Pediatric Iron Deficiency Anemia: Its Presentations and Treatments

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Abstract

Iron deficiency anemia (IDA) is the most prevalent blood disorder among children worldwide. Untreated IDA can lead to devastating health impacts. Oral iron, intravenous iron and blood transfusion are used to treat IDA.

This thesis provides comparative evaluations of prevalence, effectiveness and safety of interventions used to treat pediatric IDA. A systematic review (SR) and meta-analysis (MA) was used to synthesize evidence and examine sources of heterogeneity. Next, data from two Canadian pediatric hospitals, was used to explore associations between clinical variables and known biomarkers of IDA and examine their impact on treatment choice.

It was found that oral iron is the leading treatment of IDA, while intravenous iron and blood transfusion are reserved for more severe cases. All three treatments were safe and effective. Weak to moderate associations between vital and laboratory markers of IDA were found. Hemoglobin was found to be the dominant biomarker influencing clinical practice in Canadian hospitals.

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Chapter 1

Introduction and Thesis Structure

1.1 Pediatric Iron Deficiency Anemia

Anemia, a severe and oftentimes chronic condition, is the most prevalent blood disorder worldwide [1]. Globally, nearly 40% of children suffer from anemia, while 42% experience iron deficiency, which is the leading cause of anemia [2,3]. Although many national and international programs have been implemented to increase awareness and promote the prevention of iron deficiency anemia (IDA), the issue persists around the world, though it is most significantly felt in lower-income regions [4,5]. In Canada, it is estimated that 3.5% to 10% of children suffer from IDA, which is much lower than the global average [6]. However, in some rural and remote Indigenous communities, the prevalence of IDA is estimated to be as high as 50% [7,8].

The World Health Organization (WHO) defines IDA as low hemoglobin (<110 g/L for children under 5 years, <120 g/L for older children and adolescents) and serum ferritin levels (<12 µg/L for children under 5 years, <15 µg/L for older children and adolescents) [9,10]. IDA disproportionately affects young children, aged six to thirty-six months, and menstruating adolescent females, although its causes differ between these two sub-groups of the population [9, 11–16]. Young children often develop IDA due to poor intake of iron fortified foods or the over-consumption of dairy milk, during a period of rapid growth wherein iron is greatly needed [11,17–19]. On the other hand, adolescent females may develop IDA due to heavy menstrual bleeding (menorrhagia), wherein a major loss of blood via menses causes an overall depletion in hemoglobin and iron throughout the body [3,20,21]. Children suffering with chronic conditions, such as chronic kidney disease, inflammatory bowel disease or cancer, are also at increased risk of experiencing IDA [22, 23]. Other causes of IDA can be attributed to environmental, socioeconomic, and cultural factors, such as vegetarianism, where an individual would consume a lower quantity of iron-rich red meats, or overall poor access to nutritious food, which can be the case in lower-income regions of the world [4,5,11]. Regardless of its cause, chronic and severe IDA can have

detrimental effects on a child's cognitive, neurologic, and behavioural development if left untreated [24–28].

Other than hemoglobin and serum ferritin levels, several other laboratory and clinical characteristics can be used to identify IDA in children. Hemoglobin is primarily responsible for the transportation of oxygen throughout the body [29–31], implying that a reduction in hemoglobin concentration may lead to hemodynamic alterations and/or complications arising from chronic severe anemia [31–35]. Cardiac manifestations of IDA may include increased heart rate (tachycardia), palpitations, low blood pressure, and in severe cases, heart failure [31, 36–40]. These clinical indicators are important for decision-making pertaining to treatment of IDA.

Although mild IDA can be treated with a course of oral iron supplementation, moderate and severe IDA may require more intensive intervention, such as intravenous (IV) iron supplementation and/or the transfusion of packed red blood cells (pRBCs) [41–44]. Treatment decision is most generally based on the severity of the condition, coupled with existing co-morbidities on evaluation [11, 45]. Expert consensus, as well as the 2022 American Society of Hematology (ASH) and the American Society of Pediatric Hematology/Oncology (ASPHO) Choosing Wisely Campaign, "...advises against the transfusion of packed red blood cells (pRBCs) in asymptomatic children with iron deficiency anemia (IDA) with no evidence of hemodynamic instability or active bleeding [11, 46–48]."

1.2 Rationale and Thesis Objectives

Although there exist many different guidelines that inform clinicians on how to manage IDA, none are based on a systematic review or synthesized evidence. In fact, as to our knowledge, there exists no systematic review on interventions used to treat IDA in children. Considering that systematic reviews and synthesized evidence are the highest forms of evidence quality and that they are on the top of the evidence pool hierarchy [49], there is a need to fill this knowledge gap. The primary objective of this thesis, therefore, was to undertake a systematic review of the literature, with the goal of synthesizing all available evidence on the various treatment options for children with IDA. The key objective of this systematic review was to perform comparative evaluations of the leading treatments of pediatric IDA in regard to their prevalence as well as effectiveness and safety. Notably, the three commonly used interventions are oral iron supplementation, IV iron therapy and the transfusion of pRBCs.

The second objective was to examine the relationship between laboratory measures, clinical measures, and treatment choice, specifically blood transfusion, in children with severe IDA. The ASH-ASPHO Choosing Wisely recommendation highlights the importance of clinical assessment of hemodynamic status in

determining treatment options for patients with severe IDA [47, 48]. The guideline does not however define hemodynamic instability or the clinical thresholds at which different treatments should be considered in children with IDA. Vital signs are the most commonly used measures in the clinical assessment of hemodynamic instability [50], including blood pressure and heart rate. These measures can be obtained easily, immediately, and non-invasively, and may be repeatedly obtained to assess effectiveness of treatment. Vital signs are universally measured in emergency department settings and used to triage patients according to urgency and priority for treatment. For example, thresholds for specific vital signs have been found to help identify major trauma among injured children assessed in emergency departments at time of triage [51]. Thus, vital signs may guide clinical decision-making by identifying children with the greatest need for maximal intervention. For children with severe IDA, it is not known if vital signs (specifically heart rate and blood pressure) can identify if a child is in need of maximal intervention. In other words, whether vital signs correspond to hemodynamic status in children with severe IDA and whether vital signs or laboratory measures best guide treatment decisions is unknown.

1.3 Executive Summary

To accomplish the first objective, we performed a systematic review and meta-analysis of the existing literature on the interventions used to treat pediatric IDA. The review included 84 studies with 10991 patients, 7738 of which received either oral iron, IV iron or blood transfusion. The quality of the studies was deemed adequate through risk of bias assessments. We found that oral iron was the most prevalent of the interventions (84%), while IV iron (13%) and blood transfusion (3%) were given less often. These results were consistent throughout the sensitivity analyses we performed, however for children with severe IDA, we found that blood transfusion and IV iron became much more prevalent, while nearly all young children received oral iron. We quantitatively examined and compared the effectiveness of oral iron and IV iron and found that IV iron was significantly more effective than oral iron for the general pediatric population, while oral iron proved to be more effective for children with severe IDA. Although we could not quantitatively meta-analyze the effectiveness results for blood transfusion, the few studies included in the review indicated that blood transfusion was, for the most part, highly effective in treating IDA. All interventions were deemed adequately safe, with IV iron performing the best in terms of safety.

We identified and elucidated causes of heterogeneity among the included studies. We posited that the significant heterogeneity seen among the studies reporting on oral iron was due in part to the various formulations, regimes and dosages of oral iron given. For IV iron, moderate heterogeneity was reported, which we hypothesized was due to the fact that two formulations were often (and equally) used among the studies.

Heterogeneity was not quantified among the studies reporting on blood transfusion, as minimal quantitative data was available.

The second objective was addressed in two ways. First, we conducted canonical correlation analysis (CCA) on the laboratory and clinical measures of IDA to determine if these two sets of variables were correlated with one another. Retrospective data obtained from two major pediatric hospitals in Canada were used in our analysis. Prior to the CCA, very weak pairwise correlations between the two sets of variables were observed. The CCA revealed a maximized correlation of 0.55 between the two sets of variables, indicating moderate correlation. The two leading variables contributing to this moderate association were temperature and RBC count, while the other indicators were not influential. We hypothesized that the high correlation seen between RBC and hemoglobin affected the influence hemoglobin had in this maximized correlation, while RBC contributed greatly. Second, we constructed binary logistic regression models to examine the relationship between the various indicators of IDA and treatment choice (the binary outcome indicating having received a transfusion versus having not received a transfusion), with the objective of gaining an understanding of clinical practice in pediatric hospitals in Canada. The results from the logistic regression modelling indicated that hemoglobin was the only significant variable and was the leading determinant of treatment choice by clinicians in Canadian hospitals. Specifically, the results showed that a 10 g/L clinically meaningful decrease in hemoglobin level led to a staggering 359% increase in the odds of receiving a blood transfusion, while other covariates were held constant. Contrary to our initial hypothesis, the findings from this second study revealed that there was no significant relationship between vital signs and hemoglobin level, in general. In particular, the results showed no significant relationship between heart rate and hemoglobin level. Nevertheless, further investigation with a larger data set is required to confirm this or explore potential non-linear relationships.

The systematic review and meta-analysis contributed invaluable information to the existing literature on pediatric IDA, and can inform future clinical practice guidelines and policies for treating children with IDA. The findings from the logistic regression demonstrate that a very low hemoglobin level is the primary indicator of blood transfusion. The findings also reveal that vital signs and other clinical presentations of IDA played little to no role in the clinician's decision to give blood transfusion to children with severe IDA. This is true regardless of age and gender. Both studies and their respective results are beneficial to the medical community, in that they present novel findings with respect to the clinical and laboratory presentations and treatments of pediatric IDA.

1.4 Thesis Organization

In Chapter 2, we provide an overview of the methods used to accomplish each of the two objectives outlined above. In Chapter 3, we present our systematic review and meta-analysis including details on the methods, the review, and data collection process. We present the findings and interpret the results in light of available recommendations and highlight the contributions of our study and its potential impact. In this chapter, we also present results pertaining to quality of evidence and risk of bias. The study related to the second objective is presented in Chapter 4, wherein the methods, including a canonical correlation analysis and logistic regression, as well as the results, are presented. These results were interpreted to determine the associations both between the two sets of variables (laboratory and clinical) and treatment outcomes, as well as within these sets. Discussion, summary, and future directions are provided in Chapter 5.

Chapter 2

Background and Methods

This thesis consists of two projects addressing two related objectives. The first objective (Project I) focuses on comparative evaluations of prevalence, effectiveness and safety of interventions used to treat pediatric iron deficiency anemia (IDA). We conducted an evidence synthesis of existing literature with data collected through a systematic review. In the initial sections of this chapter, we provide a brief overview of systematic reviews and evidence synthesis methods, including meta-analysis. The second project (Project II) utilizes a retrospective dataset involving pediatric IDA patients treated at two major pediatric hospitals in Canada. The first project aims to employ multivariate exploratory techniques to determine potential associations between key hematologic biomarkers and measurements related to vital signs and clinical presentations of IDA. In this chapter, we briefly provide the specific multivariate methods we used to quantify associations between two sets of variables. Project II also aims to identify and examine potential factors associated with a patient's need of a blood transfusion for treating severe IDA. This involves modeling binary dependent variables; hence, we provide a brief overview of binary logistic regression models along with relevant goodness of fit statistics.

2.1 Systematic Reviews and Evidence Synthesis

The concept of evidence synthesis, though not entirely new, is becoming increasingly popular in the research world, specifically within the medical and health sciences realm. It consists of gathering all existing data on a certain topic and combining it together to produce a singular work that has the ability to be both concise and impactful. These works are often used to support policy and program development and, within the medical field, establish treatment guidelines.

Exceptional medical practice is always evidence-based, which is why evidence synthesis is considered to be the gold standard for informing policy, guideline

development and evidence-informed patient care [52]. Without such synthesized evidence (from a high-quality evidence pool) on which to base medical decisions, we must rely on expert consensus, which is considered to be less robust, and can often lead to a lack of treatment development progress [53, 54]. One must also account for individualized treatment planning for patients, in that every patient presenting with an illness experiences different environmental factors that may contribute to their response to treatment [53].

There are several types of reviews, from expert reviews (which are shown to be associated with the highest bias) and scoping reviews, to more intensive systematic reviews. Scoping reviews involve the systematic search of all available resources on a broad research topic, and are often used to perform background searches, identify research gaps, and help to develop a research question [55, 56]. Systematic reviews, on the other hand, use strict methodology to retrieve all available literature on a more focused (relative to scoping reviews) and specific topic, with the objective of synthesizing all available evidence, often involving quantitative synthesis leading to empirical results [57]. Systematic reviews, in their nature, also help to reduce bias associated with certain study designs, such as observational studies [58]. Randomized control studies are often able to control for confounding and bias by blinding participants and randomizing the treatment. On the other hand, it is difficult to control for certain variables in observational studies, specifically if they are retrospective in their design. Attempting to control for certain characteristics in an observational study can lead to selection bias, wherein the study population is not randomly selected and thus does not accurately represent the full population of interest [59]. For these reasons, we opted to perform a systematic review to address our research interests.

2.1.1 Systematic Review Methods

The methodology of conducting a systematic review is well-established and globally renowned. Researchers often follow the Cochrane Handbook for Systematic Reviews, as this handbook provides detailed information and tools on how to conduct a review properly and effectively, based on evidence and expert consensus within the field of evidence synthesis [60]. Although systematic reviews are used in a wide range of practical applications, our presentation in this thesis focuses on their application in biomedical sciences. As such, most of the terminology and topics discussed here are specific to biomedical applications.

Study Eligibility and Search Methods

Prior to commencing the systematic review, reviewers outline what are known as PICO criteria (population, intervention, comparator, and outcome). These criteria

help to determine which studies will be included in the review, and which will be ineligible. These criteria are often formalized into "inclusion" and "exclusion" criteria, where reviewers will compose a list of acceptable traits within each of the PICO criteria that will identify studies to be included in the review.

From there, researchers are well-equipped to develop a search strategy to identify the relevant studies that will align with their PICO criteria and thus be included in their review. The search strategy includes a compilation of keywords that align with the PICO and inclusion/exclusion criteria, that are then searched in online medical databases to retrieve relevant studies. It is a recommended and common practice to consult with a research librarian when developing the search strategy, as they can provide insights into the words to include and the databases to search to yield the best results and reduce bias in the systematic review [57]. Content experts (e.g. clinical researchers and health care providers, knowledge users and other stakeholders) are often involved in the conduct of systematic reviews, including active participation during establishment of the search and eligibility criteria. Researchers also often perform grey literature searches, wherein studies are sought for in non-traditional publications such as official government websites and theses repertoires. It is also considered a good practice to evaluate the references of articles that meet the inclusion criteria of the study to determine if there are more studies that are eligible for inclusion [61]. All of these are done in order to retrieve all available literature and reduce bias associated with omitted studies.

Screening, Data Extraction and Data Preparation

Once the search strategy is implemented, the researchers extract important study information (first author, year of publication, title) and upload these details to a systematic review software to begin sorting through the studies. Popular systematic review software include Covidence, DistillerSR, Rayyan and JBI SUMARI, which all offer semi-automated processes that allow for efficacious sorting of retrieved titles into relevant and irrelevant piles.

The sorting, reviewing, and examining for inclusion (retaining of the study) and exclusion of the articles (based on pre-prepared inclusion/exclusion criteria) occurs in two steps. In the first phase, screening is done by reviewing titles and abstracts of each of the retrieved studies. To avoid bias and eliminate subjectivity, each study is reviewed by two independent reviewers and studies are retained only if they are deemed relevant by both reviewers. Disagreements are discussed until consensus is reached. If consensus cannot be reached, the disagreement is resolved by consultation with a third reviewer, who is often a content expert. The second phase of screening begins by acquiring all of the full texts of the articles deemed relevant based on their titles and abstracts. To do so, reviewers often use a reference management software

such as Mendeley, Zotero, or EndNote, that search available online databases for full-text publications. In the case that the use of one or multiple of these platforms does not yield all of the full texts required for the review, researchers must manually search for and upload the full texts to their systematic review software of choice. Once all full texts are available, the two independent reviewers analyze each of the texts to determine if the study meets all the inclusion criteria to be included in the review. Similar to the first phase of screening, disagreements are resolved through discussions or by consulting with an independent third reviewer. The studies that are retained in this final step of screening are included in the systematic review.

Agreement Between Reviewers - The Kappa Coefficient

To ensure that the inclusion and exclusion criteria are clear and being properly implemented by both reviewers, data on agreements of inclusion or exclusion are collected and analyzed. Cohen's kappa coefficient is often used to measure agreement [62]. According to the Cochrane Handbook, monitoring the kappa coefficient throughout the screening process can ensure that the two reviewers are following the agreed upon inclusion and exclusion criteria, and are accurately applying these criteria to the retrieved articles [57].

The kappa coefficient, unlike simple proportion of agreement, accounts for agreement by chance and is a better measure of agreement. Letting p_0 represent the proportion of units that two reviewers actually agreed on and p_c represent the proportion of units expected to be agreed upon by chance, Cohen (1960) defined the kappa statistic as [63]:

$$\kappa = \frac{p_0 - p_c}{1 - p_c}.$$

To apply this statistic to the agreement between two reviewers conducting a systematic review, one first documents the votes of the two independent reviewers on the same set of variables (in this case, studies for inclusion or exclusion). Consider two reviewers voting to either include (Yes) or exclude (No) articles retrieved from the search, which is often summarized in the following two-by-two table 2.1.

Here, n_{ij} represents the number of articles in the $(i,j)^{th}$ cell, i representing the vote from Reviewer 1 (Yes or No) and j representing the vote from Reviewer 2 (Yes or No), and $n_{i.}$ and $n_{.i}$ are the row and column sums, respectively. N represents the total number of articles screened in the review process. The kappa statistic has been generalized to be applicable to two-by-two tables, wherein counts rather than proportions are provided. In this case, the proportion of units the two reviewers agree on, p_0, is calculated as [64]:

$$p_0 = \frac{1}{N}\Sigma n_{ii} = \frac{1}{N}[n_{yy} + n_{nn}].$$

Table 2.1: Two-by-two table representing agreement between two reviewers in their decision to include or exclude studies according to pre-prepared inclusion/exclusion criteria.

		Reviewer 2		Total
		Yes	No	
Reviewer 1	Yes	n_{yy}	n_{yn}	$n_{y.}$
	No	n_{ny}	n_{nn}	$n_{n.}$
	Total	$n_{.y}$	$n_{.n}$	N

The proportion of units expected to be agreed upon by chance, p_c, is [64]:

$$p_c = \frac{1}{N^2}\Sigma n_{i.} n_{.i} = \frac{1}{N^2}[n_{.y} n_{y.} + n_{n.} n_{.n}].$$

Given these values of p_0 and p_c, the kappa statistic can be simplified and rewritten as:

$$\kappa = \frac{\Sigma n_{ii} - \frac{1}{N}\Sigma n_{i.} n_{.i}}{N - \frac{1}{N}\Sigma n_{i.} n_{.i}}.$$

The interrater agreement is considered non-existent when $\kappa \leq 0.2$, minimal when $0.21 \leq \kappa \leq 0.39$, weak when $0.4 \leq \kappa \leq 0.59$, moderate when $0.6 \leq \kappa \leq 0.79$, and strong when $\kappa \geq 0.8$ [62].

Data Preparation and Imputation of Missing Data

To prepare data for the quantitative synthesis, it is imperative to first examine the data and ensure no available data is missing. Data preparation also involves ensuring that all data follows the same format and making adjustments (conversions) where necessary. For categorical outcomes, the number of events (frequency) and total sample size for all groups should be reported. Studies also report effect measures such as relative risk (RR), odds ratio (OR) or risk difference (RD) along with the total sample size for each group, from which quantitative synthesis is done. At the data preparation stage, one ensures that all data is converted to the same format (e.g. frequency and sample size, RR, OR, or RD). For continuous outcomes, mean and standard deviation (SD) for each group, along with sample size, are often reported. Mean difference (MD) and standardized mean difference (SMD) are also reported as effect measures for comparing continuous outcomes between different groups. In some cases, however, median with the corresponding measure of variation such as range or interquartile range (IQR) are reported. In such instances, it is imperative to transform such summary data to the same format, often mean and SD.

For articles reporting measures in median and range, Hozo's method is commonly used to calculate the mean and SD from the range and median. Let y_1 be the lower bound of the range, y_n be the upper bound, and $\tilde{x}$ be the median value [65]. Using Hozo's method, the mean ($\bar{x}$) and the SD (σ) are calculated as:

$$\bar{x} = \frac{y_1 + 2\tilde{x} + y_n}{4} + \frac{y_1 - 2\tilde{x} + y_n}{4n},$$

and

$$\sigma = \sqrt{\frac{1}{n-1}(y_1^2 + \tilde{x}^2 + y_n^2 + (\frac{n-3}{2})\frac{(y_1 + \tilde{x})^2 + (\tilde{x} + y_n)^2}{4} - n(\frac{y_1 + 2\tilde{x} + y_n}{4} + \frac{y_1 - 2\tilde{x} + y_n)^2}{4n}).}$$

For studies reporting median and IQR, the formula requires the median ($\tilde{x}$), the 25^{th} percentile (1^{st} quartile denoted by q_1) and 75^{th} percentile (3^{rd} quartile and denoted by q_3) to compute the estimated mean and SD of the sample [21]. This imputation method is also suggested by the Cochrane handbook [57] and the formulae are given by [21]:

$$\bar{x} = \frac{q_1 + \bar{x} + q_3}{3},$$

and

$$\sigma = \frac{q_3 - q_1}{1.35}.$$

In situations where studies report the mean and range instead of mean and SD, reviewers can follow another method by Hozo, with an improvement suggested by Wan et al, wherein normality is assumed and the SD becomes a function of the sample size and the range [66, 67]. Let y_1 be the lower bound of the range, y_n the upper bound of the range, and n the sample size of the study. Let Φ represent the cumulative distribution function (CDF) of the standard normal distribution. The formula for SD is given by:

$$\sigma = \frac{y_n - y_1}{2 \cdot \Phi(\frac{n-0.375}{n+0.25})}.$$

In some instances, reviewers are interested in change-from-baseline measures to determine the efficacy or safety of a treatment or an intervention. Apart from individual level data, the best summary data one can obtain is all summary measurements (mean, SD, frequency, sample size etc.) at baseline and follow up, along with estimates of correlation between baseline and follow up. Nevertheless, in practice, we rarely see these reported for the majority of studies. As such, the mean difference and standard error of the mean difference (if not provided) can be

imputed based on available data. Details on these are provided in the Cochrane Handbook [57].

The correlation between baseline and follow up measurements (ρ), which is needed to impute the standard error for the mean change from baseline, may not be reported in some studies. One can either borrow the correlation estimate from a similar study or use a conservative estimate of 0.5 [68, 69]. A value of 0.5 would indicate a moderate positive correlation between baseline and follow up measurements, which may not always be the case. Nevertheless, there is no established evidence as to why a value of 0.5 is used, hence it is important to conduct sensitivity analyses to explore its impact on the synthesized evidence.

2.1.2 Evidence Synthesis and Statistical Analysis

Once the studies are selected for the final systematic review and data is abstracted from the studies, the next step is to synthesize the evidence. Evidence can be synthesized narratively and/or quantitatively. Quantitative evidence synthesis primarily takes the form of a meta-analysis, wherein estimates of a particular outcome of interest is pooled from across several studies included in a review. Narrative syntheses are used to qualitatively summarize evidence in text or words, in the absence of high-quality numerical (quantitative) data [70]. Although narrative synthesis is considered a useful tool in systematic reviews lacking numerical data, it is also subject to potential subjective interpretation bias [57, 71]. Thus, quantitative evidence synthesis is often the primary objective of a systematic review when adequate data is available. It is also important to note that some form of narrative synthesis is often used in combination with quantitative syntheses, since studies provide important clinical information that may not otherwise be fully synthesized. In this section, we focus on quantitative synthesis, which is done using meta-analysis.

Meta-analysis is a statistical method used to combine data from multiple studies. Although individual patient data (IPD) meta-analysis is well established, the most common form of meta-analysis is done to combine summary statistics (known as effect sizes), such as mean difference in the case of continuous data and risk ratio (RR) or odds ratio (OR) in the discrete/binary case [72]. In non-comparative studies, effect measures for discrete variables are often given in pooled proportions or percentages (i.e. prevalence of disease). In this thesis, we focused more on meta-analysis of summary data. In addition to the benefit of synthesized evidence, which can be a high-quality evidence base for evidence-informed decision making and patient care, the purpose of pooling such data together is to both allow us to increase the sample size and hence improve precision of estimators and increase statistical power [73]. Meta-analytic approaches also offer methodological capabilities that allow us to quantify

heterogeneity within and between studies as well as identify and study potential sources of heterogeneity.

Fixed-Effect Meta-Analysis

There are two major meta-analytic approaches for pooling the estimates from similar studies: fixed-effect meta-analysis or random-effects meta-analysis. The fixed-effect model assumes one underlying true effect size and any differences between individual study estimates are due to sampling error [74, 75]. The fixed-effect model is the most appropriate when the pooled estimates are not being used to generalize outcomes outside of the set of studies included in the analysis, when the number of studies included in the analysis is small and when there is no significant heterogeneity above and beyond what is expected from a sampling error [75, 76].

In fixed-effect meta-analyses, the pooled estimates are obtained using weighted sums of study level estimates, where weights are assigned to individual studies. The standard procedure is to use the inverse-variance method, where a weight is assigned to a study based on the inverse of the study level variance. Let Y_i and $\hat{\theta}_i$ denote a study level effect size (i.e. mean difference) and its estimator from study i. The fixed-effect model can be written as $Y_i = \theta_i + \varepsilon_i$, where Y_i denotes the unknown true mean difference for study i while ε_i denotes the sampling variation, which is also referred to as the within-study variability [77]. We often assume that $\varepsilon_i \sim N(0, \sigma_i^2)$. Using the inverse-variance method, the pooled estimator is given by [77, 78]:

$$\hat{\theta}_{pooled} = \frac{\Sigma w_i \hat{\theta}_i}{\Sigma w_i},$$

with:

$$w_i = \frac{1}{var(\varepsilon_i)} = \frac{1}{var(\theta_i)}.$$

The variance for this estimator is thus [77]:

$$Var(\hat{\theta}_{pooled}) = \frac{1}{\Sigma w_i}.$$

For effect measures such as the odds ratio (OR) and the relative risk (RR), where the normality assumption is violated, the delta method is employed to provide an approximate asymptotic estimator for the variance of the pooled estimator and provide asymptotic confidence intervals and tests [79].

Random-Effects Meta-Analysis

In practical applications, significant variability between studies commonly arises, which can be due to different patient populations, settings, or a multitude of other factors, hence the assumption that there is one underlying true effect measure may not be valid in most cases [57, 80]. This variability across the studies is often referred to as heterogeneity, which must be examined and accounted for when performing a meta-analysis [81]. Some potential clinical sources of heterogeneity include varying population characteristics, severity of disease and cointerventions [80], while statistical or methodological sources of heterogeneity include study design and analytical tools used in a study [82]. When significant heterogeneity is present in the data, the fixed-effect model is no longer appropriate to pool the estimates, and rather a random-effects model must be employed [74]. If heterogeneity is left unaccounted for, this can lead to unreliable pooled estimates stemming from the meta-analysis [83].

The random-effects model, unlike the fixed-effect model, does not assume that one underlying true effect size exists among the included studies, but rather a variation of true effect sizes across the different studies [74]. The random-effects model accounts for both the within- and between-study variability [74]. This makes the pooled estimate more reliable as it considers all variability found within the studies [75]. In the absence of significant heterogeneity, the fixed-effect and random-effects models provide similar results [75].

Consider a set of studies with a global mean for the effect size (say mean difference) denoted by θ. The first measure of variability to be considered is the between-study variability (which is also referred to as heterogeneity), which describes the difference between the true mean effect size for study i (denoted by θ_i) and the global mean effect size θ. The random-effects model can be written as $Y_i = \theta_i + \xi_i$, often assuming that $\xi \sim N(0, \tau^2)$ [77]. Next, the within-study variability is considered, where a model is fitted for the within-study effect where we assume $\theta_i \sim N(\theta, \sigma_i^2)$, that is $\theta_i = \theta + \varepsilon_i$ [77].

Putting these two models (within- and between-study models) together, the observed effect size for study i can be written as [77]:

$$Y_i = \theta_i + \xi_i = \theta + \varepsilon_i + \xi_i, \text{ where } \xi_i \sim N(0, \tau^2) \text{ and } \varepsilon_i \sim N(0, \sigma_i^2).$$

The weighting strategy to pool the estimates from the individual studies is similar to what is used in the fixed-effect model; however, the random-effects model accounts for heterogeneity and within and between studies. The weight for study i is, therefore, calculated as [77, 84]:

$$w_i = \frac{1}{var(\varepsilon_i) + var(\xi_i)} = \frac{1}{\sigma_i^2 + \tau^2}.$$

Note that τ^2 is assumed to be constant for all studies, as it describes the total variability between the studies in the set. The method of calculating estimates of the pooled effect size and its corresponding variance is identical to what is used for the fixed-effect model, where the pooled estimate is given as [77, 84]:

$$\hat{\theta}_{pooled} = \frac{\Sigma w_i \hat{\theta}_i}{\Sigma w_i},$$

and

$$Var(\hat{\theta}_{pooled}) = \frac{1}{\Sigma w_i}.$$

Quantifying Heterogeneity

Meta-analyses offer the tools needed to quantify heterogeneity as well as identify and elucidate potential sources of heterogeneity. This can be done graphically by visually examining the resulting forest plots as well as quantitatively using the I^2 and Q statistics, which are measures of heterogeneity across the different studies. Consider a point estimate of a variable denoted as $\hat{\theta}_i$. Recall the weighting methodology of a meta-analysis as the inverse-variance method, where $w_i = \frac{1}{\sigma_i^2}$ with σ_i^2 denoting the variance of the study level point estimator $\hat{\theta}_i$. Now consider the pooled estimate of the variable, denoted $\hat{\theta}_{pooled}$, which is calculated by combining the point estimates and their weights of all the studies included in the meta-analysis. A common estimate of heterogeneity within a meta-analysis is Cochran's Q statistic, calculated as follows [57]:

$$Q = \Sigma \hat{\theta}_i (\hat{\theta}_i - \hat{\theta}_{pooled})^2 \overset{appr.}{\sim} \chi^2(N-1).$$

The statistic follows an approximate chi-square distribution with $N-1$ degrees of freedom, where N is the number of studies included in the meta-analysis. Using this statistic, we can perform Cochran's Q test, which tests the null hypothesis of homogeneity among the studies in the meta-analysis. Thus, when the observed value of the test statistic $Q_0 > \chi^2_\alpha(N-1)$, there is evidence of heterogeneity among the included studies, at a significance level of α.

The Q statistic is designed to detect the presence or absence of heterogeneity and does not quantify the extent of heterogeneity influencing the analysis, which is important when determining the validity of the results from a meta-analysis. To overcome this limitation, Higgins et al (2002) proposed the use of the I^2 statistic, which is a function of Cochran's Q, but provides a quantitative measure of heterogeneity that allows us to gauge the variability across the studies [85, 86]. The I^2 statistic is calculated as follows:

$$I^2 = \frac{Q - (N-1)}{Q} * 100\%.$$

In practical applications, ad hoc cut-off values are often used to determine the level of heterogeneity, where $0 \leq I^2 \leq 40\%$ is considered little or no heterogeneity, $30\% \leq I^2 \leq 60\%$ is considered moderate heterogeneity, $50\% \leq I^2 \leq 90\%$ is substantial heterogeneity, and $75\% \leq I^2 \leq 100\%$ indicates significant heterogeneity [57].

Knowledge of the presence of significant heterogeneity can be used to perform sensitivity analyses to understand whether high variability within the results of different studies contributes to possibly unreliable results of the meta-analysis [57]. Moreover, measures of heterogeneity allow us to identify and explain potential sources of heterogeneity, which in turn can inform subgroups for future considerations. When data is available, meta-regression can be used to investigate whether significant heterogeneity is caused by study design or population characteristics [83]. Meta-regression aims to combine the results of studies included in the review when a single pooled estimate does not adequately summarize the data presented within these studies [57]. These two tools are often implemented to control for heterogeneity when it is highly suspected within a systematic review.

Publication Bias

Another possible source of variability or bias in a systematic review involving comparative analysis is publication bias, which arises from the increased probability of studies that report on positive findings (statistically significant differences) being published compared to studies with negative findings [87]. Other sources of publication bias include English language bias, wherein negative findings are more likely to be published in non-English language journals, contributing to the lack of negative results included in a systematic review which uses English language publications only [88]. These factors can skew the results from a systematic review in a positive direction [87,88]. It is a common practice to visually assess publication bias using funnel plots, which plots study level summary statistics against the standard error of the effect size. Plots that are skewed or asymmetrical indicate potential publication and other biases within the set of studies included in the review [88]. The plot includes the 95% confidence interval (CI) of the effect size as well as a vertical line denoting the estimated quantity [89]. Evidence of little publication bias in the funnel plot will appear as the data points (from the studies in the review) falling within the 95% CI (acceptability region) and centred about the estimated value [90]. In the case that multiple data points are found outside the acceptability range, or the plot appears very asymmetric, publication bias is considered as an important factor influencing the outcome variables of the study [88, 90].

Subgroup and Sensitivity Analysis

To determine whether the pooled estimates are uniform across all possible subgroups of the data, it is a common practice to perform subgroup and sensitivity analyses. These analyses allow researchers to examine differences across different subgroups of the population, provide group specific estimates and/or assess whether certain systematic or methodological choices influenced the estimates. Subgroups are often determined beforehand, where researchers specify the various subgroup analyses to be conducted. These, for instance, might be according to health care settings (hospital vs outpatient), with respect to population characteristics (e.g. young children vs adolescence, male vs female) or according to type or severity of illness (e.g. chronic vs acute, mild vs severe). Some subgroups are also determined based on other factors that are known to potentially contribute to significant heterogeneity in the summary outcomes. Subgroup analysis also allows us to have more homogeneous groups of studies (alleviates the problem of significant heterogeneity) and allows us to provide more meaningful results. Sensitivity analyses are often conducted with respect of risk of bias (e.g. removing studies associated with high-risk of bias), with respect to missing data (e.g. with and without imputed studies) and other factors that are sources of heterogeneity, but not pre-determined as subgroup analyses [60]. Subgroup and sensitivity analyses can allow researchers to understand how a certain study characteristic or various components of intervention affect the pooled estimates. Sensitivity analyses can also inform the researchers of whether bias might be introduced through some of the methodological choices as well as through the quality of individual studies [57].

2.1.3 Evidence Quality and Risk of Bias Assessment

To ensure that the quality of the evidence being synthesized is of the highest standard, reviewers are required to examine each individual study for risk of bias, which arises due to various reasons. For systematic reviews involving randomized control trials (RCTs), the Cochrane Risk of Bias 2 (RoB2) tool is used, where bias is evaluated across five domains: bias related to randomization, bias due to deviations from the intended interventions, bias due to missing outcome data, bias due to the measurement of the outcome, and bias due to the selection of the reported result [57]. Each study is assessed by domain, giving each domain a rating of low risk of bias, high risk of bias, or posing some concerns of bias. Once each domain has received a rating, the overall rating for the study is determined by using the highest rating (the highest risk of bias) across the five domains.

For systematic reviews involving observational studies (case-control, cohort or cross-sectional designs, retrospective studies etc.), the Risk of Bias in Non-Randomized Studies - Intervention (ROBINS-I) tool is used to assess bias in seven

domains: bias due to confounding, bias in the selection of participants into the study, bias in classification of interventions, bias due to deviations from intended interventions, bias due to missing data, bias in measurement of outcomes and bias in selection of the reported result [57]. For each study, and each domain, the ROBINS-I tool assigns ratings of low risk of bias, moderate risk of bias, serious risk of bias, or critical risk of bias. For domains where no information is provided to make a fair judgement, a rating of no information is accorded. Overall risk of bias is accorded in the same fashion as with the RoB 2 tool, wherein the overall score is equal to the highest rating out of the seven domains. If multiple domains receive ratings of no information, the overall rating will reflect the same.

2.2 Canonical Correlation Analysis

Canonical correlation analysis (CCA), proposed by Harold Hotelling in 1936, is a multivariate method used to measure associations between two sets of variables, and is considered as a multivariate extension of Pearson's correlation coefficient [91, 92]. CCA seeks to find sets of linear combinations (referred to as canonical variates) of the variables in the two sets of data (say X variables and Y variables), with the objective of maximizing the correlation between the two sets [91]. Through the weights (loadings) of the canonical variates, CCA allows us to identify and examine variables (from the original sets of variables) that contribute the most to the associations between the two sets of variables. This can also indirectly enable us to identify variables that are not relevant to the objective at hand.

Consider two sets of variables $X : n \times p$ and $Y : n \times q$ where X and Y have the same number of rows (experimental units), n. The variance-covariance matrix for the combined dataset $\boldsymbol{(x, y)}$ is given as:

$$\begin{pmatrix} \boldsymbol{\Sigma_{11}} & \boldsymbol{\Sigma_{12}} \\ \boldsymbol{\Sigma_{21}} & \boldsymbol{\Sigma_{22}} \end{pmatrix}.$$

Here, $\boldsymbol{\Sigma_{11}}$ and $\boldsymbol{\Sigma_{22}}$ represent the variance-covariance matrices for X and Y, respectively, while $\boldsymbol{\Sigma_{12}} = \boldsymbol{\Sigma'_{21}}$ are the covariance matrices between X and Y, which is also referred to as the cross-covariance (and the correlations referred to as cross-correlations). The linear combinations (z_k and w_k, also known as canonical variates) are given as follows [91, 93]:

$$\boldsymbol{z_1} = v_{11}x_1 + v_{12}x_2 + \ldots + v_{1p}x_p = \boldsymbol{v'_1 x}$$

$$\boldsymbol{z_2} = v_{21}x_1 + v_{22}x_2 + \ldots + v_{2p}x_p = \boldsymbol{v'_2 x}$$

$$\vdots$$

$$\begin{aligned}
\boldsymbol{z_r} &= v_{r1}x_1 + v_{r2}x_2 + \ldots + v_{rp}x_p = \boldsymbol{v_r'x} \\
\boldsymbol{w_1} &= u_{11}y_1 + u_{12}u_2 + \ldots + u_{1p}y_p = \boldsymbol{u_1'y} \\
\boldsymbol{w_2} &= u_{21}y_1 + u_{22}u_2 + \ldots + u_{2p}y_p = \boldsymbol{u_2'y} \\
&\vdots \\
\boldsymbol{w_r} &= u_{r1}y_1 + u_{r2}u_2 + \ldots + u_{rp}y_p = \boldsymbol{u_r'y},
\end{aligned}$$

where $r = min(p, q)$. By design, $\boldsymbol{z_k}$ and $\boldsymbol{z_j}$ are orthogonal for all $j \neq k$, meaning that the two vectors are uncorrelated. Similarly, $\boldsymbol{w_k}$ and $\boldsymbol{w_j}$ are uncorrelated for all $j \neq k$, which means that $(\boldsymbol{z_k}, \boldsymbol{w_k})$ is uncorrelated with $(\boldsymbol{z_j}, \boldsymbol{w_j})\forall k \neq j$.

The vectors $\boldsymbol{v_k}$ and $\boldsymbol{u_k}$ represent the weights corresponding to the elements of $\boldsymbol{z_k}$ and $\boldsymbol{w_k}$, respectively, and are known as the loading vectors, the elements of which are referred to as the canonical loadings [91]. The canonical loadings describe the magnitude of the influence each variable has on the corresponding canonical variate [91].

These loading vectors are obtained by maximizing the correlations between the $\boldsymbol{z_k}$ and $\boldsymbol{w_k}$ vectors, for $k \in 1, 2, ...r$, where the maximal correlation corresponds to the correlation between $\boldsymbol{z_1}$ and $\boldsymbol{w_1}$. Thus, the process is started at $k = 1$:

$$\begin{aligned}
cor(\boldsymbol{z_1}, \boldsymbol{w_1}) &= cor(\boldsymbol{v_1'x}, \boldsymbol{u_1'y}) \\
&= \frac{cov(\boldsymbol{v_1'x}, \boldsymbol{u_1'y})}{\sqrt{Var(\boldsymbol{v_1'x})}\sqrt{Var(\boldsymbol{u_1'y})}} \\
&= \frac{\boldsymbol{v_1'}cov(\boldsymbol{x}, \boldsymbol{y})\boldsymbol{u_1}}{\sqrt{\boldsymbol{v_1'}cov(\boldsymbol{x})}\sqrt{\boldsymbol{u_1'}cov(\boldsymbol{y})}} \\
&= \frac{\boldsymbol{v_1'\Sigma_{12}u_1}}{\sqrt{\boldsymbol{v_1'\Sigma_{11}v_1}}\sqrt{\boldsymbol{u_1'\Sigma_{22}u_1}}} \\
&= \frac{\boldsymbol{v_1'\Sigma_{11}^{1/2}\Sigma_{11}^{-1/2}\Sigma_{12}\Sigma_{22}^{-1/2}\Sigma_{22}^{1/2}u_1}}{\sqrt{\boldsymbol{v_1'\Sigma_{11}v_1}}\sqrt{\boldsymbol{u_1'\Sigma_{22}u_1}}}
\end{aligned}$$

To ensure that the correlation is superiorly bounded by 1, the constraints $\Sigma\boldsymbol{v_i^2} = 1$ and $\Sigma\boldsymbol{w_i^2} = 1$ are used, meaning that the solution leads to standardized weights. Letting $\boldsymbol{a'} = \boldsymbol{v_1'\Sigma_{11}^{1/2}}$, $\boldsymbol{b'} = \boldsymbol{u_1'\Sigma_{22}^{1/2}}$ and $\boldsymbol{\Omega} = \boldsymbol{\Sigma_{11}^{-1/2}\Sigma_{12}\Sigma_{22}^{-1/2}}$, we get:

$$cor(\boldsymbol{z_1}, \boldsymbol{w_1}) = \frac{\boldsymbol{a'\Omega b}}{\sqrt{\boldsymbol{a'a}}\sqrt{\boldsymbol{b'b}}}.$$

Setting $\boldsymbol{a}'$ to be the normalized eigenvectors of $\boldsymbol{\Omega\Omega}'$ and $\boldsymbol{b}'$ the normalized eigenvectors of $\boldsymbol{\Omega}'\boldsymbol{\Omega}$, we then have:

$$(cor(\boldsymbol{z_1}, \boldsymbol{w_1}))^2 = (\frac{\boldsymbol{a'\Omega b}}{\sqrt{\boldsymbol{a'a}}\sqrt{\boldsymbol{b'b}}})^2 = (\boldsymbol{a'\Omega b})^2 = \boldsymbol{a'\Omega b b'\Omega' a'} = \boldsymbol{a'\Omega\Omega' a} = \lambda_1,$$

where, λ_1 represents the largest eigenvalue of $\Omega\Omega'$, which yields the maximum possible correlation between the two linear combinations. The canonical loading vectors can thus be calculated as $\boldsymbol{v} = \boldsymbol{\Sigma_{11}^{1/2} a}$ and $\boldsymbol{u} = \boldsymbol{\Sigma_{22}^{1/2} b}$. From here, the elements of the loading vectors can be interpreted with respect to the original datasets. It is common to use the first few leading canonical variates to determine which variables are contributing most to the relationship between the two sets of data.

2.3 Logistic Regression

The logistic regression model is a generalized linear model (GLM) used for modeling binary or proportion-dependent variables, where the assumptions of the linear regression model are not satisfied. Logistic regression assumes the binomial distribution and the mean is linked to independent variables (covariates) through a link function. The logit link, which is the canonical link, is often used, although the probit and the complementary log-log links are also used in some cases.

Consider a binary dependent variable y, taking on values 1 or 0, and suppose $Pr(y = 1) = \pi$. The systematic component (also referred to as linear predictor) of the logistic regression model is given by:

$$g(\pi(x)) = logit[\pi(x)] = \log\left(\frac{\pi(x)}{1 - \pi(x)}\right) = x'\beta.$$

Here, $g(\cdot)$ is the link function, which in this case is the logit function. The term $\pi(x)$ is used to highlight that the probability is modeled as a function of the independent variables (covariates) x. The above leads to [94]:

$$\pi(x) = \frac{exp(x'\beta)}{1 + exp(x'\beta)}.$$

The log-likelihood function of the binomial distribution, in its canonical form, is given by:

$$\mathcal{L}(\pi, y_i) = \Sigma y_i \log\left(\frac{\pi_i}{1 - \pi_1}\right) + \log(1 - \pi_1) = \Sigma y_i x_i' \boldsymbol{\beta} - \log(1 + e^{x_i'\boldsymbol{\beta}}).$$

Here, x_i is the covariate vector for individual (or subject) i and $\boldsymbol{\beta}$ is a vector of the regression coefficients. Newton-Raphson methods with Fisher Scoring are often used to maximize the log-likelihood, which leads to estimators that are equivalent to those obtained using iteratively re-weighted least squares (IRWLS), where the weights correspond to Fisher's information matrix, which is equivalent to the inverse of the variance of the binomial outcome [95]. When the logit link is used, the coefficients of the regression ($\boldsymbol{\beta}$) correspond to the log odds (for the intercept) and log odds ratio (log-OR) [96]. When the covariate is a continuous variable, the odds ratio corresponds to a unit change in the covariate [97, 98].

Assessing Goodness of Fit of the Model

Once a model is built, it is imperative to test whether it is accurately fit to the data to determine if it can be implemented for its intended use. Traditional methods for examining goodness-of-fit (e.g. Pearson's residuals), although useful tools in logistic regression models involving proportions, are not appropriate for binary outcomes [95]. Nevertheless, there are many other well-established tests to confirm whether the logistic model is well-fit to the dataset, including the Hosmer-Lemeshow Goodness-of-Fit (GoF) test, McFadden's pseudo-R^2 statistic, and the area under the receiver operating characteristic (AUROC) curve [94, 99]. It is common practice to select several diagnostic tools to determine whether a model is well-fit to the dataset for which it was based upon.

The Hosmer-Lemeshow GoF test, for instance, is an extension of Pearson's GoF test when indicator variables are not categorical [94, 100]. The hypotheses for this test are formulated as follows, where we test whether the estimated odds of a "success" outcome accurately reflect the true odds of success seen in the dataset:

$$H_0 : \pi_i = \frac{e^{\boldsymbol{x'}\hat{\boldsymbol{\beta}}}}{1 + e^{\boldsymbol{x'}\hat{\boldsymbol{\beta}}}} \; vs. \; H_\alpha : \pi_i \neq \frac{e^{\boldsymbol{x'}\hat{\boldsymbol{\beta}}}}{1 + e^{\boldsymbol{x'}\hat{\boldsymbol{\beta}}}}.$$

The Hosmer-Lemeshow test compares the expected and observed values for the outcome variable to determine whether the fitted model is accurate in its predictive ability [94, 100]. To employ this test, the data are partitioned into G groups of equal size (standard practice uses G = 10) and the squared difference between the observed values (y_g) and the expected values ($E_g = n_g\hat{\pi_g}$) is calculated. The test statistic is calculated as follows [94, 101]:

$$HL = \Sigma_{g=1}^{G} \frac{(y_g - n_g\hat{\pi_g})^2}{n_g\hat{\pi_g}(1 - \hat{\pi_g})} \approx \chi^2(G - 2).$$

Asymptotically, the test statistic approximately follows a chi-square distribution with $G - 2$ degrees of freedom. A large value of HL, such that $HL > \chi^2_\alpha(G - 2)$, would indicate that there is significant evidence towards rejecting the null hypothesis, hence suggesting that the model does not fit the data well.

Chapter 3

Systematic Review and Meta-Analysis of Interventions for Pediatric Iron Deficiency Anemia

3.1 Introduction

Iron deficiency anemia (IDA) is a major health risk for children both in North America and around the world. It is estimated that nearly 40% of children worldwide suffer from anemia, with iron deficiency (ID) being the most prevalent cause of anemia [2]. In Canada, a lower incidence of pediatric IDA is observed, with the proportion of Canadian children afflicted with IDA estimated between 3.5% and 10.5% [6]. However, much higher incidence rates of childhood IDA (estimated to be up to 50%) is seen in remote Indigenous communities within Canada, such as First Nations living on reserve and Inuit residing in Inuit Nunangat [6–8].

IDA knows no borders and it is not limited to specific socioeconomic classes, as it can be felt in both high- and low-income regions, though the cause of IDA varies from region to region [11]. What does not appear to vary geographically is the fact that young children, between the ages of 6 and 36 months, are disproportionately affected by IDA, most notably during the period of rapid growth where antenatal iron stores are often not replaced through adequate dietary intake [11]. Menstruating adolescent girls are also often victims of IDA due to heavy menstrual bleeding (menorrhagia) [11]. In socioeconomically poorer regions of the globe, such as Southeast Asia and Southwestern Africa, IDA is often caused by nutritional deficiencies, wherein a child does not have access to adequate food, which leads to undernutrition [4,11]. Nutrition-based IDA can also be caused by excessive intake of dairy milk, which is a poor source of iron and can lead to satiation and inadequate consumption of other iron-rich foods, or, in a more serious case, microscopic blood loss from the gut [18,19]. Culture-based

nutrition practices also may have an impact on the prevalence of IDA in children, such as vegetarianism, a common practice in some parts of the world including Southeastern Asia, which excludes the consumption of red meats and other iron-rich foods, which can negatively impact a child's iron store [11]. Non-environmental causes of IDA include chronic illness such as chronic kidney disease, inflammatory bowel disease and cancers [11, 41, 47, 102–104].

IDA is associated with both short-term and long-term health impacts. For young children, IDA may be associated with poor cognition, emotional, and behavioural problems [24, 26, 27, 105]. In adolescent females with IDA caused by menorrhagia, poor cognition, fatigue, and reduced endurance and work productivity are common manifestations of IDA [20, 21]. In more common and less severe cases of IDA, children may present asymptomatic or with mild symptoms such as pallor, fatigue, muscle weakness and decreased appetite [9, 11]. Left untreated, chronic IDA may also lead to long-term health problems including devastating and irreversible neurological, cognitive, and physiological consequences [25, 28]. Severe IDA may be associated with cardiovascular and respiratory issues, including tachycardia, hypotension, heart failure, and stroke [106]. It is therefore imperative that IDA in children be detected and treated in its early stages.

Iron status is commonly defined according to serum ferritin levels, which are considered to have the highest sensitivity and specificity for detecting ID [12, 107]. Anemia is defined according to hemoglobin levels. For preschool children (< 5 years) the World Health Organization (WHO) defines ID as a serum ferritin level of <12 µg/L and IDA as a hemoglobin level of <110 g/L [9]. For adolescents, the WHO defines ID as a serum ferritin level of <15 µg/L and IDA as a hemoglobin level of <120 g/L [10]. Nevertheless, literature shows that several other cut-off values are being used to diagnose ID and IDA, specifically in select sub-populations, such as children living at high altitude [108, 109]. Considering the high prevalence and severity of health outcomes for children and youth with ID/IDA, early identification and intervention are important. Treatments include oral iron, intravenous (IV) iron, and packed red blood cell (pRBC) transfusion [41–44]. However, there are significant limitations to currently available guidelines for diagnosis, treatment, and management of ID/IDA [110]. Peyrin-Biroulet and colleagues conducted a systematic review of guidelines (published between 1 January 2004 and 30 June 2014) on the management of ID [111]. Of the 29 guidelines included in the study, only 3 were targeted at children. Similarly, Numan and Kaluza undertook a systematic review of guidelines on the treatment of IDA, with the aim of exploring current recommendations on the use of IV iron. The study reported the lack of guidelines in pediatric care [112].

While pRBC transfusion is a commonly used treatment among children with severe IDA, recent AABB (Association for the Advancement of Blood & Biotherapies) international guidelines highlight the importance of offering transfusion "only when

benefits outweigh harms" [46]. More recently, the American Society of Pediatric Hematology/Oncology (ASPHO) published the Choosing Wisely guideline with recommendations against pRBC transfusion in asymptomatic children with IDA and in those with no evidence of hemodynamic instability or active bleeding [47, 48]. Instead, the guideline recommends oral or IV iron supplementation. In order to increase the quality of the evidence base for pediatric guidelines involving the diagnosis and treatment of IDA, a systematic literature review and evidence synthesis is needed.

The objective of this study was to conduct a systematic review of the literature with the aim of identifying, evaluating, and synthesizing evidence related to interventions used to treat pediatric IDA. The study aimed to qualitatively and quantitatively synthesize evidence related to prevalence of use of the various treatment options as well as provide comparative evaluations of effectiveness and safety of the interventions. We also quantified heterogeneity across the studies as well as identified and elucidated sources of heterogeneity with respect to prevalence, effectiveness, and safety. The evidence was synthesized quantitatively, when possible, as well as narratively using qualitative methods.

3.2 Methods

The methods used in this systematic review and meta-analysis closely followed the Cochrane Handbook for Systematic Review of Interventions [60]. During the conception of the study and development of the protocol and methodology for the systematic review, we ensured that our approaches met all the criteria specified in the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2020 checklist [113]. This systematic review was registered with the International Prospective Register of Systematic Reviews (PROSPERO) (ID: CRD42023456480).

3.2.1 Eligibility Criteria, Data Sources and Search Strategy

Prior to developing the search strategy to acquire the relevant studies for our review, we outlined our PICO (population, intervention, comparator and outcome) criteria that would inform the search development and screening process. These criteria were defined as follows:

- Study Population: Studies involving children and youth between the ages of 6 months and 18 years, with IDA confirmed by laboratory tests were eligible.

- Intervention: The three commonly used treatment options for IDA (oral iron, IV iron, blood transfusion) were considered.
- Comparators: Placebo or usual care, or any of the 3 treatments.
- Outcomes: For effectiveness outcomes, we used laboratory outcomes (hematologic measures and iron indices such as hemoglobin level, transferrin saturation, serum ferritin and serum iron concentrations and mean corpuscular volume). For safety outcomes, we used any reported adverse effects (total adverse events and treatment-related adverse events). For prevalence outcomes, we used the number and proportion of children treated using the 3 treatment options.

The search strategy was prepared in consultation with a University of Ottawa librarian (Peter Farrell), who provided the necessary knowledge and tools to properly develop and implement the search strategy. The strategy centered around three main terms: children, treatment, and iron deficiency anemia. Prior to the finalization of the search strategy, we used several tools within the databases to identify supplemental search terms that would aid in retrieving as many articles as possible. These tools included subject headings, medical subject headings (MeSH) and keywords. Once we had considered all possible search terms within the four databases, a preliminary search was conducted to confirm that the included search terms would yield sufficient results.

A search of major medical databases (MEDLINE (Ovid), EMBASE (Ovid), SCOPUS and Cumulative Index to Nursing and Allied Health Literature (CINAHL)) was conducted on June 26th, 2023. A grey literature search was also subsequently conducted, including reference searches (i.e. reviewing the references of the retained articles from the preliminary search). Retrieved systematic reviews on similar topics were reviewed for their contents, in the hopes of acquiring more relevant evidence for our review.

3.2.2 Inclusion and Exclusion Criteria, Screening, and Data Extraction

Two reviewers independently reviewed all the retrieved studies (from the search) and decided on eligibility (for inclusion) according to the PICO related eligibility criteria outlined above and with respect to the detailed inclusion and exclusion criteria (developed pre-search) provided in Table 3.1 below.

Table 3.1: Inclusion and exclusion criteria for determining the relevance of articles to our study.

Criteria	**Inclusion**	**Exclusion**	**Rationale for Exclusion**
Population	Children between 6 months and 18 years of age diagnosed with IDA.	Children under 6 months of age, adults of 18 years or older, or children that either do not have IDA or have a chronic comorbidity.	Patients younger than 6 months of age still retain the iron stores provided to them throughout the gestational period and therefore are not a reflection of true IDA. Patients without IDA (that did not serve as controls) do not reflect the study objective of this project. Chronic illness as a co-morbidity to IDA could interfere with both the presentations of IDA as well as the treatment options for IDA.
Intervention	Oral iron, IV iron therapy and red blood cell transfusions, or a combination of these treatments.	Holistic treatments, preventive measures (for children who do have IDA), intramuscular administration of iron.	Preventive measures are more likely to be given to children who do not have IDA and therefore do not meet the inclusion criteria for the population. Intramuscular administration is not considered one of the main treatments of pediatric IDA and therefore is irrelevant to our study objective.
Comparator	Placebo or usual care, or any of the 3 treatments. For observational studies, no comparator is necessary.	Studies were not excluded based on the comparator used so long as the primary treatment was one of oral iron, IV iron, or blood transfusion.	We were interested in outcomes for the three defined target treatments, regardless as to whether they were compared against another treatment, placebo, or usual care.
Outcomes	Articles must include a prevalence outcome and/or at least one of the effectiveness or safety related outcomes.	Articles that do not provide substantive evidence for prevalence, effectiveness, or safety outcomes.	Articles that did not provide information on prevalence, effectiveness or safety did not meet the objective of our study.

Table 3.1: Inclusion and exclusion criteria for determining the relevance of articles to our study.

Criteria	**Inclusion**	**Exclusion**	**Rationale for Exclusion**
Settings	In-patient, out-patient or community-based (with the appropriate health care professionals involved) settings.	Home-based treatments (over-the-counter supplements and/or holistic treatments that are not administered by a health care professional).	Treatments had to be prescribed and/or administered by a health professional. Over-the-counter and holistic treatments cannot be monitored nor are they always given to children with IDA. For this reason, we were unable to include studies pertaining to these treatments.
Study Design	All study designs involving original research were considered. Editorials, abstracts, and commentaries containing data were retained.	Case-studies, case-series, systematic reviews, guidelines, or recommendations were not considered. Conference abstracts, commentaries and editorials providing insufficient data.	Case-studies and case series do not provide adequate evidence on prevalence, and usually do not meet the population inclusion criterion of patients having no co-morbidities. Systematic reviews or other forms of unoriginal research cannot be included in a systematic review. All forms of study designs that provided no data concerning the treatment of IDA do not serve the objective of the project.
Language	Studies written in English or that have translations available in English.	Articles written in a language other than English that have no available English translation.	We did not have access to sophisticated translation software and were therefore unable to translate articles that did not have an English translation available.

Two reviewers (Autumne Cadieux and Michael Pham) began with level one screening, where the titles and abstracts (when available) of the retrieved articles were reviewed, independently and in duplicates, against the inclusion and exclusion criteria. We used Covidence software to sort the articles based on eligibility, where

studies deemed eligible were retained for level two screening, while those deemed ineligible were removed. The level two screening involved full-text review of studies retained from the level one screening, where screening was also done independently by the two reviewers and in duplicates. The reviewers again consulted the inclusion and exclusion criteria to determine if an article met the required criteria to be included in the systematic review. During this phase of screening, for each excluded article, a reason for exclusion was required in the Covidence software, wherein the reason for exclusion had to match from both reviewers to avoid a conflict requiring resolution. Covidence has pre-set categories for the exclusion criteria, including wrong outcomes, wrong study population, and wrong intervention. We utilized these built-in tools, while adding our own exclusion criteria as well: abstract only and text unavailable in English. Articles that met all inclusion criteria were retained for the final stage. Articles that did not meet the criteria were removed and the reasons for exclusion were documented and summarized.

During both levels of the screening process, the reviewers monitored their kappa coefficient to ensure that it was at a reasonable standing. The goal was to maintain a kappa coefficient of 0.6 or higher, which indicates moderate agreement [62]. Disagreements were resolved through discussions until a consensus was reached. In the case that the two reviewers were unable to agree on the inclusion or exclusion of a particular article, a third reviewer (Dr. Patricia Parkin) was consulted for input and decisions were made based on consensus.

To extract the data from the retained articles, we followed the Cochrane Handbook for Systematic Reviews of Interventions to create a standardized data extraction form. The form was pilot tested on 5 randomly selected studies to ensure the reviewers were collecting identical data. Extracted data included key information required to perform comparative evaluation of prevalence, effectiveness, and safety of IDA treatments. Covidence software was also used as the main reference organization tool at this stage of the review. When available, the following data was collected from each study:

- Publication information: author, title, year of publication, country of publication.
- Study/Population Characteristics: age of population (mean and standard deviation; median and interquartile range (IQR) or range), interventions used, total number of participants in each treatment arm, number of female/male participants for each treatment arm, clinical setting (in-patient, out-patient or community-based), study design (eg. RCT, observational etc.), devices or tools used for measurement of each biomarker.
- Intervention: important information related to all treatment arms (eg. drug type, dose, period of use).

- Effectiveness Outcomes: for each effectiveness outcome, measure of effectiveness (eg. mean or median in each treatment arm, mean-difference between treatment arms) along with the corresponding measure of variability (eg. SD, IQR, range, standard error and or 95% confidence intervals (CIs)) were abstracted. For studies involving pre- vs post-intervention measurements, the reviewers extracted mean change for each intervention arm along with the corresponding standard error (or 95% CI). When these values were not provided, baseline and follow-up means or medians with the corresponding measures of variabilities were abstracted. When available, the covariance or correlation between baseline (pre-treatment) and follow-up (post-treatment) values were abstracted.
- Safety Outcomes: for each safety outcome, the total number of events in each treatment arm were extracted.

3.2.3 Evidence Synthesis, Subgroup, and Sensitivity Analyses

Data was synthesized both narratively and quantitatively. Descriptive statistics were first used to summarize all available data related to study and population characteristics, outcome measures and interventions. Mean (SD) or median (IQR, range) were used to summarize continuous variables. Categorical data were summarized using frequencies and percentages. The mean change-from-baseline differences (for studies involving pre- vs post- measures) were used as effect measures for effectiveness outcomes. For safety outcomes, relative risk (RR) or incidence rate (for non-comparative studies) was used as an effect measure. Heterogeneity across the different studies was examined graphically (using forest plots) and quantitatively using the I^2 statistic [114]. We identified and elucidated sources of heterogeneity with respect to study and population characteristics including severity of IDA and healthcare settings. We anticipated significant heterogeneity between the studies given the vast geographical, methodological and intervention-related differences (i.e. dosage, formulation, regime). As such, we used random effects meta-analysis to account for both the within-study and between-study heterogeneity. The results of the meta-analysis were presented using forest plots, and when appropriate, pooled estimates along with the corresponding 95% CIs were provided for the prevalence, effectiveness, and safety outcomes (Section 3.3.4). All statistical analyses were performed using the R statistical software [115]. When quantitative synthesis was not possible, a narrative synthesis was done to qualitatively synthesize information (evidence) using words and text.

Subgroup analyses were performed on the following pre-defined groups of the population: children between the ages of 6 and 36 months, and children of any age between 6 months and 18 years with severe IDA, defined as a hemoglobin count

of less than 70 g/dL [46]. The first subgroup analysis was performed on children experiencing severe IDA. We sought to understand whether the severity of IDA had a large impact on the treatment course a child may follow. This subgroup analysis also aligned with the recommendations from the Choosing Wisely Campaign, as it aided in determining whether blood transfusion is indicated for children with severe IDA. Next, we performed subgroup analysis on children aged 6 to 36 months. Children between the ages of 6 and 36 months are especially susceptible to IDA, as their growing bodies require large quantities of iron [11]. When a child is lacking in iron either due to dietary habits, blood loss or malabsorption, detrimental effects to their physical and neurological development can arise [28]. This subgroup analysis determined whether special considerations needed to be made when deciding on treatment options for young children suffering with IDA.

Sensitivity analyses were performed to see if there were any differences in the pooled estimates when only studies that followed the WHO definition of IDA were included in the meta-analysis [2]. We found that some of the studies (retained in the screening process) considered the children to have IDA, but in fact had hemoglobin levels higher than the WHO threshold of 110 g/L for children under 5 years of age or 120 g/L for older children and adolescents [2]. As such, we investigated whether these alternative definitions of IDA were significant sources of heterogeneity and sought to determine if these had an impact on the outcomes for prevalence, effectiveness, and safety. We also performed sensitivity analysis with respect to imputations. In certain cases, we had to convert measures from one format (median and one of either range or IQR) to another (mean and SD) to include all studies with viable data in the meta-analysis. To do so, we employed the imputation techniques discussed in Section 2.1.1. We sought to determine whether these imputative measures influenced the results of our analyses on prevalence, effectiveness, and safety, and therefore conducted sensitivity analysis on the subgroup of studies that did not require imputation of the results used for the meta-analysis. To do so, we removed the studies that required such measures and re-evaluated the subgroup for the three measures of interest. Lastly, we sought to determine whether studies that screened at a high risk of bias (denoted as "high" for RCTs and "serious" or "critical" for observational studies) had an effect on our estimates for prevalence, effectiveness, and safety. Thus, we removed the studies that screened at a high or serious risk of bias and performed our analyses again to see if the estimates were in fact influenced by the inclusion of potentially highly biased studies.

3.2.4 Quality of Evidence and Risk of Bias Assessment

To ensure that the overall quality of the data being synthesized was adequate, we performed risk of bias (RoB) assessments on each of the studies that are included in the final systematic review [60]. We followed the recommendations in the Cochrane

Handbook for Systematic Reviews of Interventions in our RoB assessments. For RCTs, we used the recent version of Cochrane Collaboration's tool for assessing risk of bias, often referred to as RoB 2 [60, 113]. For non-RCTs, the ROBINS-I tool was used to assess the quality of the data and the risk of bias within the data [116]. As outlined in Chapter 2, RoB 2 provides ratings of low, moderate, or high risk of bias with respect to 3 domains, while the ROBIN-I tool provides ratings of low, moderate, serious or critical risk of bias across 7 domains. The results of the RoB assessment are presented graphically using bar plots and heatmaps. Publication bias was examined graphically using funnel plots bias and using Egger's statistical test [88].

3.3 Results

3.3.1 Search and Screening

The initial search yielded 3440 results, and two more articles were added afterward following a grey literature search, of which 631 were removed because they were duplicates. At the first stage of screening, titles and abstracts of 2811 articles were screened in duplicates by two independent reviewers, determining that 2571 articles did not meet the inclusion criteria. At the level two screening, full texts of 240 articles were reviewed, of which 84 met the inclusion criteria and were included in the final systematic review. Figure 3.1 presents the PRISMA flow diagram of the screening process, including the major reasons for exclusion at the full-text screening phase. The final kappa statistic corresponding to the title and abstract screening was 0.57, indicating weak interrater agreement, while the kappa statistic for the full text screening was 0.83, indicating strong agreement between the two independent reviewers.

During the title and abstract screening phase, 2571 articles were deemed irrelevant and thus excluded from the study. We documented the reasons for excluding each article, which we categorized into 9 groups (Table 3.2). We saw that nearly 40% of the articles excluded at the first stage of screening were on the basis of IDA patients having co-morbidities. The two major comorbidities we identified were irritable bowel disease and chronic kidney disease. The next most prevalent reasons for exclusion were age being out of the eligible range (either younger than 6 months of age or older than 18 years of age) or treatment of IDA was not considered in the article (i.e. an article centred on the diagnosis of pediatric IDA).

The results of the full text screening led to the inclusion of 84 articles and the exclusion of 136 articles. The breakdown of the exclusion criteria used in this phase of screening, along with frequency and percentage of excluded articles are presented in Figure 3.1. Overall, nearly 25% of the articles (n = 39) were excluded because of

wrong outcomes being reported. Some studies in this category were excluded because they did not provide useful insights into treatment outcome, and/or did not report on prevalence, effectiveness, or safety outcomes with respect to treatment. About 20% (n = 31) of the articles excluded at the second phase of screening were deemed ineligible due to wrong patient population, for instance patients who were either outside of the age range (i.e. adults or newborns) or the study population included a co-morbidity alongside IDA. We also excluded 41 (26.8%) abstracts (no full text available) on the basis that they did not provide substantial data pertaining to one or more of prevalence, effectiveness, or safety. Abstracts that provided data on the prevalence, safety, or effectiveness of the treatments for IDA were included.

3.3.2 Study Characteristics

A total of 84 studies consisting of 10991 patients were included in the final systematic review, 7738 of the patients having received one of the three treatment options while the remaining 3253 received either placebo, usual care, or an alternative treatment. Of these 84 studies, 26 (31%) were randomized control trials (RCTs) consisting of 4128 (37.5%) patients, 27 (32%) were case-control with 4432 (40.3%) patients, 30 (36%) were cohort (prospective and retrospective) consisting of 2341 (21.8%) patients and 1 (1%) was cross-sectional involving 90 (0.8 %) patients. Table 3.3 provides the key pooled characteristics of the studies grouped by intervention, and more details on individual studies, including author and year of publication as well as the country and region in which the study was conducted, and is provided in the Appendix A.

All 26 RCTs considered oral iron supplementation and comparisons were made with placebo, usual care or between different formulations, doses, or regime of oral iron, while all studies considering blood transfusion were cohort studies. All case-control studies pertained to oral iron, while IV iron was studied through cohort studies and one cross-section study. Including both RCTs and observational studies, there were a total of 63 articles (9354 patients) involving oral iron supplementation (either as a lone treatment (non-comparative) or comparative). A total of 14 observational studies (939 patients) considered IV iron as an intervention and all were non-comparative (i.e. IV iron was the sole treatment) and only 2 studies (both observational) with 172 patients combined included blood transfusion as the sole intervention. We also retained two articles that reported on both oral iron and blood transfusion (97 patients), two that reported on oral and IV supplementation (142 patients), and one with IV iron and blood transfusion (27 patients). The studies including two of the interventions did not report adequate data to perform pairwise or three-way comparison for neither effectiveness nor safety.

Overall, most (n = 77, 10328 patients) articles reported hematologic indicators of IDA (most commonly hemoglobin and serum ferritin), while five studies (330 patients)

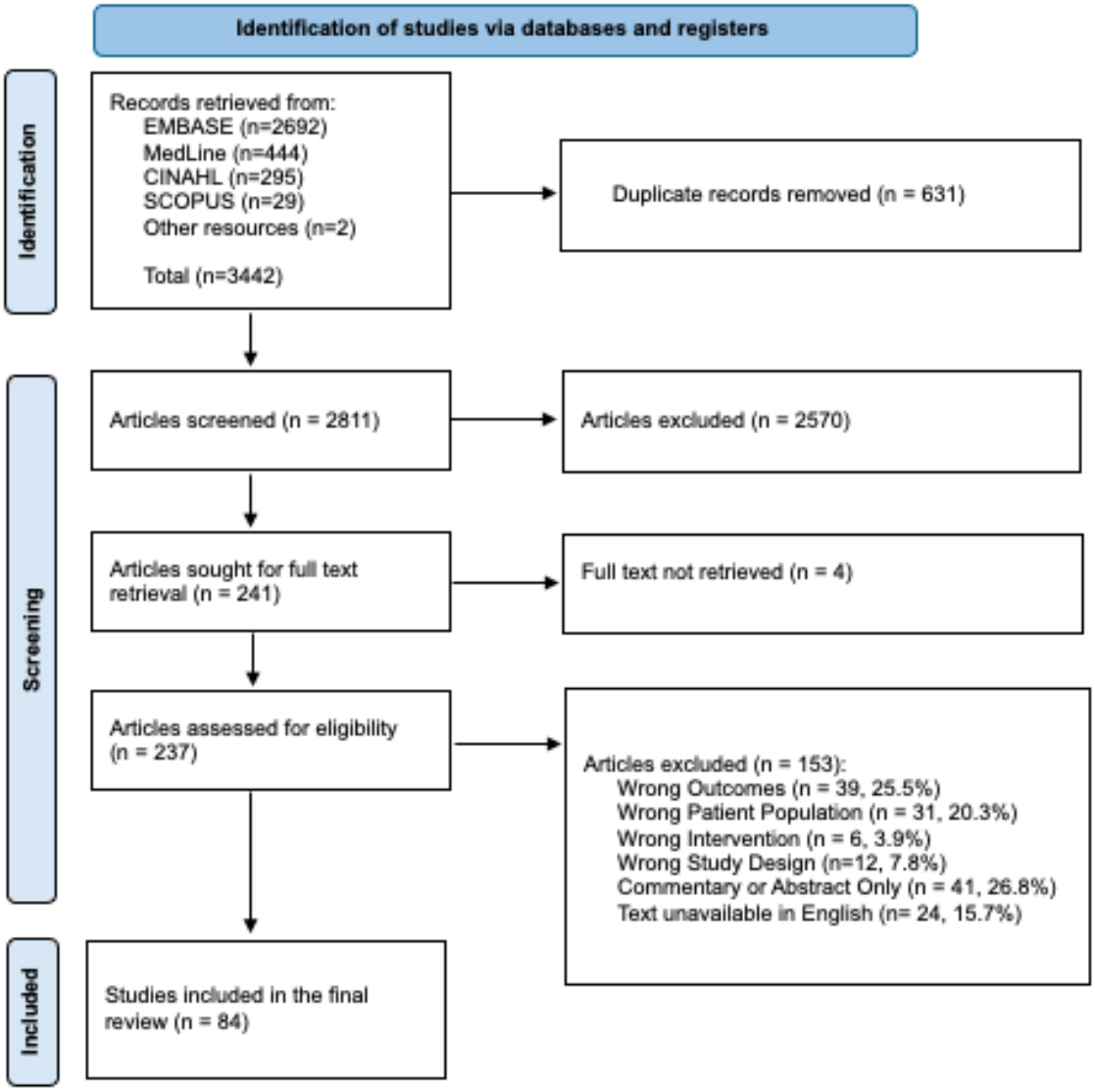

Figure 3.1: PRISMA Flow Diagram of the Screening Process.

reported outcomes related to vital signs and 25 studies (3239 patients) reported on adverse reactions stemming from treatments. Geographically, about 25% of the studies (n = 20) were conducted in Turkey, followed by India (n = 12, 14.3%) and the United States of America (n = 12, 14.3%). The remaining studies were conducted either in Southeast Asia (n = 7, 8.3%), Africa (n = 8, 9.5%), South America (n = 8, 9.5%), Western Asia (n=5, 6.0%) or Europe(n = 6, 7.1%), while 3 (3.6%) were conducted in the Middle East and only one study was conducted in each of Canada and New Zealand (See Table A, in Appendix for details).

Table 3.2: Exclusion criteria descriptions used during title and abstract screening.

Exclusion criteria	Description	Frequency (%)
IDA not the sole illness	Patients were suffering from co-morbidities as well as IDA, or the patients did not appear to have IDA at all.	967 (37.6)
Treatments not a theme	The articles did not appear to discuss the treatment of IDA, most commonly the articles focused on information and diagnostic materials.	376 (14.6)
Outside age range	The patients were either adults (>18 years of age) or were newborns/infants (<6 months of age)	384 (14.9)
Non-ID anemia	Patients had anemia that was not caused by iron deficiency. For example, thalassemia trait or sickle cell anemia.	107 (4.2)
Incompatible study design	Case study or series, editorials, abstracts, or guidelines with insufficient primary data.	246 (9.6)
Non-anemic ID	Patients had iron deficiency but were not considered anemic.	159 (6.2)
Not the treatment of interest	Treatments were discussed but were centred on alternative interventions	138 (5.4)
Non-human study	The participants in the study were not human.	32 (1.2)
Irrelevant	Studies that do not meet any of the inclusion criteria are deemed completely irrelevant.	161 (6.3)

The majority of the studies (n= 44, 52.4%) were conducted in out-patient settings, although a considerable number of studies (n=10, 11.9%) involved in-patient settings, and 5 (6.0%) studies included a mix of in-patient and out-patient

Table 3.3: Characteristics of the studies included in the systematic review.

Characteristic	**N (%)**
Treatment Type	
Oral Iron	63 (75)
IV Iron	14 (16.7)
Blood Transfusion	2 (2.4)
Oral Iron & IV Iron	2 (2.4)
Oral Iron & Blood transfusion	2 (2.4)
IV iron & Blood transfusion	1 (1.2)
Clinical Setting	
In-patient	10 (11.9)
Out-patient	44 (52.4)
Community-based	18 (21.4)
Mixed	5 (6)
Not identified	7 (8.3)
Study Design	
Randomized Control Trial	26 (31)
Cohort Study	30 (36)
Case-Control	27 (32.1)
Cross-Sectional	1 (1.2)
Number of Patients	
<50	28 (33.3)
50 - <100	25 (29.8)
100 - <500	28 (33.3)
> 500	3 (3.6)

settings. A considerable proportion (n=18, 21.4%) of the studies were also conducted in a community setting (treatment prescribed by a physician or community healthy nurse but given in a community setting such as at school) and 7 studies (8.3%) did not specify the clinical setting where treatment took place.

3.3.3 Risk of Bias Assessment

Risk of Bias Assessment of Randomized Control Trials

Overall, low to moderate risk of bias was observed for the RCTs, specifically within the domains 2 (risk due to deviations from intervention) and 4 (risk due to the measurement of the outcome), where all but one screened at a low or moderate risk of bias (Figure 3.2). Bias due to the selection of the reported result (Domain 5) caused

the most trouble among the RCTs, with two articles screening at a high risk of bias and 4 causing some concern for the domain. With respect to overall bias (combined across all 5 domains), 11 RCTs (42.3%) screened at a low risk of bias, while 13 (50%) posed some concerns and only 2 (7.7%) screened at a high of risk of bias.

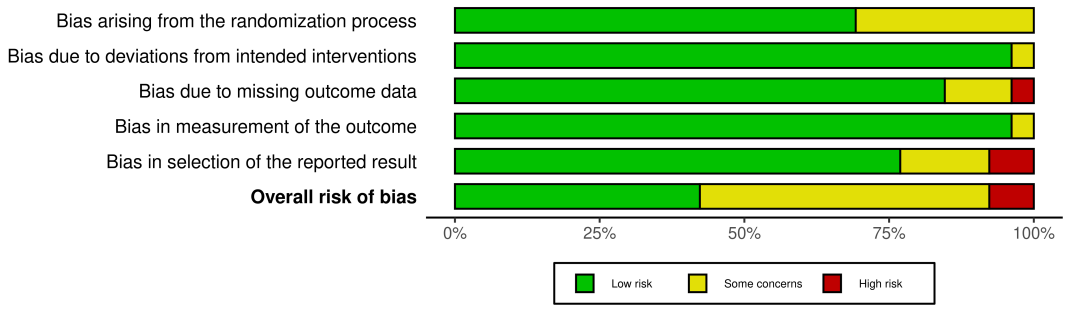

Figure 3.2: Distribution of RoB for the 26 randomized control trials, across the five domains described in RoB 2 tool and an overall RoB representing the highest of the 5 domains.

Figure 3.2 presents the distribution of ratings across the five domains, while Figure 3.3 provides the individual domain-level and overall ratings of each of the 26 RCTs. We saw from both figures a high level of green (indicating a low risk of bias) across all domains, while yellow (some concerns) was the predominant colour seen in the overall (domain combined) column. This is due to the fact that, if an article posed some concerns of risk of bias in any of the domains, the article automatically was considered as posing some concerns overall.

Risk of Bias Assessment for Observational Studies

Relatively, RoB assessment results showed higher risk of bias for observational studies compared to RCTs, which was to be expected. Given that our review included many (n = 19) retrospective cohort studies, and that by their nature, these studies included many barriers to controlling various factors, we expected to find that most observational studies would screen at either a moderate or serious risk of bias. The results indeed showed that only two articles (3.4%) screened at a low risk of bias (these studies strictly controlled for confounding, a domain that set them apart from the others), while the other 56 screened at either a moderate risk of bias (n = 39, 67.2%) or high risk of bias (n = 17, 29.3%).

Figure 3.4 presents the distribution of RoB ratings across the seven domains and an overall rating combined across the 7 domains. From this figure, we saw high

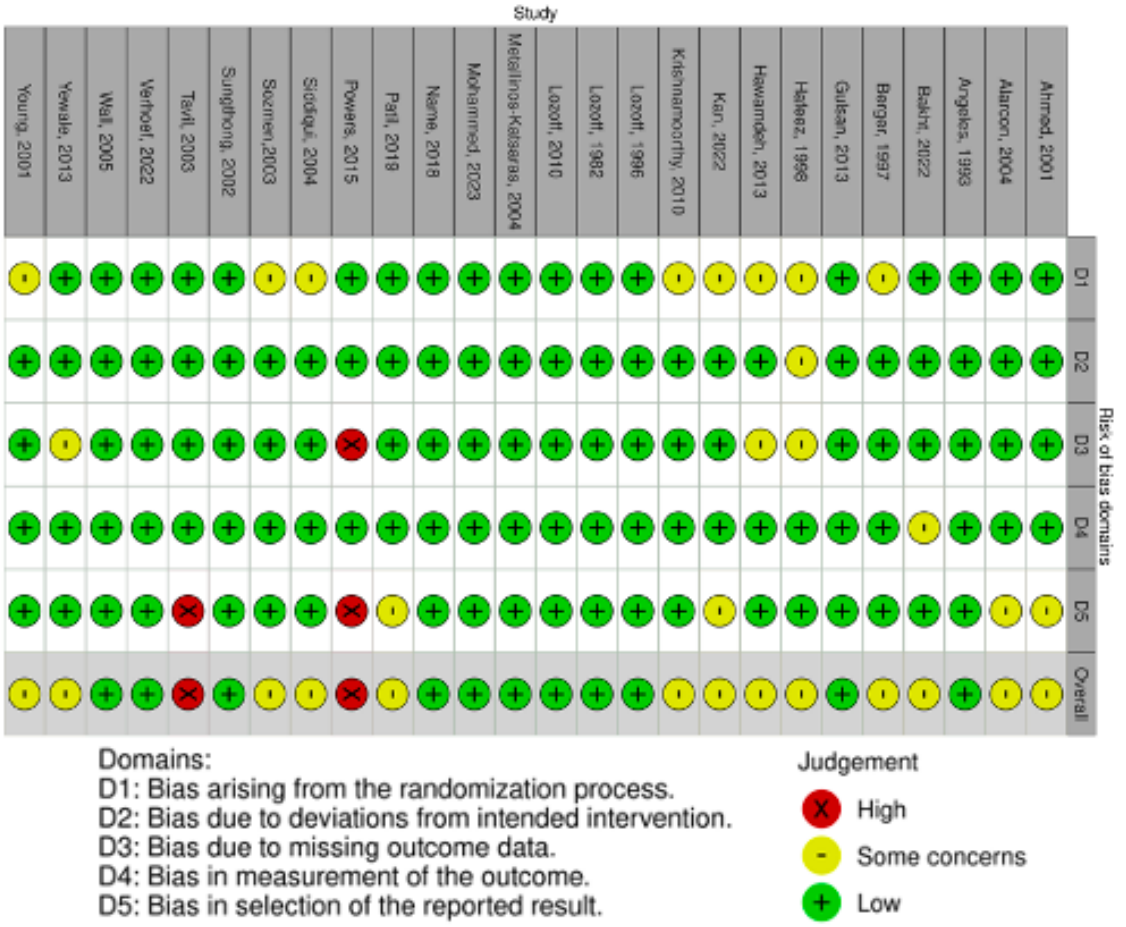

Figure 3.3: Individual study RoB assessment using the RoB 2 tool.

levels of green (low risk) and yellow (moderate risk) across the board, indicating that most studies performed generally well across the majority of domains. The articles performed best in domains 5 (bias due to missing data) and 7 (bias in selection of the reported result), while domains 1 (bias due to confounding) and 6 (bias in the measurement of the outcomes) posed the most problems. As discussed in Section 2.1.3, if an article screened at a serious RoB in even one of the domains, the rating was considered as serious risk with respect to the overall RoB rating, which explains why there appeared to be minimal red (serious risk) among the seven domains but a larger portion of studies screening at a serious risk in the overall rating. Figure A.3 (Appendix) presents individual study RoB assessments for all the 58 observational studies, using the seven domains and overall rating.

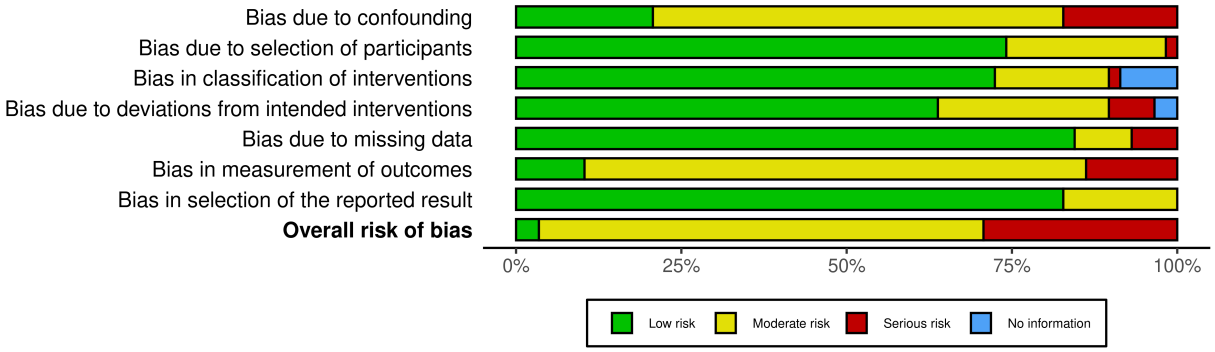

Figure 3.4: Distribution of RoB results for the 58 observational studies based on the 7 domains of ROBINS-I and an overall rating representing the highest rating of the 7 domains.

3.3.4 Evidence Synthesis and Meta-Analysis

Prevalence Outcomes

The results from our systematic review showed that oral iron supplementation is the most common treatment option for pediatric patients with IDA, where 84.14% (n = 6511 out of the total 7738) [95% CI: 83.33% to 84.96%] were treated with oral iron. This is in agreement with what is considered as the primary course of treatment for pediatric IDA patients who are not experiencing cardiac or respiratory complications [42–44, 46, 47]. Among children who were treated using oral iron, 64% (n=4143) received daily oral iron, while 19% (n = 1250) received a weekly administration. The majority of children received ferrous sulphate as the form of oral iron (n = 2580, 40%), while only 2.5% (n = 166) received iron polymaltose complex (IPC). The remaining children received a variety of different formulations, such as ferrous ascorbate and ferrous gluconate.

Children who received IV iron accounted for 13.16% [95% CI: 12.40% to 13.91%] of the patient population, while patients who received blood transfusion made up only 2.7% [95% CI: 2.34% to 3.06%] of the population. Among patients treated with IV iron, the percentage of children who received iron sucrose and the percentage who received ferric carboxymaltose were nearly identical (48.8%, n = 497 and 47.8%, n = 487, respectively). Dosages and regimes were dependent on each patient medical condition and bodily weight.

We wished to see if there was a statistically significant difference in the percentages we obtained, for which we used the 3-way Pearson Goodness of Fit (GoF) test to assess the equality of the proportions. We found that there was a significant difference between the three prevalence estimates ($\chi^2 = 13675$, p-value < 0.0001), which confirmed our preliminary observations.

The estimates provided above reflect the interventions used across all study designs in this review, including both the RCTs and observational studies. Nevertheless, treatment choices in the RCTs are done by design, and thus do not reflect the treatment choices in clinical practice. Moreover, our systematic review revealed no RCTs involving IV iron and blood transfusion, which is to be expected, considering the nature of these treatments and comparing prevalence (frequency of use) of the treatments based on RCTs is misleading. Therefore, subsequent prevalence comparisons were made using non-randomized studies only.

We sought to determine whether the prevalence estimates we obtained in this section compared well with what is seen in a natural clinical environment. As such, we retrieved the retained articles that did not consider treatment type as an inclusion criterion for their population, but rather examined certain characteristics of the patients (i.e. severity of IDA) and determined the distribution of treatment given in this population. There were very limited data in this respect, and were therefore only able to meta-analyze the prevalence of each treatment for a small subgroup of the overall population.

All four studies included in this meta-analysis provided data on oral iron, three provided data on blood transfusion, and only one study provided data on IV iron. We meta-analyzed the prevalence of oral iron and blood transfusion (Figures 3.5, 3.6). From our analyses, we found that the pooled estimate of prevalence of oral iron was 72.26% [95% CI: 58.59% to 85.93%], and for transfusion was 22.15% [95% CI: 12.83% to 31.47%]. For IV iron, the singular prevalence estimate provided was 18.97% [95% CI: 8.88% to 29.05%]. It was clear from a preliminary comparison that this breakdown of prevalence distribution was different from what was seen in the analysis using all studies included in the review. We used the chi-square test to check equality of proportions and the results showed significant evidence that the proportions were not equal ($\chi^2 = 122.72$, p-value < 0.0001). We also tested the proportions of IV iron and transfusion for equality, assuming that the results of the three-way test were significant due to the high proportion of children receiving oral iron, and found that these two proportions were not statistically different ($\chi^2 = 0.127$, p-value $= 0.7214$). We found significant heterogeneity within the oral iron subgroup ($I^2 = 76.9\%$, p-value $= 0.002$), and some evidence of heterogeneity within the transfusion subgroup ($I^2 = 51.2\%$, p-value $= 0.129$).

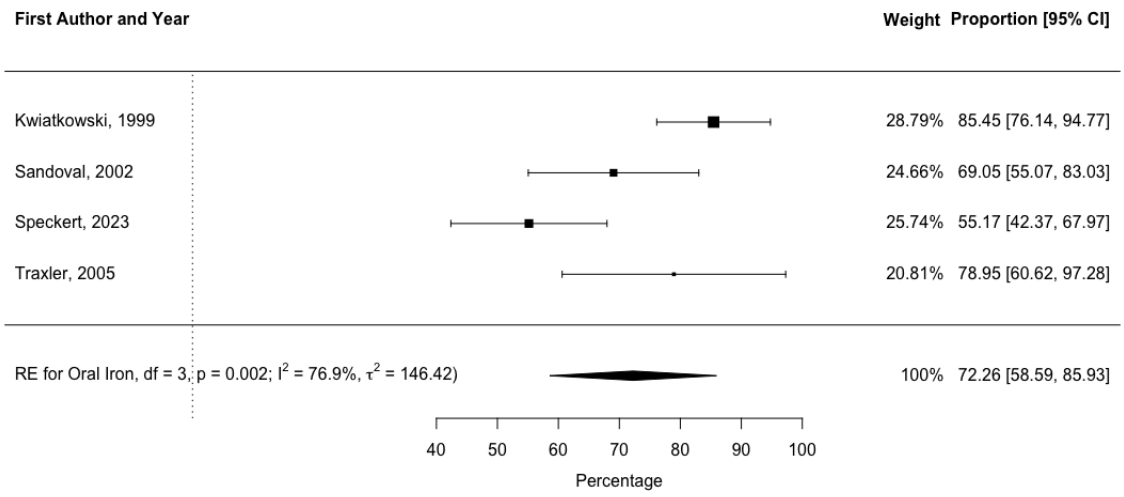

Figure 3.5: Percentage of pediatric patients who received oral iron supplementation for IDA in four non-randomized studies.

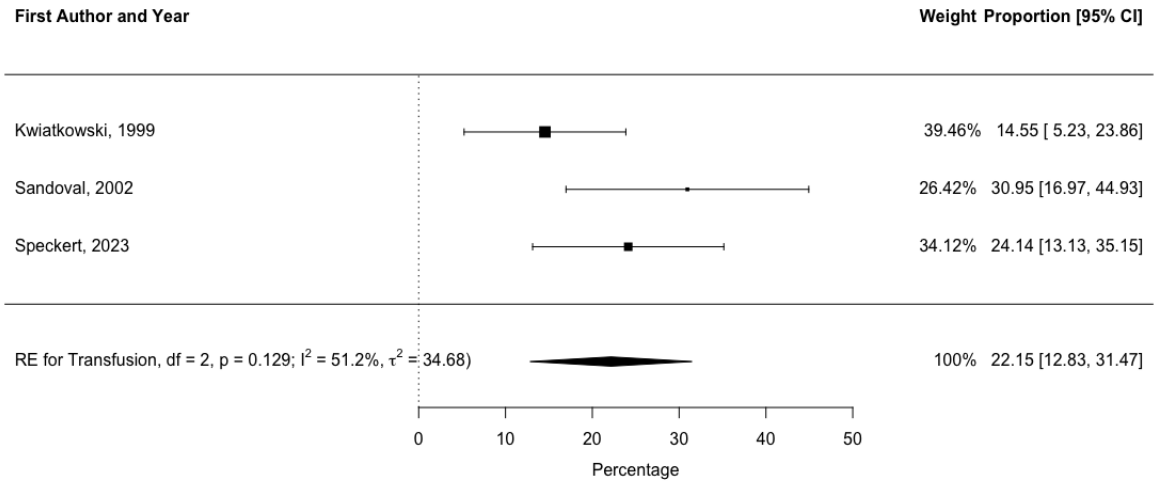

Figure 3.6: Percentage of pediatric patients who received a blood transfusion for IDA in three non-randomized studies.

Subgroup Analysis

The first subgroup analysis we considered was with respect to severity of IDA. Severe anemia in children is often defined as a hemoglobin level less than 70 g/L and a serum ferritin concentration of less than 12 ng/L [9,46]. However, there is no consensus on a

standardized cut-off point for either of these indicators. As previously discussed, severe, chronic IDA can negatively impact a child's cognitive, neurological, and behavioural development, and therefore must be treated in order to avoid irreversible harm [28]. We wanted to see if prevalence of the different interventions for severe IDA differs from the overall estimates we saw above.

Fourteen non-randomized studies (with 794 patients) were included in this subgroup analysis. Of the total 794 children included in this subgroup, 351 (44.21%, 95% CI: 40.75% to 47.66%) received oral iron supplementation, 234 (29.47%, 95% CI: 26.30% to 32.64%) received IV iron and 209 (26.32%, 95% CI: 23.26% to 29.39%) received blood transfusion.

The results for the severely ill children showed significantly larger prevalence of use of IV iron and blood transfusion (Figure 3.7), where the majority (n=443, 55.79%) received either IV iron or blood transfusion. The chi-square test showed significantly different prevalence among the three treatments ($\chi^2 = 65.135$, p-value <0.001). However, when compared pair-wise, we saw that the proportion of children receiving IV iron did not differ significantly from the proportion of children receiving blood transfusion ($\chi^2 = 1.8033$, p-value $= 0.1793$).

The second subgroup analysis we considered was with respect to age, where we aimed to investigate whether the treatment choice differed for younger children. Although children of all ages can be affected by IDA, children between 6 and 36 months are especially susceptible [11]. This is due in part to the rapid growth phase children experience during this time frame, in conjunction with a depletion of the iron stores from birth and a possible lack of replenishment of these stores for one reason or another [11].

Of the total of 1586 children who received treatment for IDA, 1545 (97.41%, 95% CI: 96.63% to 98.19%), received oral iron, 28 (1.77% , 95% CI 1.12% to 2.41%)received IV iron, and only 13 0.82% (95% CI: 0.38% to 1.26%) received blood transfusion. We noted that the prevalence of oral iron was much higher in this subgroup and the prevalence of both IV iron and blood transfusion were significantly lower, indicating that oral iron was the preferred course of treatment for younger children. We did not have adequate data to perform a proper meta-analysis for this subgroup.

Sensitivity Analysis

The WHO defines IDA in young children (<5 years) as having a hemoglobin concentration of less than 110 g/L, while for older children and adolescents, IDA is defined as a hemoglobin concentration of less than 120 g/L [9]. The results from our systematic review showed that most of the studies used these thresholds, however, there were four studies that identified children as having IDA although their

hemoglobin levels were higher than these cut-off values. We therefore performed sensitivity analysis by excluding these four studies.

All four studies that defined IDA differently from the WHO pertained to oral iron administration. This sensitivity analysis included a total of 10281 children, of whom 7301 received treatment for their IDA. The majority 6074 (83.19% , 95% CI: 82.34% to 84.05%) received oral iron, followed by 1018 (13.94% , 95% CI: 82.34% to 84.05%) receiving IV iron, with the remaining 209 (2.86%, 95% CI: 2.48% to 3.25%) receiving blood transfusion. The distribution of treatment seemed similar to what was seen in the full sample (Figure 3.7). We also performed sensitivity analysis with respect to the results of the RoB assessment, where we removed 19 studies with high or serious RoB. The estimates of prevalence from this analysis were 82.51% [95% CI: 81.45% to 83.57%] for oral iron, 13.97% [95% CI: 13.01% to 14.94%] for IV iron and 3.51% [95% CI: 3.01% to 4.03%] for transfusion. These estimates also appeared very similar to what was found in the full sample, indicating that the prevalence estimates are consistent and reliable.

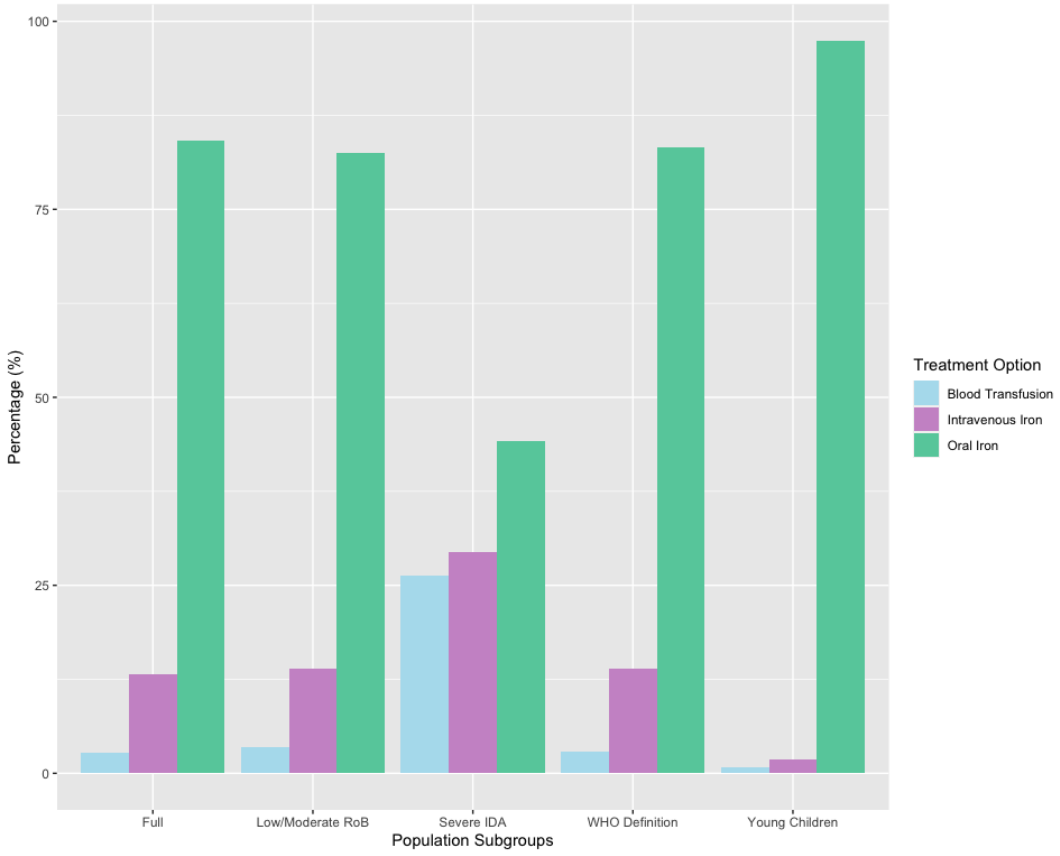

Figure 3.7: Percentage of pediatric patients who received a blood transfusion for IDA in three non-randomized studies.

Effectiveness Outcomes

Data appropriate for comparative effectiveness and safety analysis were only available for oral iron, where oral iron was either compared with placebo or different formulations, doses, or regimens of oral iron. As such, our traditional meta-analysis on effectiveness and safety outcomes involved oral iron, and all studies in this analysis were RCTs. The results from this analysis are presented in Figures 3.8, 3.9, 3.10, and 3.11.

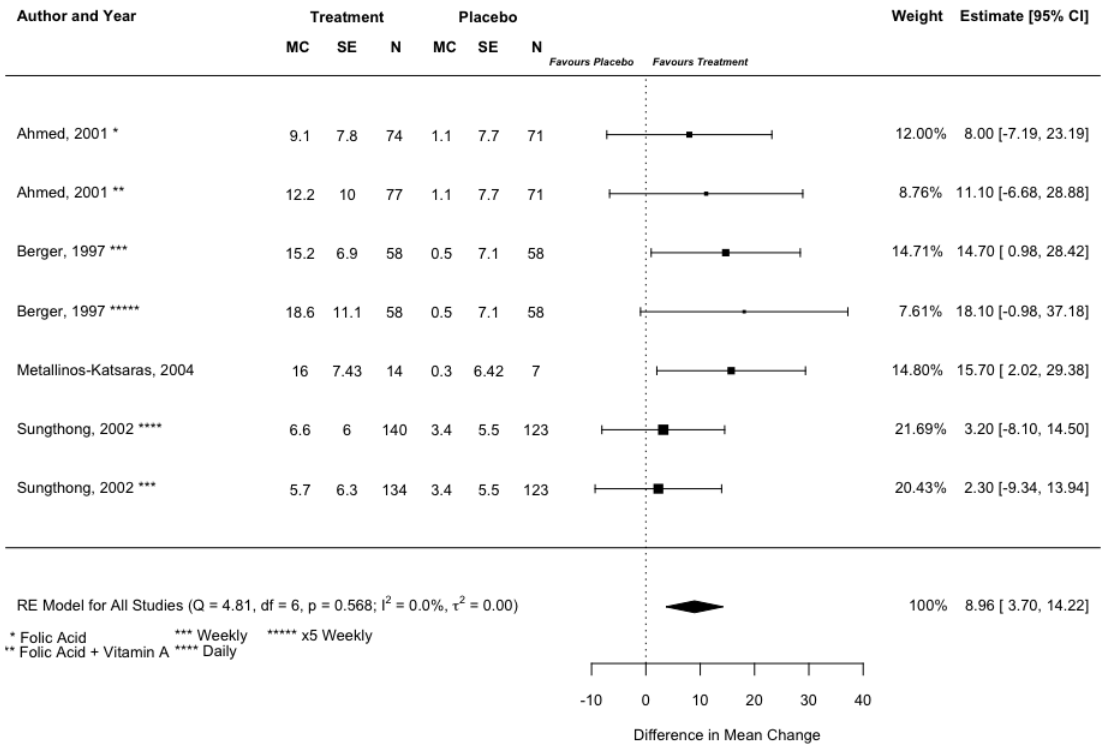

Figure 3.8: Results of random effects meta-analysis comparing difference in mean change from baseline between oral iron or placebo.

The forest plot presented in Figure 3.8 presents the results of a random effects meta-analysis on hemoglobin levels of children with IDA from RCTs comparing oral iron and placebo. Change in hemoglobin from baseline for each treatment arm as well as the difference in mean change between the two arms (placebo and oral iron) are provided in the Figure. As we can see from the figure, the pooled estimate for the difference in the mean change between the oral iron and placebo groups is 8.96 g/L [95% CI: 3.70, 14.22], indicating that oral iron is an effective treatment in significantly (p-value= 0.008) increasing hemoglobin levels of children with IDA. The

results also show that there was no heterogeneity ($I^2 = 0\%$, p-value $= 0.568$) across the different studies, indicating that the results are consistent, where all the studies included in this meta-analysis showed that oral iron was effective in increasing hemoglobin. Nevertheless, only one of the studies was able to show statistical significance, indicating that the other studies were under-powered. This shows the benefit of meta-analysis, where the pooled analysis provides increased power and precision. The funnel plot for this analysis (A.1) demonstrated that publication bias was not a concern for these studies.

We also synthesized data from RCTs comparing different regimens of oral iron, specifically whether giving oral iron daily is more effective in increasing hemoglobin levels than weekly administration (Figure 3.9). The results show that daily use of oral iron is more effective in increasing hemoglobin level compared to a weekly supplement (pooled estimate of 4.88 g/L, 95% CI: 2.44, 7.33, p-value < 0.0001). We observed no evidence of heterogeneity across the studies ($I^2 = 0\%$, p-value $= 0.892$). The funnel plot (A.2) showed minimal evidence of publication bias for these studies.

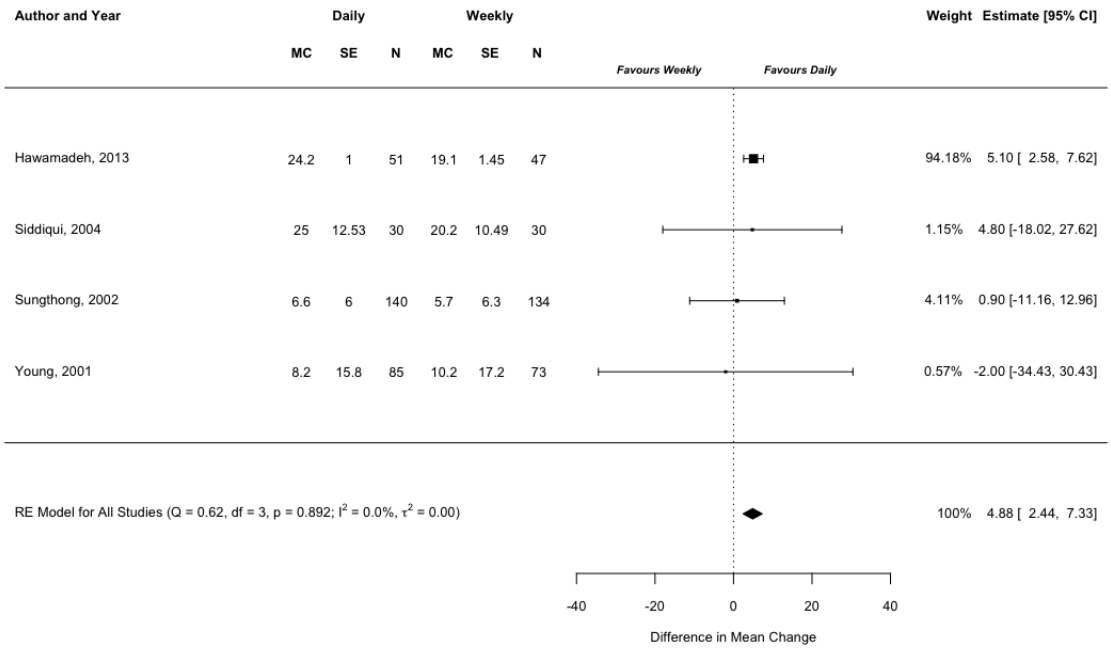

Figure 3.9: Results from random effects meta-analysis comparing daily or weekly oral iron supplementation for children with IDA.

Some of the RCTs compared oral iron as a lone treatment option and oral iron with additional supplements, such as vitamin A, zinc, multivitamins, or a combination of zinc and vitamin A. The results of the meta-analysis involving these RCTs (Figure

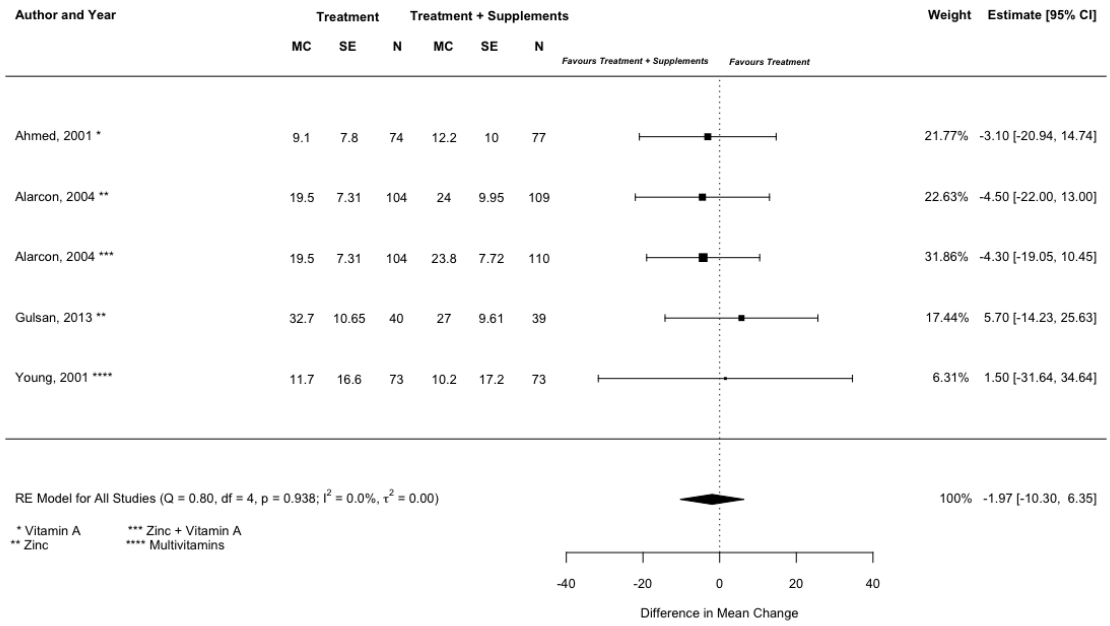

Figure 3.10: Results from a random effects meta-analysis comparing oral iron as the sole treatment with oral iron and additional supplements for children with IDA.

3.10) showed that oral iron with additional supplements was slightly more effective than using oral iron alone (pooled estimate: -1.97 g/L, 95% CI: -10.30, 6.35, p-value = 0.642). However, the difference was very small and statistically insignificant. There was no concern of heterogeneity for this meta-analysis (I^2 = 0%, p-value = 0.938), where all the studies showed small differences between the two groups. The funnel plot (A.4) also showed little evidence of publication bias for this subset of studies.

Similarly, we considered different formulations of oral iron, and the results (Figure 3.11) showed that iron polymaltose complex (IPC) to be more effective in increasing hemoglobin level compared to alternative formulations (pooled estimate: 1.98 g/L, 95% CI: -4.16, 8.13, p-value); however, the difference was not statistically significant. Moderate heterogeneity was observed (I^2 = 8.3%, p-value = 0.493), which could be attributed to the various alternative formulations used among the studies. We saw little evidence of publication bias arising from the subset of studies (A.5).

Although traditional meta-analysis was not possible for comparing the 3 major IDA treatment options in our objective (oral iron, IV iron and blood transfusion) with respect to effectiveness outcomes, we considered indirect comparative analysis, whereby we first synthesized evidence for each treatment separately and compared

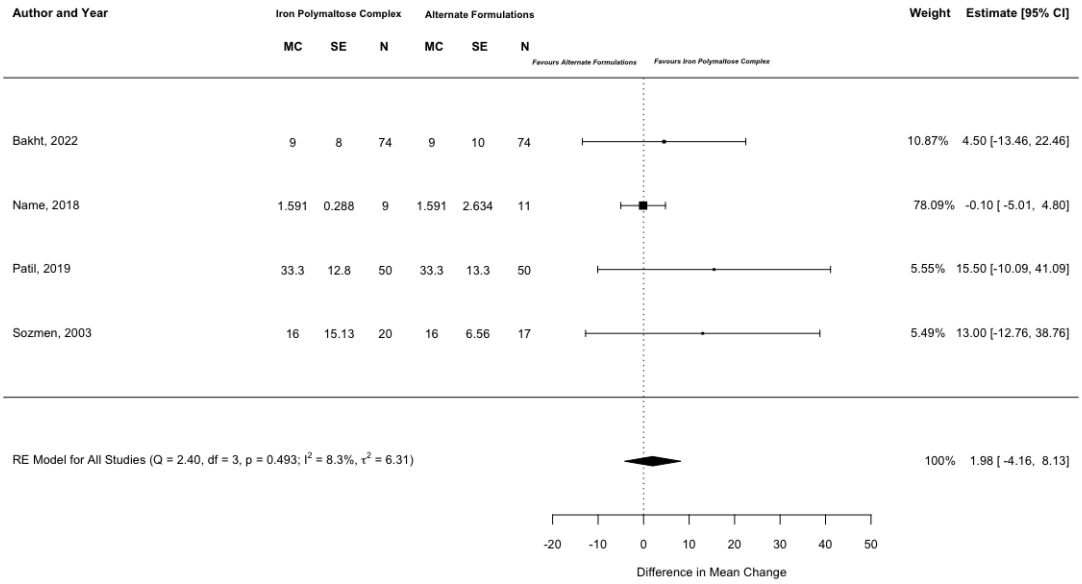

Figure 3.11: Results of random effects meta-analysis to compare the effectiveness of iron polymaltose complex compared to another formulation, in increasing hemoglobin level of children with IDA.

the pooled mean change from baseline between the different treatments. There was no adequate data to analyze improvements in hemoglobin for blood transfusion; hence the results presented here are for oral iron and IV iron. We would also like to highlight here that data for IV iron is available only in the non-randomized studies, and as such, we first considered non-randomized studies for both oral iron and IV iron, and the results are presented in Figures 3.12 and 3.13, below.

The results from this analysis showed that both oral and IV iron were effective in improving hemoglobin levels in children with IDA, where oral iron yielded a pooled mean hemoglobin change of 22.30 g/L [95% CI: 17.71, 26.88, n = 38) from baseline compared to a mean hemoglobin change of 26.66 g/L [95% CI: 20.99, 32.33, n = 18] for children treated with IV iron. Since these summary measures were obtained from independent studies, we were able to use the t-test to compare these two pooled estimates. The result showed a significant difference (difference = 4.36, 95% CI: 4.16, 4.56, p-value < 0.0001) in effectiveness between the two treatments, where IV iron is shown to be slightly more effective than oral iron. High heterogeneity was observed across the studies reporting on oral iron ($I^2 = 76.96$ %, p-value < 0.0001), while moderate heterogeneity was observed for IV iron ($I^2 = 43.59\%$, p-value $= 0.087$).

Next, we looked at the full set of studies (RCTs and observational designs) reporting on effectiveness measures with respect to hemoglobin (A.6). We found

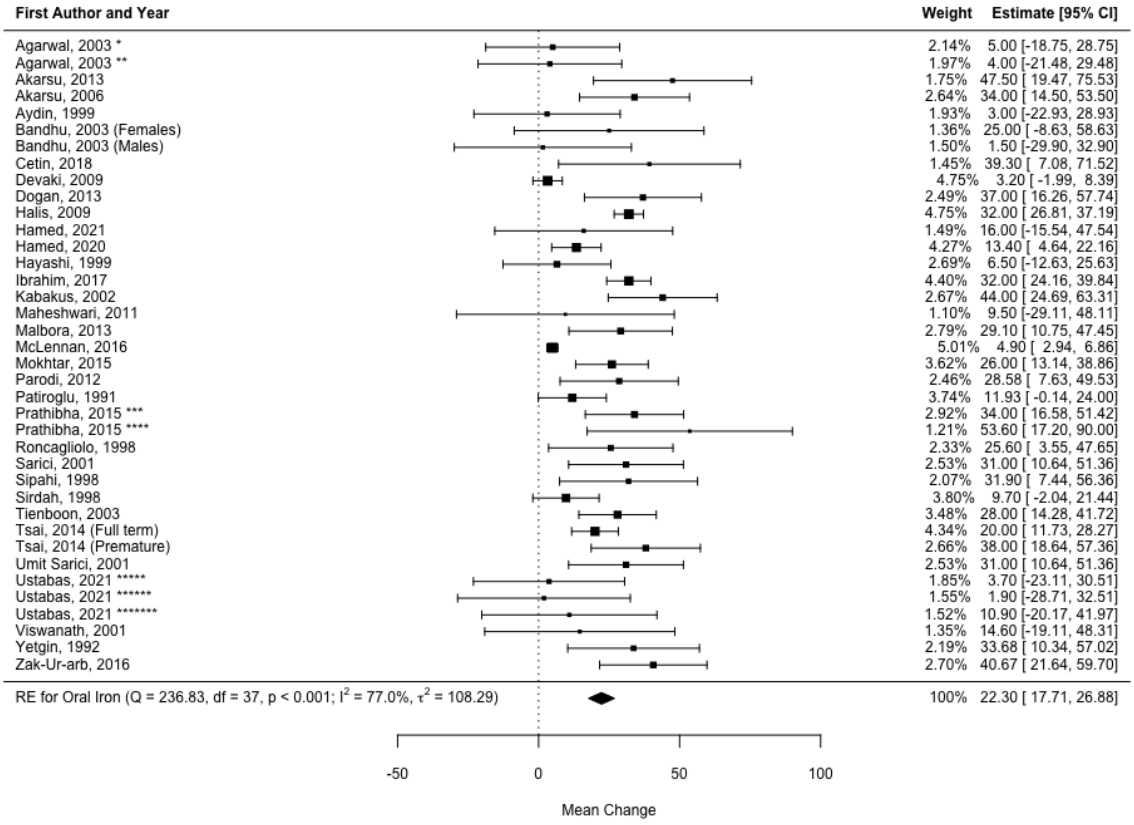

Figure 3.12: Results of random effects meta-analysis to pool hemoglobin change from baseline after use of oral iron supplementation to treat children with IDA.

a pooled estimate for oral iron of 21.16 g/L [95% CI: 18.22, 24.09], slightly lower than what we found based on only non-randomized studies. Nevertheless, the difference is negligible, indicating that the effectiveness estimates are stable. We observed significant heterogeneity ($I^2 = 93\%$, p-value < 0.0001), higher than what was observed for non-randomized studies. The slight difference in the estimates and increased heterogeneity can be attributed to the difference in settings, formulations, administrations, and doses.

There were insufficient quantitative data to meta-analyze the effectiveness outcomes for children who received transfusion to treat severe IDA. However, our systematic review included 5 studies in which children were treated with blood transfusion. The studies indicated that blood transfusion was an effective intervention in promoting the re-establishment of hemoglobin levels in children

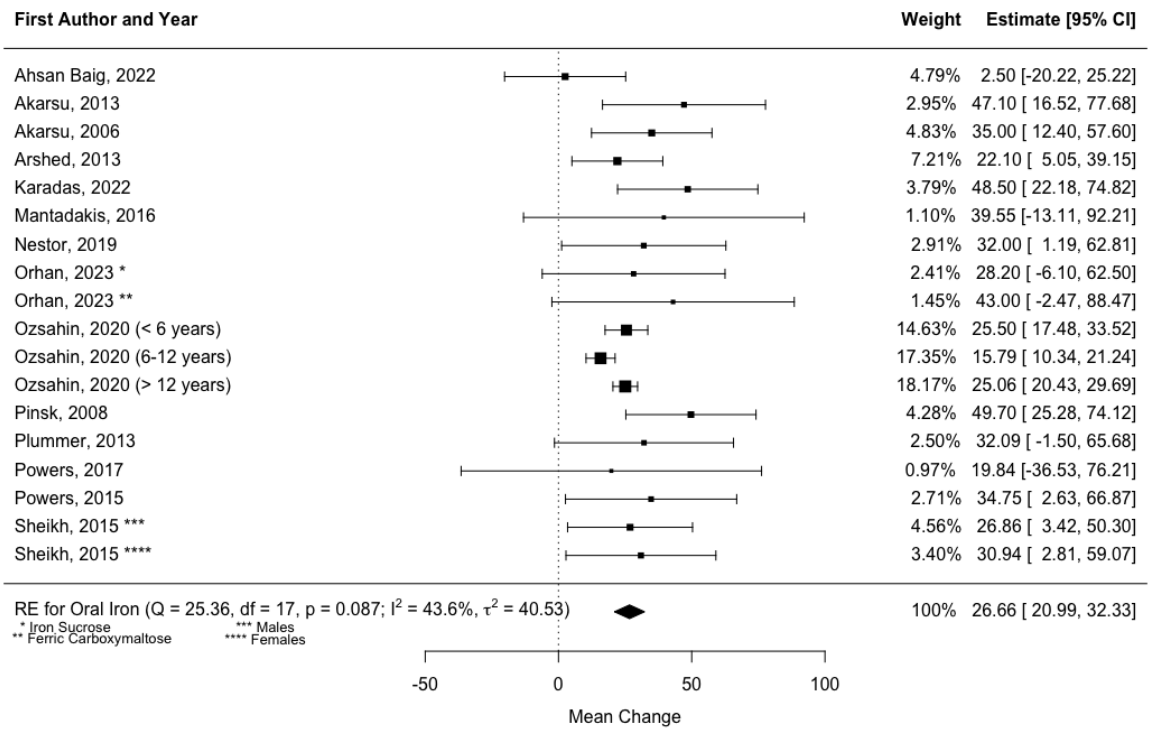

Figure 3.13: Results of random effects meta-analysis to pool hemoglobin change from baseline after use of IV iron to treat children with IDA.

with severe IDA [117]. We also found that blood transfusion was a highly effective intervention in reducing heart rate in children with severe IDA causing tachycardia [117]. Adjacently, blood transfusion was associated with positive outcomes for children with congestive cardiac failure due to IDA [118]. In one study, it was reported that all children, except one, who received a transfusion to treat severe IDA did not require further intervention in the form of a transfusion after the initial treatment [119]. Similarly, it was reported in the same study that no patients who received IV iron required further intervention to treat severe IDA [119].

Subgroup Analysis

The first subgroup analysis we considered is with respect to severity of IDA. Similar to what we did above, we first synthesized evidence for oral iron and IV iron separately using random effects meta-analysis, and then made indirect comparisons between the two pooled estimates (Figures 3.14 and 3.15). The results showed that oral iron was very effective in significantly increasing hemoglobin levels of children with severe IDA, with a pooled mean hemoglobin change from baseline of 26.00 g/L [95% CI: 1.84, 50.52, n = 3]. We found a similar effectiveness result for IV iron as compared

to the full sample, with a pooled estimate of 25.00 g/L [95% CI: -20.07, 70.07, n = 2]. We observed moderate heterogeneity for the oral iron group ($I^2 = 67.2\%$, p-value = 0.055) and significant heterogeneity for the IV iron group ($I^2 = 85.1\%$, p-value = 0.010). This is likely due to varying treatment dosages, formulations and regimens observed across the treatment groups. Statistical comparison between oral and IV iron in treating severe IDA showed that the two treatments were not significantly different in effectiveness (difference = 1, 95% CI: -4.16, 6.16, p-value < 0.703). We would, however, like to highlight that there are limited number of studies in this subgroup and caution should be taken in interpreting the results from this meta-analysis. Heterogeneity estimates and tests of homogeneity are not reliable in this circumstance as well.

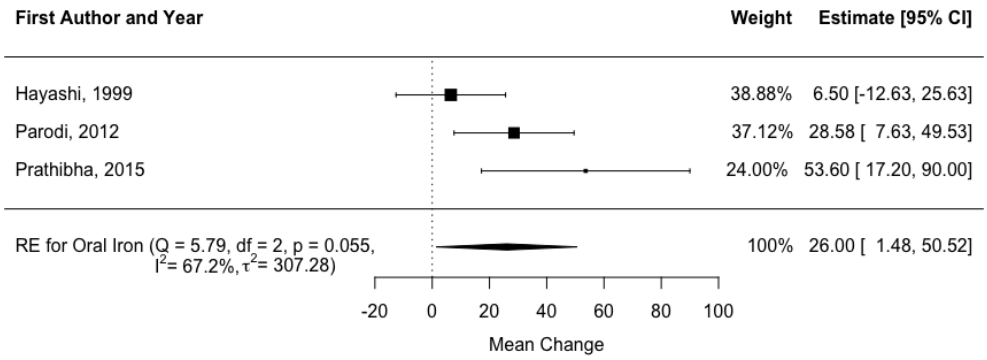

Figure 3.14: Results of random effects meta-analysis to pool mean hemoglobin change from baseline after use of oral iron to treat severe IDA in children.

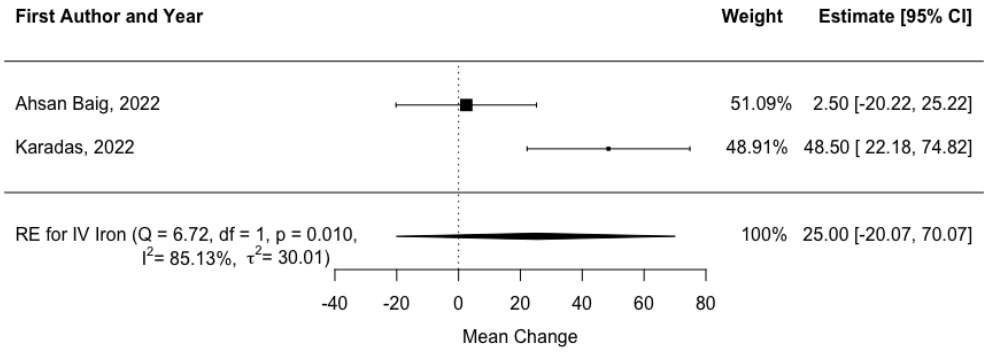

Figure 3.15: Results of random effects meta-analysis to pool mean hemoglobin change from baseline after using IV iron to treat severe IDA in children.

The second subgroup analysis we performed was with respect to age, where we synthesized evidence from studies involving young children between the age of 6 months and 3 years old. This subgroup only contained one study for IV iron, thus we performed meta-analysis only on oral iron and made indirect comparisons between the pooled estimate (Figure 3.16) for oral iron and the single estimate for IV iron. We found a pooled change in hemoglobin level for oral iron of 23.75 g/L [95% CI: 14.56, 32.94, n = 8], while the single estimate for IV iron was 25.5 g/L [95% CI: 0.09, 0.29, n = 1]. We compared these two estimates and found that the difference was not statistically significant (difference = 1.75, 95% CI: -0.07, 3.57, p-value = 0.06). We also observed significant heterogeneity for the oral iron studies (I^2 = 77.6%, p-value < 0.0001).

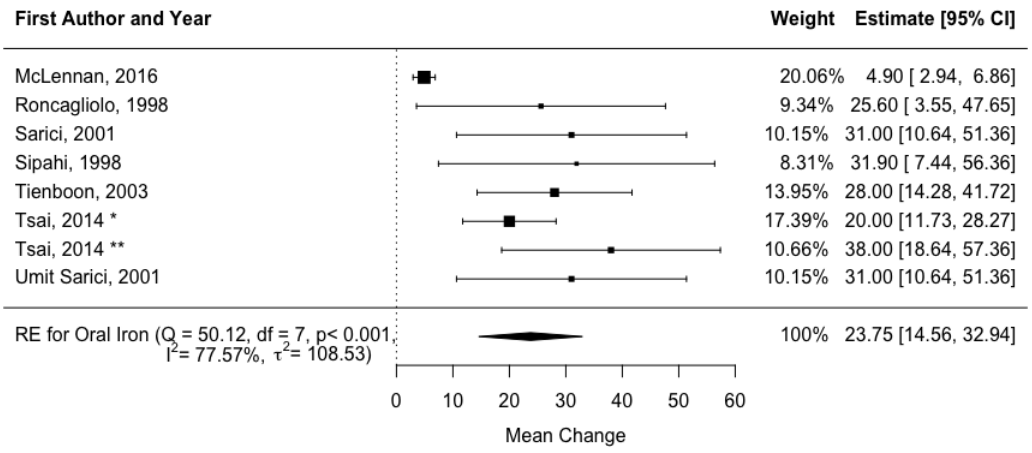

Figure 3.16: Results of random effects meta-analysis to pool mean hemoglobin change from baseline after use of oral iron to treat IDA in children between the age of 6 and 36 months.

Sensitivity Analysis

We considered sensitivity analysis with respect to WHO's definition of IDA (based on hemoglobin cut-off value of 110 g/L for children <5 years old and 120 g/L for older children). All studies involving IV iron followed WHO's definition; hence meta-analysis was done only on oral iron studies where we removed studies that did not follow WHO's definition (Figure A.7 in the appendix). The analysis led to a pooled mean hemoglobin change of 23.19 g/L [95% CI: 18.46, 27.92, n = 35], a slight increase from the pooled estimate (21.19 g/L) obtained from the full analysis. Comparing this estimate with that of the IV iron group (26.66 g/L), we found that the effectiveness between the two interventions was significantly different (difference = 3.47, 95% CI:

3.27, 3.67, p-value < 0.0001). Between-study heterogeneity reduced by about 7%, with I^2 value of 86.2% compared to 93.02%, indicating that some of the heterogeneity in oral iron studies might have been because of the use of different cut-off values in their definition of IDA.

Next, we considered sensitivity analysis with respect to imputations of missing summary statistics (Figures A.8 and A.9 in the appendix). The results showed that for oral iron, the mean hemoglobin change was 22.65 g/L [95% CI: 17.71, 27.59, n = 33] slightly more than 21.19g/L, which we found based on the full sample. For IV iron, we obtained a mean hemoglobin change of 26.37 g/L [95% CI: 20.30, 32.44, n = 14], which was almost the same as what we found using the full sample (which was 26.66 g/L). These results, therefore demonstrated that our imputations did not lead to significant changes in the pooled estimates, and are therefore reliable. We confirmed again that the two interventions were statistically significantly different (difference = 3.72, 95% CI: 3.49, 3.95, p-value < 0.0001) in their effectiveness with IV iron showing slightly better patient outcome. As was seen in the full model, significant heterogeneity was detected in the oral iron group ($I^2 = 79.5\%$, p-value < 0.0001) and moderate heterogeneity was found in the IV iron group ($I^2 = 51.86$, p-value = 0.0307).

Lastly, we considered the sensitivity analysis of studies screening at a high or serious risk of bias. We removed these studies from our analysis and compared the two interventions for effectiveness (Figures A.10 and A.11 in the appendix). We found slightly higher pooled estimates (than the estimate from the full sample) for oral iron (23.50 g/L, 95% CI: 18.38, 28.62, n = 29). On the other hand, we found slightly lower pooled estimates for IV iron (24.76 95% CI: 19.26, 30.26, n = 14). We compared these pooled estimates, and the results showed a significant difference in effectiveness (difference = 1.26, 95% CI: 0.99, 1.53, p-value < 0.0001), although the difference seemed to be very small. We observed moderate heterogeneity in the oral iron group ($I^2 = 67.2\%$, p-value < 0.0001) and some evidence of heterogeneity within the IV iron group ($I^2 = 41.0\%$, p-value = 0.082).

Safety Outcomes

Similar to our analysis of the effectiveness outcomes, we first performed meta-analysis of incidence of adverse events for studies involving the three treatments. We did not have adequate data to meta-analyze safety outcomes for oral iron, as only one study reported adverse reactions related to oral iron use. As such, we meta-analyzed the results for IV iron and blood transfusion separately (Figures 3.17 and 3.18) and compared these pooled estimates with the single estimate reported for oral iron. The results show that an estimated 7% [95% CI: 4% to 10%, n = 12] of patients receiving IV iron experienced an adverse reaction due to treatment. On the other hand, 12% [95% CI: 0% to 31%, n = 2] of children who received blood transfusion

had some sort of reaction. For the one study that reported adverse reactions due to oral iron treatment, 11% [95% CI: 4% to 17%] of children were reported to have adverse reaction. We noted moderate heterogeneity for the IV iron analysis (I^2 = 51.9%, p-value = 0.013), while substantial heterogeneity was found across the studies reporting adverse reactions related to use of blood transfusion (I^2 = 70.9%, p-value = 0.064). However, we would like to highlight that the meta-analysis for blood transfusion only involves two studies, and caution should be taken when interpreting the results. Estimates of heterogeneity are also not reliable when we have small number of studies in a meta-analysis.

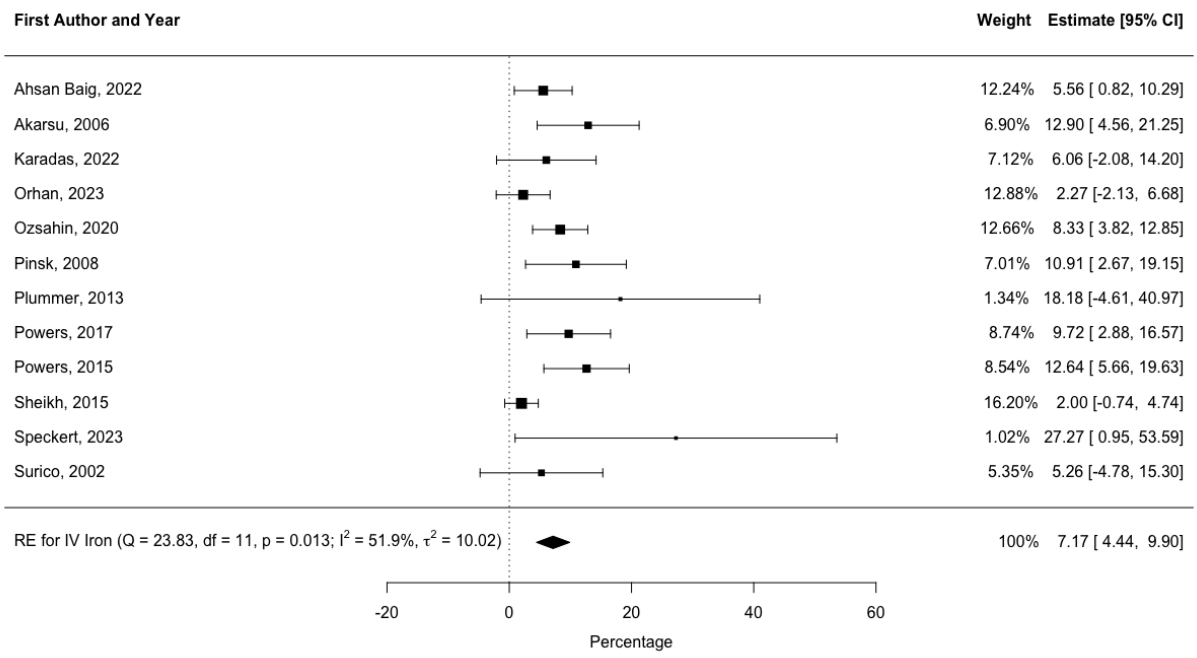

Figure 3.17: Adverse reactions related to use of IV iron to treat children with IDA.

Significant differences in incidence of adverse events between the 3 treatments were observed (χ^2 = 81.803, p-value < 0.0001). The results showed that IV iron posed the lowest adverse reactions, a difference of 4% (95% CI: 0% to 11%, p-value = 0.3124) compared to oral iron and a difference of 5% (95% CI: 0% to 12%, p-value= 0.04451) compared to blood transfusion. Oral iron, despite its effectiveness in increasing hemoglobin levels and successfully treating pediatric IDA (even in severe cases) was associated with a significantly higher incidence rate of adverse reactions compared to IV iron, while it was relatively equal to blood transfusion.

We would like to highlight that we did not stratify safety outcome estimates by

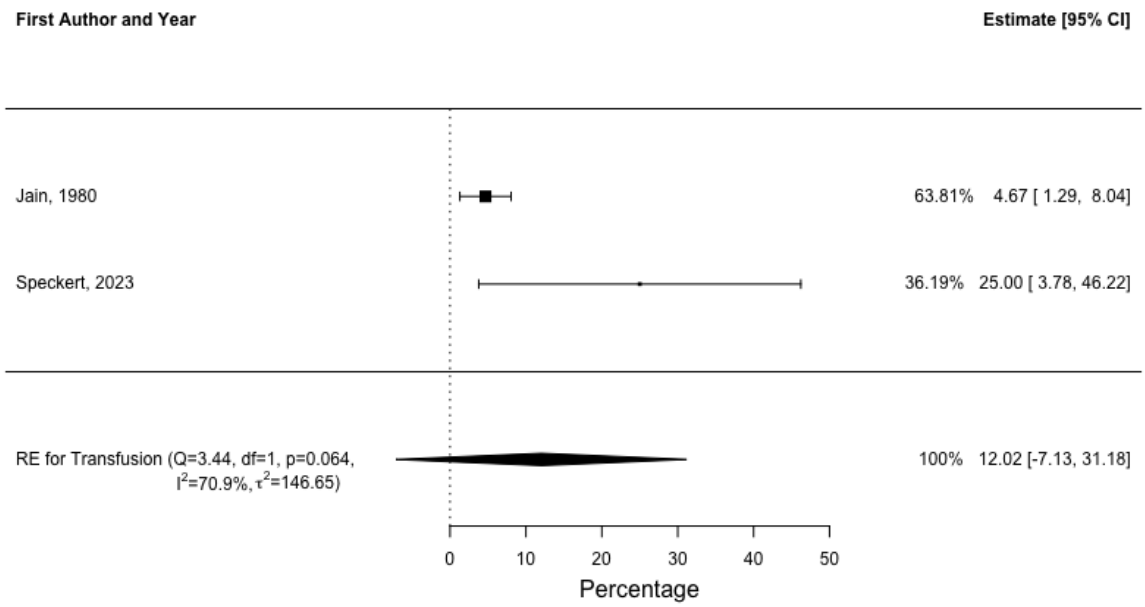

Figure 3.18: Adverse reactions related to use of blood transfusions to treat children with IDA.

reaction type, so the incidence rate included varying severities of reactions, from mild skin irritation to life-threatening altercations. For children who received oral iron, 33% (n = 3) of the reported adverse reactions were fever, with the remaining 67% (n = 6) being a non-specified reaction. For children who received IV iron and reported a reaction (n = 60), the most prominent adverse reactions were dermatological manifestations such as rash or irritability at the injection site (28%, n = 17). Other reactions experienced by children who received IV iron included fever (15%, n = 9) and gastrointestinal issues such as gas (10%, n = 6). Of the 12 children who received one or more transfusions and experienced a reaction, the reactions were much more severe than what was seen for oral iron and IV iron, with 5 children (42%) experiencing cardiac complications and 4 (33%) having died due to treatment-related complications. Thus, the percentage estimates we obtained in this section are not comparable, as reactions experienced by children receiving a transfusion were much more serious than those experienced by children receiving either oral or IV iron.

Subgroup Analysis

To determine whether any of the treatments were considered safe for children with severe IDA, we looked at the percentage of children with severe IDA who experienced adverse reactions. The studies retained in this review did not provide safety data on children who received oral iron to treat severe IDA, and all studies regarding blood

transfusion as an intervention for IDA pertained to the case of severe IDA. As such, no meta-analysis could be performed for the oral iron treatment group, and the results of such an analysis for the blood transfusion treatment group were identical to what was seen in the full study population. As such, we only meta-analyzed and compared the results for the IV iron group with the results found for the full population

The results from the subgroup analysis showed (Figure 3.19) that a small percentage 4% [95% CI: 1%, 7%, n = 4] of children who received IV iron experienced adverse reactions. We noted very small heterogeneity within this subgroup of studies ($I^2 = 26.54\%$, p-value = 0.1470).

Due to the lack of adequate data to estimate similar percentages for oral iron, we were unable to perform a three-way comparison for safety outcomes between the three interventions. We did, however, test the difference in proportions between the safety outcomes for IV iron and blood transfusion and found statistically significant evidence that the safety outcomes differed between the two treatments ($\chi^2 = 8.5338$, p-value = 0.003486). Nevertheless, the results should be interpreted with caution because some adverse reactions are more serious than others.

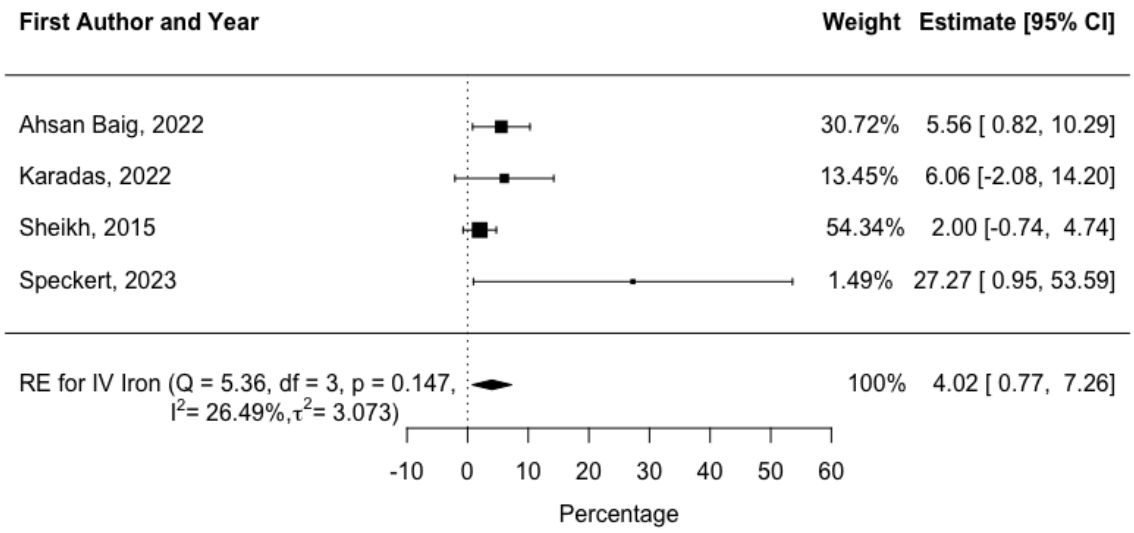

Figure 3.19: Adverse reactions after receiving IV iron to treat severe IDA in children.

Similarly, we performed a subgroup analysis on young children between the ages of 6 and 36 months to determine whether safety outcomes were different in this population. However, only one of the studies involving IV iron provided data on

adverse reactions in younger children, while no studies pertaining to young children receiving either oral iron or blood transfusion reported on adverse effects. Thus, we were only able to provide the estimated percentage of children who experienced adverse reactions in the one study that provided IV iron as treatment. This study found that no young children experienced adverse reactions due to treatment when given IV iron therapy to treat IDA. This differed slightly from the estimate stemming from the full study population (7%).

Sensitivity Analysis

All studies that reported on adverse reactions in this review followed the WHO guidelines for diagnosing IDA. These studies also did not require any imputative measures with respect to safety outcomes. As such, no sensitivity analysis was required for these subgroups of studies. We did, however, perform a sensitivity analysis on the studies that screened at either low or moderate RoB, in order to determine whether studies with high RoB had affected our estimates. We noted that none of the studies that reported on adverse reactions due to receiving transfusion were removed in this sensitivity analysis, and thus the results remained the same from what we found in the full population. We found that the pooled estimated percentage of children who received IV iron was 7% [95% CI: 4%, 10%] (Figure 3.20), which did not differ from the results found using the full sample. Again, we found some evidence of heterogeneity ($I^2 = 44.99\%$, p-value = 0.0367). Thus, the comparison for safety outcomes between the children who received IV iron and those who received blood transfusion were identical to what was seen in the full sample.

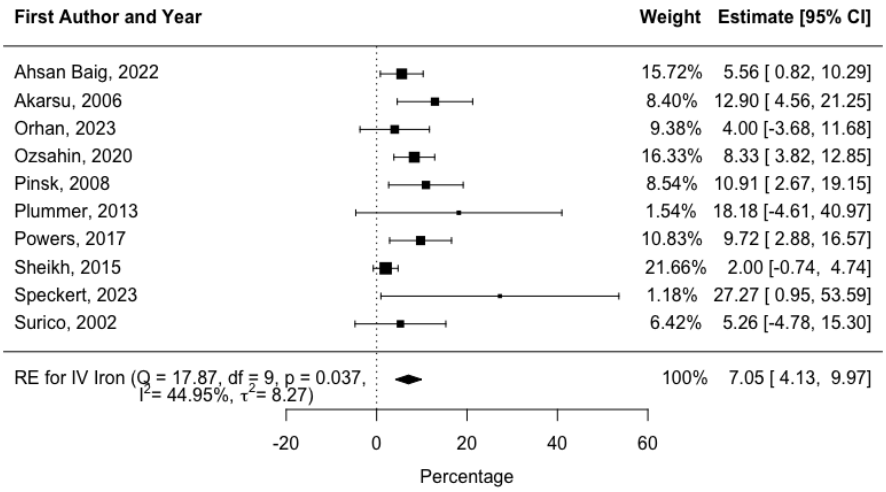

Figure 3.20: Adverse reactions after receiving IV iron to treat IDA, where only studies with low or moderate RoB were used.

3.4 Discussion

This review provided insights into the prevalence, effectiveness, and safety outcomes of the three key interventions used to treat pediatric IDA: oral iron, IV iron therapy and the transfusion of RBCs. Prior to this review, there existed no literature comparing these three treatments to determine when each intervention would be most appropriate to be used to treat a child with IDA. There was, however, a plethora of existing research concerning each of the three treatments individually, indicating that a systematic review was possible and a good opportunity to bring together the data from the multiple individual sources into one synthesized document. Given that synthesized data is considered the highest quality of evidence, we found it necessary to provide clinicians with this review to support evidence-based clinical practice and develop evidence-informed guidelines. It was our objective to conduct a review that could be used to develop treatment guidelines for clinicians treating children of all ages with IDA, regardless of the severity of the illness.

Using all the studies included in this systematic review, we found that oral iron is by far the most prevalent of the treatment options for children of all ages and severity of anemia (84%), followed by IV iron (13%) then blood transfusion (3%). Of the studies that examined treatment outcome in a natural clinical environment, the proportion of children receiving oral iron per study was 72%, for IV iron this figure was 19% and for transfusion it was 22%. These figures more accurately reflect the breakdown of treatment given to children with IDA and are therefore more reliable, as the inclusion criteria for these studies did not include parameters on treatment administered. It was shown that the prevalence of use of the treatments depends greatly on the age of the child, as younger children are much more likely to receive oral iron (97%) than IV iron (2%) or blood transfusion (1%). These results were expected, as oral iron administration is the less invasive approach to treating IDA, and therefore is expected to be more well-received by younger children compared to more invasive approaches such as IV iron or blood transfusion. Severity of IDA was also an important factor for the prevalence of use of treatments, as the distribution became much more uniform across the treatment types in children with severe anemia (44%, 30% and 26% for oral iron, IV iron, and blood transfusion, respectively). Again, these results came as no surprise considering blood transfusion and IV iron are habitually used for more severe cases of IDA, as they provide a more rapid uptake of iron, which is often required for very ill children [120, 121]. Our sensitivity analyses showed that neither alternative definitions of IDA nor high RoB in retained studies influenced these estimates, demonstrating the stable nature of our prevalence estimates from the systematic review.

We also studied the comparative effectiveness of both oral iron and IV iron but did not have adequate data to evaluate comparative effectiveness of blood transfusion compared to oral and/or IV iron. We used mean change-from-baseline of hemoglobin

as our effect measure and made indirect comparisons of pooled effect sizes when direct comparisons were not possible. The results showed that IV iron was indeed slightly more effective in re-establishing hemoglobin levels in children with IDA compared to oral iron (mean change-from-baseline hemoglobin levels of 26.66 g/L for IV iron and 21.16 g/L for oral iron). We found that the relative effectiveness of oral iron increased significantly in children with severe IDA and in younger children aged between 6 to 36 months (26.00 g/L and 23.75 g/L, respectively), while IV iron did not appear to be more or less effective in either of these subgroups of populations.

We obtained adequate data from the retained RCTs to compare oral iron in four ways: placebo versus oral iron, daily versus weekly administration, iron polymaltose complex versus other formulations, and oral iron given alone versus given with supplemental vitamins. In terms of effectiveness, we found that oral iron was more effective than placebo, while daily administration was slightly more effective than weekly administration. We also found that iron polymaltose complex was the most effective formulation of oral iron, and that adding vitamins to an oral iron regime increases the effectiveness of the intervention.

The last element we examined was the safety outcomes of each of the three treatments, which we defined as the proportion of children experiencing adverse reactions due to treatment. We found that oral iron and blood transfusion had higher incidence rates of adverse reactions (11% and 12%, respectively) than IV iron (7%). However, we noted that the disparity in incidence rates for adverse reactions may have been due in part to the severity and types of reactions that stem from each of the treatments, where reactions to oral iron are often less severe than those seen in patients receiving IV iron or blood transfusion. We found that safety outcomes did not differ for children with severe anemia nor for young children, however we were only able to compare safety outcomes for oral iron for the subgroup of young children, and therefore cannot comment on the safety of IV iron and blood transfusion in young children.

Through the sensitivity analyses we conducted, we found that studies that used alternative definitions of IDA only had a significant impact on the effectiveness measure for oral iron, whereas the distribution of prevalence, the effectiveness of IV iron and all safety outcomes remained unaffected. For articles that had significant imputation, the distribution of prevalence was affected for both oral iron and IV iron but not for blood transfusion, while only the safety outcomes for oral iron were impacted by the removal of these studies. Effectiveness measures, as well as safety outcomes for IV iron and transfusion, were unaffected by the imputation measures used in the review.

With respect to the quality of evidence used in this review, we obtained vastly different results for risk of bias between the RCTs and observational studies. For the RCTs, we found that most studies screened at a low risk of bias across the domains,

with 11 screening low overall, 13 posing some concerns with respect to bias, and only 2 screening at a high risk of bias. For the observational studies, we found that the majority of studies screened at a moderate risk of bias (n = 39), while only 2 screened at a low risk of bias and 17 screened at a serious risk of bias. We posited that the disparity in risk of bias between these study designs was most likely due to the fact that RCTs are able to control for confounding and other factors that could influence the outcome of a study, whereas in certain observational study designs, such as retrospective cohort studies, researchers are unable to control for many variables, leaving these studies at a higher risk of bias. Overall, we were satisfied with our results from the risk of bias assessment.

There are significant limitations to currently available guidelines for diagnosis and management of ID/IDA [122]. Currently published guidelines are based on expert consensus, which is considered to be a low level of evidence [53]. These include guidelines published by professional organizations and regional or hospital guidelines. One of the key reasons we decided to undertake this review was to review the existing data that supports the ASPHO Choosing Wisely campaign that advises against the use of pRBC transfusion for children with IDA that have no evidence of hemodynamic instability or bleeding [48]. These guidelines appear to be based on expert consensus. Furthermore, the guideline provides some evidence on the effectiveness of oral iron (type, dose, dosing interval) and IV iron in children. However, the guideline does not provide guidance on the indications for each of these three treatments; for example, should transfusion be considered at a specific hemoglobin threshold; how is hemodynamic instability defined; when should clinicians consider oral versus IV iron?

The systematic review of guidelines by Peyrin-Biroulet et al. (1 January 2004 to 30 June 2014) identified three targeting or including children [111]. These include guidelines from the province of British Columbia, Canada (now updated and effective April 17, 2019), the Nutrition Committee of the American Academy of Pediatrics (published 2010), and the Canadian Paediatric Surveillance Program (published 2011) [107,123,124]. These guidelines, however, are also based on expert concensus. Numan and Kaluza undertook a systematic review of guidelines on the treatment of IDA to explore current recommendations on the use of IV iron published between 1 January 1990 and 24 January 2020 and reported on the lack of guidelines in the area of pediatric care [112]. They identified 35 guidelines for IDA across different therapeutic areas, of which 7 (20%) made no mention of the role for IV iron in the management of IDA and approximately 60% were outdated [112]. The United States Preventive Services Task Force (USPSTF) did undertake a systematic review of trials of oral iron treatment. These trials reported inconsistent findings for hematologic measures, and no benefit on growth and developmental outcomes. Thus, the USPSTF concluded that there was insufficient evidence to assess the balance of benefits and harms of screening for iron deficiency anemia in children 6 to 24 months of age [125].

In 2013, Powers et al. undertook a survey of American Pediatric Hematologists two hypothetical cases of IDA: a toddler with nutritional IDA and an adolescent with heavy menstrual bleeding and IDA [110]. The survey results provided expert decision making regarding the role of oral iron, IV iron and RBC transfusion. The authors noted the lack of evidence-based guidelines for management of IDA and reported that survey respondents indicated that experience, rather than evidence, guided their decision-making. In 2022, McEvoy et al. published their experience with the development, implementation and evaluation of a standardized algorithm for management of IDA for young children in the emergency department [126]. The algorithm was developed following a search of the literature for existing guidelines and a survey of clinical experts with consensus agreement. While a paucity of consensus guidelines was noted by the authors, they cited the published guidelines of the British Columbia Government, Alberta Medical Association, and ASPHO [47, 48, 124, 127]. The final algorithm provides a hemoglobin-based risk-stratification approach to the use of oral iron, IV iron, and RBC transfusion.

More recent guidelines which target or include children include the Alberta Medical Association Iron Deficiency Anemia Clinical Practice Guideline (2018); the Canadian Paediatric Society Iron Requirements in the First 2 Years of Life (2019); the UK National Health Service Anaemia in Childhood (2021); and the Royal Children's Hospital Melbourne Iron Deficiency Clinical Practice Guideline (2023) [127–130]. Clinical experts have published recommendations in clinical journals affiliated with professional organizations, for example Powers and O'Brien published: "How I approach iron deficiency with and without anemia" (published in 2018 in the official journal of the American Society of Pediatric Hematology/Oncology), and Parkin and Borkhoff published: "Practical tips for paediatricians: Assessment and management of young children with iron deficiency" (published in 2018 in the official journal of the Canadian Paediatric Society) [131, 132].

A limitation of this review was the lack of evidence for all three of the interventions in certain domains, such as safety for young children. In this domain, we only had adequate data to synthesize an estimate for oral iron, while we were unable to comment on the outcomes for both IV iron and blood transfusion. We also did not have adequate data to compare blood transfusion for effectiveness in the full sample nor within our subgroups. This lack of data involving head-to-head comparisons between any two of the three interventions did not allow us to make traditional meta-analysis or network meta-analysis, instead we used indirect approaches to compare pooled effect sizes. As such, we were not able to draw more concrete conclusions with respect to the pairwise and three-way effectiveness and safety comparisons between the treatments. Nevertheless, our indirect comparisons still provided important findings that might provide insight into current practice and treatment choice as well as effectiveness and safety of the treatments used.

Another limitation of this study was the significant heterogeneity seen in both the oral iron and IV iron treatment groups, which could have caused unreliable pooled estimates for effectiveness and safety of treatment. To overcome this, we used random effects meta-analysis. We also attempted to elucidate sources of this heterogeneity, and posited that variation in treatment regimen, formulation and dosage within each treatment category was most likely the source of such heterogeneity, however, other underlying factors may have also influenced these high rates of heterogeneity. Regardless of observed heterogeneity, we still chose to provide pooled estimates, since the objective was to gauge the direction of evidence much more than the actual pooled estimate. Since within and between variance estimates, as well as the heterogeneity estimates, are provided, the researcher can make an informed decision in examining the validity of the various synthesized evidence.

This review contributes to the existing and growing stock of literature surrounding IDA and the interventions to treat the condition. What is unique about this review is its comparative analysis of the three treatment types by prevalence, effectiveness, and safety; facets that had not been analyzed in previous research works. This review employed novel indirect comparisons of pooled estimates to provide comparative analysis, which otherwise is not possible to do using traditional meta-analysis. The review provides a synthesis of all existing evidence concerning the treatment of IDA, which will provide important insights to clinicians actively treating children with IDA.

Chapter 4

Current Practice in Treating Iron Deficiency Anemia in Two Canadian Pediatric Hospitals.

4.1 Introduction

Although many public health policies and programs have been implemented in recent years to provide the necessary education and resources to help stem the growth of IDA as a disorder seen in children, nearly 40% of children continue to experience IDA worldwide [2]. Young children, specifically those between 6 and 36 months of age, are disproportionately affected by IDA, due in part to the rapid growth a child experiences during this time, where iron stores are often not replenished as quickly as they are depleted [11].

In infants and toddlers (6 to 23 months), iron deficiency is defined as a hemoglobin level of less than 105 g/L, while in children aged 24 to 59 months, this threshold is 110 g/L. For children aged 5 to 11 years, a hemoglobin level of less than 115 g/L is indicative of iron deficiency, and for adolescents (12 to 18 years, non-pregnant for females), this threshold is 120 g/L [3, 9]. Anemia is defined by serum ferritin, wherein an otherwise healthy young child (<5 years) with a serum ferritin level of less than 12 µg/L, or an older child/adolescent (5 years and older) with a serum ferritin level of less than 15 µg/L, would be considered anemic [9, 16]. The case of IDA in a child is considered severe if the hemoglobin levels drop below 70 g/L [46].

Globally, a common cause of IDA is a lack of adequate intake of iron-rich food, such as red meats, which can be caused by both cultural practices, such as vegetarianism, which is prevalent in Southeast Asia, as well as a lack of access to nutritious food, which can lead to undernutrition [4]. In young children, excessive

intake of dairy milk is also a prevalent cause of IDA, as the overconsumption satiates the child and does not promote the intake of other iron-rich foods in its place [4, 11, 18, 19]. IDA can also be caused by comorbidities, such as chronic kidney disease, inflammatory bowel disorder and certain cancers [22, 23, 41, 102–104].

If left untreated, severe IDA can cause both short-term and long-term effects on a child's health, such as delayed neurological, cognitive, emotional, and behavioural development [24, 26, 27, 105], thus it is important that a child with severe IDA receives the proper intervention to halt the progression of the condition. For mild to moderate cases of IDA, a child is usually treated with oral iron supplementation, whereas a child with a more severe case of IDA may receive intravenous (IV) iron [41, 43, 44]. If a child presents with severe IDA and is considered hemodynamically unstable, a clinician may opt to treat the child with a transfusion of red blood cells (RBCs) to rapidly increase the child's hemoglobin and stabilize the child's hemodynamic state [42]. The results from our systematic review and meta-analysis, presented in Chapter 3, showed that, for children with severe anemia, roughly 44% received oral iron, 30% received IV iron and 26% received a transfusion. In young children, the distribution of treatments was found to be dramatically skewed toward oral iron, with 97% receiving oral iron for IDA, while only 2% received IV iron and 1% received a transfusion.

Recently, many guidelines have advised against the use of transfusion as a treatment of IDA in non-extenuating circumstances. One such guideline is the American Society of Hematology (ASH) and the American Society of Pediatric Hematology/Oncology (ASPHO) joint Choosing Wisely Campaign, which advises "against the transfusion of packed red blood cells (pRBCs) in asymptomatic children with iron deficiency anemia (IDA) with no evidence of hemodynamic instability or active bleeding [23, 48]." The guideline instead recommends the use of oral or IV iron to treat IDA in otherwise healthy children. Similar recommendations are made in the Alberta Medical Association Iron Deficiency Anemia Clinical Practice Guideline (2018) and the Canadian Paediatric Society Iron Requirements in the First 2 Years of Life (2019) [127, 128].

In addition to hemoglobin and serum ferritin, other laboratory measures can be used to identify and diagnose the severity of IDA in children. Decreased values of mean corpuscular volume (MCV), serum iron, transferrin saturation, and RBC count are all indications of IDA [23, 133, 134]. Increased values of total iron-binding capacity (TIBC) are also indicative of IDA [134]. Nevertheless, most of these variables are shown to be highly correlated with one another, although some might provide additional information related to disease presentations and severity. Some of the variables (eg. MCV) are also important markers related to different types of anemia, for example sickle cell anemia. In conjunction with laboratory values, clinical manifestations, including vital signs, can also be used to identify the severity of IDA. Upon entry to the emergency department, children are often assessed for heart rate,

blood pressure, respiratory rate, oxygen saturation and temperature, which can all be used to identify a child who is severely ill [17, 135, 136]. However, there is lack of evidence related to quantifying the associations between the IDA-related laboratory measurements and clinical presentations.

Given that IDA is defined in part by a reduction in hemoglobin concentration, and that hemoglobin is primarily responsible for the transportation of oxygen throughout the body [29–31], it is no surprise that there is evidence of hemodynamic alterations and/or complications arising from chronic severe anemia [31–35]. Cardiac manifestations of IDA can include increased heart rate (tachycardia), alterations in blood pressure, palpitations, and in severe cases, heart failure [31, 35–40, 137]. Although there exists extensive evidence of the impacts of IDA on cardiac and respiratory health for adult humans and in animal studies, there is a lack of information on the subject in pediatric patients.

Moreover, there is no guideline relating clinical manifestations of IDA and course of treatment. Links have been drawn between laboratory measures of severe anemia and the treatment of choice. For instance, a recent study by Sun et al examined hemoglobin threshold values as potential predictors of RBC transfusion in young children hospitalized with IDA [138]. The authors concluded that hemoglobin levels could predict receipt of RBC transfusion with an area under the receiver operating characteristic (ROC) curve of 0.89; and a hemoglobin of 39 g/L had a sensitivity of 92% and specificity of 72% for transfusion [138].

The objective of the project presented in this Chapter was to first examine pairwise and multivariate relationships between the clinical manifestations of IDA, including vital signs and laboratory measures known to be indicators of IDA. Second, we investigated current practice in two Canadian pediatric hospitals, with the aim to study how the various clinical and laboratory measurements might have influenced the clinician's decision to treat pediatric IDA using RBC transfusion.

4.2 Materials and Methods

4.2.1 Study Design and Patient Population

The data for this analysis was collected retrospectively through independent chart reviews at two major Canadian tertiary pediatric hospitals: The Hospital for Sick Children (SickKids) in Toronto, Ontario, and the Children's Hospital of Eastern Ontario (CHEO) in Ottawa, Ontario. Relevant data were collected from children diagnosed with severe IDA, who presented to CHEO between 1 September 2017 and 1 June 2021 and to SickKids between 1 January 2001 and 15 May 2022. The health records of these children were examined for eligibility, which included a hemoglobin level of less than 70 g/L for children presenting to CHEO and 110 g/L for children presenting to SickKids. These two data sets were originally collected for other studies, hence the difference in their eligibility criteria, including the hemoglobin threshold.

4.2.2 Data Preparation

As indicated above, the dataset used in the analysis was created by combining two independent datasets from two similar, but separate, studies. Only the variables/information that were available in both datasets were combined. In total, we retained 19 independent variables that are indicators of IDA and its clinical presentations, and one dependent variable (treatment received for IDA). Of the independent variables, all were continuous except for sex, which was categorical male/female. The outcome variable was dichotomized to transfusion received versus no transfusion received.

4.2.3 Statistical Analysis

Descriptive statistics were calculated to assess key characteristics of the study population, such as age, sex, weight, medical history, clinical presentations, and laboratory measurements upon initial assessment at the hospitals. To address the primary objective of the investigation, we first conducted pairwise correlation analysis, wherein we investigated the correlations within the pairs of laboratory measurements that are indicators of IDA and clinical presentations including vital signs. We also performed canonical correlation analysis (CCA) to investigate multivariate associations, and the results were presented in tables and graphs. Details on CCA are presented in Chapter 2.

To address the second objective, we fit a logistic regression model where treatment (transfusion vs no transfusion) was used as a dependent variable. We first fit two models: one large model where we fit variables that were potentially associated with treatment choice, where variables were mostly selected based on

content expertise and a preliminary assessment of strengths of their relationship with the dependent variable and a second smaller model where variables from the full model were excluded based on their contributions to the model, using established goodness of fit assessment tools. To examine the overall goodness of fit of the models, we used the Hosmer-Lemeshow test. Details on the methods are presented in Chapter 2.

4.3 Results

The combined data from the two hospitals consisted of 183 pediatric patients (33% male) with severe IDA, the median age being 20.4 months (IQR=15.0, 29.0), and the mean age was 40.7 (SD = 51.6) months. Mean hemoglobin at diagnosis was 42.8 g/L (SD = 15.8), which was well below the WHO standard for diagnosing severe IDA. The mean heart rate upon admission was 132 (SD = 22.9) beats per minute (bpm). These and other key patient characteristics, including weight, temperature, heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), respiratory rate (RR), oxygen saturation, red blood cell (RBC) count, platelet, mean corpuscular volume (MCV), ferritin, iron and albumin are presented in Table 4.1.

Table 4.1: Summary statistics on patient characteristics, clinical presentations and laboratory measurements.

	Overall (N=183)		IV Iron (N=15)		Transfusion (N=76)**		Oral Iron (N=92)	
Characteristic	Mean (SD) or count (%)	N	Mean (SD) or count (%)	N	Mean (SD) or count (%)	N	Mean (SD) or count (%)	N
Age (months)*	20.4 (15.0, 29.0)	183	22.8 (14.0, 157)	15	21.0 (17.0, 28.0)	76	19.5 (14.3, 30.0)	92
Sex, male	61 (33.3)	183	3 (20)	15	26 (34)	76	32 (35)	92
Weight (kg)	11.6 (10.3, 13.3)	182	12.2 (10.9,46.9)	14	16.4 (15.6)	76	16.6 (14.7)	92
Temp (C)	37.0 (36.7, 37.4)	176	37.0 (36.8, 37.2)	15	37.1 (36.6, 37.4)	74	36.9 (36.7, 37.4)	87
HR (bpm)	132.0 (22.9)	183	128.4 (25.9)	15	137.5 (23.4)	76	128.1 (21.4)	92
SBP (mmHg)	104 (95, 115)	177	107 (102, 127)	15	108 (96, 115)	75	100 (94, 114)	87
DBP (mmHg)	59.1 (11.3)	152	63.5 (13.3)	15	57.5 (10.4)	68	59.7 (11.6)	69
RR (br/min)*	28.0 (24.0, 32.0)	183	30.0 (21.0, 36.0)	15	28.0 (24.0, 32.0)	76	26.0 (24.0, 32.0)	92
Oxygen (%)*	1.0 (1.0, 1.0)	180	1.00 (0.99, 1.00)	15	1.0 (1.0, 1.0)	76	1.0 (1.0, 1.0)	89
RBC count	3.0 (0.9)	182	3.0 (0.6)	15	2.4 (0.8)	75	3.5 (0.8)	92
Hemoglobin (g/L)	42.8 (15.8)	183	48.0 (32.0, 52.5)	15	32.3 (12.5)	76	51.2 (13.2)	92
Platelet count*	428 (313, 706)	182	482 (320, 595)	15	413 (290, 691)	76	438 (326, 753)	91
MCV (fl)	54.4 (8.4)	183	54.4 (8.4)	15	53.9 (8.4)	76	54.5 (8.3)	92
Ferritin (μg/L)*	2.0 (1.0, 3.9)	157	2.0 (2.0, 3.0)	13	1.45 (1.0, 3.0)	68	1.9 (1.0, 5.7)	76
Iron (mg/L)*	2.15 (1.1, 3.0)	112	2.0 (1.9, 3.0)	11	2.3 (1.4, 3.1)	45	2.0 (1.0, 3.0)	56
Albumin (g/L)*	25.0 (20.0, 33.0)	112	29.5 (23.5, 34.8)	10	25.0 (22.0, 46.0)	48	24.0 (19.0, 32.8)	54

* values given in median and IQR.

** 4 patients also received IV iron

4.3.1 Pairwise and Multivariate Associations

The results from pairwise correlations are presented using correlation heatmaps (Figure 4.1) and pairwise scatter plots (B.1, B.2). The results show that within the set of clinical measurements, diastolic and systolic blood pressure as well as respiratory rate and heart rate were moderately correlated (r = 0.507 and r = 0.465, respectively), while the other pairings had either weak or no correlation. Nevertheless, diastolic blood pressure has significant missing data (n=31, 17%) and this might have underestimated the correlation, which is known to be much higher than 0.51 [139]. For the laboratory measures, we found that RBC and hemoglobin were strongly correlated (r = 0.768) as expected, while MCV and hemoglobin were moderately correlated (r = 0.432). All other pairings were either weakly or not significantly correlated.

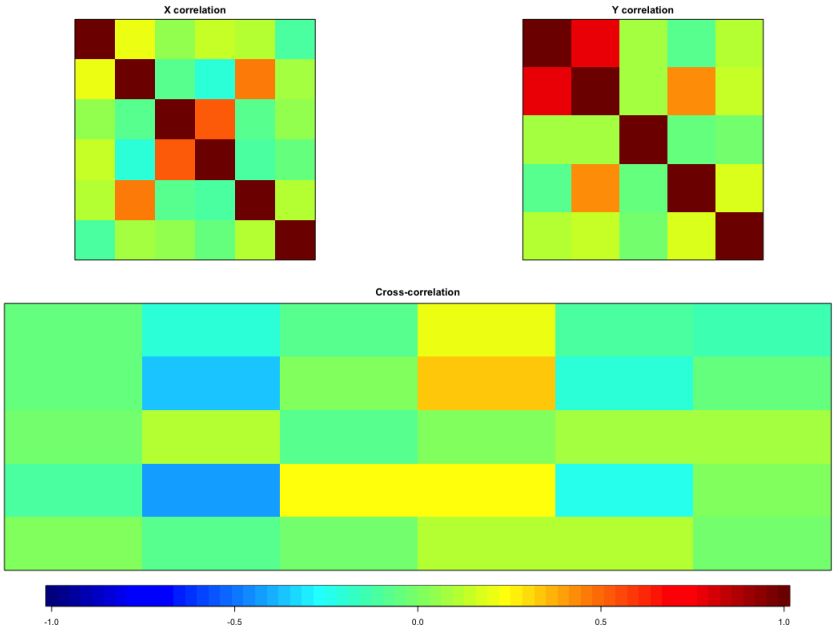

Figure 4.1: Heatmap of the correlations within and between the clinical and laboratory measures.

The results from the pairwise cross-correlations between the two groups of variables show that weak to moderate correlations (Figure 4.1, Table 4.2), where the 3 largest correlations observed were between diastolic blood pressure and each of RBC, hemoglobin and platelet count (r = 0.35, r = 0.29, and r = 0.24, respectively). These results further reinforced our notion that CCA was required to uncover multivariate relationships between these two sets of variables.

Table 4.2: Pairwise cross-correlations between the clinical variables and laboratory measurements.

		Clinical Measures					
		Temperature	Heart Rate	Systolic Blood Pressure	Diastolic Blood Pressure	Respiratory Rate	Oxygen Saturation
Laboratory Measures	RBC	-0.01	-0.14	0.07	0.35	-0.03	0.07
	Hemoglobin	0.02	-0.12	0.09	0.29	0.02	0.04
	Platelet	-0.02	-0.08	0.18	0.24	-0.09	0.06
	MCV	-0.03	-0.15	0.07	0.01	0.01	-0.13
	Serum Ferritin	-0.08	-0.11	-0.08	0.06	-0.01	0.00

4.3.2 Canonical Correlation Analysis

The results from the canonical correlation analysis showed a relatively larger correlation (ρ=0.5538) than the largest pairwise cross-correlation we saw earlier (which was 0.35). The correlations between the second and third canonical variates were 0.3706 and 0.1938, respectfully.

The canonical loadings of the first canonical variate, stratified by clinical and laboratory variables, are presented in Figure 4.2. The results showed that, among clinical markers of IDA, temperature was by far the most influential variable in the dataset, followed by diastolic blood pressure and heart rate, although it was quite clear that the importance of these two variables paled in comparison to temperature. For the laboratory markers of IDA, we found that RBC was the most influential variable, again by steep margins. We saw that MCV and hemoglobin followed RBC, but neither were remotely as important as RBC to the first canonical variate.

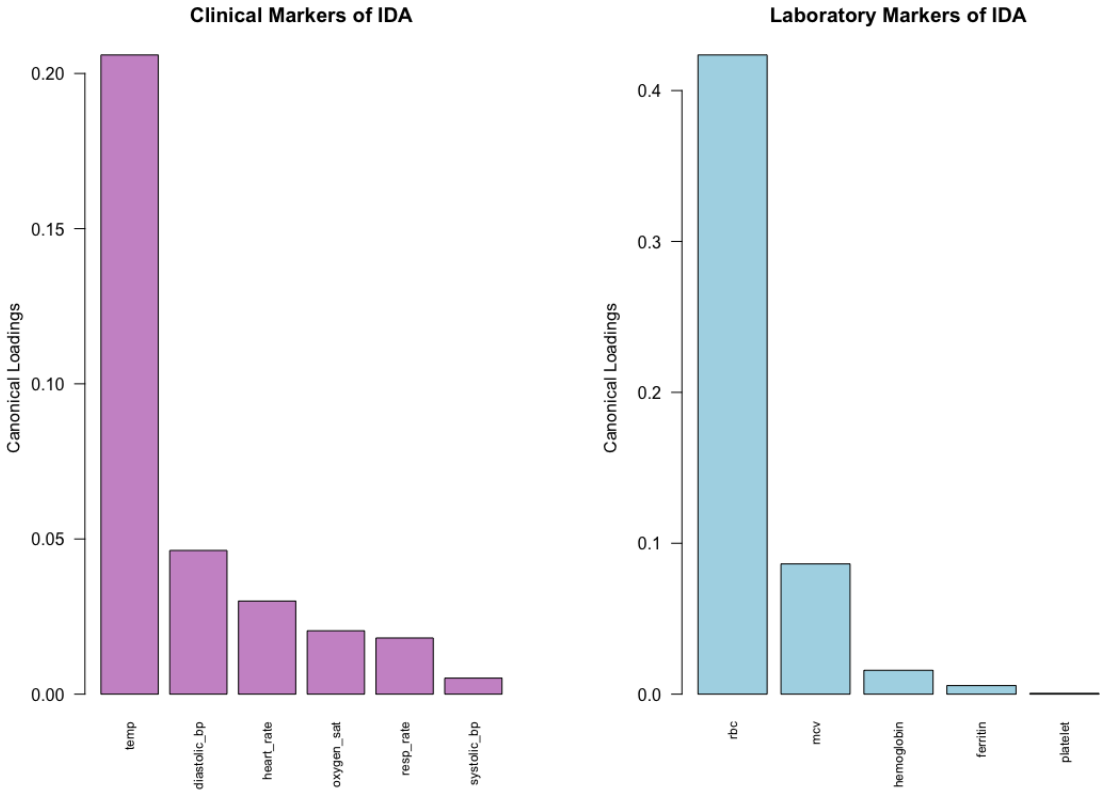

Figure 4.2: Bar plots of the canonical loadings for the clinical and laboratory markers of IDA stemming from the first canonical variate.

From the CCA (Figure 4.2), we found that temperature and RBC were the leading variables responsible for the relationship between the laboratory and clinical indicators of IDA, even though the pairwise correlation between these two variables was nearly nil (r = -0.01). We had hypothesized that heart rate and hemoglobin would be moderately to strongly correlated in children with IDA, as a reduction in hemoglobin would seemingly imply that the heart would need to pump the blood faster around the body to meet the oxygenation needs of the tissues. This did not end up being the case in this analysis, as hemoglobin and heart rate were very weakly correlated (r = 0.12) and neither contributed greatly to the association between the clinical and laboratory measures of IDA.

4.3.3 Logistic Regression

We had 72 patients who received exclusively transfusion, 15 who received only IV iron, four who received both, and 92 who received oral iron. Because of the sparseness of the data, we used a binary dependent variable (transfusion vs no-transfusion), where oral iron and IV iron are coded as no-transfusion. The 4 children who received both transfusion and IV iron were removed from the analysis.

We found that albumin, iron, transferrin, and diastolic blood pressure were all missing significant data (>20%). As such, these variables were removed from the analysis. Next, we looked at variables that had significantly low variability and hence were non-informative, and we opted to remove them from the analysis. These variables included oxygen saturation, temperature, RBC, and the weight Z-score (adjusted for age), all of which had very little variability (<1.5). Before fitting the model, we also investigated the presence of multicollinearity, but the results showed little to weak correlation between the independent variables and overall, very low variance inflation factor, indicating that there was no concern of a multicollinearity problem.

The full logistic regression model consisted of the independent variables age, sex, hemoglobin, MCV, heart rate, respiratory rate, platelet count, and SBP. The adjusted and unadjusted ORs with the corresponding 95% confidence intervals (CIs) and p-values are provided in Table 4.3. The results showed that hemoglobin and heart rate were statistically significant to the one-to-one models, while hemoglobin, age, and MCV were significant in the full model. For hemoglobin, we found in both models that as hemoglobin increases, the odds of receiving a transfusion for IDA decreases ($OR_{unadjusted} = 0.90$, $OR_{adjusted} = 0.87$). From the unadjusted model, this was interpreted as every 1 g/L increase in hemoglobin diminishes a patients odds of receiving a transfusion for IDA by 10%. In the case of heart rate, we saw that as tachycardia (high heart rate) increases in severity, the odds of receiving a transfusion for IDA increase slightly ($OR_{crude} = 1.02$). We noted that the results for

sex, respiratory rate, and systolic blood pressure were insignificant in both models, as 1 was contained within their respective 95% CIs.

Table 4.3: Unadjusted and adjusted odds ratios for the full logistic regression model.

	Unadjusted		**Adjusted**	
Variable	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Age	1.00 (0.99, 1.00)	0.6530	1.02 (1.00, 1.03)	0.0255
Sex (male)	0.88 (0.46, 1.70)	0.7120	1.80 (0.73, 4.41)	0.2018
Hemoglobin	0.90 (0.87, 0.93)	< 0.0001	0.87 (0.83, 0.91)	< 0.0001
MCV	0.98 (0.95, 1.02)	0.3130	1.07 (1.00, 1.15)	0.0370
Heart Rate	1.02 (1.00, 1.03)	0.0187	1.02 (0.99, 1.04)	0.1580
Respiratory Rate	1.03 (0.98, 1.07)	0.2053	1.03 (0.97, 1.01)	0.3206
Platelet Count	1.00 (1.00, 1.00)	0.2120	1.00 (1.00, 1.00)	0.8060
Systolic blood pressure	1.01 (0.99, 1.03)	0.4280	0.99 (0.96, 1.02)	0.4029

We also examined the probability of a patient receiving a transfusion (based on the full model) with respect to some of the clinically relevant covariates in the full model (Figures 4.3, 4.4, and 4.5). The results showed that hemoglobin had a strong negative relationship with the probability of receiving a transfusion, while heart rate only had a weak association, where a slight increase in the probability of transfusion was observed with increasing heart rate. Of the remaining variables; age, MCV, respiratory rate and systolic blood pressure had little to no impact on the probability of receiving a transfusion.

Next, we fit a reduced model with four variables (sex, age, hemoglobin and heart rate), where the variables were selected with respect to clinical relevance and their contributions in the full model. The results (Table 4.4) showed that sex and hemoglobin were the most indicative of treatment outcome (OR = 1.44 and OR = 0.88, respectively), although the estimate for sex was deemed insignificant as 1 was contained within the 95% CI. For hemoglobin, the OR=0.88 indicates a 14% increase (1/0.88=1.14) in the odds of receiving transfusion for every 1 g/L decrease in hemoglobin level. For a more clinically meaningful decrease, say a 10 g/L decrease in hemoglobin level, the odds of receiving transfusion nearly double (i.e. OR=3.59 for a 10 g/L decrease in hemoglobin). Age and heart rate were again found to be not indicative of treatment outcome (OR = 1.02 and OR = 1.01). Together with the unadjusted ORs, we concluded that hemoglobin was the only variable that is consistently indicative of a patient receiving a transfusion for IDA. This meant that, based on the adjusted estimated estimate, with all other covariates held fixed, every

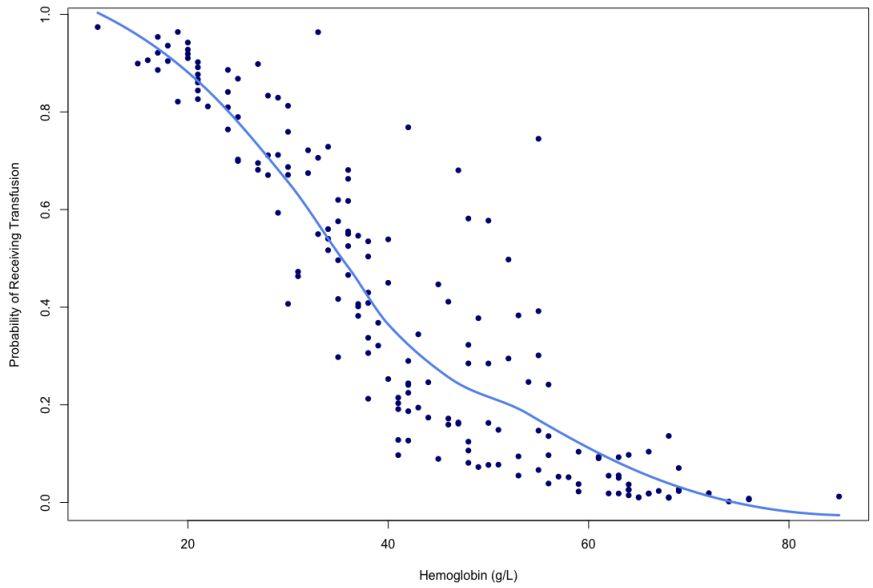

Figure 4.3: Probability of receiving a transfusion versus no transfusion for severe IDA based on a patient's hemoglobin level.

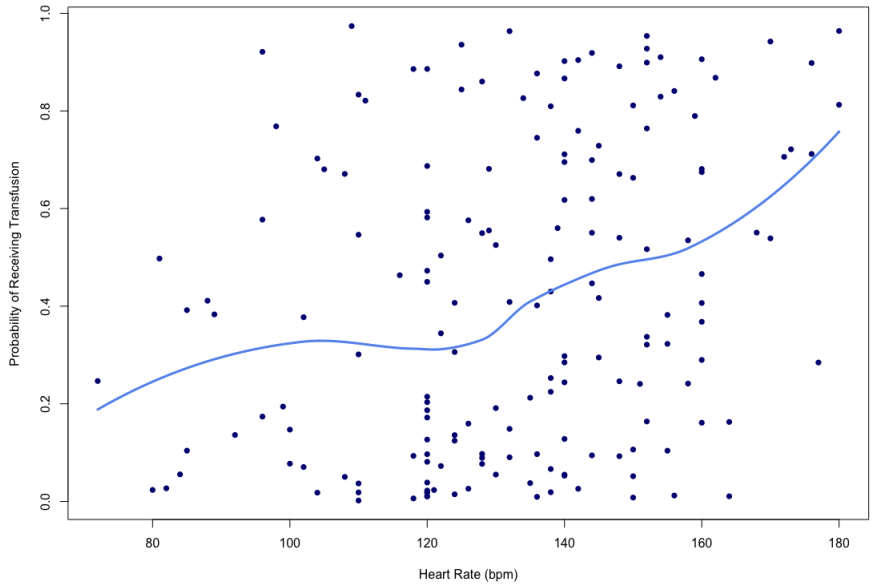

Figure 4.4: Probability of receiving a transfusion versus no transfusion for severe IDA based on a patient's heart rate.

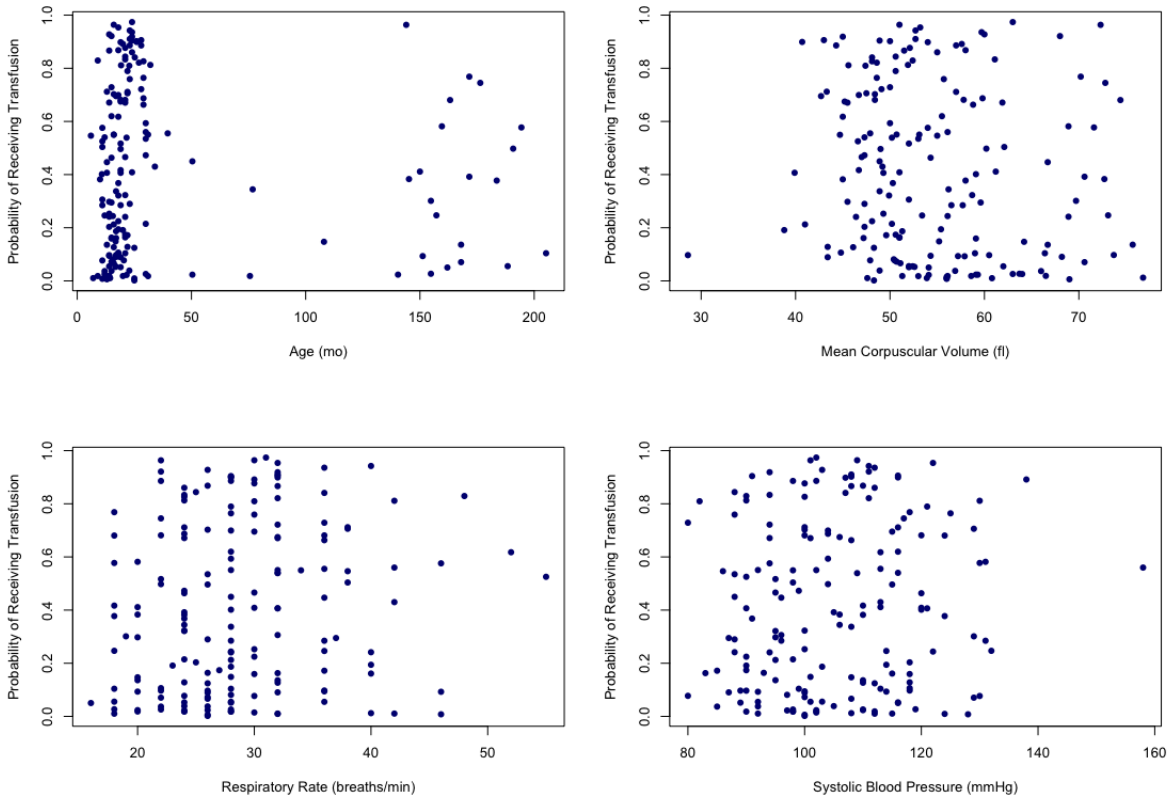

Figure 4.5: Probability of receiving a transfusion versus no transfusion for severe IDA based on a patient's (clockwise from top left) age, MCV, respiratory rate and systolic blood pressure.

1 g/L increase in hemoglobin translated to a decrease of 12% in the odds of receiving a transfusion for IDA.

Table 4.4: Unadjusted and adjusted odds ratios for the reduced logistic regression model.

	Unadjusted		**Adjusted**	
Variable	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Age	1.00 (0.99, 1.00)	0.653	1.02 (1.01, 1.03)	0.0024
Heart Rate	1.02 (1.00, 1.03)	0.0187	1.01 (0.99, 1.04)	0.2264
Hemoglobin	0.90 (0.87, 0.93)	< 0.0001	0.88 (0.85, 0.92)	< 0.0001
Sex (male)	0.88 (0.46, 1.70)	0.712	1.44 (0.62, 3.38)	0.3968

We then tested the final reduced model for goodness of fit, using the Hosmer-Lemeshow test, and found insignificant evidence of lack of fit ($\chi^2 = 11.33$, p-value $= 0.1839$). However, we were unable to trust the results of this analysis due to the sparsity seen in specifically the age variable, implying that the results we have obtained may be unreliable.

4.4 Discussion

Throughout the numerous analyses seen in this section, it was simple to identify hemoglobin as the sole variable truly indicative of the clinician's decision to treat children with severe IDA with a transfusion. We observed that, as hemoglobin levels decreased, the odds of receiving transfusion increased significantly. In fact, the results showed that the OR estimates for hemoglobin were stable, regardless of which variables were included in the model, and the probability of receiving a transfusion were not impacted that much by the other variables in the model, including heart rate and other vital signs. This indicated that very low levels of hemoglobin are perhaps the only factor that might have influenced the clinician's decision to order blood transfusion. We also observed that females were at much lower odds of receiving a transfusion than males, with all other covariates equal. However, this might be due to other differences not included in the data. Additional studies are required to investigate this further.

Our results showed that heart rate and age were not particularly indicative of transfusion as a course of treatment for children with severe IDA. These results were unexpected, as we had hypothesized that heart rate and other vital signs, as well as other relevant clinical presentations, would influence the clinician's decision to order a blood transfusion. We also hypothesized that age would play more of role, as IDA peaks in early childhood [140]. A possible reason for this lack of significance for age could be the sparseness of the data for ages over 36 months. The data used to fit the final reduced model included mostly children between the ages of 6 and 36 months (n=150, 84%), with few adolescents and older children (n=29, 16%). Further investigation using more older children is needed to determine whether transfusion to treat IDA is used differently in older children than those younger than 36 months.

Little is known regarding associations between IDA-related laboratory measurements, such as hemoglobin, and clinical markers in pediatric patients with severe IDA. However, several studies have looked at the effect of treatment for IDA with respect to clinical markers. In 1999, Hayashi, Ogawa et al published on the cardiovascular function in children with IDA before and after undergoing 4-10 weeks of oral iron supplementation [141]. They found that initial hemoglobin concentrations of less than 60 g/L were associated with circulatory congestion and

ventricular dysfunction, although they did not assess whether such factors were associated with known markers of IDA [141]. A 1993 study by Jayabose, Ruddy and Ciavarella studied the effects of transfusion on heart rate in children with severe IDA and found that the average heart rate decreased by 28% after receiving a transfusion of RBCs [117]. They did not, however, use heart rate to diagnose IDA, nor did they study the association between hemoglobin levels and heart rate. A 2020 study by Hamed et al found that children with moderate to severe IDA had higher resting heart rates than their healthy, age-matched counterparts [32]. Our results showed a weak association between heartrate and hemoglobin, which is the primary marker used to determine severity of IDA. However, our results from the CCA showed moderate associations between clinical presentations (including vital signs) and IDA-related laboratory markers. Nevertheless, further analysis using larger and more representative data is required, including data from healthy children as well as children with mild to moderate cases of IDA to confirm these results.

Recent studies have also sought to build predictive models to identify cut-off points for common indicators of IDA to predict when blood transfusion would be necessary to treat severe IDA. The 2023 study by Sun et al. sought to identify a threshold hemoglobin value that indicated the need for transfusion in children with severe IDA [138]. They determined that a hemoglobin level of less than 39 g/L indicated the need for transfusion with a sensitivity of 92% and a specificity of 72% [138]. The results of the present study support this finding, as children with severe IDA become increasingly at higher odds for receiving a transfusion as their hemoglobin levels decrease. We saw that a 5 g/L decrease in hemoglobin was associated with nearly double the odds of requiring a transfusion for severe IDA, confirming that hemoglobin is an excellent indicator of treatment outcome.

This study provided insights into the key clinical and laboratory markers that are known indicators of IDA. Our results provided overwhelmingly strong evidence that hemoglobin is the most informative indicator of a clinician's decision to use blood transfusion as a course of treatment for severe IDA, whereas other laboratory and clinical variables were not informative. This study adds to the existing literature and ongoing scientific discussions regarding use of blood transfusion, and existing recommendations on using transfusion conservatively and instead resorting to less invasive approaches to treatment, such as IV iron. The results of this study can be used to inform practicing clinicians on the main clinical and laboratory indicators of IDA and how they may have influenced treatment choice in two Canadian hospitals.

Chapter 5

Summary and Future Directions

5.1 Summary and Conclusions

This thesis covered both the topics of biomarkers of IDA and its clinical presentations in pediatric populations, as well as the interventions used to treat pediatric IDA. Our systematic review provided insights into the existing literature related to current clinical practices in pediatric IDA care and revealed the status of research on pediatric IDA of all severities. Our analysis, using data from two major Canadian pediatric hospitals, examined the current practice in Canada, and evaluated associations between various clinical presentations, vital measures, and laboratory biomarkers of IDA in severely ill children. These two studies set the groundwork for further comprehensive research in the field and have the potential to provide recommendations and guideline development, which can, in turn, impact clinical practice in treating pediatric IDA.

Our systematic review showed that oral iron supplementation was, by far, the most prevalent treatment for pediatric IDA and is the most researched treatment option, even when only non-randomized studies are considered. In children with severe IDA, we found that more intensive interventions, such as IV iron therapy and blood transfusion, were required to treat the patient. However, oral iron is still the most prevalent treatment option, including in hospital settings. This was consistent with our results from the analysis of data from two Canadian pediatric hospitals, where oral iron is one of the most commonly used interventions to treat pediatric IDA in hospital settings. We found that 50% of the children in our dataset were given oral iron, while 8% received IV iron and 42% received one or more transfusions to treat severe IDA. This differed from the results of our systematic review, where the distribution of treatment was more uniform in this population. This suggests that, in hospital settings in Canada, IV iron is not as commonly used as it is globally. Our systematic review also revealed that, in younger children, aged 6 to 36 months, nearly all patients received oral iron to treat IDA, which we posited was due to the fact that

oral iron is less intensive than IV iron or blood transfusion and is thus easier to give to a young child who is not critically ill. This is consistent with what we found from our analysis of data from Canadian hospitals.

The results from our meta-analysis of existing evidence showed that IV iron and oral iron were both effective in increasing hemoglobin levels of children with IDA of all severities. The results indicated that IV iron was slightly more effective than oral iron in the general pediatric population with IDA, however oral iron was marginally more effective than IV iron in children with severe IDA. Nevertheless, there were a limited number of studies involving severely ill children and the results should be interpreted cautiously. There were no quantitative effectiveness data on blood transfusion, hence we were not able to compare effectiveness of blood transfusion with that of oral and IV iron. We would also like to highlight that IV iron and transfusion are not treatment options that can be studied using randomized studies. As such, there is a need for comprehensive retrospective studies aimed at investigating patient outcomes for pediatric patients with IDA treated with these three main interventions.

Regarding safety of IDA treatments, we found that all three treatment options were relatively low risk, where the safety outcome used was the percentage of children with adverse reactions to treatment. The results showed that oral iron was associated with the highest proportion of adverse reactions amongst the three groups. These results were stable within our subgroup analyses of young children and children with severe IDA. We posited that oral iron was associated with higher risk of adverse reactions as the reactions stemming from this form of intervention are generally very mild, such as rash or gastrointestinal issues. This contrasts greatly with the potentially serious adverse reactions stemming from IV iron or blood transfusion, implying that patients are probably more likely to report small reactions from oral iron than severe reactions from IV iron or transfusion.

Our pairwise and multivariate analyses of the clinical and laboratory measures of pediatric IDA demonstrated that there are little to weak pairwise correlations within and between the different groups of variables. This result provided evidence against our initial hypothesis that some of the vital signs, in particular heart rate, are strongly associated with laboratory markers of pediatric IDA such as hemoglobin. However, the results from the canonical correlation analysis uncovered a moderate correlation between clinical and laboratory measurements, where temperature and RBC count were the most influential variables in this association. To a lesser extent, we found that diastolic blood pressure, heart rate, MCV and hemoglobin were also contributing to the association between these two sets of variables. However, we would like to emphasize that we only considered linear relationships, and further investigation is needed to explore if there are non-linear relationships between vital signs and laboratory measurements, in particular heart rate and hemoglobin. This will allow us to confirm or reject the current hypothesis that these two groups of

variables are highly correlated, hence treatment choices might have been influenced by clinical presentations and not solely on hemoglobin levels of children.

In fact, the results from the logistic regression model showed that hemoglobin indeed seems to be the only variable that is highly associated with a clinician's choice related to whether or not to provide blood transfusion. Our results showed this finding was stable, regardless of the model choice and the number and type of variables entering the model. From the various logistic regression models that we fit, we saw a stable estimate of high odds ratio (OR) with respect to hemoglobin, where children with very low hemoglobin levels were highly likely to get transfusion. There was no variability in the predicted probability of a child receiving transfusion for low and high level of hemoglobin. However, we observed some variability in between, where for instance two children with similar hemoglobin levels received different treatments (transfusion vs oral iron). This may be because of other differences including sex, where being a female was shown to be associated with lower probability of transfusion. Other factors that might have caused the heterogeneity in transfusion probability include heart rate and age. However, additional data with a wider age range would be needed to confirm or refute these findings.

Our studies faced several limitations. The systematic review, although it retained eighty-four articles, did not have adequate data to make comparative effectiveness evaluations of blood transfusion, compared to oral and IV iron, and as such, we were unable to conduct three-way comparisons as we originally planned in our objectives. Moreover, our systematic review included all children below eighteen years of age, however the mean age in each treatment category was around five years old, indicating that the population was heavily skewed toward younger ages. To understand the full scope of IDA throughout childhood, it would have been beneficial to have more data on older children and adolescents. Nevertheless, it is known that IDA is more prevalent in younger children, thus explaining why the retained studies focused more on this patient population.

Another limitation of our systematic review was the fact that IDA was defined differently among the included studies, with four studies not aligning their diagnostic criteria with that of the WHO. We overcame this limitation by performing a sensitivity analysis on the subset of studies that did follow the WHO criteria and found that the effectiveness measure for oral iron was indeed affected by these studies using alternative definitions of IDA.

Although not a limiting factor, we would like to highlight that our review focused on only IDA caused by environmental and nutritional factors. As such, we excluded studies related to different forms of anemia, such as aplastic anemia, sickle cell anemia and thalassemia. We excluded these patients on the basis that there were underlying reasons for their anemia, rather than controllable factors such as excessive intake of cow's milk and other nutritional causes, which is a common cause of pediatric IDA

and was the objective of our study. As such, our findings may not be generalizable to pediatric anemia caused by factors other than nutritional deficiencies.

For our study evaluating current practice in Canadian pediatric hospitals, we had a relatively small sample size (n = 183), which might have contributed to low statistical power. However, the prevalence of severe IDA in Canada is very low, hence this was a good sample size for such a study. Data was originally collected for different purposes, thus did not contain any patient outcomes post-intervention, and as such, we were not able to perform comparative effectiveness or safety of the different treatment options. A follow-up study is needed to collect important patient outcomes to study if different treatment options led to different outcomes and allow clinicians to use not only clinical presentation upon hospital admission, but also evidence-informed treatment choices that incorporate patient outcomes.

5.2 Discussion and Future Directions

The studies in this thesis and the corresponding findings will contribute to the growing discussion within the field of pediatric hematology, in particular, discussions related to the diagnosis, treatment, and management of IDA in children. To our knowledge, there existed neither a study that synthesized the current evidence pertaining to the treatments of pediatric IDA, nor a study examining the relationship between clinical presentations of IDA and known laboratory biomarkers. Our studies individually drew on existing literature on the topic of pediatric IDA, while actively filling the knowledge gaps to better inform evidence-based recommendations and clinical practice. Furthermore, our systematic review is the first review that provided a quantitative synthesis of, not only prevalence, but also effectiveness and safety. Our study also provided an in-depth look at the heterogeneity across the different studies, by determining quantitative estimates of heterogeneity, and identifying and elucidating potential sources of heterogeneity. When direct meta-analysis was not possible, we used novel indirect approaches to compare the interventions with respect to their prevalence and effectiveness. Our systematic review is also the first of its kind to collect and synthesize data on quality of evidence and risk of bias in studies involving pediatric IDA.

Our systematic review joins many other reviews that have brought together evidence on the guidelines pertaining to the treatment of IDA, mostly in the adult population. None of these studies compared the three treatment types for prevalence, effectiveness, or safety, which was the gap in knowledge that our review sought to fill. Numan and Kaluza (2020), for instance, studied the guidelines of using IV iron for the treatment of IDA, although their study did not focus on the pediatric population [112]. The USPSTF performed a similar review, wherein they examined the current guidelines for prescribing oral iron supplementation to children aged six to

24 months diagnosed with IDA, however the results were varied [125]. Peyrin-Biroulet et al (2015) identified three guidelines concerning children in their systematic review of guidelines pertaining to the treatment and diagnosis of IDA worldwide [111]. They found that oral iron is often cited as the primary course of treatment for IDA, whereas IV iron may be used in more severe cases of IDA or in the case of intolerance to oral iron [111]. This is in agreement with the findings from our systematic review.

Our study involving the clinical and laboratory measures of IDA was unique in that it examined both the relationship between these two sets of variables, as well as their association with treatment choice. Prior to our study, there existed both current and outdated evidence linking some clinical and laboratory measures of IDA. A 1972 publication by Varat and colleagues found that cardiac output (quantity of blood pumped by the heart per minute) at rest was elevated in patients with anemia [31,142], although their study did not focus on children with IDA. A more recent study by Hamed et al (2020) studied the effects of IDA on heart rate and other autonomic functions in children and found that children with IDA had significantly higher heart rates than healthy children, but a three-month course of oral iron helped to reduce these elevated heart rates [32]. These results are consistent with a similar study performed by Hayashi, Ogawa et al in 1999 [141]. This was also observed in our data, although the associations between heart rate and hemoglobin were moderate, and that heart rate and any other vital signs are not the leading factors influencing treatment choice. Nevertheless, our data was limited to two Canadian pediatric hospitals, and as such, a larger multicenter study is needed to make generalizations of our findings. Although there existed literature on the subject of clinical manifestations of IDA, no study sought to understand how these variables could be used to diagnose IDA, how they were connected with laboratory indicators of IDA, or how they influenced treatment choice. We believe that our study filled that knowledge gap.

We have identified and presented some of the limitations that could be addressed in subsequent studies. We have also highlighted follow-up studies that can broaden the scope or allow generalizability of our findings. Considering the high prevalence of IDA in rural and remote Indigenous communities in Canada, a possible future research area could be to study current practice in treating pediatric IDA in these communities, in a culturally sensitive manner. Indigenous communities are often overlooked or underfunded when it comes to interventions in healthcare, which leads to gross inequality in the care that children receive for usually very treatable disorders, such as IDA. To ensure that the full scope of the issue is examined, it would be necessary to look at not only the clinical indicators of IDA and treatment choices within these communities, but also the socioeconomic factors that may lead an Indigenous community to have high rates of pediatric IDA. Common socioeconomic disparities within remote and rural Indigenous communities that are well-documented, include significantly higher food prices for fresh produce and meats (leading to food

insecurity), as well as lack of access to adequate health care services and reduced infrastructure capacity, which can cause a barrier to Telehealth options for health care [143–145]. We believe such a study would be prudent, as the incidence of pediatric IDA within Indigenous communities is much higher than the incidence rate across Canada, indicating a major disparity in healthcare policies and programs that work to prevent and treat IDA in children [6–8].

Another potential future research topic is to look into the effects of chronic eating disorders in adolescents and its relations to IDA, such as anorexia nervosa and bulimia nervosa. Such eating disorders often include the restriction of food intake or the rapid expulsion of eaten food through the use of purging techniques or laxatives [146]. Given that there is considerable evidence indicating an exponential increase in the prevalence of eating disorders among teens due to the COVID-19 pandemic, it would be interesting to determine whether such disorders also contributed to an increase in adolescent IDA, and whether one treatment option is more suitable for treating IDA in these patient populations [147–149]. There currently exists limited literature on the topic of anemia in patients with eating disorders.

Lastly, there is a knowledge gap related to IDA caused by heavy menstrual bleeding (menorrhagia) in adolescent females. Given that recent estimates have put the prevalence of menorrhagia in adolescent females at up to 37%, we believe a study aimed at investigating the prevalence, clinical presentations and treatment options of IDA for this subpopulation is needed [150]. Our systematic review did not reveal any articles studying the effects of treatment on IDA due to menorrhagia, although such articles would have met our inclusion criteria. A 2018 study by Mishra et al found that IV ferric carboxymaltose was effective in treating IDA in adult women with menorrhagia [151]. We believe that a similar study in an adolescent population would benefit the pediatric hematology community, as no evidence is currently available on how to best treat IDA in adolescents concurrently experiencing menorrhagia.

Appendix A

Appendix for Chapter 3

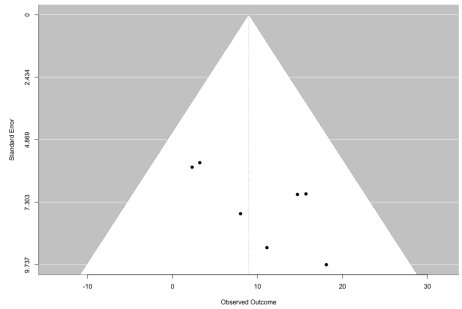

Figure A.1: Funnel plot of RCTs comparing oral iron to placebo.

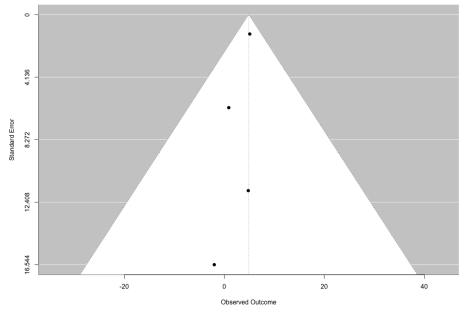

Figure A.2: Funnel plot of RCTs comparing daily versus weekly administration of oral iron.

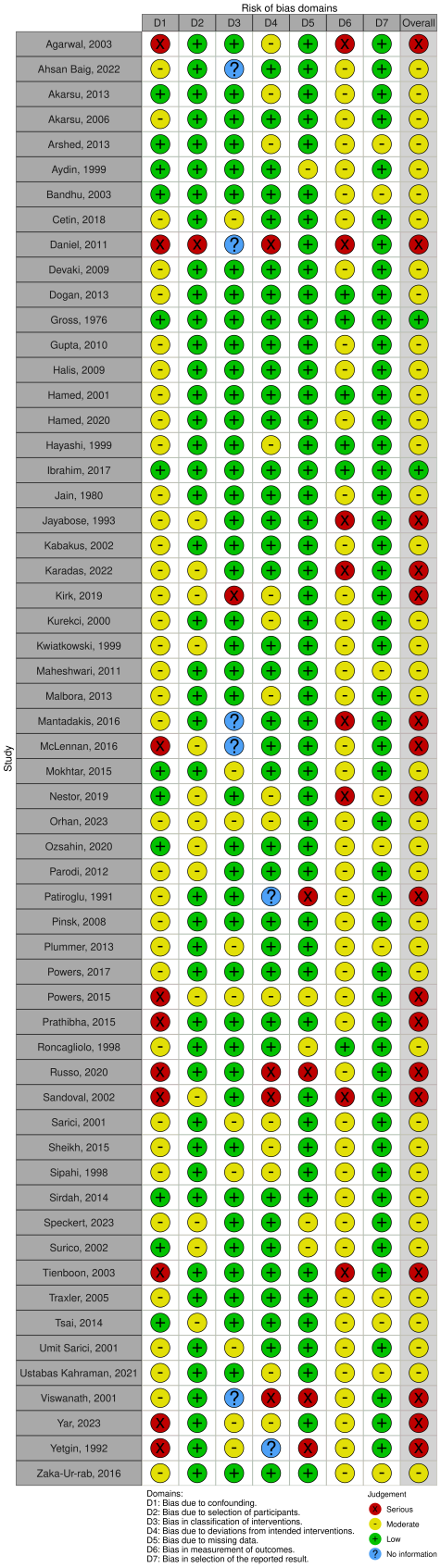

Figure A.3: Individual study RoB assessment using the ROBINS-I tool

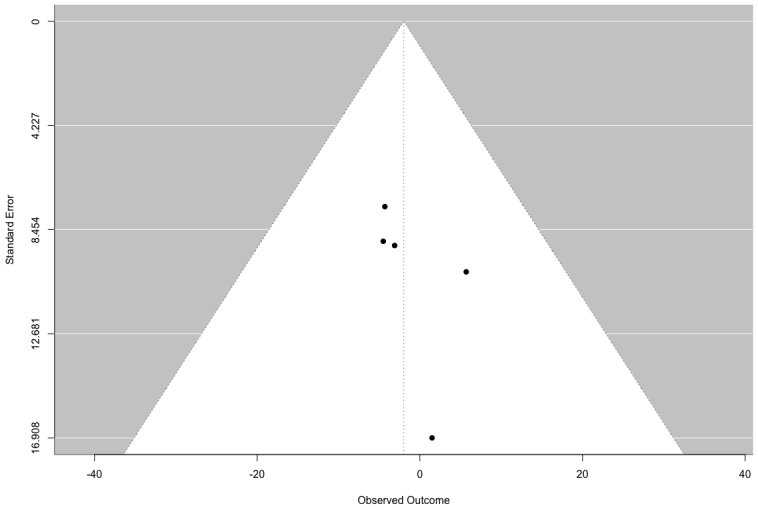

Figure A.4: Funnel plot of RCTs comparing oral iron to oral iron given with supplements.

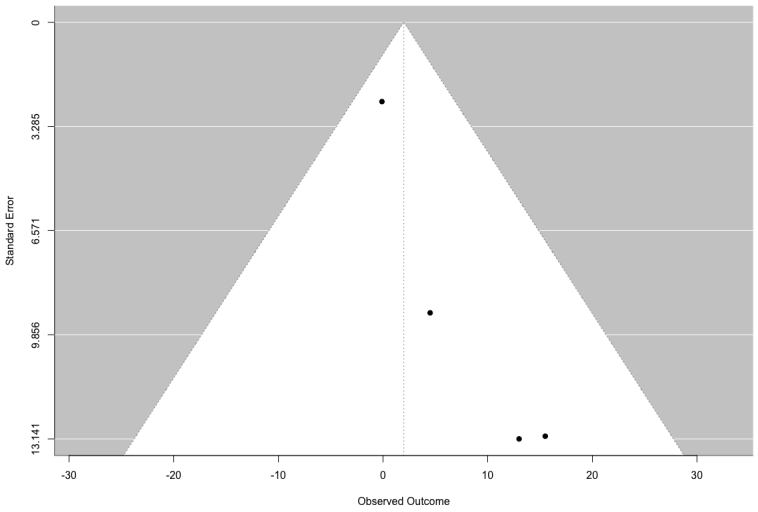

Figure A.5: Funnel plot of RCTs comparing iron polymaltose complex with alternative formulations of oral iron.

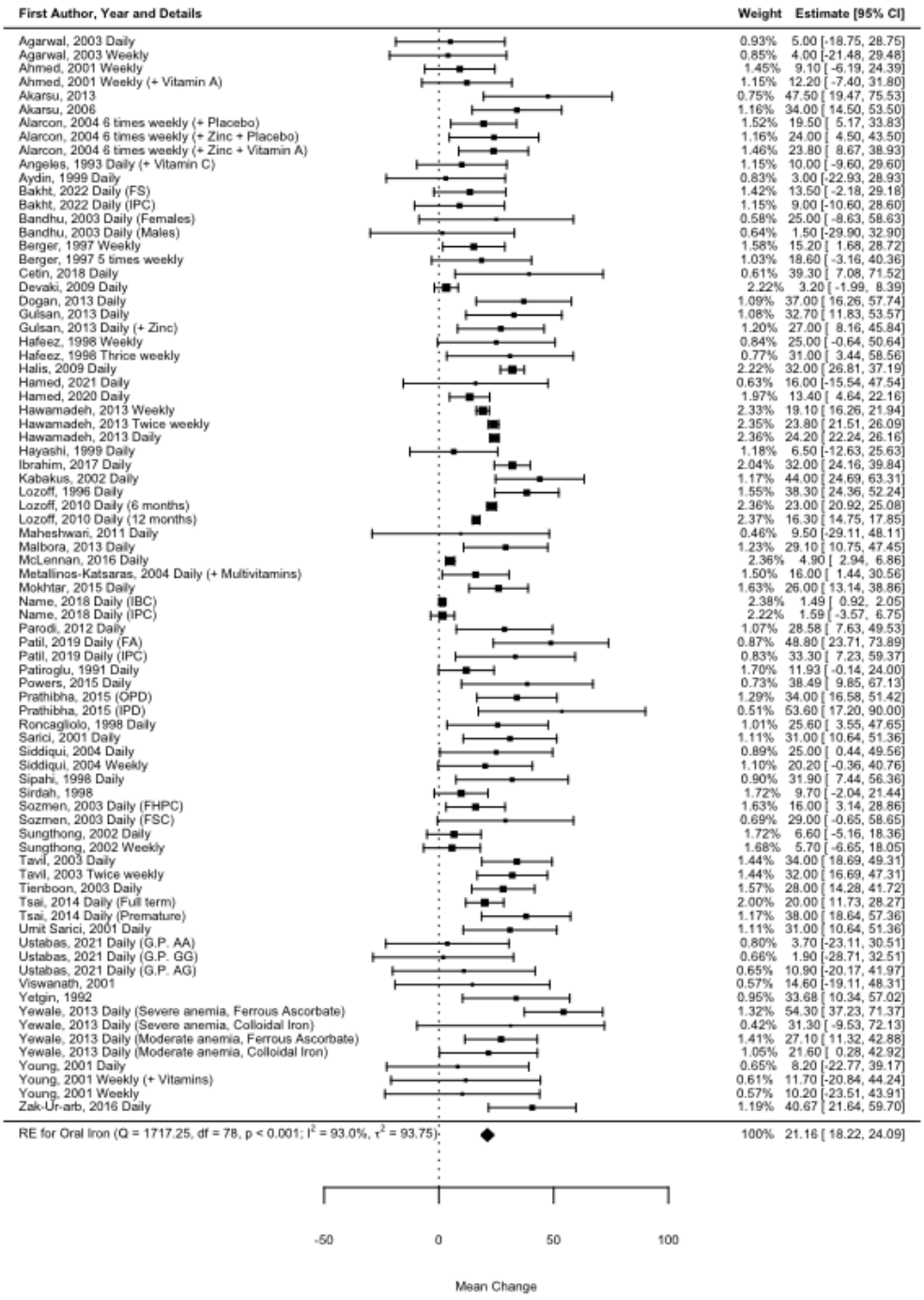

Figure A.6: Results of random effects meta-analysis to pool mean hemoglobin change from baseline after use of oral iron to treat IDA in children using both RCTs and observational studies.

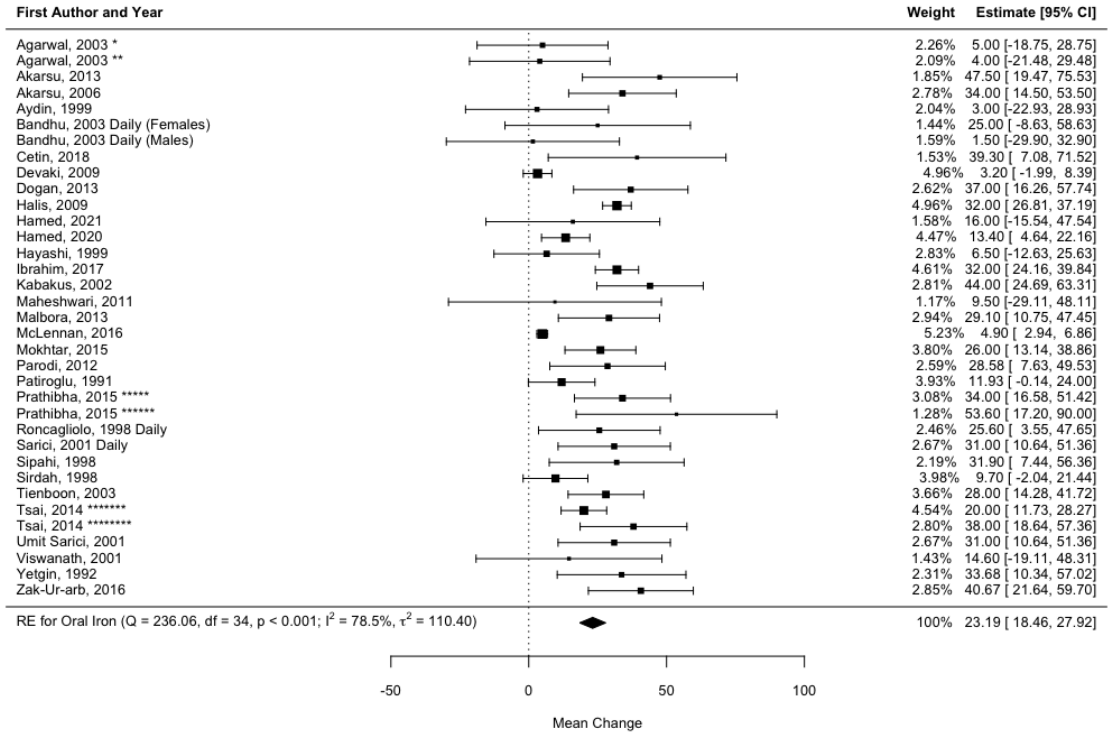

Figure A.7: Results of random effects meta-analysis to pool mean hemoglobin change from baseline after use of oral iron to treat IDA in children for studies following the WHO definition of IDA.

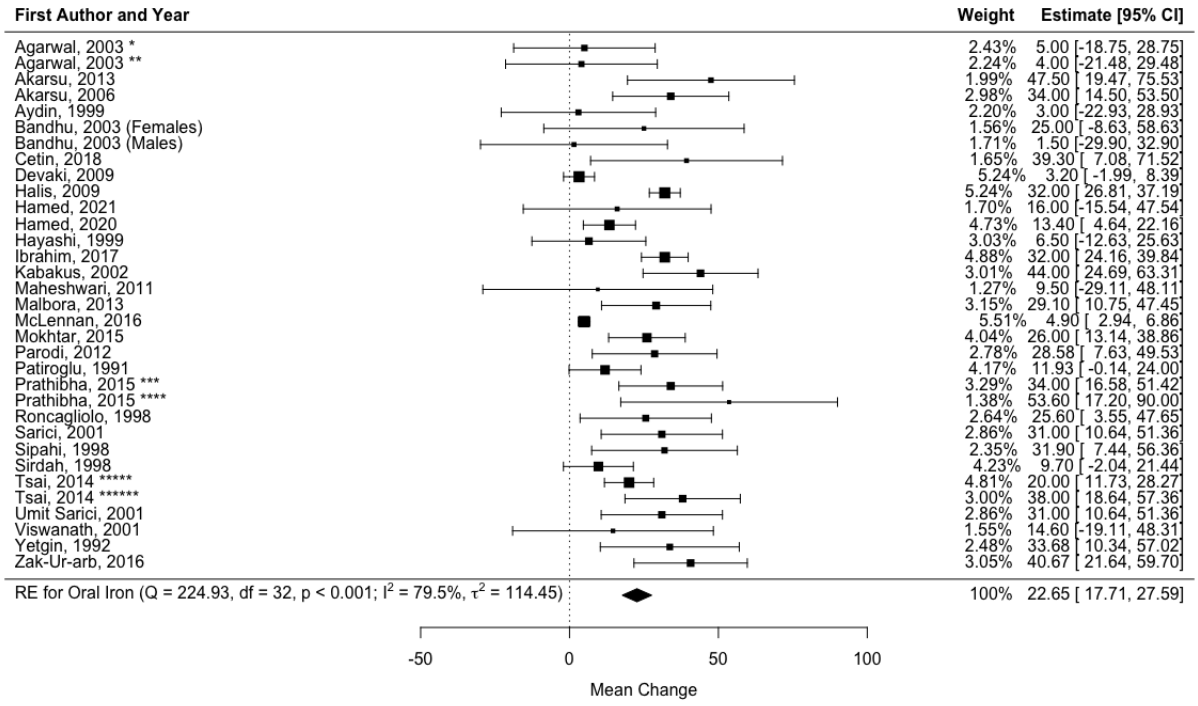

Figure A.8: Results of random effects meta-analysis to pool mean hemoglobin change from baseline after use of oral iron to treat IDA in children for studies requiring minimal imputation.

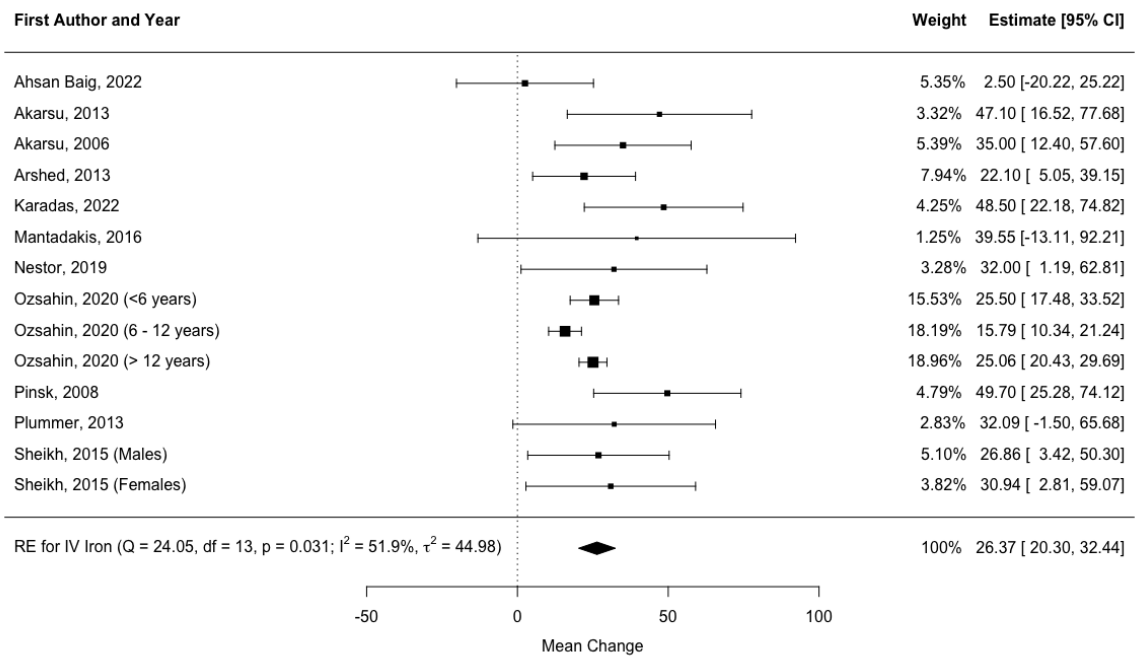

Figure A.9: Results of random effects meta-analysis to pool mean hemoglobin change from baseline after use of IV iron to treat IDA in children for studies requiring minimal imputation.

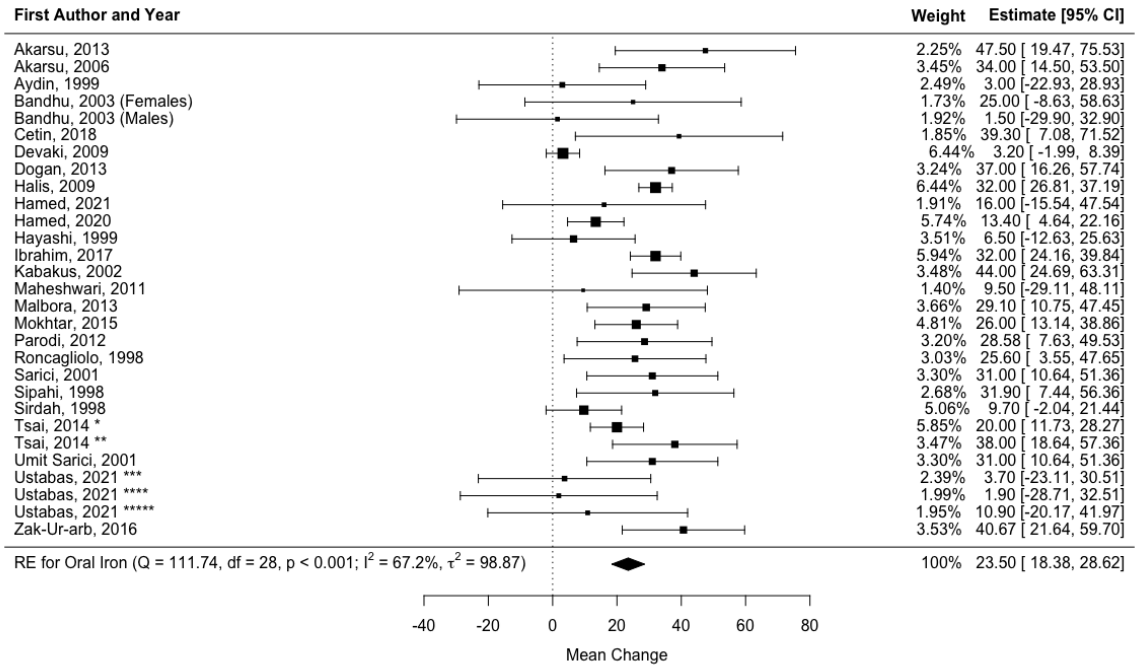

Figure A.10: Results of random effects meta-analysis to pool mean hemoglobin change from baseline after use of oral iron to treat IDA in children for studies with low or moderate risk of bias.

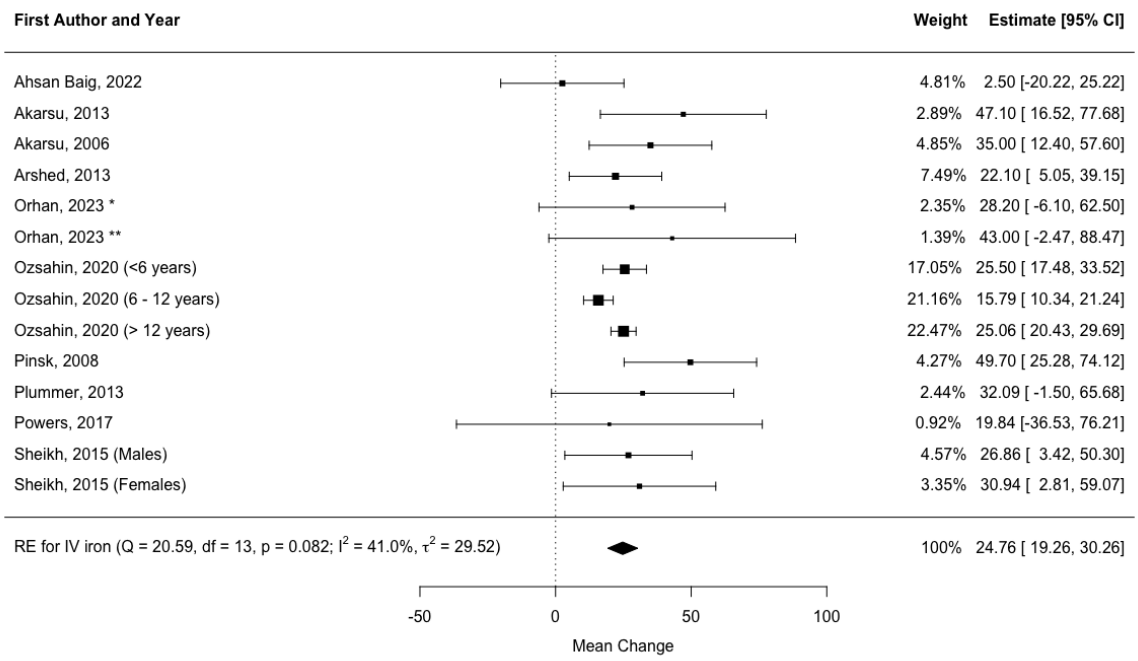

Figure A.11: Results of random effects meta-analysis to pool mean hemoglobin change from baseline after use of IV iron to treat IDA in children for studies with low or moderate risk of bias.

Table A.1: Individual study characteristics

Study ID	Country	**Population age (mean,sd), range (months)**	**Intervention(s)**	**Sample Size (Number of Females)**	**Study Design, Study Setting**	**Risk of Bias Assessment**
Agarwal (2003) [152]	India	120 to 240	Placebo Daily oral iron (100 mg elemental iron and 500 µg of folic acid) Weekly oral iron (100 mg elemental iron and 500 µg of folic acid)	691 (691) 702 (702) 695 (695)	CC Community-Based	S,L,L,M,L,S,L Serious Risk
Ahmed (2001) [153]	Bangladesh	16.2 , 1.5 168 to 228	Placebo Vitamin A (2.42 mg retinol) Oral iron + Folic Acid (120 mg ferrous sulphate + 3.5 mg folic acid) Oral iron + Folic Acid + Vitamin A (120 mg ferrous sulphate + 3.5 mg folic acid+ 2.42 mg retinol)	71 (71) 67 (67) 74 (74) 77 (77)	RCT Community-Based	L,L,L,L,S Some concerns
Ahsan Baig (2022) [154]	Pakistan	23.1 , 10.73 12 to 60	Intravenous iron sucrose (Total dosage of iron = (required Hb - observed Hb) x 80 ml x body weight x 0.034	90 (43)	CS In-Patient	M,L,N,L,L,M,L Moderate Risk
Akarsu (2013) [155]	Turkey	95.9 , 3.07 6 to 186 56.35 , 46.28 6 to 144 104.5 , 78.66 6 to 204 62.6 , 64.42 7 to 180	Placebo Oral Iron (4–6 mg/kg ferrous sulphate) Intramuscular Iron (5.9 , 3.8 mg/kg ferro III hydroxy polymaltose on alternate days) Intravenous Iron (20 mg/mL iron sucrose added into 75–100 mL physiologic saline, and infused in 2 h at a rate of 13.8 , 6.2 mg/kg)	20 (9) 20 (8) 20 (9) 20 (8)	CC Mixed	L,L,L,M,L,M,L Moderate Risk
Akarsu (2006) [156]	Turkey	46.9 , 61.5 8 to 180	Intravenous (iron sucrose std. dose) Oral iron (ferroglycine sulfate dose unknown)	62 40	PCH In-patient	M,L,L,L,L,M,L Moderate Risk
Alarcon (2004) [157]	Peru	17.7 , 7.6 6 to 35 17.2 , 7.9 6 to 35 16.9 , 7.4 6 to 35	Oral iron (3 mg/kg/day ferrous sulphate) Oral iron + zinc (3 mg/kg/day ferrous sulphate + 3 mg/kg/day zinc sulphate) Oral iron + zinc + Vitamin A (3 mg/kg/day ferrous sulphate + 3 mg/kg/day zinc sulphate + 100 000 IU retinol palmitate)	104 109 110	RCT Community-Based	L,L,L,L,S Some concerns
Angeles (1993) [158]	Indonesia	37.4 , 9.5 24 to 60 37.2 , 10.5 24 to 60	Oral iron + Vitamin C (30 mg ferrous sulphate + 20 mg Vitamin C) Vitamin C (20 mg Vitamin C)	39 (19) 37 (18)	RCT Out-Patient	L,L,L,L,L Low Risk

Arshed (2013) [159]	Pakistan	6.75 , 2.69 12 to 120	Intravenous iron sucrose (7 mg/kg given over 2 hours with 1:1 dilution in 250 ml normal saline)	50 (20)	PCH Out-Patient	L,L,L,M,L,M,M Moderate Risk
Aydin (1999) [160]	Turkey	36 pm 15.6 18 to 84 40.8 , 22.8 18 to 96	Oral iron (6 mg/kg/day unknown composition) Control group (no intervention)	56 (26) 60 (34)	CC Out-Patient	L,L,L,L,M,M,L Moderate Risk
Bakht (2022) [161]	Pakistan	107.16 , 23.16 72 to 144 100.2 , 22.56 72 to 144	Oral iron (6 mg/kg/day ferrous sulphate) Oral iron (6 mg/kg/day iron polymaltose complex)	74 (42) 74 (37)	RCT Out-Patient	L,L,L,S,L Some concerns
Bandhu (2003) [162]	India	N/A	Control Oral iron (ferr sulph 3-4 mg/kg/day)	37 (23)	CC Community-Based	L,L,L,L,L,M,M Moderate Risk
Berger (1997) [163]	Bolivia	67.2 , 9.3 39.6 to 99.6 67.8 , 7.6 39.6 to 99.6 69.6 , 12.2 39.6 to 99.6	Placebo Oral Iron (3-4 mg/kg/week ferrous sulphate) Oral Iron (3-4 mg/kg 5 days/wk ferrous sulphate)	57 (30) 58 (28) 58 (31)	RCT Community-Based	S,L,L,L,L Some concerns
Cetin (2018) [35]	Turkey	159.48 , 35.52 152.52 , 43.92 123.48 , 27.84 136.56 , 48.12	IDA - oral iron (dosage and composition unknown) ID (no intervention) Thalassemia (no intervention) Control	25 (18) 25 (19) 25 (11) 25 (13)	CC Out-Patient	M,L,M,L,L,M,L Moderate Risk
Daniel (2011) [164]	United States	N/A	Oral iron (3-6mg/kg ferrous sulphate)	195	RCH Out-Patient	S,S,N,S,L,S,L Serious Risk
Devaki (2009) [165]	India	180 to 216	Control Placebo Group Control Supplementation - Oral iron (100 mg elemental iron per day for 6 days a week over 8 months.) ID - Oral iron (100 mg elemental iron per day for 6 days a week over 8 months.) IDA - Oral iron (100 mg elemental iron per day for 6 days a week over 8 months.)	30 (15) 30 (15) 30 (15) 30 (15)	CC Community-Based	M,L,L,L,L,M,L Moderate Risk
Dogan (2013) [166]	Turkey	33.4 , 15.3 8 to 68 20 , 12 7 to 67	Control Oral Iron (iron II-glycine-sulphate complex 6 mg/kg/day)	28 (14) 30 (16)	CC Out-Patient	M,L,L,L,L,L,L Moderate Risk
Gross (1976) [167]	Kenya	N/A	Control Oral iron (ferrous sulphate 9 mg/kg/day) Intramuscular iron (composition and dosage unspecified)	5 24 7	CC Not identified	L,L,L,L,L,L,L Low Risk
Gulsan (2013) [168]	United States	23 , 16 6 to 72 26.3 , 17.6 6 to 72 Total: 89 (30)	Oral iron (4 mg/kg/d ferrous sulfate) Oral iron + zinc sulphate (4 mg/kg/d ferrous sulfate + 5 mg/d zinc sulphate)	40 49	RCT Out-Patient	L,L,L,L,L Low Risk
Gupta (2010) [169]	Turkey	22.3 , 14.3 7 to 53	Oral iron (ferrous sulphate 4 mg/kg/day)	35 (10)	PCH Out-Patient	M,L,L,L,L,L,M Moderate Risk

Hafeez (1998) [170]	Pakistan	34.8 , 16.8 12 to 72 26.25 , 16.79 12 to 72	Oral iron (6 mg/kg/day ferrous gluconate syrup) Oral iron (6 mg/kg 3 consecutive days per week ferrous gluconate syrup)	27 28	RCT Out-Patient	S,S,S,L,L Some concerns
Halis (2009) [171]	Turkey	42 , 6.7 35.5 , 6.5	Control Oral iron (5 mg/kg/day composition unknown)	20 (10) 20 (10)	CC Not identified	M,L,L,L,L,M,L Moderate Risk
Hamed (2021) [172]	Egypt	110.4 pm 42 60 to 168 122.4 , 38.4 60 to 168 130.8 , 39.6 60 to 168	IDA - Oral iron (10 mg/day iron syrup) ID - Oral iron (10 mg/day iron syrup) Control	39 (22) 45 (26) 60 (32)	CC Community-Based	M,L,L,L,L,L,L Moderate Risk
Hamed (2020) [32]	Egypt	184.2 , 27.36 120 to 216 174 pm 24.48 132 to 216	Control Oral iron (6 mg/kg/day ferrous sulphate)	40 (28) 60 (40)	CC Out-Patient	M,L,L,L,L,M,L Moderate Risk
Hawamdeh (2013) [173]	Jordan	24.8 , 7.6 6 to 60 26.98 , 8.4 6 to 60 25.42 , 8.56 6 to 60	Oral iron - once weekly (6 mg/kg elemental iron) Oral iron - twice weekly (6 mg/kg elemental iron) Oral iron - daily (6 mg/kg elemental iron)	47 (17) 50 (16) 51 (16)	RCT Out-Patient	S,L,L,L,L Some concerns
Hayashi (1999) [141]	Japan	139 , 29.5 75 to 184 124.6 , 35.45 42 to 169	Control Oral iron (3-4 mg/kg/day composition unknown)	38 (24) 30 (19)	CC Out-Patient	M,L,L,M,L,L,L Moderate Risk
Ibrahim (2017) [174]	Egypt	32.4 , 13.2 72 to 60 24 , 9.6 72 to 60	Control Oral Iron (6 mg/kg/day ferrous sulphate)	40 (15) 40 (6)	CC Out-Patient	L,L,L,L,L,L,L Low Risk
Jain (1980) [118]	India	1 to 144	Transfusion of packed red blood cells	150	RCH In-Patient	M,L,L,L,L,L,M Moderate Risk
Jayabose (1993) [117]	United States	23.28 pm 26.73 3 to 144	Transfusion of packed red blood cells (2 cc/kg/hr)	22 (14)	PCH In-Patient	M,M,L,L,L,S,L Serious Risk
Kabakus (2002) [175]	Turkey	29 , 1.3 25 to 36 31 , 1.3 25 to 42	Control Oral Iron (6 mg/kg/day ferrous sulphate)	12(6) 18 (8)	CC Out-Patient	M,L,L,L,L,M,L Moderate Risk
Kan (2022) [176]	Turkey	0 to 12	Oral iron + Vitamin D given at the same time (3mg/kg/day ferrous sulphate + 400 IU Vitamin D) Oral iron + Vitamin D given 12 hours after (f3mg/kg/day ferrous sulphate +400 IU Vitamin D) Oral iron + Vitamin D given at the same time (3mg/kg/day ferric carboxymaltose + 400 IU Vitamin D) Oral iron + Vitamin D given 12 hours after (3mg/kg/day ferric carboxymaltose + 400 IU Vitamin D)	15 15 15 15	RCT Out-Patient	S,L,L,L,S Some concerns

Karadas (2022) [177]	Turkey	133.08 pm 56.04 36 to 216	Intravenous iron sucrose (total cumulative dose (mg) = [target Hb (12 g/dL) - actual Hb] × weight (kg) × 0.24 + [15 × weight (kg)])	33 (17)	RCH In-Patient	M,M,L,L,L,S,L Serious Risk
Kirk (2019) [178]	United States	90.61 , 62.08 4 to 247.2	Intravenous ferric carboxymaltose (dosage unspecified)	165 (92)	RCH In-Patient	M,M,S,M,L,M,L Serious Risk
Krishnamoorthy [179] (2010)	India	10 to 192	Oral iron (6 mg/kg/day ferric ammonium citrate) Oral iron + Vitamin C (6 mg/kg/day ferric ammonium citrate + 250 mg Vitamin C tablet) Oral iron + Vitamin E (6 mg/kg/day ferric ammonium citrate + 200 micrgram Vitamin E tablet)	7 7 7	RCT Out-Patient	S,L,L,L,L Some concerns
Kurekci (2000) [180]	Turkey	9 to 22 7 to 24	Control Oral iron (4 mg/kg/day ferrous sulphate)	12 (6) 25 (10)	CC Out-Patient	M,L,L,M,L,M,L Moderate Risk
Kwiatkowski (1999) [181]	United States	21.6 , 8.16 0 to 48	Oral iron (6 mg/kg/day composition unspecified) Transfusion of packed red blood cells Total: 55 (31)	47 8	RCH Mixed	M,M,L,L,L,M,L Moderate Risk
Lozoff (1996) [182]	Costa Rica	12 to 23	Control Oral iron (3 mg/kg/dose composition unspecified)	54 (26) 32 (16)	RCT Community-Based	L,L,L,L,L Low Risk
Lozoff (1982) [183]	Guatemala	6 to 24	Control – Placebo Control - Oral iron (5 mg Fe/Kg ferrous ascorbate) IDA - Placebo IDA - Oral iron (5 mg Fe/kg ferrous ascorbate)	18 17 13 12	RCT Community-Based	L,L,L,L,L Low Risk
Lozoff (2010) [184]	Chile	9.23 , 3 6- and 12-months 6 to 12	Control - Placebo Control - Oral iron (15 mg/day ferrous sulphate) IDA - Placebo IDA - Oral Iron (15 mg/day ferrous sulphate)	61 (34) 69 (42) 80 (29) 77 (17)	RCT Community-Based	L,L,L,L,L Low Risk
Maheshwari (2011) [185]	India	72 to 168	Mild IDA - Oral Iron (6 mg/kg/day ferrous sulphate + folic acid) Moderate IDA - Oral iron (6 mg/kg/day ferrous sulphate + folic acid) Severe IDA - Oral iron (6 mg/kg/day ferrous sulphate + folic acid)	45 23 32	PCH Community-Based	M,L,L,L,L,M,M Moderate Risk
Malbora (2013) [186]	Turkey	29.04 , 14.88 25.2 , 17.52 6 to 72	Control Oral iron (4 mg/kg/d ferrous sulphate)	31 (13) 30 (9)	CC Out-Patient	M,L,L,M,L,M,L Moderate Risk
Mantadakis (2016) [187]	Greece	98.8 , 55.83 14.4 to 168	Intravenous iron sucrose (100-200 mg, total dosage per patient varies)	12 (6)	RCH In-Patient	M,L,N,L,L,S,L Serious Risk
McLennan (2016) [126]	Dominican Republic	Non-anemic: 38.64, 18.72 Anemic: 21.6 , 13.92 9 to 72	Oral iron (5 mg/kg/day ferrous sulphate) Oral iron (5 mg/kg/day ferrous sulphate)	Non-anemic: 83 (43) Anemic: 182 (72)	RCH Community-Based	S,M,N,L,L,M,L Serious Risk
Metallinos-Katsaras	Greece	44.52 , 3.9 36 to 48	Control - Placebo (multivitamins)	10	RCT Community-Based	L,L,L,L,L Low Risk

(2004) [188]		44.64 , 3.34 43.32 , 4.4 41.64 , 3.51	Control - Oral iron (15 mg iron + multivitamins) IDA - Placebo (multivitamins) IDA - Oral iron (15 mg iron + multivitamins)	17 7 14		
Mohammed (2023) [189]	The Gambia	22 , 10.4 22.33 , 11.1 21.83 , 10.37	Placebo Oral iron (38 mg ferrous sulphate) Oral iron (74 mg IHAT powder)	214 (120) 214 (96) 214 (120)	RCT Out-Patient	L,L,L,L,L Low Risk
Mokhtar (2015) [40]	Egypt	91.2 , 48 68.4 , 50.4	Control IDA - Oral iron (6 mg/kg/ day ferrous sulphate)	20 20	CC Out-Patient	L,L,M,L,L,M,L Moderate Risk
Name (2018) [190]	Brazil	46.8 , 14.4 12 to 156 46.8 , 8.4 12 to 156	Oral iron (iron bisglycinate chelate 3 mg iron/kg/day) Oral iron (polymaltose iron 3 mg iron/kg/day)	9 (3) 11(4)	RCT Out-Patient	L,L,L,L,L Low Risk
Nestor (2019) [191]	United States	153.6 , 61.2 12 to 252	Intravenous iron sucrose	33 (26)	RCH In-Patient	L,M,L,M,L,S,M Serious Risk
Orhan (2023)	Turkey	116.93 , 57.65 6 to 222	Intravenous iron sucrose (7 mg/kg/dose) Intravenous ferric carboxymaltose (500 mg/dose max) Total:44 (28)	25 19	RCH In-Patient	M,M,M,M,L,M,L Moderate Risk
Ozsahin (2020) [192]	Switzerland	18 to 216	Intravenous ferric carboxymaltose (max 20 mg/kg/dose)	44 (96)	RCH Out-Patient	L,M,L,L,L,M,M Moderate Risk
Parodi (2012) [193]	Italy	46.24 , 65.41 1.2 to 196	Oral iron (ferrous sulphate 2 mg/kg/day)	14 (4)	RCH Out-Patient	M,M,L,L,L,M,L Moderate Risk
Patil (2019) [194]	India	36.12 , 25.92 12 to 144 27.72 , 15.48	Oral iron (ferrous ascorbate 6 mg/kg/day elemental iron) Oral iron (iron polymaltose complex 6 mg/kg/day elemental iron)	50 (26) 50 (25)	RCT Not identified	L,L,L,L,S Some concerns
Patiroglu (1991) [195]	India	IDA: 67.25 , 50.45 16.8 to 168	Control Oral iron (6 mg/kg/day composition unknown)	13 (6) 24 (13)	CC Not identified	M,L,L,N,S,M,L Serious Risk
Pinsk (2008) [196]	Israel	90.5 , 48.33 79 11 to 192	Intravenous iron sucrose (5 mg Fe+++ per kilogram per day)	45 (22)	PCH Out-Patient	M,L,L,L,L,M,L Moderate Risk
Plummer (2013) [197]	United States	IDA: 26.82 , 14.34 Overall: 104.16 , 74 11 to 60	Intravenous Low Molecular Weight Iron Dextran	IDA: 11 Other: 20 Total: 31	PCH Out-Patient	M,L,M,L,L,M,M Moderate Risk
Powers (2017) [198]	United States	138.09 , 51.15 Median 164.6 9 to 216	Intravenous ferric carboxymaltose (15 mg/kg; maximum 750 mg per dose)	72 (49)	RCH Out-Patient	M,L,L,L,L,M,L Moderate Risk
Powers (2015) [199]	United States	15.97 , 1.07 11 to 41	Oral iron (ferrous sulphate 3 mg/kg/day) Oral iron (NovaFerrum 3 mg/kg/day)	62 (27)	RCT Out-Patient	L,L,H,L,H High Risk
Powers (2015) [200]	United States	148.43 , 62.32 Median 168 9 to 249.6	Intravenous ferric carboxymaltose (15 mg/kg, max 750 mg)	87 (62)	RCH Out-Patient	S,M,M,M,M,M,L Serious Risk
Prathibha (2015) [201]	India	Out-patient: 75.1 , 34.9	Oral iron (ferrous sulphate dosage unknown)	Out-patient: 52 (23)	PCH Mixed	S,L,L,L,L,M,L Serious Risk

		In-Patient: 86.8 , 35.5 12 to 144	Oral iron (ferrous sulphate dosage unknown)	In-patient: 40 (27)		
Roncagliolo (1998) [202]	Chile	All 6 months	Control - oral iron (15 mg elemental Fe/d ferrous sulphate) IDA - oral iron (15 mg elemental Fe/d ferrous sulphate)	26 (12) 29(5)	CC Community-Based	M,L,L,L,M,L,L Moderate Risk
Russo (2020) [203]	Italy	33.7 , 25.67 Median : 20.4 2.4 to 88.8 24.9 , 12.58 13.2 to 46.8 42.7 , 41 7.2 to 115.2 47.55 , 36.68 6 to 144 53.22 , 45.4 4.8 to 147.6	Oral iron (ferrous gluconate/sulfate 2 mg/kg) Oral iron (ferrous gluconate/sulfate 4 mg/kg) Oral iron (ferric iron salts 2 mg/kg) Oral iron (bis-glycinate ferrous iron 0.45 mg/kg) Oral iron (liposomal iron 0.7-1.4 mg/kg)	18 (7) 7 (3) 7 (2) 62 (22) 13 (8)	PCH Not identified	S,L,L,S,S,M,L Serious Risk
Sandoval (2002) [204]	United States	74.95 , 70.39 Median: 21 7 to 240 17.54 , 3.68 12 to 24	Oral iron (composition and dosage unknown) Transfusion (pRBCs)	29 13	RCH Mixed	S,M,L,S,L,S,L Serious Risk
Sarici (2001) [205]	Turkey	14.05 , 5.18 6 to 24 14.79 , 4.88 7 to 24	Control IDA - oral iron (4 mg/kg ferrous sulphate)	20 (8) 20 (7)	CC Out-Patient	M,L,M,M,L,M,L Moderate Risk
Sheikh (2015) [206]	Pakistan	50.16 , 44.16 8 to 180	Intravenous iron sucrose (Single dose was diluted in 100cc 0.9% normal saline solution.)	100 (33)	PCH Out-Patient	M,L,L,M,L,M,L Moderate Risk
Siddiqui (2004) [207]	Pakistan	82.8 , 2.88 60 to 120 98.04 , 3.24 60 to 120	Oral iron - daily (200 mg ferrous sulphate) Oral iron - weekly (200 mg ferrous sulphate)	30 (15) 30 (15)	RCT Community-Based	S,L,L,L,L Some concerns
Sipahi (1998) [208]	Turkey	IDA: 16.69 , 7.7 6 to 36	Control IDA - Oral iron (elemental iron 6 mg/kg/d) Total: 35 (10)	10 25	CC Out-Patient	M,L,M,M,L,M,L Moderate Risk
Sirdah (2014) [209]	Palestine	48 to 60	Control IDA - Oral iron (50 mg ferrous carbonate + 100 mg vitamin C /5 mL)	489 (240) 246 (111)	CC Out-Patient	L,L,L,L,L,L,M Moderate Risk
Sozmen (2003) [210]	Turkey	8 to 168	Oral iron (ferric hydroxide–polymaltose complex 6mg/day) Oral iron (ferrous sulfate complex 6 mg/day)	14 11	RCT Out-Patient	S,L,L,L,L Some concerns
Speckert (2023) [119]	Canada	21.77 , 8.52 Median: 144 Median: 30	Transfusion (pRBCs) Intravenous iron sucrose (7 mg/kg average dosage)	16 (11) 11 (10)	RCH Mixed	M,M,L,L,M,M,L Moderate Risk
Sungthong (2002) [211]	Thailand	116.4 , 20.4 72 to 156 115.2 , 20.4	Placebo Oral iron - daily (ferrous sulphate 300 mg/tablet)	140 (71) 134 (70)	RCT Community-Based	L,L,L,L,L Low Risk

		116.4 , 22.8	Oral iron - weekly (ferrous sulphate 300 mg/tablet)	123 (71)		
Surico (2002) [212]	Italy	30 , 33.6 8.4 to 162 49.2 , 488.4 to 162	Intravenous iron sucrose (28 mg/kg/day) Intramuscular iron polymaltose complex (20 mg/kg twice weekly)	19 (11) 14 (8)	RCH Mixed	L,M,L,L,M,M,L Moderate Risk
Tavil (2003) [213]	Turkey	18.78 , 12.3 5 to 72 17.42 , 11.17 5 to 72	Oral iron (ferrous sulphate 6 mg/kg/day) Oral iron (ferrous sulphate 6 mg/kg twice weekly)	48 (16) 46 (9)	RCT Out-Patient	L,L,L,L,H High Risk
Tienboon (2003) [214]	Thailand	23.9 , 7 18.4 , 6 12 to 36	Control IDA - oral iron (ferrous sulphate 6 mg/kg/day)	8 (2) 9 (2)	CC Out-Patient	S,L,L,L,L,S,L Serious Risk
Traxler (2005) [215]	United States	9 to 132	Oral iron (composition and dosage unknown)	ID: 36 IDA: 15	RCH Out-Patient	M,L,L,L,L,M,M Moderate Risk
Tsai (2014) [216]	Taiwan	9 , 2.2 7 to 12 10.8 , 3.8 6 to 17	Full-term - oral iron (4-6 mg/kg/day composition unknown) Premature - oral iron (4-6 mg/kg/day composition unknown)	3 (0) 12(5)	RCH Out-Patient	L,M,L,L,L,M,M Moderate Risk
Umit Sarici (2001) [217]	Turkey	14.79 , 4.88 Median: 14 7 to 24	Oral iron (4 mg/kg/day iron sulphate)	20 (8)	PCH Out-Patient	M,L,M,L,L,M,L Moderate Risk
Ustabas Kahraman [218] (2021)	Turkey	137.8 , 27.02 89 to 191 128.42 , 24.72 90 to 183	Control IDA - oral iron (4 mg/kg/day composition unknown)	50 (31) 50 (22)	CC Out-Patient	M,L,L,M,L,M,M Moderate Risk
Verhoef (2022) [219]	Kenya	19.6 pm 9.3 2 to 36 17.8 , 9.5 17.3 pm 9.8 18.4 , 9.7	Placebo Oral malaria intervention Oral iron (6·25 g/L ferrous fumarate twice weekly) Oral iron + malaria intervention (6·25 g/L ferrous fumarate x2/week)	82 (41) 82 (34) 82 (36) 82 (37)	RCT Community-Based	L,L,L,L,L Low Risk
Viswanath (2001) [220]	India	6 to 60	Oral iron (6 mg/Kg of elemental iron composition unknown)	100 (53)	PCH Out-Patient	M,L,N,S,S,M,L Serious Risk
Wall (2005) [221]	New Zealand	15.5 , 3.6 9.4 to 23.3 14.5 , 3.1 8.9 to 20.8 14.4 , 3.5 9.2 to 22.8	Oral iron (3 mg/kg/day ferrous gluconate) Iron fortified follow-on milk (12.0 mg/l iron as ferrous caseinate, and vitamin C 124–195 mg/l) Iron fortified partially modified cows' milk (12.9 mg/l iron as ferrous sulphate, and vitamin C 124–195 mg/l)	84 (31) 74 (32) 76 (35)	RCT Out-Patient	L,L,L,L,L Low Risk
Yar (2023) [222]	Pakistan	36 , 10.41 24 to 144	Oral iron (dosage and composition unknown)	100 (48)	RCH Not identified	S,L,M,M,L,M,L Serious Risk
Yetgin (1992) [223]	Turkey	6 to 192	Control IDA - oral iron (composition and dosage unknown) Total: 80 (9)	40 40	CC Not identified	S,L,M,N,S,M,L Serious Risk
Yewale (2013) [224]	India	24.6 , 28 6 to 144	Oral iron (ferrous ascorbate 3 mg/kg body weight/day)	40 (15)	RCT Out-Patient	L,L,S,L,L Some concerns

		32.64 , 34	Oral iron (colloidal iron suspension 3 mg/kg body weight/d)	33 (16)		
Young (2001) [225]	Malawi	15 to 60	Oral iron (ferrous sulphate 6 mg/kg/day) Oral iron (ferrous sulphate 6 mg/kg once weekly) Oral iron + supplements (iron (60 mg) plus vitamins A (7 500 IU), C (45 mg), D3 (600 IU), B1 (3 mg), Bi (1.5 mg) and B3 (22.5 mg).)	85 73 73	RCT Community-Based	S,L,L,L,L Some concerns
Zaka-Ur-rab (2016) [226]	India	49.45 pm 29.85 12 to 144	Control	31 (20)	CC Out-Patient	M,L,L,L,L,M,M Moderate Risk

Appendix B

Appendix for Chapter 4

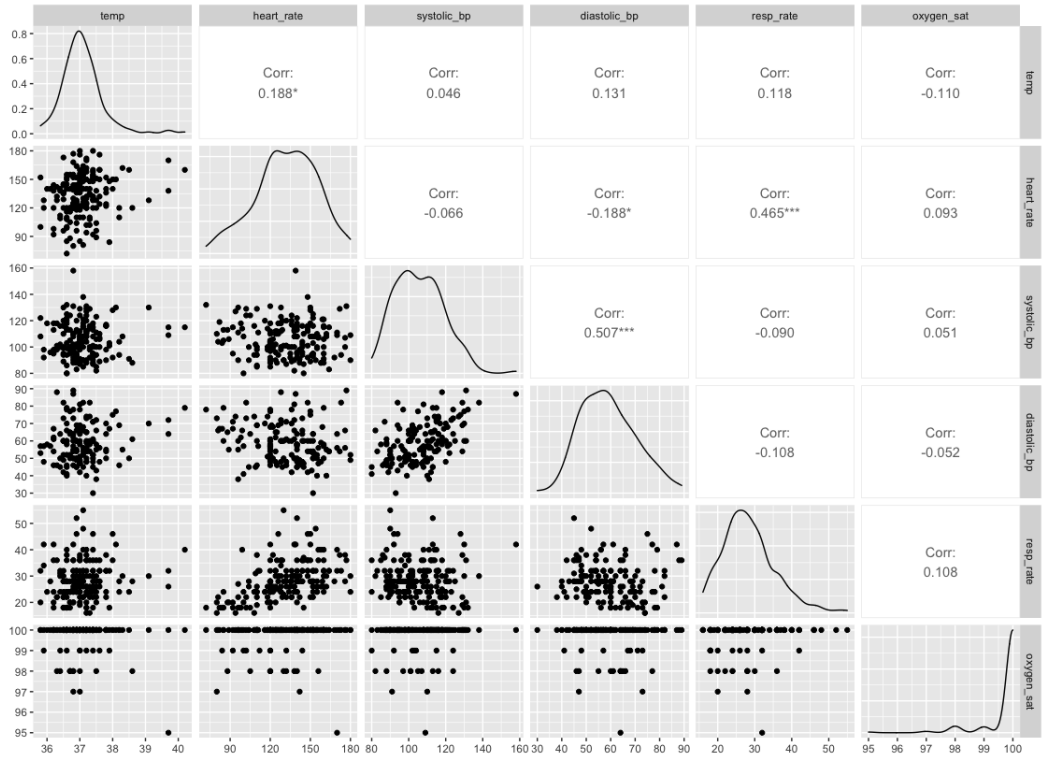

Figure B.1: Scatter plot matrix and pairwise correlations between the clinical markers of IDA.

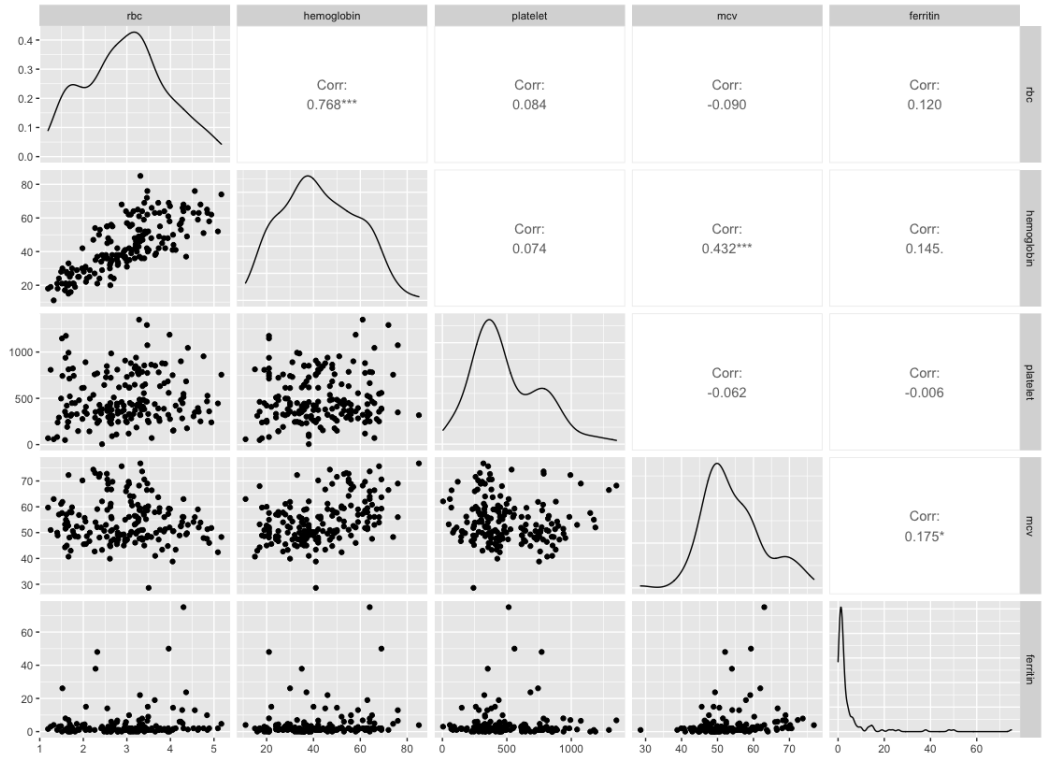

Figure B.2: Scatter plot matrix and pairwise correlations between the Laboratory markers of IDA.

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