical and post-analytical phases. Data encoding standards like Health Level 7 (HL7) are necessary for this transmission (McCudden et al., 2020). HL7 is a widely recognised standard for exchanging medical information between healthcare providers. Its scope encompasses various aspects such as order entry, test result reporting, prescriptions, and patient admit/discharge/transfer processes. HL7 has emerged as one of the most successful medical standards in the industry (Kimura, 1999).

In addition to the LIS, there is also important information about document management, quality control, and handling semi-structured data in the testing process (McCudden et al., 2020). By utilising all this data, laboratories can improve operational efficiency and quality, and conduct research.

Figure 20

LIS process at different phases



Note. Figure adapted from Sysmex Corporation (2020).

Figure 20 shows the steps of a patient sample process from collection stages until the transcription of patient results from laboratory analysers to the ME health information system. Therefore, the figure shows the operational workflow of the RHL patient results from the beginning (sample registration/collection) to the reporting stage (the end-phase of the process).

Chapter 3 Research Results/Analysis

3.1 Study Data (Clinical Data)

Once ethics approval from AUTEC and CIREC was granted, along with permission to undertake research in the Cook Islands, data collection for this study began. There were two forms of data collected for this study, one which were the data was collected during the study period and the already archived data. Data collected during the study period from the haematology laboratory analyser was obtained between July 2023 and February 2024. Secondly, archived data was collected from June 2021 to June 2023. These were randomly selected data but fitting the selection criteria.

The master copy dataset consisted of 1,463 sets of patient FBC results; however, exclusion was carried out prior to data analysis being performed. This exclusion involved the removal of 589 patient FBC results that were identified as duplicate patient results. These 589 results were excluded to allow accurate estimation and evaluation of the microcytic anaemias. Therefore, the dataset in this study consisted of a total of 874 (unique values – non-repeated patient) de-identified results, also called the 'Study Data'. The dataset was re-categorised into six ethnicities before data analysis was performed. Data analysis includes using different analysis tools such as descriptive analysis, simple parametric statistical analysis, MI calculation and the use of web-based tools for the evaluation of the type of anaemia present.

Re-categorising of ethnicities in the Study Data was based on the ethnic groups residing in the Cook Islands as identified in *The World Factbook* (CIA, n.d.). Therefore, the categorisation of the Study Data into different ethnicities is set out below. Table 13 displays the breakdown of this dataset under different characteristics were:

- Cook Islands Maori (n=748) = 86% of the population (includes full and half inheritance of Cook Islands Maori ancestry)
- Fijian (n=43) = 4.9% of the population (includes Fijian and Fijian Indian)
- New Zealand/European (n=14) = 1.6% of the population (includes New Zealanders, New Zealand Māori and those specified on the ME as New Zealand/European)
- Filipino (n=12) = 1.4% of the population
- Other Pacific islands (n=17) = 1.9% of the population (includes Tahiti, Kiribati, Tonga, Vanuatu, Samoa and Other Pacific)

- Others (n=40) = 4.6% of the population (includes Indonesia, Burma, China, USA, Australia, Canada, Other European, Other ethnicity not specified, and Unknown (not specified))

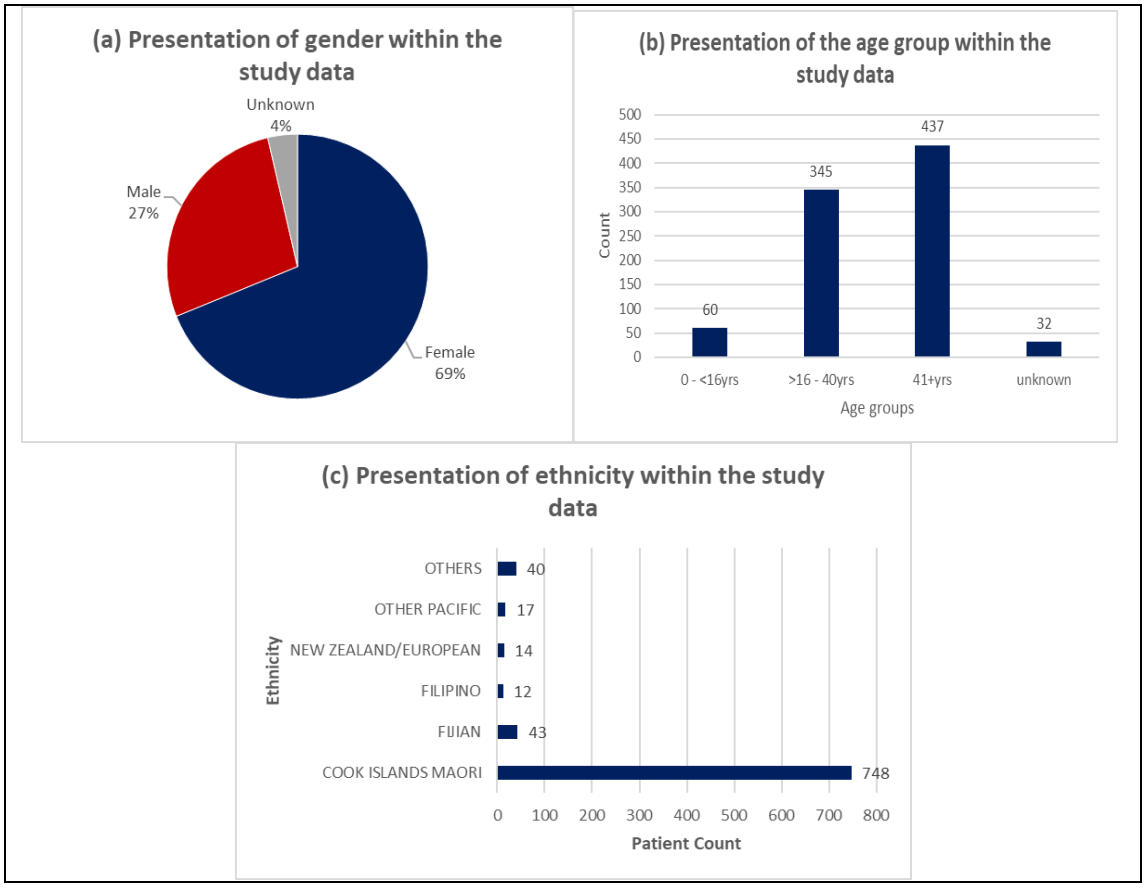
Table 13

Descriptive statistics of the 874 patients selected for this study specified by gender, ethnicity and age

Characteristics	**Frequency (n=874)**
Gender:	
Female	602
Male	240
Unknown	32
Ethnicity:	
Cook Islands Maori	748
Fijian	43
New Zealand/European	14
Filipino	12
Other Pacific islands	17
Others	40
Age groups (years)	
0 – <16	
Female	29
Male	30
Unknown	1
≥ 16–40	
Female	276
Male	68
Unknown	1
41^{+}	
Female	296
Male	141
Unknown	0
Unknown Age	
Female	1
Male	1
Unknown	30

Figure 21

Descriptive statistics of the 874 patients selected for this study specified by gender, ethnicity and age



Note. The pie chart reveals the presentation of gender within the Study Data. The top right bar chart shows the number of patients under different age groups. The bottom bar chart displays the number of patients in different ethnic groups.

Figure 21 above shows that a majority of patients were female, making up 69% while males made up 27%. Only 4% were unidentified. In terms of age groups, the figure shows that almost half of the patients in this Study Data are within the age group of 41 years and over (n=437 patients). There seems to be lower number of patients in the dataset within the age group of 0-<16yrs (n=60 patients), with 32 patients identified as unknown for their age. Cook Islands Maori make up 86% (n=748) of the dataset as compared to the other ethnic groups. Fijian is the second highest with 43 patients, followed by Other ethnic group of 40 patients. Other Pacific becomes the fourth highest with 17 patients, leaving New Zealand/Europeans the second least at 14 patients after Filipinos (n=12).

3.1.1 Breakdown of the Study Data into Numerical Response Variable Categories

The Study Data is further separated into different variables to allow statistical comparison and analysis among them. Out of these 874 sets of patient FBC results, 598 patients had low MCV, 861 had low MCH only and 585 patients had both low MCV and low MCH. Table 14 shows a detailed breakdown of the samples collected for this study, categorised by their respective characteristics. This is provided to show an initial clear indication of the spread of data among respective characteristics. All the data for this study was selected based on the study data specified criteria of MCV and/or MCH, following the RHL lower cut-off point of normal standardised patient reference ranges.

Table 14

Breakdown of the Study Data into different variables such as MCV, MCH and the combination of both MCV and MCH

Characteristics	Frequency (n=874)		
	Low MCV <80 fL (n = 598)	Low MCH <27 pg (n = 861)	Low MCV with low MCH (n = 585)
Gender:			
Female	413	595	406
Male	153	234	147
Unknown	32	32	32
Ethnicity			
Cook Islands Maori	494	742	488
Fijian	34	39	30
New Zealand/ European	12	14	12
Filipino	9	12	9
Other Pacific islands	12	15	10
Others	37	39	36
Age Group (years)			
0 – <16			
Female	21	29	21
Male	24	30	24
Unknown	1	1	1
≥ 16 – 40			
Female	196	273	193
Male	43	66	41
Unknown	1	1	1
41^{+}			
Female	195	292	191
Male	85	137	81
Unknown	0	0	0
Unknown Age			
Female	1	1	1
Male	1	1	1
Unknown	30	30	30

Note. Out of the 874 non-repeated de-identified results, there are 276 results with normal MCV (≥ 80 fL) and 13 results with normal MCH (≥ 27 pg). These data were collected for exclusively both low MCV and/or low MCH , which explains why there is data for normal MCV and normal MCH. These normal MCV patients have low MCH, and these low MCH patients have normal MCV.

Graphical presentation of the Study Data

The dataset of this study is further presented in graphs to show more understanding of the data. Figure 22 and Figure 23 depict the spread of response variables of MCV and MCH against gender and ethnicity.

Figure 22

Analysis of the spread of MCV among genders and ethnicities specified in the Study Data

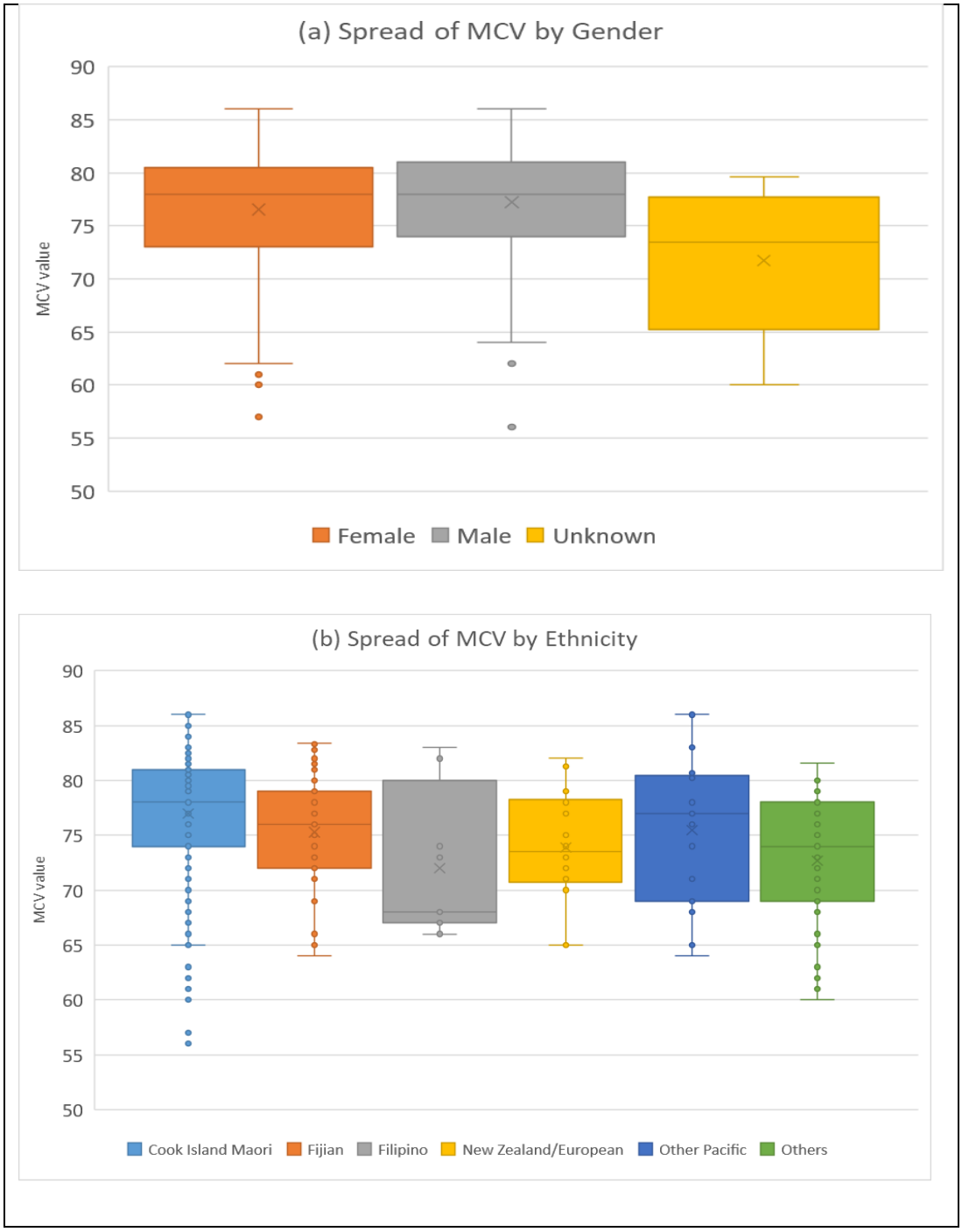


Figure 22 above contains box plots that visually represent the variability in MCV measurements, broken down by three gender categories: female, male, and unknown

and further categorised by various ethnic groups; this allows for a detailed analysis of how the distribution of MCV differs across these diverse populations.

The MCV values for the female group show a mean of approximately 78 to 79 fL, with the median slightly lower at around 77 fL, indicating a slight rightward skew in the distribution. The interquartile range (IQR) spans from about 73 fL (lower quartile (LQ)) to 81 fL (upper quartile (UQ)), while the overall range extends from approximately 63 fL to 86 fL. Notably, there are several outliers with very low MCV values, around 60 fL or below, which are marked as dots below the lower whisker on the box plot. These outliers likely represent microcytic cases, suggesting severe presentation of conditions such as IDA or thalassemia within the patient population.

The MCV values for the male group have a mean of approximately 78 to 79 fL, with the median nearly equal at around 78 fL, similar to that of the female group. This is indicating a symmetrical distribution. The IQR extends from about 74 to 75 fL (LQ) up to 82 to 83 fL (UQ), while the whiskers span from roughly 64 fL to 86 fL. There are a few outliers with MCV values below 60 fL, which may indicate a few male patients with more severe anaemia or abnormal RBC morphology.

The unknown group exhibits a lower MCV compared to others, with a mean around 73 to 74 fL and a median clearly below the mean at approximately 72 fL, suggesting a slight right skew in the distribution. The IQR spans from about 65 fL (LQ) to 78 fL (UQ), while the whiskers extend from 60 fL to 80 fL. Notably, no clear outliers are present in this group, indicating a relatively consistent range of MCV values without extreme deviations.

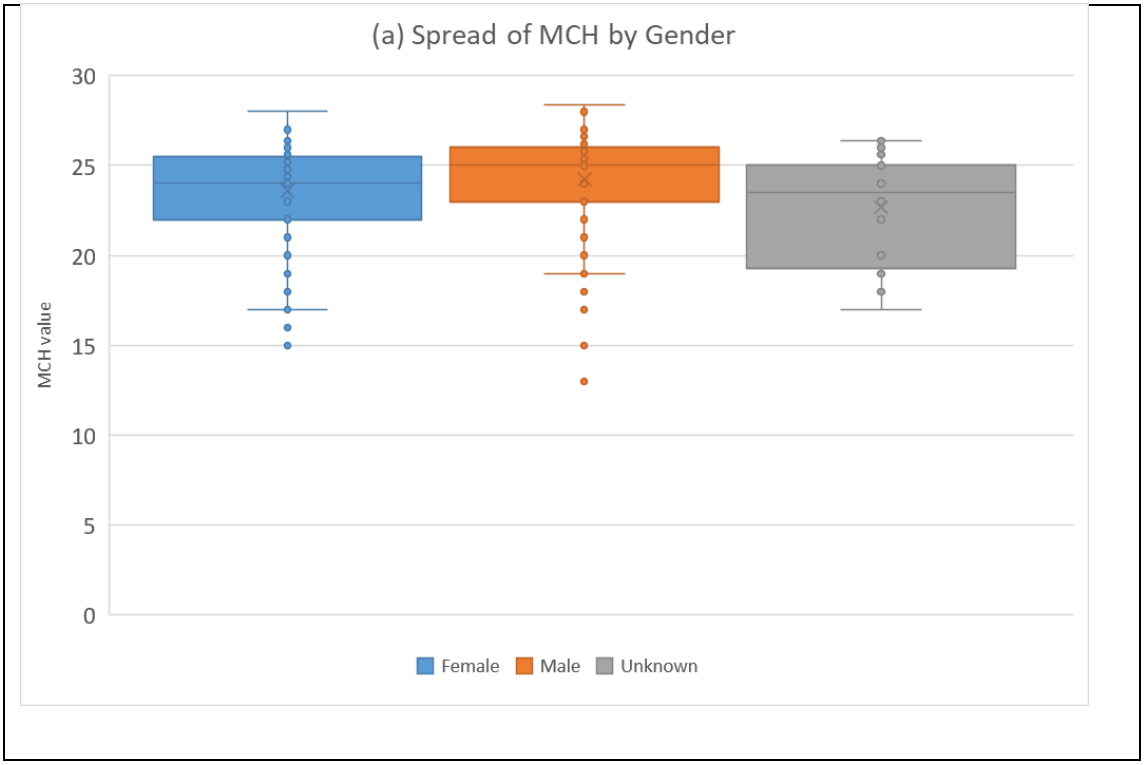
The distribution of MCV varies across ethnic groups. Cook Island Maori exhibit a mean MCV around 78–79 fL with many low outliers reaching as low as 55 fL, likely indicating severe microcytosis or anaemia. The Fijian group has a mean and median near 76 fL, with a few low outliers around 65 fL, reflecting a slightly lower average MCV. Filipinos show a lower mean and median (around 73–74 fL and 72 fL, respectively) with some outliers near 65 fL. New Zealand/Europeans have a tighter MCV range (mean ~75 fL, median ~74 fL) with no observed outliers. The Other Pacific group has similar central tendencies to Cook Island Maori (mean ~78 fL, median ~77 fL) with no obvious low outlier. Lastly, the Others category presents a mean and median near 74 fL, with a few very low outliers (~60 fL), indicating a wider spread of MCV values. Overall, groups with low outliers may reflect subpopulations with

microcytic anaemia or abnormal RBC morphology. A clear pattern of disparity in MCV measurements exists when comparing different ethnic groups, indicating notable ethnic differences. Some possible reasons for the disparity in MCV measurements among ethnicities, could be that Filipino and New Zealand/European would possibly have dietary differences to the Cook Islands Maori and Other Pacific groups or they could be depiction of microcytic disorders such as thalassaemias in the Filipino and New Zealand/European ethnic groups.

In summary, analysis of microcytosis in the Study Data reveals that certain ethnic populations, such as the Cook Island Maori and other Pacific Islanders, exhibit higher MCV values with greater variability, in contrast to groups like Filipinos and Fijians, whose MCV distributions are characterised by lower values and a narrower range of variation. Given the variations observed, it is plausible that genetic factors, dietary influences, and pre-existing health issues could all play a significant role, suggesting the need for further investigation to unravel their specific contributions.

Figure 23

Analysis of the spread of MCH among genders and ethnicities specified in the Study Data



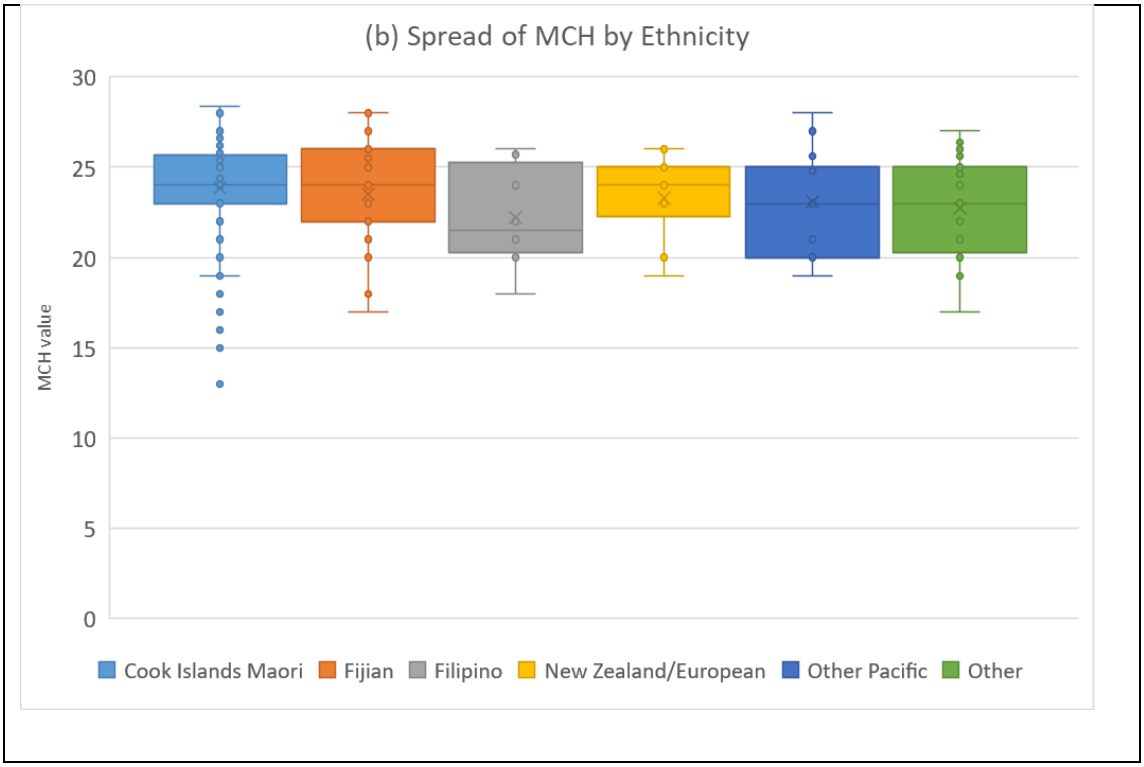


Figure 23 above shows box plots that effectively demonstrate the distribution and range of MCH values, with the data clearly segmented and presented according to gender and ethnicity.

The boxplots show the distribution of MCH values by gender. For females, the mean MCH is slightly below 24.5, with a median around 24.5. Females IQR spans from approximately 22.5 (LQ) to 25.5 (UQ). There are several low outliers below 18 and a few high outliers close to 28. Males have a slightly higher mean MCH, just above 25, and a median near 25. Their IQR is narrower, from about 23.5 (LQ) to 26 (UQ). They show a few low outliers down to 13–15, as well as high outliers near 28. The unknown gender group has the lowest mean at around 23.5 and a median just below 24, with a wider IQR from roughly 20 (LQ) to 25 (UQ). This group also has a few low outliers near 18 and high outliers close to 27. Overall, males tend to have slightly higher MCH values, while the unknown group displays a wider spread and lower central tendency.

The boxplots show that MCH values vary slightly across ethnic groups, with the Fijian group having the highest mean (~25 fL) and a few low outliers. The Filipino group has the lowest mean and median, with fewer outliers, suggesting generally lower MCH levels. Cook Islands Maori display a similar central tendency to Fijians but have the widest spread and the most low outliers (as low as 13 fL), indicating potential severe microcytosis. New Zealand/Europeans show the tightest distribution with minimal

outliers, while Other Pacific and Other groups have comparable spreads and a few low values. Overall, most groups cluster around similar median MCH values, but the Cook Islands Maori group stands out for its variability and frequency of low results.

Parametric statistical analysis of the Study Data

To investigate the relationships between MCV, MCH and RBC against age, gender and ethnicity in the cohort, a multiple linear model analysis was conducted. The null hypothesis for this statistical analysis was that MCV, MCH and RBC had no statistical effect or relationship with age, gender and ethnicity. The p-value of 0.01 was used, where a p-value of <0.01 reject the null hypothesis and accept the alternative hypothesis that MCV, MCH and RBC have an effect or relationship with age, gender and ethnicity. NZ/European ethnicity was used as a baseline for comparison among other ethnicities against MCV, MCH and RBC.

Table 15

Multivariate linear model analysis of MCV against age, gender and ethnicity

MCV	Estimate	Std Error	t-value	P-value
Intercept	71.58	1.46	48.93	< 2e-16
AGE	0.04	0.01	4.13	0.00
GenderM	0.55	0.40	1.39	0.16
Cook Island Māori	3.60	1.39	2.59	0.01
Fijian	2.04	1.58	1.29	0.20
Filipino	-1.44	2.02	-0.71	0.48
Other Pacific	2.56	1.86	1.38	0.17
Others	2.05	1.86	1.11	0.27

Based on this multivariate analysis, age was an important predictor of MCV (β=0.04±0.01, t=4.13, p<0.01). Every one year increase in age was associated with an average increase of 0.04 MCV, therefore age had a statistical effect on MCV. In addition, males on average had higher MCV compared to females (β=0.55±0.40, t=1.39, p>0.01). When comparing ethnicity against MCV, NZ/Europeans were used as a baseline for comparison. From Table 15 above, Cook Island Māori (β=3.6±1.39, t=2.6, p<0.01), Fijian (β=2.04±1.58, t=1.29, p>0.01), other Pacific (β=2.56±1.86, t=1.375, p>0.01) and other ethnicity (β=2.05±1.86, t=1.105, p>0.01) had on average higher MCV when compared to NZ/Europeans. However, Filipino had on average lower MCV (β=-1.44±2.02, t=-0.71, p>0.01).

Table 16

Multivariate linear model analysis of MCH against age, gender and ethnicity

MCH	Estimate	Std Error	t-value	P-value
Intercept	22.53	0.66	34.18	< 2e-16
AGE	0.01	0.00	2.32	0.02
GenderM	0.62	0.18	3.50	0.00
Cook Island Māori	0.71	0.63	1.14	0.25
Fijian	0.42	0.71	0.59	0.56
Filipino	-0.96	0.91	-1.06	0.29
Other Pacific	0.10	0.84	0.12	0.90
Others	0.22	0.84	0.26	0.80

Based on this multivariate analysis, age did not appear to be an important predictor of MCH (β=0.01±0.00, t=2.32, p>0.01). Every one year increase in age was associated with an average increase of 0.01 MCH, therefore age had no statistical effect on MCH. In addition, males on average had higher MCH compared to females (β=0.62±0.18, t=3.50, p<0.01). When comparing ethnicity against MCH, NZ/Europeans were used as a baseline for comparison. From Table 16 above, Cook Island Māori (β=0.71±0.63, t=1.14, p>0.01), Fijian (β=0.42±0.71, t=0.59, p>0.01), other Pacific (β=0.10±0.84, t=0.12, p>0.01) and Other ethnicity (β=0.22±0.84, t=0.26, p>0.01) had on average higher MCH when compared to NZ/Europeans. However, Filipino had on average lower MCH (β=-0.96±0.91, t=-1.06, p>0.01).

Table 17

Multivariate linear model analysis of RBC against age, gender and ethnicity

RBC	Estimate	Std Error	t-value	P-value
Intercept	4.94	0.19	25.73	<2e-16
AGE	0.00	0.00	0.65	0.51
GenderM	0.55	0.05	10.61	<2e-16
Cook Island Māori	0.07	0.18	0.37	0.71
Fijian	0.10	0.21	0.47	0.64
Filipino	0.22	0.27	0.83	0.41
Other Pacific	0.19	0.24	0.80	0.43
Others	0.20	0.24	0.84	0.40

Lastly, age did not appear to be an important predictor of RBC (β=0.00±0.00, t=0.65, p>0.01). Every one year increase in age was associated with an average increase of 0.00 RBC, therefore age had no statistical effect on RBC. In addition, males on average had higher RBC compared to females (β=0.55±0.05, t=10.61, p<0.01). This likely reflects the higher normal range for RBC in males versus females. When comparing ethnicity against RBC, NZ/Europeans were used as a baseline for comparison. From Table 17 above, Cook Island Māori (β=0.07±0.18, t=0.37, p>0.01), Fijian (β=0.10±0.21, t=0.47, p>0.01), Filipino (β=0.22±0.27, t=0.83, p>0.01) Other Pacific (β=0.19±0.24, t=0.80, p>0.01) and Other ethnicity (β=0.20±0.24, t=0.84, p>0.01) had on average higher RBC when compared to NZ/Europeans.

3.1.2 Other analysis method of the Study Data

MI calculation of 874 dataset for IDA and thalassaemia

The MI calculation formula used is:

Mentzer Index (MI) = MCV/RBC

Out of the 874 patient FBC results, the evaluation of possible IDA (where MI >13) and thalassaemia (where MI ≤ 13) indicates 74% IDA individuals (n=646) and 26% thalassaemia individuals (n=225).

In these IDA estimates, there were (Table 18) 485 (80.6%) females and 142 (59.2%) males, indicating more females than males have IDA. However, thalassaemia estimates are dominant in males, with an evaluation of 40.4% compared to females at an estimate of 19.1%. The overall male to female ratio in the study population was 28.5% to 71.5%.

Table 18

Breakdown of thalassaemia and IDA among different genders

Gender	Thalassaemia (MI ≤ 13)		IDA (MI > 13)		No MI results	
Female (n=602)	115	(19.1%)	485	(80.6%)	2	(0.3%)
Male (n=240)	97	(40.4%)	142	(59.2%)	1	(0.4%)
Unknown (n=32)	13	(40.6%)	19	(59.4%)	0	(0%)

Note: No MI result indicate missing RBC value (only MCV is available) for three patients in the Study Data. MI calculation requires MCV and RBC values. This is revealing incomplete recording of data.

Table 18 above provides ethnicities breakdown of this sub-group dataset.

Table 19

Breakdown of thalassaemia and IDA among the different ethnicities

Ethnicity	Thalassaemia (MI ≤ 13)		IDA (MI > 13)		No MI results	
Cook Islands Maori (n=748)	181	(24.2%)	564	(75.4%)	3	(0.4%)
Fijian (n=43)	10	(23.3%)	33	(76.7%)	0	(0%)
Filipino (n=12)	6	(50%)	6	(50%)	0	(0%)
New Zealand/European (n=14)	4	(28.6%)	10	(71.4%)	0	(0%)
Other Pacific (n=17)	6	(35.3%)	11	(64.7%)	0	(0%)
Others (n=40)	18	(45%)	22	(55%)	0	

Note: No MI result indicate missing RBC value (only MCV is available) for three patients in the Study Data. MI calculation requires MCV and RBC values. This is revealing incomplete recording of data.

Table 19 above shows that Cook Islands Maori rank second at 75.4% in the estimate of IDA and the second lowest in thalassaemia at 24.2% (relatively similar to that of the Fijian population at 23.3% estimates of thalassaemia). Estimates of IDA in the Cook Islands Maori ethnic group, as compared to Fijian and New Zealand/European do not show a significant difference between them. They are relatively the same. Filipinos appear to have the highest estimates of thalassemia at 50% compared to the other ethnicities. The estimates of thalassaemia in Filipino in this Study Data relate to the fact that this ethnicity has already been identified in literature to have a significant rate of 20.8% for α-thalassaemia trait (Capanzana et al., 2018).

Web-based tools analysis of Study Data evaluating pregnant mothers for thalassaemia risk

Analysis of the Study Data using the web-based tools (see Figure 12 on page 50) was performed on identified pregnant women in the dataset. A total of 27 pregnant women were found and 26 of them were identified to be at risk of thalassaemia using the web-based tool. The recommendation given with this finding is to perform confirmatory testing to exclude/confirm thalassaemia. Analysis using the tool was performed on different gestational ages such as first trimester, second trimester and late pregnancy ≥ 28 weeks, due to the lack of specific pregnancy gestational information available on ME from the clinicians. The ranges of MCV within this sub-group of the dataset is within MCV of 67– <80 fL with MCH normal ranges within 19–26 pg.

In these 27 pregnancies, there are 25 Cook Islands Maori, one Fijian and one Kiribati identified.

On further investigation, it was found that one mother had an existing alpha thalassaemia result in the ME, one pregnancy was found with the woman's mother diagnosed with alpha thalassaemia and one pregnancy was found with persistent low MCV/MCH with normal haemoglobin.

The use of this tool is computerised (using RBC parameters of Hb, RBC, Hct) and will provide clinicians a hint of possible thalassaemia risk versus IDA in pregnancy. This tool is inexpensive, and it is valuable to have an indication of these before further laboratory testing is applied.

3.1.3 Other clinical information of the Study Data

Frequency of iron studies in the dataset

Iron studies including serum ferritin were also investigated and it was found that 64 out of the 874 patients had serum ferritin results.

Among the 64 patients with serum ferritin tested, seven patients had high ferritin ranging from 460– > 2,000 ug/L. Thirteen patients were identified to have low serum ferritin level (<20 ug/L) and they were all female of Cook Islands Maori ethnicity. Six females were between the ages of 19–38 years and seven females were between the ages of 44–56 years. Forty-four patients were identified to have normal ferritin results (thirty females and fourteen males). In the presence of microcytosis/hypochromia, a normal ferritin level is very useful as it excludes IDA unless there is inflammation.

Frequency of CRP testing

CRP is a measure of inflammation and can be an indicator of anaemia of chronic conditions. From our cohort of 874 patients, 57 had CRP results on ME. It was found that 41 patients had CRP results ≤ 5 and 16 had CRP of > 5, ranging from 6 to 138 mg/L (above the RHL normal reference range of CRP <5.0 mg/L). This result indicates that 16 patients have underlying inflammation that may contribute to their microcytosis/hypochromia.

Frequency of reticulocytes results

Among the 874 patients, 36 patients were found to have reticulocytes result available. These reticulocyte results include reticulocyte parameters of the absolute, percentage and ret-he (reticulocyte haemoglobin equivalent). Thirty patients with normal reticulocyte results (normal reference range of 10–100 x 10^9/L) and six patients with abnormal results (i.e., above the normal reference range of 10-100 x 10^9/L).

Reticulocyte counts/measurements reflects bone marrow's erythropoietic activity (meaning, the rate of production of RBCs, which can be hypoerythropoietic (reduced)/hypererythropoietic (increased)) (Keohane et al., 2016). A reticulocyte count aids in diagnosing different types of anaemia. In cases of microcytic hypochromic anaemias, the reticulocyte parameters provide valuable insight into iron status, proving particularly useful in the diagnosis of IDA (Aedh et al., 2023). Ret-he provides a rapid and efficient method for evaluating the body's available iron stores. An abnormal low ret-he means an ID state (Brugnara et al., 2006).

Frequency of blood film examination

There was a total of nine out the 874 patients found to have record of blood film examination, with six blood film results indicating the diagnosis of microcytic anaemia. Comments include microcytosis, hypochromia, RBC targeting, polychromasia and pencil cells, and two of them have extra information regarding the patients' persistent low MCV/MCH. The other three blood film examination results were classified as being suspected bone marrow diseases such as leukaemia and myeloproliferative diseases.

Although there were insufficient blood film examination results, flag/abnormality comments from the haematology XN1000 analyser were collected which were found to be helpful in detecting any abnormality from the analysis of the patient's EDTA sample for FBC analysis. There were 358 out of the 874 patients with XN1000 analyser comments indicating some anaemia abnormalities. Comments such as microcytosis, hypochromia, anaemia, polychromasia, anisocytosis, HGB defects and iron deficiency are indications of RBC abnormalities in the patients, leading to a first step in analysing what medical condition that patient may have.

Existing thalassaemia/haemoglobinopathy testing in the dataset (alpha-thal testing)

There were 75 patients tested with alpha-thal test strip out of the 874 patients in the Study Data. Out of these 75 patients, 50 patients had a positive test (24 had MI lower $\leq$ 13 and 26 had MI > 13) and 25 had a negative test.

Chapter 4 Discussion

Iron deficiency the commonest cause of anaemia worldwide (Camaschella, 2019) significantly affects vulnerable populations of children and women (Williams et al., 2023) and alarms health authorities to act by implementing preventable measures to solve or minimise the problem. It is usually at a microcytic hypochromic state that IDA appears to be problematic, thus, causing severe health complications which can be life-threatening (Kumar et al., 2022). Other significant health conditions also depict the same picture of microcytosis and hypochromia as seen Figure 1 (Keohane et al., 2016). Of these, thalassaemias are identified as a common monogenic disorder worldwide (Capanzana et al., 2018; Kattamis et al., 2022) and are most clinically significant. Whilst heterozygous states are mild or asymptomatic (Keohane et al., 2016) whereas in the homozygous or compound heterozygous state, the clinical consequences range from moderate or severe lifelong dependence on blood transfusions (Marengo-Rowe, 2017). Those who are transfusion dependent will likely have a diagnosis made and/or be referred for genetic counselling for family members (Bain et al., 2023). Lifelong transfusions are not without risk, especially those of iron overload and transfusion-associated infections (Shah et al., 2019).

Heterozygous inheritance of thalassaemias (Table 7 and Table 8) are likely to remain well in the presence of a mild microcytic anaemia (Keohane et al., 2016; McKenzie et al., 2015). These individuals are at risk of being mis-diagnosed as iron deficient and given iron supplements which may be harmful (Purcell, 2021). In addition, they may partner with another asymptomatic individual and produce a transfusion dependent offspring (Langer, 2024).

The current approach to microcytic anaemia at the RHL begins with an analysis of a EDTA sample which provide an FBC result that includes measurement of RBC indices such as RBC count, Hb concentration, Hct, MCV, MCH, reticulocyte count etc. RBC indices are the initial focus for identification of possible RBC disorder. Blood film examination and biomarkers such as ferritin and CRP are also available in the RHL that is literally utilised for further microcytic anaemia for confirmation/exclusion. However, it is clear from this study that these initial investigations are not being performed in most instances. Of the 874 data, there were only nine blood film report available, sixty-four serum iron, 57 CRP results and alpha thalassaemia strip have 75 existing reports. It appears that abnormal RBC indices from an FBC result are often overlooked. This

suggests possible staff ignorance regarding the importance of further testing, laboratory staff incapabilities to perform further testing (especially in blood film examination which may requires high knowledge and competency in blood film interpretation) and staff (i.e. laboratory staff and requesting health practitioners) limited knowledge of the importance of distinguishing different causes of microcytic hypochromic red cells. There are no specific thalassaemia screening guidelines/protocols currently in place in the Cook Islands, nor are there sufficient resources in the RHL for carrying out further testing to confirm or exclude these conditions.

Thalassaemia has become a global concern because of widespread migration of people from regions where thalassaemia is prevalent (World Health Organisation Southeast Asia, 2021). Cook Islands populations consist of significant numbers of individuals who identify as belonging to different ethnic groups (CIA, n.d.). The migration and settlement (due to employment) in the Cook Islands of those of ethnicities recognised to have high prevalence of thalassaemia indicate a potential risk of having these inherited disorder present within local communities. Thus, mixed marriages/mating among local Cook Islanders and these migrants enables these hereditary conditions to be amongst local Cook Islands communities. Within the Cook Islands current population, it is recorded that the Fijian ethnicity at 3.6% is the second highest community residing in the Cook Islands, followed by New Zealand Maori/European at 3.4%, Filipinos at 2.9%, other Pacific countries (includes Tahiti, Kiribati, Tonga, Vanuatu, Samoa and Other Pacific) at 1.8% with 2.6% identified as people from Southeast Asia region (such as Indonesia, Burma (Myanmar)), China, United States of America (USA), Australia and Canada (CIA, n.d.). Southeast Asia is reported to have a high prevalence of α^0-thalassaemia, β-thalassaemia and high occurrence of co-inheritance of Hb E with β-thalassaemia (which is mainly seen in eastern regions of Myanmar and throughout Southeast Asia) (Capanzana et al., 2018; Kattamis et al., 2022). It is to be expected that cases with these inherited disorders exist within our populations. Additionally, in the Philippines, a study was carried out and found that 27.8% of the Philippines population in Manila have haemoglobinopathy. Among these haemoglobinopathy the prevalent form was α-thalassaemia trait (20.8%), followed by β-thalassaemia trait (5%), IDA with concomitant Hb E at 1% and β-thalassaemia Hb E interacting conclude at 1% (Capanzana et al., 2018). According to WHO region, the Western Pacific region (which includes all the Pacific countries) reported 11% of pregnant women were found to be α^+-thalassaemia carriers (Modell et al., 2007). This further highlights the likelihood that

these conditions might be present in our local Cook Islands communities. Currently, no published data exists regarding the prevalence (frequencies) and existence of these diverse group of conditions in the Cook Islands, nor is there any diagnostic laboratory process in place to identify and subsequently manage them appropriately.

This study attempts to highlight the probable existence of thalassaemias amongst microcytic red cell disorders in patients referred to the RHL for laboratory testing. The rationale for undertaking this study is the concern that hereditary haematological RBC disorders, particularly thalassaemias, are associated with considerable morbidity, potentially severely taxing the nation's health resources.

The aim is thus to raise awareness amongst Cook Islands health authorities of these probabilities and suggest the implementation of a more robust diagnostic process beyond what is current practice.

In this study of 874 FBC results showing microcytosis (low MCV) and/or hypochromia (low MCH), in the absence of further diagnostic testing, they were evaluated for the possible likelihood of being due to IDA or thalassaemia (using MI, web-based calculation for pregnant women) and other microcytic conditions (CRP to indicate ACI) as laid out in Figure 24.

A distinction between IDA and thalassaemia among the studied population was achieved by using the Mentzer Index (MI). MI method has been identified in literatures to be a reliable tool in distinguishing between IDA and thalassaemia (Sherali, 2023). Numerous studies have used this method, as it is instant and simple. In addition, the RBC indices used for this tool is always part of the RBC panel from FBC results. Out of the 874 FBC results it is found that there is 74% of individual with possible IDA and 26% with thalassaemia and among these significant figures Fijian ethnicity is dominant in IDA at 76.7% with Cook Islands second at 75.4%. Filipinos appear to be the least of IDA estimates in the Study Data at 50%. However, Filipinos is dominant in thalassaemia estimates at 50% compared to the other five ethnicities included in this study. With high prevalence of these two conditions globally, the estimation of these two conditions in other ethnicities is also considered significant. Females at 80.6% dominant within the estimates of IDA as compared to the males at 59.2%. It is expected of females to dominant in this condition given woman in the childbearing age lose iron through menstruation (Fernandez-Jimenez, 2020). However, thalassaemia estimates are dominant in males, with an evaluation of 40.4% males and 19.1% females. With 24 out

of the 50 patients identified to have existing positive alpha thalassaemia results (see section 3.1.3.5 subheading 4-Existing thalassaemia/haemoglobinopathy testing in the dataset (alpha-thal testing)), it shows all 24 patients have MI ≤13. This support to say that MI is valuable and reliable. Twelve out of these 24 patients had existing normal ferritin level. There are 7 patients with no serum ferritin results, 4 patients have high and 1 patient that could not be identified if serum ferritin is normal/abnormal due to missing age (date of birth) on the ME.

Furthermore, 57 (6.5%) patients out of the 874 had record of CRP results, with 41 (72%) patients had CRP results ≤ 5 and 16 (28%) patients had CRP of > 5, ranging from 6 to 138 mg/L (see section 3.1.3.2 subheading 4-Frequency of CRP testing). Of these 16 patients, 9 patients had normal and 7 with high results. One within these 7 patients high had a very high reading of >2000 mg/L. This shows that 16 patients in the dataset might be patients diagnosed with ACI. Further investigation of these patients showed only 36 patients in the dataset had reticulocytes results.

This study reveals significant deviations from the recommended evaluation of microcytic hypochromic anaemia. Firstly, it highlights the underuse or misuse of laboratory testing. A total of 598 patient with low MCV (microcytic) alone were identified with 276 normal MCV (normocytic) (see Table 14 on page 66) in the dataset. Out of these 598 with a low MCV, 90% patient (n=540) had no iron studies (i.e. serum iron and serum ferritin level) performed to confirm/exclude IDA, to indicate the presence/absence of thalassaemia and/or other microcytic anaemia disorders (see Section 1, Figure 1), even though iron studies are recognised as part of the first line testing (BPAC, 2013) for microcytic anaemia after FBC analysis (see Section 2.1.7, Figure 13). In addition to this, out of the 540 patients who failed to have iron studies, only one patient had a blood film examination report according to archived results, with no report of iron studies.

In males, the MCV values are observed to be both the highest and most consistent compared to other groups. When comparing the average MCV between males and females, females exhibit a slightly lower MCV, and the distribution of their MCV values shows a greater number of data points at the lower end of the range. Across the lifespan, the incidence of IDA is notably lower in males when contrasted with females. This disparity stems mainly from the fact that women of reproductive age experience regular blood loss associated with their menstrual cycle, which results in depletion of

their iron stores (Fernandez-Jimenez et al., 2020). In contrast to other groups, the Unknown group shows more dispersion in its data and typically has lower MCV readings, signifying a notable difference in the distribution and magnitude of MCV values. This dispersion in the Unknown group might likely be caused by the presence of the two genders (male and female), with the likelihood that females MCVs could be the cause of this dispersion. The observed discrepancies among the three groups could potentially be attributed to several factors, including inherent physiological variations among the subjects, inconsistencies in data quality, or unique characteristics of the samples themselves, with the Unknown group exhibiting particularly noteworthy distinctions.

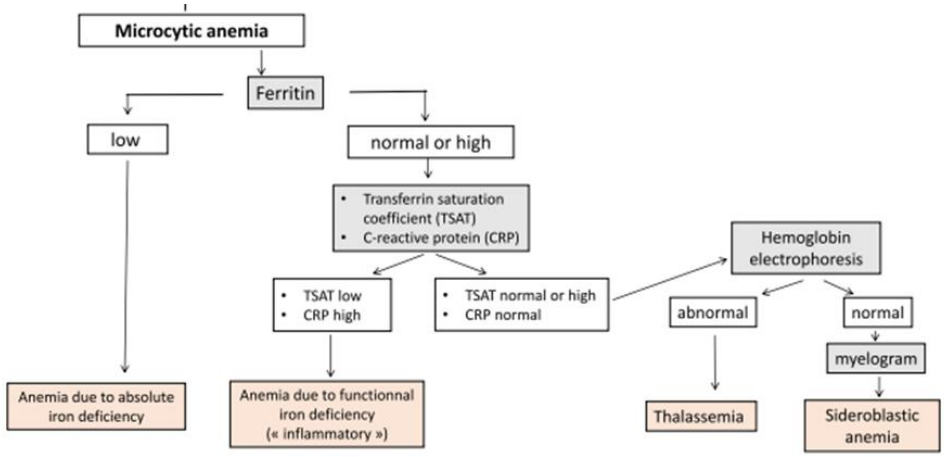
Numerous reviews and articles have discussed how anaemia assessment necessitates the use of a reticulocyte count and a peripheral blood film examination. In this study only nine patients had blood film report due to some abnormality indicated in the FBC results and 36 patients with reticulocyte results. It is unclear why these tests were not performed more often but could possibly be due to the sample integrity. When considering the available tests in the RHL for anaemia diagnosis, this study shows a significant underutilisation of recommended laboratory tests, resulting in no clear cause for the microcytosis among IDA, thalassaemia or other microcytic anaemia. To improve the diagnostic process of anaemias in the RHL a comprehensive diagnostic algorithm should be followed as described in Figure 24 for diagnosis completion.

Secondly, the study was considerably hampered by ethical constraints. Even though it is recommended best practice worldwide, no further laboratory testing was permitted without approval and consent from the participant. Since the data was selected from results already reported, if the recommended further tests were not requested by the referring practitioner, then no further evaluation was permitted.

Lastly, evaluation of the data was limited by the sparse clinical patient information available in the ME. No clues could be obtained as to why a particular result might have occurred.

Figure 24

Step-by-step algorithm laboratory tests in microcytic anaemia



Note: The grey boxes indicate the tests to be performed, and the pink boxes indicates the main diagnoses. Diagram adapted from Halfon et al. (2024).

The algorithm provided in Figure 24 shows a good diagnostic pathway for microcytic anaemias that the RHL staff should strictly followed after FBC analysis. This will ensure to show a reliable diagnostic programmed for microcytic anaemias. With the significant number of possible thalassaemia cases in the local Cook Islands population, the application of the MI tool to the diagnostic algorithm will be useful by pointing out the next steps. In this instance it will help save cost by misusing laboratory tests. This can be implemented but it is still best practice to ensure a possible diagnosis is excluded and/or confirmed. Current available biomarkers in the RHL such as serum ferritin, CRP, blood film examination remain as part of the screening pathway for microcytic anaemia. However, when the diagnosis is unclear the alpha thalassaemia test strip, supravital stains for HbH inclusions and electrophoresis should be implemented.

Due to the significant possible thalassaemia identified from this study, a further study is recommended urgently whereby proper thalassaemia screening is undertaken in any suspected case. Thereafter, preventive approaches in the Cook Islands should be implemented such as widespread screening programs, genetic counselling initiatives and comprehensive prenatal diagnosis policies. Other nations long since implemented proactive measures, to mitigate the incidence and resulting burden of disease (Cao & Kan, 2013). These screening programs aim to identify healthy individuals who are unknowingly carrying recessive genes, thus preventing the possibility of passing these genes on to their offspring, who may then acquire a homozygous recessive genotype (Cousens et al., 2010). To ensure carrier partners are well-informed, subsequent genetic

counselling sessions must thoroughly address reproductive risks and explore the full range of reproductive options available to them. As early as the 1970s, this measure was being promoted within the Mediterranean region (Cao & Kan, 2013). I believe it is time for the Cook Island health authorities to take notice.

Although a mandatory screening program has been surrounded by controversy, the implementation of screening regimens in regions where thalassemia is prevalent has resulted in a significant reduction in the number of thalassemia cases, as evidenced by Cousens et al. (2010). In the region of Sardinia, a significant decrease in the incidence of β-thalassemia was observed over a twenty-year period, with the prevalence rate falling from one in 250 individuals to one in 4000 (Cao et al., 1989). In the year 1973, on the island nation of Cyprus, a voluntary premarital screening program was first put into place. The number of affected births decreased significantly, falling from fifty-one in 1974 to only eight by 1979. Following the nationwide implementation of mandatory screening in 1980, a dramatic reduction in new β-thalassemia major cases was observed, with just five cases reported from 1991 to 2001. Significantly, the absence of reported cases of affected births, continuing up to and including the year 2007, highlights the profound effectiveness of the screening measures that were put into place (Bozkurt, 2007).

Preventive approaches should be accompanied by continuous thalassaemia awareness programs to ensure Cook Islands communities are knowledgeable and aware of the condition.

Due to the unavailability of other laboratory tests for thalassaemia, RHL refer patients' samples to New Zealand reference laboratory for diagnostic testing of thalassaemia. These shipments are costly as they involve payments for transportation and test charges by the New Zealand reference laboratories. In addition, this sample referral process experiences various disadvantages such as, sample integrity lost through the time of transportation resulting in the rejection of samples to be analysed, the uncertainty of sample reaching to the correct destination and delayed diagnostic management and monitoring for the suspected patient.

It is likely that the cost of establishing simple thalassaemia screening tests in RHL would be less than that of sending samples to NZ. Funds could then be reserved for genetic testing, which would best be referred to a specialist centre.

A comprehensive prospective study to assess the size of the problem of thalassaemia and other haemoglobin gene abnormalities in the Cook Islands is strongly recommended. This will enable future evaluation of best practices in the Cook Islands.

Chapter 5 Conclusion

This study has provided a preliminary evaluation of microcytic anaemias in the Cook Islands population presenting to RHL. It raises the likelihood that a significant number of cases of thalassaemia/haemoglobinopathy may be present in the population. Current diagnostic guidelines of microcytic anaemia must be reviewed. A strict guideline for the investigation- of cause of microcytic anaemias should be implemented to avoid overlooked and misdiagnosis of patients that may have these conditions. In addition, RHL should implement investigative protocols for thalassaemia/haemoglobinopathy. Additionally, thalassaemia/haemoglobinopathy prenatal screening should be included as part of the antenatal screening of pregnant mothers. If action is taken now, before the problem expands, the Cook Islands will save greatly, both in terms of preventing severe morbidity due to these conditions, and financially due to a reduction in the costs of lifelong expensive management of affected patients.

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Appendices

Appendix A Ethics and Research Approval

a) AUTEC approval letter

7 July 2023

Jill Meyer
Faculty of Health and Environmental Sciences

Dear Jill

Ethics Application: 23/107 **The prevalence of microcytic anaemias in patients reporting to a tertiary care centre in the Cook Islands.**

The Auckland University of Technology Ethics Committee (AUTEC) has **approved** your ethics application at its meeting of 3 July 2023.

This approval is for three years, expiring 3 July 2026.

Non-Standard Conditions of Approval

1. The data post analysis needs to be stored with the supervisor at AUT. Please review the AUT data storage guidelines and provide confirmation that these will be adhered to https://aut.ac.nz.libguides.com/RDM/introduction#s-lg-box-22079032

Non-standard conditions do not need to be submitted to or reviewed by AUTEC unless requested but must be completed before commencing your study.

Standard Conditions of Approval

1. The research is to be undertaken in accordance with the Auckland University of Technology Code of Conduct for Research and as approved by AUTEC.
2. All public facing documents must have the AUTEC approval number and be of a high standard of spelling and grammar. Dates on the Information Sheet(s) and Consent Form(s) must be consistent.
3. Any amendments to the project must be approved by AUTEC prior to being implemented.
4. A progress report is due annually on the anniversary of the approval date.
5. A final report is due at the expiration of the approval period, or, upon completion of project.
6. Any serious or adverse events must be reported to AUTEC, this includes unforeseen issues that might affect continued ethical acceptability of the project.
7. AUTEC grants ethical approval only. You are responsible for obtaining management permission for access from any institution or organisation at which your research is being conducted and you need to meet all ethical, legal, public health, and locality obligations or requirements for the jurisdictions in which the research is being undertaken.

The application number and title need to be referenced on all correspondence related to this project.

All forms are available online http://www.aut.ac.nz/research/researchethics

For any enquiries, please contact ethics@aut.ac.nz

(This is a computer-generated letter for which no signature is required)

The AUTEC Secretariat
Auckland University of Technology Ethics Committee

Cc: vwuatai@ymail.com

b) Cook Islands Research Committee Approval Letter

COOK ISLANDS RESEARCH COMMITTEE
OFFICE OF THE PRIME MINISTER
PRIVATE BAG, RAROTONGA, COOK ISLANDS
Phone +682 29454
Email: research.secretariat@cookislands.gov.ck Web: https://www.pmoffice.gov.ck/

File ref: **510.3**
Letter no: **17-023**

19th July 2023

Victoria Wuatai
School of Science
AUT University
Auckland
New Zealand
Fiji.

Kia Orana Victoria,

RE: APPROVED RESEARCH APPLICATION

I am pleased to advise that the National Research Committee has granted approval for your research titled **"The prevalence of microcytic anaemias in patients reporting to a tertiary care centre in the Cook Islands."**

Enclosed is your research permit issue **# 17/23**

The following conditions listed below have been imposed by the National Research Committee

- **The researcher complies with the Cook Islands Immigration**
- **The researcher provides a preliminary report to the Office of the Prime Minister at the earliest**
- **The researcher provides three (3) hard copies + one (1) e-copy of the final output generated from this research to the Office of the Prime Minister by June 2024.**

Kia Manuia

Ben Tonia
CHAIRPERSON

c) Cook Islands Research Permit

PERMIT TO UNDERTAKE

Research in the Cook Islands

This is to certify that: **Victoria Wuatai**

Has permission from the Cook Islands National Research Committee to conduct research in the Cook Islands from: **May 2023-May 2024.**

On the islands of: **Rarotonga**

The topic of research is: **"The prevalence of microcytic anaemias in patients reporting to a tertiary care centre in the Cook Islands."**

The Cook Islands Researcher is: **Mr Douglas Tou**

The following special conditions apply to this research:

- The researcher complies with the Cook Islands Immigration, Ministry of Marine Resources and National Environment Services requirements

- The researcher provides a preliminary report to the Office of the Prime Minister at the earliest

-The researcher provides three (3) hard copies + one (1) e-copy of the final output generated from this research to the Office of the Prime Minister by June 2024.

Permit Issued on: ***19 July 2023*** Issued by: **Ben Ponia**

CHAIRPERSON

Receipt Number: **308487**

Reference Number: **17-23** **Signed:** _____

For enquiries concerning this permit, please quote the Name of the Researcher and the Reference Number to the Secretariat of the National Research Committee at the Office of the Prime Minister, Rarotonga, Cook Islands. Phone (682) 25 494, or Email: research.secretariat@cookislands.gov.ck Website: www.pmoffice.gov.ck

Appendix B Supporting Documents

a) Support letter from the Director of Hospital Health Services

TE MARAE ORA
Ministry of Health
COOK ISLANDS

HEAD OFFICE
PO Box 109
Rarotonga
Cook Islands

Tel: 682 29 664
Fax: 682 23 109
Website: www.health.gov.ck

29th March 2023

RE: Ethics Evidence

Dear Dr Jill,

I write to provide my full support to Victoria Wuatai's Research Proposal on "The prevalence of microcytic anaemias in patients reporting to a tertiary care centre in the Cook Islands".

Victoria has consulted her research proposal with me. She has really emphasised and clearly explained in her research proposal the need of choosing such topic. I discussed with several of my fellow clinicians, and they agreed for Victoria's study to proceed. I do agree with her that this research study will be vital to the multitudes of people living in the Cook Islands with undiagnosed and diagnosed blood disorders as no study on these diseases were carried out in the past. This is the first study conducted for blood disorders in the Cook Islands, and this will allow to identify whether Cook Islands local population have a high frequency of red blood cell disorders. As one of her objectives "local guidelines for targeted red blood cell disorders diagnostics will be developed and recommended to Cook Islands health authorities", this is an example of achieving sustainable development outcomes and improvements in the health system for the local Cook Islands communities.

I wish Victoria the best with her research study and look forward to the final findings and recommendations.

Yours sincerely,

Dr Yin Yin MAY | Director of Hospital Health Services
Hospital Health Services | Te Marae Ora - Ministry of Health phone: +(682) 22664 | fax: +(682) 23109 | mobile: +682 55965 | PO Box 109 | Avarua, Rarotonga, Cook Islands web: http://www.health.gov.ck | mail: yin.may@cookislands.gov.ck

TE MARAE ORA
COOK ISLANDS Ministry of Health

b) Overseas supervisor

TE MARAE ORA
Ministry of Health
COOK ISLANDS

HEAD OFFICE
PO Box 109
Rarotonga
Cook Islands

Tel: 682 29 664
Fax: 682 23 109
Website: www.health.gov.ck

11th April 2023

Re: Approval letter from line manager

Dear Dr Jill,

I write to inform and provide confirmation of my full approval and support as being the Laboratory Manager and the external supervisor/advisor for Victoria's postgraduate research.

I am overwhelmed of what she has specified in her research proposal and I look forward to the final findings and her recommendations for improvements in the diagnostic process of red blood cell disorders.

Yours sincerely,

Douglas TOU | Laboratory Manager
Hospital Health Services | Te Marae Ora - Ministry of Health
Rarotonga, Cook Islands | PO Box 109
phone: (+682) 22664
web: www.health.gov.ck | email: douglas.tou@cookislands.gov.ck

TE MARAE ORA
COOK ISLANDS Ministry of Health

c) Consultations letters with other communities

- Traditional communities

6 June 2023

RE: Consultation evidence for Ethics Application

Dear Dr Jill Meyer,

Victoria Wuatai has consulted with me about her research study titled — "The prevalence of microcytic anaemias in patients reporting to a tertiary care centre in the Cook Islands". She has explained the reason of carrying out this research and future potential benefits that will be achieved from her research.

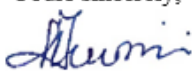
I am a traditional leader invested under traditional custom of the Cook Islands. As the spokesperson of the paramount chief Makea Vakatini Philip Ngamatoa Ariki, I speak on behalf of the Ariki as well as his tribe.

It is with great pleasure that I voice our paramount chief and his tribe full support of the research Victoria is carrying out using existing personal health data of the people of the Cook Islands. We truly believe that the health of our people will benefit as the prevalence of these health diseases in our population can be determined and may offer some alternatives understanding to our physicians.

Being able to speak the local language will eventually be a bonus as our people can readily understand and accept one who can relate to them their health condition easily. So, we fully support her using of our people's existing health data for her research study (thesis). We also find that consent seeking using these existing health data is unnecessary where she has discussed that there will not be any identities exposure in the research with data strictly use only for che purpose of the research. Additionally, having to have our own Cook Islands people conducting research based in the Cook Islands such as Victoria, people of the Cook Islands would willingly volunteer to participate in everything they would benefit from.

Once again, we give Victoria our full support for her research to proceed and wish her afl the best.

Yours sincerely,



Meleaone Tumii (Itaaka Rangatira)
Traditional Leader
Spokesperson of Makea Vakatini Phillip Ngamatoa Ariki and his tribe

- Religious communities

10th June 2023

RE: Consultation evidence for Ethics Application

Dear Dr Jill Meyer,

On behalf of my church congregation and my fellow church leaders, I voice our support for Victoria's research study on "The prevalence of microcytic anaemias in patients reporting to a tertiary care centre in the Cook Islands". She has explained the reason of carrying out this research and its implications.

I can see there's virtually no risk in her research study as she will only be retrieving existing data from laboratory patient results databases. She will be de-identifying her study data and just basically analysing study data to achieve what she want to find out. From her final research findings I surely believe that her research will benefit the Cook Islands people in terms of better management of these health diseases if it is found to be highly present in the population, especially hereditary red blood cell disorders.

With no hesitation, once again, we give Victoria our full support for her research to proceed.

Yours sincerely,

Mr Vakaroto Ngaro
Reverend for Avarua Cook Islands Christian Church
Rarotonga
Email: 10romana4.11@gmail.com

- Filipino communities

30th May 2023

RE: Consultation evidence for Ethics Application

Dear Dr Jill,

I am writing to inform you that Victoria Wuatai has consulted with me about her research studies about "The prevalence microcytic anaemias in patients reporting to a tertiary care centre in the Cook Islands."

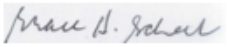
She has discussed important points, hence the reason why she chose to do this research study. For instance, there were no previous studies performed on these diseases within the local Cook Islands population which could lead to the possibility of having these red blood cell disorders such as Thalassaemias discovered in the local population that the health system and the people (i.e. patient) may not be aware of. Out of her research final findings, I can see her research would benefit the Cook Islands people in improving the current health system in relation to the management of patients discovered with these underlying red blood cell diseases. If there are gaps in the management of these diseases in the current health system, then this research study would provide some recommendations to implement for the management of these diseases.

Victoria's research will be collecting existing data from laboratory result databases, and she has explained that confidentiality and privacy of patient details will be maintained, where no identities will be exposed by the research, likewise, no opportunity for discrimination to result from the study.

I am speaking on behalf of my Philippines community settling in the Cook Islands, we fully support for this research to proceed with waiving the need for having informed consent from patient whose data belongs to, as she is only retrieving existing data only, and no exposure of identities.

I wish Victoria all the best with her research study and look forward to what she will contribute to the Cook Islands people.

Yours sincerely,



Grace Scheel (President of Filipino Community)

d) IT Data Management Plan

10th June 2023

RE: IT Data Management Plan

Dear Dr Jill Meyer,

I write to provide evidence and support for Victoria Wuatai's request for a study Data Management plan under the advice and approval of the Director of Hospital Health Services.

Study Data Management Plan is as follows:

I. Retrieval of patient FBC results (data) meeting the selection criteria from Laboratory patient result databases. Included in those patient data are: Patient name, DOB, Gender, FBC results. However, patient personal details such as name, DOB will be not included in the research data set. Study data will be de-identified, and the confidentiality and privacy of patients will be maintained. No identities will be exposed in the research.

II. IT personnel (i.e., IT manager) will create an electronic secure folder to store Study Data for Research. No-one other than Victoria will have access to it, and it will be strictly secured. Only the primary researcher and IT personnel Level 3 (i.e., IT manager & second in charge) can access this stored data. IT personnel will only be involved if there are any technical issues which need to be solved. Permission to access the secure folder by IT personnel must be obtained from the primary researcher.

III. Study Data will be stored for 10 years for health data, as recommended by AUTEC. The Cook Island Ministry of Health is in agreement with this duration.

IV. After 10 years of storage the secure folder will be fully destroyed from the Health electronic system by the primary researcher or IT personnel. The primary researcher must provide the permission for Study Data secure folder to be deleted.

From the understanding, support and the approval of the Director of Hospital Health Services understands the intent and scope of Victoria's research, and gives their full support and approval to proceed. Under these stipulated conditions, approval is granted for sharing of data with AUT, restricted to the said purpose of the research.

Yours sincerely,

Ralema Makena Geno (Ms) | Manager ICT
Te Marae Ora | TMO ICT |
Te Marae Ora - Ministry of Health
Avarua, Rarotonga, Cook Islands | PO Box 109
phone: **+682 22664**
web: **www.health.gov.ck** | email: **ralema.geno@cookislands.gov.ck**

e) Data Sharing Agreement

TE MARAE ORA
Ministry of Health
COOK ISLANDS

HEAD OFFICE
PO Box 109
Rarotonga
Cook Islands

Tel: 682 29 664
Fax: 682 23 109
Website: www.health.gov.ck

12th June 2023

RE: Data Sharing Agreement

Dear Dr Jill Meyer,

Victoria Wuatai has discussed her planned research (entitled, "The prevalence of microcytic anaemias in patients reporting to a tertiary care center in the Cook Islands") with our Director of Hospital Health Services (Dr May) and other medical staff.
A letter of support and approval was provided with the first application for ethics approval.

Victoria has returned to consult with Dr May concerning some issues raised by her first ethics submission. These concern an acceptable Data Management Plan and Data Sharing Agreement. She was advised by Dr May to consult IT department to create a secure folder, including a revised Data Management Plan, which has now been approved by the IT manager.

We have no problem with sharing of study data between Victoria (the researcher) and AUT, as will be de-identified and given numerical codes prior to analysis. Thus, we anticipate no exposure of patient identities.

On behalf of the Director of Hospital Health, sharing of de-identified data with AUT is approved for the purpose of Victoria's research study only and for her research to proceed.

Yours sincerely

(Acting Director of Hospital Health Services)
Dr Deacon TEAPA | Surgical Consultant
Hospital Health Services | Hospital Health Services |
Te Marae Ora - Ministry of Health
Avarua, Rarotonga, Cook Islands | PO Box 109
phone: **+(682) 22664 | fax: +(682) 23109**
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