UNIVERSITY OF TWENTE.

# Evaluating overuse of laboratory diagnostics:

## A case study into diagnosing anaemia in Dutch general practice

Master Thesis



**M.L.J. (Martijn) Oonk**

Master Health Sciences
Faculty of Science and Technology

**Graduation Committee**
First Supervisor – dr. ir. H. (Erik) Koffijberg
Second Supervisor – dr. M.M.A. (Michelle) Kip

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## Abstract

**Background**: Physicians often order laboratory tests of which the results do not contribute to medical decision-making. Such unnecessary test ordering is partially caused by uncertainty of physicians about which laboratory tests they need to order. In general practice, many laboratory tests are often ordered simultaneously in diagnosing the underlying cause of anaemia. This study assesses whether each of these tests adds value to this diagnostic process and determines the most efficient subset of tests for (correctly) diagnosing an underlying cause of anaemia.

**Methods**: Logistic regression models were fitted to data about diagnoses established by general practitioners, collected through a previously performed survey including cases of real-world anaemia patients. A stepwise backward selection process was performed: laboratory tests with the lowest added value were iteratively eliminated by selecting subset models, including a subset of the laboratory tests, with the best performance score (Akaike Information Criterion).

**Results**: Eight laboratory tests have a statistically significant impact on diagnosing an underlying cause of anaemia: ferritin, leukocytes, ESR, CRP, reticulocytes, MDRD, folic acid and serum iron. With regard to the correct diagnosis, two tests have a statistically significant impact: ferritin and MCV. The most efficient subset of laboratory tests for diagnosing an underlying cause contains nine laboratory tests: ferritin, CRP, reticulocytes, serum iron, ESR, MDRD, haemoglobin, leukocytes and folic acid. With regard to diagnosing the correct underlying cause, this subset contains five tests: ferritin, CRP, MCV, transferrin and folic acid.

**Conclusion**: Only a subset of the investigated laboratory tests impacts the ability of the general practitioner to (correctly) diagnose an underlying cause of anaemia. Therefore, general practitioners may order the most efficient subset of tests without limiting this ability. Whether such a subset is acceptable and cost-effective in daily practice has to be further investigated.

**Keywords**
Diagnostic testing, general practice, anaemia, efficiency, logistic models

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## Introduction

Laboratory testing often adds essential information to anamnesis and physical examination in the process of setting a diagnosis and choosing a disease management approach[1]. Physicians' options in the ordering of laboratory tests continuously grow, as many more, relatively cheap, tests become readily available[2]. However, this growing availability of tests contributes to the increasing use of laboratory tests by physicians[3]. In addition, it adds to the complexity, experienced by the physicians, of determining the added value of tests and subsequently deciding which tests to order. As a consequence, the existing uncertainty of the physicians in deciding which laboratory tests to order only grows[4]. All these issues stress the importance of determining the added value of individual laboratory tests for medical decision-making to support physicians in deciding which tests to order[1].

It has been observed that physicians, in practice, often order laboratory tests of which the results do not affect medical decision-making. Such tests may be seen as unnecessary and may amplify the increase in the use of laboratory tests by physicians[5]. Actually, they even appear to be one of the main causes of this increasing use[6]. One of the factors contributing to this unnecessary ordering of laboratory tests is the abovementioned uncertainty that physicians have in deciding which laboratory tests to order[7]. General practitioners (GPs) report this uncertainty in 14.7% of the patients for which they order laboratory tests[2].

Some direct negative effects may arise from this unnecessary laboratory testing. For example, unnecessary patient discomfort and additional healthcare costs[6]. Further, it potentially results in missed or delayed diagnoses as the results of those unnecessary tests may divert the attention of the physician away from the clinically relevant information[8]. In addition, it contributes to the growing problem of overdiagnosis which leads to downstream activities that may impact patient safety and well-being. In turn, these activities pose indirect additional costs to the healthcare system[6, 9]. All in all, one can infer from these points that unnecessary test ordering by physicians eventually can have substantial consequences for both patients and society.

Determining whether a laboratory test can be seen as unnecessary, only based on the added value of this test for medical decision-making, is impeded by the fact that multiple factors may influence physicians in their decision to order a test[10]. This decision does not necessarily emanate from an intention to support medical decision-making (e.g. establishing a diagnosis or selecting a disease management approach). Other intentions, like reassuring the patient, may (partly) underlie such a decision[5]. Additionally, the reasoning behind a decision to order a laboratory test may vary per physician and opinions about justified test ordering also depend on the context of the patient for whom a test is ordered. As a consequence, it is challenging to determine whether a laboratory test can be considered unnecessary, from a medical point of view, when strictly handling the abovementioned definition of unnecessary laboratory testing[5].

Objective research into the added value of laboratory tests specifically for medical decision-making could provide a base for determining the necessity of these laboratory tests in daily practice. Based on such research, a subset of the available laboratory tests can be selected that can be considered optimal, from a statistical point of view, to support medical decision-making by physicians. The current study specifically focuses on the ordering of laboratory tests, in general practice, to determine the underlying cause of anaemia, one of the conditions in which commonly many tests are ordered. It is determined to what extent individual laboratory tests add value to the GP in diagnosing the underlying cause of anaemia. Further, it is assessed which laboratory tests can be considered unnecessary for this diagnostic process.

Anaemia is a haematologic condition, frequently encountered in general practice, associated with an increased morbidity and mortality. Since anaemia is regarded to be a sign of an underlying disease, diagnosing patients with the correct underlying cause of anaemia is of great importance to timely start the appropriate treatment[11]. Laboratory testing is an essential supplement to anamnesis and physical examination in diagnosing this underlying cause[12]. Decisions regarding which laboratory tests to order in diagnosing the underlying cause of anaemia are supported by the anaemia guideline of the Dutch College of General Practitioners (DCGP)[13]. However, the underlying cause of anaemia remains unknown in 52% of the anaemia patients when using the laboratory protocol included in this guideline[14]. If cause specific laboratory testing (i.e.

focussing on one specific underlying cause) does not sufficiently explain the anaemia, the guideline recommends physicians to consider the simultaneous ordering of all laboratory tests included in this same protocol[13]. Based on the DCGP guideline, 14 laboratory tests are identified that should be ordered in such situation and which should allow to diagnose the most prevalent underlying causes of anaemia that are encountered in general practice[15]. A previous study published by Schop et al. showed that simultaneously ordering this complete set of 14 laboratory tests is more effective for diagnosing the underlying cause of anaemia compared to leaving the ordering of (a subset of) these 14 tests to the choice of the GP[15].

However, it is currently unclear to what extent these 14 tests may or may not individually contribute to the ability of the GP to (correctly) diagnose an underlying cause of anaemia. Consequently, an optimal subset of these 14 tests for (correctly) diagnosing an underlying cause of anaemia is also not yet known[12]. In this study, the individual impact of the abovementioned laboratory tests on the ability of the GP to diagnose an underlying cause of anaemia was quantitatively assessed. Further, the added value to the GP was determined of these tests for diagnosing the *correct* underlying cause of anaemia. Finally, the statistically most efficient subset of laboratory tests was determined for both diagnosing an underlying cause of anaemia as well as for diagnosing the *correct* underlying cause of anaemia.

## Methods

Study Design

A statistical analysis was performed during this research. Approval for this research was granted by the ethics committee of the faculty Behavioural, Management and Social Sciences of the University of Twente.

Data for this analysis were obtained in several ways. Data about diagnoses established by GPs in diagnosing the underlying cause of anaemia were obtained from an online survey conducted by Schop et al[15]. In this survey, cases of real-world anaemia patients were presented to and diagnosed by participating GPs. Data about these cases, concerning patient characteristics (age and gender) and the results of the laboratory tests performed in these patients, were obtained from a prospective database of anaemia patients[16]. Finally, data about the correctness of the abovementioned diagnoses were obtained by comparing each diagnosis established by the GPs with the (presumably) correct diagnosis per case, as established by an expert panel[15].

All patients in the abovementioned prospective database were included between the 1$^{st}$ of February 2007 and the 1$^{st}$ of February 2015, were 50 years and older and presented with newly diagnosed anaemia in general practice (n = 3325)[16]. A set of laboratory tests, the 14 tests identified based on the DCGP guideline, was performed in all of these patients. More specifically, this set of tests involved haemoglobin, mean corpuscular volume (MCV), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), vitamin B12, folic acid, creatinine, lactate dehydrogenase (LDH), ferritin, transferrin, serum iron, leukocytes, thrombocytes and reticulocytes. In addition, the kidney function of each patient was calculated based on a patient's creatinine level using the MDRD-formula. In this study, MDRD is therefore considered as the 15$^{th}$ laboratory test. Patient characteristics, age and gender, were known for each of the patients in the database. An expert panel, consisting of a GP, internist and clinical chemist, established the (presumably) correct underlying cause of anaemia for each patient in the database[15]. Cases in the database were divided, based on the diagnoses established by the expert panel, into four groups of underlying causes: anaemia of chronic disease (ACD), iron deficiency anaemia (IDA), renal anaemia and other underlying causes (including unknown cause). Schop et al. randomly selected 201 cases from the abovementioned prospective database for the survey described below[15]. These cases were selected from the database based on the distribution of the underlying causes of anaemia in this database. As a consequence, this set of 201 cases reflected the actual prevalence of each of the underlying causes of anaemia in the prospective database, as shown by Table 1 of their previously published study[15].

In January 2016, a survey was conducted by Schop et al. for which a representative sample of Dutch GPs (n=836) was invited, by e-mail, to participate[15]. As part of this survey, the participating GPs received three cases in which information was presented about the laboratory test results and characteristics (age and gender) of an anaemia patient. Each of these three cases was randomly selected from the abovementioned set of 201 cases of anaemia patients. In each of these three cases, the GPs were asked to select one underlying cause of anaemia

from the abovementioned groups of underlying causes or to state that no underlying cause could be established based on the information provided. Correctness of the diagnoses established by the GPs was determined by comparing the underlying cause of anaemia diagnosed by the GP with the underlying cause diagnosed by the expert panel. If the underlying cause in a case was “ACD”, “IDA” or “renal anaemia”, the diagnosis of the GP had to be in accordance with the diagnosis of the expert panel to be correct. If the underlying cause in a case was “other underlying causes” or “unknown”, the diagnosis of the GP had to be either “other underlying causes” or “unknown” to be correct. This answer was considered as the correct diagnosis regardless of which of the two options was chosen, because both options indicate that the GP considers that the underlying cause is neither IDA, ACD nor renal anaemia. Schop et al. describe that the use of this method in classifying the correctness of the diagnosis is common practice as diagnosing or excluding the three most prevalent underlying causes is clinically the most relevant[15]. Altogether, 378 cases were diagnosed by the GPs via this survey. In summary, the diagnoses in these cases were divided over the five underlying causes as follows: ACD (n=117, 31.0%), IDA (n=76, 20.1%), renal anaemia (n=50, 13.2%), other underlying causes (n=22, 5.8%) and unknown (n=113, 29.9%). A total of 234 (61.9%) diagnoses was correct. More specifically, 62.4% of the ACD diagnoses (n=73) was correct, 61.8% of IDA (n=47), 58.0% of renal anaemia (n=29), 77.3% of other underlying causes (n=17) and 60.2% of unknown (n=68). A more extensive description of the prospective database, case selection from this database, the (response to the) survey and the classification of correctness is provided elsewhere[15].

Several models were built using the data obtained from the database and collected through the survey. Each model simulated the process of diagnosing the (correct) underlying cause of anaemia by the GP, based on the available information about the laboratory test results and patient characteristics (age and gender). Age and gender of the patient were included in these models to more realistically represent daily practice, as these variables influence the diagnostic decision by the GP[16].

Data Preparation

Data about the laboratory test results and patient characteristics (age and gender) of the 201 unique cases selected by Schop et al, were inspected and, if necessary, subsequently corrected to the analytical process. Missing values were observed for the ESR laboratory test (n=20). Each of these missing values was imputed by single regression imputation. Non-numerical values of CRP (i.e. “smaller than 5”, n=109) and vitamin B12 (“smaller than 111”, n=2) tests were observed. For the CRP test, these non-numerical values were replaced by the numerical value 4. For the vitamin B12 test, these non-numerical values were replaced by the numerical value 110. Outlier values within the data were identified. Outlier values were defined as values exceeding plus or minus one and a half times the interquartile range of the corresponding variable across all 201 cases. Outlier values that were identified were evaluated with the help of a consulted expert on the prospective database of anaemia patients. Cases with outlier values were maintained in the dataset if these values could be physiologically explained.

Numerical values of the results of five laboratory tests (ferritin, leukocytes, MCV, transferrin and thrombocytes) in the dataset were converted to categorical values. Although many laboratory tests have one single cut-off value to indicate an abnormal test result, for example a value <8.1 mmol/L for haemoglobin in male patients[13], these five tests can have a too low as well as a too high test result. For example, a value <80 fL for MCV is considered too low, whereas a value >100 fL is considered too high[17]. Often these different areas of abnormality point towards different underlying causes of anaemia. Models built during the analysis can more clearly demonstrate a specific effect of each of these different areas of abnormality after conversion of the numerical results of these five tests to categorical results. This conversion was based on the reference values of these tests that are relevant for diagnosing the underlying cause of anaemia, described as such in the DCGP guideline and in the flowchart for “additional laboratory testing in anaemia” included in this guideline. The results of the leukocytes test and thrombocytes test were categorized into the categories normal and abnormal. When using the results of these two tests in diagnosing the underlying cause of anaemia, it is only relevant whether the results of these tests are normal or abnormal[13]. In other words, it is not relevant to this diagnostic process whether the results of these tests are too low or too high. An overview per test of the reference values used to categorize its results and the result categories to which the numerical values were converted is included in Table 1. For this analysis, the results of the 10 other laboratory tests were maintained numerical, but the reference values and categories of these laboratory tests are also shown by Table 1 to display the distribution of their result categories in the data.

Data Analysis

Standard descriptive statistics were used to describe the abovementioned data. The impact on the ability of the GP to diagnose an underlying cause of anaemia was assessed for each of the 15 laboratory tests. This was determined by investigating how the result of a laboratory test affects the probability that a GP diagnoses an underlying cause of anaemia instead of stating that no underlying cause could be established. This was determined by fitting a multinomial logistic regression model (MLR) with a logistic link function to the data. The dependent variable in this model was the diagnosis of the GP. This was a categorical variable with five non-ordered categories, matching the abovementioned four diagnostic groups (i.e. ACD, IDA, renal anaemia and other underlying causes) plus the diagnostic group "unknown". This diagnostic group "unknown" indicated that the GP stated that no diagnosis could be establish based on the provided information. This category "unknown" was set as the reference outcome category in this MLR. Predictors in this model (n=17) were the results of the 15 laboratory tests, supplemented with the patient characteristics age and gender. In four of the five laboratory tests of which the numerical results were converted to categorical results, MCV, leukocytes, transferrin and thrombocytes, the "normal" test result was set as the reference result category. For the fifth laboratory test, ferritin, the "low normal" test result was set as reference result category. By fitting this MLR to the data, the regression coefficients and their corresponding significance level (p-value) were estimated, through maximum likelihood estimation, for each of the predictors in the model. Regression coefficients estimated for the laboratory tests show the relationship between the results of these tests and the *log odds* of choosing an outcome category (i.e. diagnostic group IDA, ACD, renal anaemia or other underlying causes) over the reference outcome category of the model (i.e. diagnostic group "unknown"). By way of example, one the regression coefficients estimated for the haemoglobin laboratory test shows how a one unit change in the result of haemoglobin influences the *log odds* of the GP diagnosing the underlying cause "IDA" over "unknown" when all other predictors stay the same. After estimating these coefficients, they were exponentiated in order to improve their interpretability. Exponentiated regression coefficients are easier to interpret as they allow to observe the relationship between the result of a laboratory test and the *odds* of choosing an outcome category over the reference category "unknown". The estimated regression coefficients were evaluated for their significance. Subsequently, the significant regression coefficients were selected and reported.

A laboratory test may improve the ability of the GP to diagnose an underlying cause of anaemia, as described in the previous paragraph. However, this does not necessarily mean that the selected underlying cause is also correct. Therefore, the added value to the GP for diagnosing the *correct* underlying cause of anaemia was assessed of each of the laboratory tests. This was determined by investigating how the result of a laboratory test affects the probability that a GP diagnoses the correct underlying cause of anaemia. This was determined by fitting a binomial logistic regression model (BLR) to the data. The dependent variable in this model was the correctness of the diagnosis of the GP, a binary variable with categories "correct" and "incorrect". The incorrect category was set as the reference category in this model. Predictors in this model were equal to the MLR described earlier. As in the MLR, the regression coefficients and their corresponding significance level were estimated for each of the predictors by fitting the model to the data. Afterwards, the resulting regression coefficients were exponentiated. To illustrate this, the regression coefficient for the haemoglobin laboratory test shows how a one unit change in the result of haemoglobin, when all other predictors stay the same, influences the *(log) odds* of the GP diagnosing the correct underlying cause of anaemia over an incorrect underlying cause. The estimated regression coefficients were evaluated for their significance and all estimated regression coefficients were reported.

When assessing the impact of each individual laboratory test as described above, this impact is assessed for a context in which GPs diagnose the (correct) underlying cause of anaemia while they are provided with the results of *all* 15 laboratory tests. However, an overlap could exist between multiple laboratory tests, concerning their impact on the ability of the GP to (correctly) diagnose an underlying cause of anaemia, as often multiple laboratory tests are ordered and interpreted simultaneously to diagnose a specific underlying cause of anaemia[13]. Therefore, presence or absence of the results of individual laboratory tests may influence the impact that other tests have on the ability of the GP to (correctly) diagnose an underlying cause of anaemia. As a consequence, it could be the case that the results of a (more efficient) *subset* of the 15 laboratory tests suffice for the GPs to diagnose the same underlying cause of anaemia as they would do when provided with the results of all 15 laboratory tests. For this reason, the statistically most efficient subset of laboratory tests was determined

for diagnosing an underlying cause of anaemia by the GP. Additionally, the statistically most efficient subset of laboratory tests was determined for diagnosing the *correct* underlying cause of anaemia by the GP. Both of these statistically most efficient subsets of laboratory tests were determined by performing a (separate) stepwise backward selection process[18]. In this process, MLR models were used to find the most efficient subset of laboratory tests for diagnosing an underlying cause of anaemia, whereas BLR models were used to find the most efficient subset for diagnosing the *correct* underlying cause. Compared to the (MLR and BLR) models used in the previous analyses, these models included only a subset of the predictors (i.e. results of laboratory tests and patient characteristics). Therefore, these models are referred to as "subset models". Subset models containing different subsets of predictors were built throughout the stepwise backward selection process and, from all of these subset models, the most efficient subset model was selected. Subsequently, this most efficient subset model was used to determine the most efficient subset of laboratory tests. Below, these steps are described in more detail.

A performance score, the Akaike Information Criterion (AIC), was determined for each subset model built in the stepwise backward selection process to enable the selection of the most efficient subset model[19]. The AIC is a performance metric balancing model complexity and goodness-of-fit (GOF) of the model: it favours models with the best balance between the number of predictors (i.e. laboratory tests and patient characteristics) included in the model and the extent to which the model explains the observations in data. Specifically, the AIC decreases with a better GOF of the model to the data and increases with a larger model complexity[18, 19]. Selection of the most efficient subset model based on this AIC statistic was done through a stepwise process of which a typical "step" (or iteration) is described by Figure 1 (Appendix I). In determining the most efficient subset model, an initial set of predictors, that consisted of all 15 laboratory test results and both patient characteristics, was iteratively reduced by eliminating the predictors that had the lowest impact on the ability of the GP to (correctly) diagnose an underlying cause of anaemia. In each iteration of the process, a certain N predictors of the initial set of predictors were not (yet) eliminated. During each iteration, the AIC was determined of each of the N possible subset models that contained N-1 of the remaining predictors. Subsequently, the subset model with the lowest, most favourable, AIC was selected to be the most efficient subset model *of that iteration*. In fact, selecting this subset model was solely based on the GOF of this model as, with N-1 predictors included in each subset model, the model complexity is equal for each subset model in an iteration. As a result of this selection, the predictor with the lowest impact on the ability of the GP to (correctly) diagnose an underlying cause of anaemia was identified since this predictor was not included in the most efficient subset model of this iteration. Next, this predictor was eliminated for the remainder of the process by continuing to a next iteration with the most efficient subset model of the current iteration. A next iteration would follow, to continue the selection process, until the AIC of the most efficient subset model in the *current* iteration, named "AIC (N-1)" in Figure 1, was larger than the AIC of the most efficient subset model in the *previous* iteration, "AIC (N)" in Figure 1. In that case, the selection process ended: eliminating an additional predictor had resulted in a model of which the reduced complexity did not compensate for the decrease in GOF. As a consequence, the most efficient subset model in the second last iteration was designated to be the most efficient subset model overall. Subsequently, the most efficient subset of laboratory tests was determined by selecting the laboratory tests from the predictors in this most efficient subset model overall.

Finally, several assumptions were tested that underlie the logistic regression model. Multicollinearity between the predictors of the selected most efficient subset models was assessed by determining the variance inflation factor (VIF) of each predictor. A VIF larger than five indicated a problematic amount of multicollinearity, influencing the estimation of the regression coefficients when fitting the model[20]. Perfect separation of predictors by the outcome was evaluated while fitting each model as the coefficient estimates of the logistic regression models would not converge to finite values if this condition was present[21]. More specifically, it was evaluated whether (a certain range of values of) a predictor was associated with only one of the outcome values (i.e. one of the underlying causes of anaemia). A Hausman-McFadden diagnostic test was performed to determine whether the independence of irrelevant alternatives (IIA) assumption held[22]. More concrete, this test assessed the independence of the dependent variable choices, meaning that the choice to diagnose a specific underlying cause of anaemia should not depend on the presence or absence of a third underlying cause. The analysis was performed in R version 3.5.0 using the mice-package for imputation and the mlogit-package and nnet-package for the MLR[23-26].

## Results

Description of the Selected Patient Cases

Table 1 describes the data about the 201 cases selected from the prospective database of anaemia patients by Schop et al. It describes, first, the age and gender of the patients and, second, the results of the laboratory tests performed in these patients. As mentioned earlier, Table 1 also shows the reference values and the result categories of the laboratory tests. The identified outlier values could all be physiologically explained and all 201 cases were therefore maintained.

| Patient Characteristics | | | | |
|---|---|---|---|---|
| Gender | Male | | Female | |
| Frequency | 47% (94/201) | | 53% (107/201) | |
| | Mean (95% CI) | Standard Deviation | Range | IQR |
| Age | 74.7 (72.9 – 76.4) | 12.7 | 50 – 102 | 20 |
| Laboratory Tests with Numerical Results (Descriptive Statistics) | | | | |
| | Mean (95% CI) | Standard Deviation | Range | IQR |
| ESR | 34.7 (30.9 – 38.5) | 27.3 | 0 – 120 | 34 |
| CRP | 26.5 (20.0 – 32.9) | 46.9 | 4 - 290 | 18 |
| Haemoglobin | 7.28 (7.17 – 7.40) | 0.85 | 4.20 - 8.40 | 1 |
| Reticulocytes | 1.04 (0.96 – 1.11) | 0.53 | 0.30 - 4.80 | 0.5 |
| Creatinine | 91.5 (84.6 – 98.3) | 49.4 | 42 - 449 | 34 |
| MDRD | 70.9 (67.4 – 74.5) | 25.8 | 8 – 184 | 32 |
| LDH | 400 (309 – 489) | 653 | 126 - 9385 | 115 |
| Serum Iron | 10.06 (9.32 – 10.80) | 5.35 | 1.90 - 25.40 | 7.8 |
| Folic Acid | 21.7 (18.9 – 24.4) | 20.1 | 3 - 227 | 15 |
| Vitamin B12 | 335 (310 – 360) | 183 | 102 – 1408 | 204 |
| Laboratory Tests with Numerical Results (Reference Values & Frequencies) | | | | |
| ESR | Abnormal | | Normal | |
| Male < 70 years | > 25 mm/h | | ≤ 25 mm/h | |
| Male ≥ 70 years | > 35 mm/h | | ≤ 35 mm/h | |
| Female < 70 years | > 30 mm/h | | ≤ 30 mm/h | |
| Female ≥ 70 years | > 35 mm/h | | ≤ 35 mm/h | |
| Frequency | 40% (81/201) * | | 60% (120/201) * | |
| CRP | Abnormal | | Normal | |
| | > 10 mg/L | | ≤ 10 mg/L | |
| Frequency | 33% (66/201) | | 67% (135/201) | |
| Haemoglobin | Abnormal | | Normal | |
| Male | < 8.5 mmol/L | | ≥ 8.5 mmol/L | |
| Female | < 7.5 mmol/L | | ≥ 7.5 mmol/L | |
| Frequency | 100% (201/201) | | 0% (0/201) | |
| Reticulocytes | Abnormal | | Normal | |
| | ≥ 2.5% of Red Blood Cells | | < 2.5% of Red Blood Cells | |
| Frequency | 2% (4/201) | | 98% (197/201) | |
| Creatinine | Low | Normal | High | |
| Male | < 59 µmol/L | 59 – 104 µmol/L | > 104 µmol/L | |
| Female | < 45 µmol/L | 45 – 84 µmol/L | > 84 µmol/L | |
| Frequency | 3% (6/201) | 67% (135/201) | 30% (60/201) | |
| MDRD | Abnormal | | Normal | |
| | ≤ 60 mL/min/1,73m$^2$ | | > 60 mL/min/1,73m$^2$ | |
| Frequency | 38% (77/201) | | 62% (124/201) | |

| LDH | Abnormal | | Normal | |
|---|---|---|---|---|
| | ≥ 450 E/L | | < 450 E/L | |
| Frequency | 16% (32/201) ** | | 84% (169/201) ** | |
| Serum Iron | Abnormal | | Normal | |
| Male | < 14 µmol/L | | ≥ 14 µmol/L | |
| Female | < 10 µmol/L | | ≥ 10 µmol/L | |
| Frequency | 64% (128/201) | | 36% (73/201) | |
| Folic Acid | Abnormal | | Normal | |
| | ≤ 5 nmol/L | | > 5 nmol/L | |
| Frequency | 2% (4/201) | | 98% (197/201) | |
| Vitamin B12 | Abnormal | | Normal | |
| | < 130 pmol/L | | ≥ 130 pmol/L | |
| Frequency | 3% (6/201) | | 97% (195/201) | |
| Laboratory Tests with Categorized Results (Reference Values & Frequencies) | | | | |
| Ferritin | Low | Low Normal | High Normal | High |
| Male | < 25 µg/L | 25 – 100 µg/L | 100 – 250 µg/L | > 250 µg/L |
| Female | < 20 µg/L | 20 – 100 µg/L | 100 – 150 µg/L | > 150 µg/L |
| Frequency (M+F) | 12.5% (25/201) | 35% (70/201) | 24% (49/201) | 28.5% (57/201) |
| Leukocytes | Abnormal | | Normal | |
| | < 4.3 x $10^9$/L or > 10 x $10^9$/L | | 4.3 – 10 x $10^9$/L | |
| Frequency | 22% (44/201) | | 78% (157/201) | |
| Thrombocytes | Abnormal | | Normal | |
| | < 150 x $10^9$/L or > 400 x $10^9$/L | | 150 – 400 x $10^9$/L | |
| Frequency | 18% (36/201) | | 82% (165/201) | |
| MCV | Low | Normal | High | |
| | < 80 fL | 80 – 100 fL | > 100 fL | |
| Frequency | 9% (18/201) | 83.5% (168/201) | 7.5% (15/201) | |
| Transferrin | Low | Normal | High | |
| | < 2 g/L | 2 – 3.6 g/L | > 3.6 g/L | |
| Frequency | 18% (36/201) | 75.5% (152/201) | 6.5% (13/201) | |
| * = in 22 patients (10%) an alternative reference value was used – in male: 20 mm/h instead of 25 or 35 mm/h – in female: 30 mm/h instead of 35 mm/h<br>** = in 29 patients (14%) an alternative reference value was used – 250 E/L instead of 450 E/L | | | | |

*Table 1 Descriptive statistics of laboratory tests and patient characteristics, reference values and result categories.*

Impact on the Ability of the GP to Diagnose an Underlying Cause

Table 2 shows the laboratory tests of which the results have a statistically significant impact on the ability of the GP to diagnose an underlying cause of anaemia. For these tests, the significant regression coefficients, the exponentiated regression coefficients and the significance level of these regression coefficients are shown. In each case, these statistics show the effect of a change in the result of the laboratory test on the probability that the GP diagnoses the *specific* underlying cause of anaemia mentioned in the table. In the case of laboratory tests with categorized results, the table also mentions the specific shift in the result category of the laboratory test (e.g. normal to abnormal result) for which the statistics show the effect on the probability that the GP diagnoses this specific underlying cause.

A shift from a low normal ferritin to a high ferritin has a larger impact on the ability of the GP to diagnose IDA (Odds Ratio (OR) 4.343) than on the ability of the GP to diagnose ACD (OR 2.791). An exponentiated coefficient with the value 38.47 was found for the reticulocytes laboratory test, indicating the ability of the GP to diagnose "other underlying causes". Results of multiple laboratory tests, for example LDH, do not significantly contribute to the ability of the GP to diagnose any underlying cause, including "other underlying causes".

| Laboratory Tests with Categorical Results | | | | | |
|---|---|---|---|---|---|
| Laboratory Test | Shift in the Result Category | Underlying Cause instead of Unknown | Coefficient (Log Odds) | Exp. Coefficient (Odds) | p-value |
| Leukocytes | Normal to Abnormal | Other | 2.352 | 10.50 | 0.0050 ** |
| Ferritin | Low Normal to Low | IDA | 3.049 | 21.09 | < 0.001 *** |
| Ferritin | Low Normal to High Normal | ACD | 1.310 | 3.706 | 0.0028 ** |
| Ferritin | Low Normal to High Normal | IDA | 1.364 | 3.911 | 0.031 * |
| Ferritin | Low Normal to High | ACD | 1.026 | 2.791 | 0.014 * |
| Ferritin | Low Normal to High | IDA | 1.469 | 4.343 | 0.029 * |
| Ferritin | Low Normal to High | Other | -4.690 | 0.0092 | 0.0015 ** |
| Laboratory Tests with Numerical Results | | | | | |
| Laboratory Test | Underlying Cause instead of Unknown | Coefficient (Log Odds) | Exp. Coefficient (Odds) | p-value | |
| ESR | ACD | 0.031 | 1.032 | < 0.001 *** | |
| ESR | Renal Anaemia | -0.044 | 0.957 | 0.047 * | |
| ESR | Other | -0.060 | 0.941 | 0.022 * | |
| CRP | Renal Anaemia | -0.065 | 0.937 | 0.043 * | |
| Reticulocytes | Other | 3.650 | 38.47 | < 0.001 *** | |
| MDRD | Renal Anaemia | -0.250 | 0.779 | < 0.001 *** | |
| Folic Acid | Other | -0.091 | 0.913 | 0.023 * | |
| Serum Iron | IDA | -0.158 | 0.854 | 0.024 * | |
| Signif. codes: *** = 0.001 ** = 0.01 * = 0.05 | | | | | |

*Table 2 Impact of laboratory tests on the ability of the GP to diagnose an underlying cause of anaemia.*

Added Value to the GP for Diagnosing the Correct Underlying Cause

Table 3 shows the impact of all investigated laboratory tests on the ability of the GP to diagnose the *correct* underlying cause of anaemia. For each test, the regression coefficients, the exponentiated regression coefficients and the significance level of these regression coefficients are shown. In each case, these statistics show the effect of a change in the result of the laboratory test on the probability that the GP diagnoses the *correct* underlying cause of anaemia. In the case of laboratory tests with categorized results, the table also mentions the specific shift in the result category of the laboratory test (e.g. normal to abnormal result) for which the statistics show the effect on the probability that the GP diagnoses the correct underlying cause. Table 3 includes multiple rows for each of the laboratory tests with categorized results as there are multiple ways to shift between result categories.

The results high MCV (OR 4.954), high normal ferritin (OR 0.531) and low ferritin (OR 3.425) have a statistically significant impact on the ability of the GP to diagnose the correct underlying cause of anaemia. In the table, the significance level (p-value) of the statistically significant regression coefficients, belonging to these three results, are accompanied by an asterisk (*).

| Laboratory Tests with Categorical Results | | | | |
|---|---|---|---|---|
| Laboratory Test | Shift in the Result Category | Coefficient (Log Odds) | Exp. Coefficient (Odds) | p-value |
| MCV | Normal to High | 1.600 | 4.954 | 0.0057 ** |
| MCV | Normal to Low | -0.176 | 0.838 | 0.785 |
| Ferritin | Low Normal to High | -0.123 | 0.884 | 0.725 |
| Ferritin | Low Normal to High Normal | -0.634 | 0.531 | 0.0471 * |
| Ferritin | Low Normal to Low | 1.231 | 3.425 | 0.0302 * |
| Leukocytes | Normal to Abnormal | -0.169 | 0.844 | 0.584 |
| Thrombocytes | Normal to Abnormal | 0.126 | 1.134 | 0.730 |
| Transferrin | Normal to High | 0.246 | 1.279 | 0.741 |
| Transferrin | Normal to Low | -0.7806 | 0.4581 | 0.05054 |

| Laboratory Tests with Numerical Results | | | |
|---|---|---|---|
| Laboratory Test | Coefficient (Log Odds) | Exp. Coefficient (Odds) | p-value |
| ESR | -0.0038 | 0.996 | 0.578 |
| CRP | 0.0077 | 1.008 | 0.0698 |
| Haemoglobin | -0.216 | 0.806 | 0.395 |
| Reticulocytes | 0.176 | 0.192 | 0.560 |
| Creatinine | 0.0062 | 1.006 | 0.222 |
| MDRD | 0.015 | 1.015 | 0.0937 |
| LDH | 0.00047 | 1.001 | 0.641 |
| Serum Iron | 0.024 | 1.024 | 0.482 |
| Folic Acid | 0.013 | 1.014 | 0.075 |
| Vitamin B12 | -0.00046 | 0.9995 | 0.509 |
| Signif. codes: *** = 0.001 ** = 0.01 * = 0.05 | | | |

*Table 3 Added value of laboratory tests for the GP in diagnosing the correct underlying cause of anaemia.*

Best Subset of Laboratory Tests for Diagnosing an Underlying Cause of Anaemia

Table 4 (Appendix II) shows the most efficient subset of laboratory tests for diagnosing an underlying cause of anaemia. It describes the development of the stepwise backward selection process through which this subset was determined. Per iteration of the selection process, the table shows: the number of predictors included in a subset model (Subset Size), AIC of the most efficient subset model (AIC Model) and the predictor eliminated compared to the previous iteration. A total of 7 predictors was eliminated from the initial set of 17 predictors. Eliminating an eighth predictor (the laboratory test haemoglobin) caused an increase in AIC (771.45) of the resulting subset model compared to the previous iteration. Table 4 shows the 10 predictors that were not eliminated during the selection process, including the patient characteristic age. The most efficient subset to diagnose an underlying cause of anaemia contains nine laboratory tests: ferritin, CRP, reticulocytes, serum iron, ESR, MDRD, haemoglobin, leukocytes and folic acid.

Best Subset of Laboratory Tests for Diagnosing the Correct Underlying Cause of Anaemia

Table 5 (Appendix III) shows the most efficient subset of laboratory tests for diagnosing the *correct* underlying cause of anaemia. It describes the development of the stepwise backward selection process through which this subset was determined. Per iteration of the selection process, the table shows: the number of predictors included in a subset model (Subset Size), AIC of the most efficient subset model (AIC Model) and the predictor eliminated compared to the previous iteration. A total of 11 predictors was eliminated from the initial set of 17 predictors. Eliminating a twelfth predictor (the laboratory test folic acid) caused an increase in AIC (482.80) of the resulting subset model compared to the previous iteration. Table 5 shows the 6 predictors that were not eliminated during the selection process, including the patient characteristic age. The most efficient subset to diagnose the correct underlying cause of anaemia contains five laboratory tests: ferritin, CRP, MCV, transferrin and folic acid.

Assumption Testing

All VIFs determined were smaller than five as shown by Table 6 (Appendix IV). No problems occurred with regard to the convergence of the maximum likelihood estimates for the coefficients. The IIA assumption was not rejected.

## Discussion

If GPs follow the anaemia guideline of the DCGP when diagnosing the underlying cause of anaemia, they will have the results of 15 laboratory tests to their disposal during this diagnostic process. In this study, it was shown that, from a statistical point of view, only a subset of these 15 tests is likely to be valuable to the GP in diagnosing the (correct) underlying cause of anaemia. When all 15 laboratory tests are simultaneously ordered, eight of these laboratory tests have a statistically significant impact on the ability of the GP to diagnose an underlying cause of anaemia: ferritin, leukocytes, ESR, CRP, reticulocytes, MDRD, folic acid and serum iron. In the same context, only two of the 15 laboratory tests have a statistically significant impact on the ability of the GP to diagnose the *correct* underlying cause of anaemia: ferritin and MCV. These findings indicate that an overlap exists between multiple laboratory tests concerning the insights that their results provide as, given the results of all other tests, the result of one single test rarely provides additional crucial information to the GP in diagnosing the (correct) underlying cause of anaemia. In the selection process, the number of laboratory tests was iteratively reduced, changing the contribution of the remaining tests to these insights provided to the GP. The statistically most efficient subset of laboratory tests for diagnosing an underlying cause of anaemia, found through this process, contained nine tests: ferritin, CRP, reticulocytes, serum iron, ESR, MDRD, haemoglobin, leukocytes and folic acid. Therefore, six tests were excluded from the original set of 15 tests: transferrin, MCV, thrombocytes, creatinine, vitamin B12 and LDH. The statistically most efficient subset of laboratory tests found for diagnosing the *correct* underlying cause of anaemia contained five tests: ferritin, CRP, MCV, transferrin and folic acid. In this case, ten tests were excluded from the original set of 15 tests: ESR, thrombocytes, creatinine, vitamin B12, LDH, leukocytes, reticulocytes, haemoglobin, serum iron and MDRD.

Of the eight laboratory tests for which a statistically significant impact was found on the ability of the GP to diagnose an underlying cause of anaemia, two tests have a significant impact on this ability in more than one underlying cause. The ferritin laboratory test has a significant impact on diagnosing ACD, IDA or “other underlying causes”. The ESR laboratory test has a significant impact on diagnosing ACD, renal anaemia or “other underlying causes”. This plural impact may be explained by the fact that these two laboratory tests play a central role, according to the DCGP guideline, in diagnosing the two most frequently encountered underlying causes of anaemia: ACD and IDA. Regarding the ferritin laboratory test, the DCGP guideline states that a high ferritin provides an indication for the underlying cause ACD. In this study, the positive impact of a high ferritin on the probability that the GP diagnoses ACD was confirmed. The DCGP guideline does not include a statement which mentions that a high ferritin provides an indication for the underlying cause IDA. In this study however, a positive impact was found for a high ferritin on the probability that the GP diagnoses IDA. A possible explanation for this unexpected finding might be that GPs considered multiple aetiologies (e.g. ACD *and* IDA) underlying the anaemia, as multimorbidity is common in elderly[27]. However, patients with multiple aetiologies were excluded from the study performed by Schop et al. to avoid statistical complexity and because diagnosing anaemia with multiple underlying causes is not supported by the DCGP guideline[15]. Therefore, the GPs had to select *one* underlying cause that they considered the most likely for each case in the survey. In practice, a GP maybe would have diagnosed multiple underlying causes. In such cases, the GPs may have selected IDA as the underlying cause while considering ACD as a second contributor to the anaemia. Further, a remarkably high exponentiated regression coefficient was found for the reticulocytes laboratory test. This coefficient showed the impact of this test on the ability of the GP to diagnose “other underlying causes”. Numerically, this coefficient seems to indicate a very large impact of this test. However, it has to be interpreted with the variation of the results of the reticulocytes test in mind. Typically, the result of the reticulocytes laboratory test varies between one percent and two percent of the total number of red blood cells[28]. With the exponentiated regression coefficient showing how a one percent change in the result of the reticulocytes laboratory test influences the odds of the GP diagnosing “other underlying causes”, the impact of the result of this test will often be limited.

A statistically significant impact was found for a high MCV on the ability of the GP to diagnose the correct underlying cause of anaemia. Based on the results of this study, it can therefore be said that a high MCV adds value to the GP in diagnosing the correct underlying cause of anaemia. However, it must be noted that a high MCV occurred in only 7.5% of the selected cases, so the impact of MCV in diagnosing the full spectrum of anaemia patients might still be limited. Additionally, the used definition of a correct diagnosis leads to determining the average added value of the laboratory tests over all groups of underlying causes. Therefore, it cannot be said,

based on these results, for which *specific* underlying cause of anaemia a high MCV adds value to the GP in correctly diagnosing this underlying cause. Using an MCV-based algorithm in diagnosing the correct underlying cause of anaemia was discouraged in a previous study[17]. Results of this research partially confirm this discouragement as no statistically significant impact was found for a low MCV result. However, the added value found for a high MCV may partially explain the fact that MCV is still one of the most often requested laboratory tests by GPs in diagnosing the underlying cause of anaemia[15]. A low ferritin and a high normal ferritin also have a statistically significant impact on the ability of the GP to diagnose the correct underlying cause of anaemia. Based on the results of this study, it can be said that a low ferritin adds value to the GP in diagnosing the correct underlying cause of anaemia. However, a high normal ferritin decreases the ability of the GP to diagnose the correct underlying cause of anaemia. This negative influence may be explained by the fact that, according to the DCGP guideline, this result contributes to both diagnosing IDA and ACD. This multi-interpretability possibly increases the risk of a GP diagnosing the wrong underlying cause of anaemia.

The statistically most efficient subset of laboratory tests found for diagnosing an underlying cause of anaemia contains all eight laboratory tests for which a statistically significant impact was found on the ability of the GP to diagnose an underlying cause of anaemia. The fact that no statistically significant impact was found for haemoglobin, the ninth test included in this most efficient subset, is in accordance with the fact that the DCGP guideline does not describe that a certain haemoglobin level provides an indication for a specific underlying cause of anaemia. However, haemoglobin is essential to the GP in actually determining that an anaemia is present, which could explain its presence in the most efficient subset of laboratory tests. Apparently, haemoglobin is indispensable for the ability of the GP to diagnose an underlying cause of anaemia. The statistically most efficient subset of laboratory tests found for diagnosing the correct underlying cause of anaemia contains both of the two laboratory tests, ferritin and MCV, for which a statistically significant impact was found on the ability of the GP to diagnose the correct underlying cause of anaemia. Additionally, three other laboratory tests (CRP, transferrin and folic acid) are included in this most efficient subset. Although no statistically significant impact was found for these three tests, they were still included in this subset as their impact appeared to be indispensable to the ability of the GP to diagnose the correct underlying cause of anaemia. The fact that all laboratory tests for which a statistically significant impact was found are included in both of the most efficient subsets suggests that the selection process used was appropriate to find the subset of laboratory tests with the largest impact on the ability of the GP to diagnose the (correct) underlying cause of anaemia. Based on this study, it appears that multiple laboratory tests can be eliminated from the full set of 15 tests recommended by the DCGP guideline as a subset of these laboratory tests suffices to provide the GP with a similar ability to diagnose the (correct) underlying cause of anaemia.

Strengths

Several aspects of the analysis performed during this research contribute to the representativeness of the impact found for the laboratory tests on the ability of the GP to diagnose the (correct) underlying cause of anaemia. Statistical models used in this analysis were built using data collected from a representative sample of Dutch GPs. Patient cases diagnosed by these GPs contained real-world patient data and were randomly selected from a prospective database of anaemia patients, mimicking daily practice where anaemia patients present themselves to the GP with a random underlying cause. Further contributing to the representativeness of the results is the fact that patient characteristics age and gender were included into the statistical models, since these influence the diagnosis of the GP.

Limitations

This study has certain limitations. Several factors limit the representativeness of the results of this study. Besides the abovementioned exclusion of patients with multiple aetiologies, another limiting factor is the limited information, provided in the survey, based on which the GPs had to diagnose the underlying cause of anaemia. Besides the laboratory test results and the patient characteristics (age and gender), no further information was provided to the GPs in any of the survey cases. In daily practice, GPs obviously have additional information at their disposal as information about anamnesis, medical history and physical examination of patients is available to them in most cases. Further, this research only considers the impact that laboratory tests have on the ability of the GP to (correctly) diagnose the underlying cause of anaemia. As mentioned earlier, other (valid) reasons (e.g. reassurance) may underlie the decision of a GP to order a laboratory test and therefore deviate from the

laboratory protocol proposed by the DCGP guideline. The impact of the laboratory tests on the ability of the GP to diagnose the (correct) underlying cause of anaemia found in this study may have been different if the abovementioned factors could have been integrated into the analysis. Another limitation, inherent to the set-up of this study, is the fact that GPs diagnose an underlying cause of anaemia based on the results of *all* laboratory tests that they are provided with in the survey. The impact of some laboratory tests may therefore not have been detected in this analysis as these tests, in terms of (correctly) diagnosing an underlying cause of anaemia, possibly represent overlapping information. Presence (or absence) of laboratory tests during this analysis therefore may have affected the detectability of the impact of the results of other laboratory tests. Results of this study have to be interpreted with this limitation in mind. A limitation of the data used during this analysis may be the large number of cases (n=109, 54%) for which the result "smaller than 5" of the CRP laboratory test was replaced by the numerical value 4. However, the effect of the choice to replace each of these results with the numerical value 4 probably did not have a large effect on the ability to correctly estimate the impact of the (CRP) laboratory test result(s) as this result certainly falls between the values 0 and 5. Therefore, this range of values is relatively small compared to the range of the results of the CRP laboratory test over all cases (i.e. 4-290 mg/L).

Implications for Practice

GPs are often the first physicians to whom anaemia patients present themselves. On a yearly basis, 57.000 patients are newly diagnosed with anaemia in general practice[15]. If the DCGP guideline is followed in each of these patients, a complete set of 15 laboratory tests would be ordered in 27.360 of these patients as no underlying cause can be diagnosed in 52% of the anaemia patients based on the laboratory protocol in the DCGP guideline[14]. GPs may reduce their test ordering in these patients with up to 67% when they apply the most efficient subset of laboratory tests, determined for diagnosing the correct underlying cause of anaemia, instead of directly ordering the complete set of 15 laboratory tests. If no clear underlying cause can be found based on this subset of laboratory tests, the GPs can still decide to order additional laboratory tests. Such a reduction in test ordering may contribute to decreasing the number of unnecessarily ordered laboratory tests. It must be noted that laboratory testing in anaemia patients does not involve the largest expenses in healthcare, so the impact of realising such a reduction in test ordering on the direct costs of laboratory testing in these patients may be limited. Additionally, such reduction in test ordering will change little to the direct impact of laboratory testing on anaemia patients as it probably will not change the number of blood samples drawn from these patients. However, reducing the total number of laboratory tests ordered by GPs may, as mentioned earlier, prevent unnecessary downstream activities that potentially involve much higher indirect costs and place a higher burden on patient well-being. Knowing the impact of the results of these laboratory tests on the ability of the GP to (correctly) diagnose an underlying cause of anaemia may further help to decrease the complexity that GPs face in ordering laboratory tests and reduce the existing uncertainty of GPs about which laboratory tests they need to order.

Results of this study may support more efficient laboratory test ordering by GPs. At the same time, these results may contribute to decreasing the existing variability in laboratory test ordering by GPs[11]. Nowadays, the decision about which laboratory tests are performed in diagnosing anaemia patients is not always made by the GP. In the Netherlands, clinical chemistry labs often offer reflex testing in which the GPs request for an "anaemia protocol"[29]. The laboratory will then decide about performing (a sequence of) tests to diagnose an underlying cause of anaemia or reject the suspicion of anaemia. However, the set of tests performed in reflex testing for anaemia varies per laboratory[29]. Consequently, the results of this study may also be valuable for laboratories to establish an optimal subset of laboratory tests for reflex testing in anaemia, to standardize reflex testing in Dutch laboratories.

Further research may be performed to study the cost-effectiveness, in practice, of applying the most efficient subset of laboratory tests for diagnosing the correct underlying cause of anaemia instead of directly ordering the complete set of 15 laboratory tests proposed by the DCGP guideline. Studies similar to this research may be performed in other countries as the most efficient subset of laboratory tests (and its effects in practice) may differ per country. These results may differ since, amongst other factors, laboratory protocols for diagnosing the underlying cause of anaemia and the average number of laboratory tests ordered by GPs possibly differ from the Netherlands[30]. Additional research may be performed, similar to this study, to find opportunities to,

responsibly, decrease the number of (laboratory) tests in other medical fields. Such a study, in another field, may lead to results that have a larger positive impact on cost reduction or preserving patient well-being.

Conclusions

In conclusion, of the full set of 15 laboratory tests, recommended by the DCGP guideline, only a subset impacts the ability of the GP to (correctly) diagnose an underlying cause of anaemia, from a statistical point of view. Therefore, this study suggests that the laboratory tests included in the most efficient subset(s) of laboratory tests, determined in this study, provide a similar ability to the GP to (correctly) diagnose an underlying cause of anaemia. Whether such a subset of laboratory tests is acceptable and cost-effective in daily practice should be further investigated.

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## Appendix

Appendix I

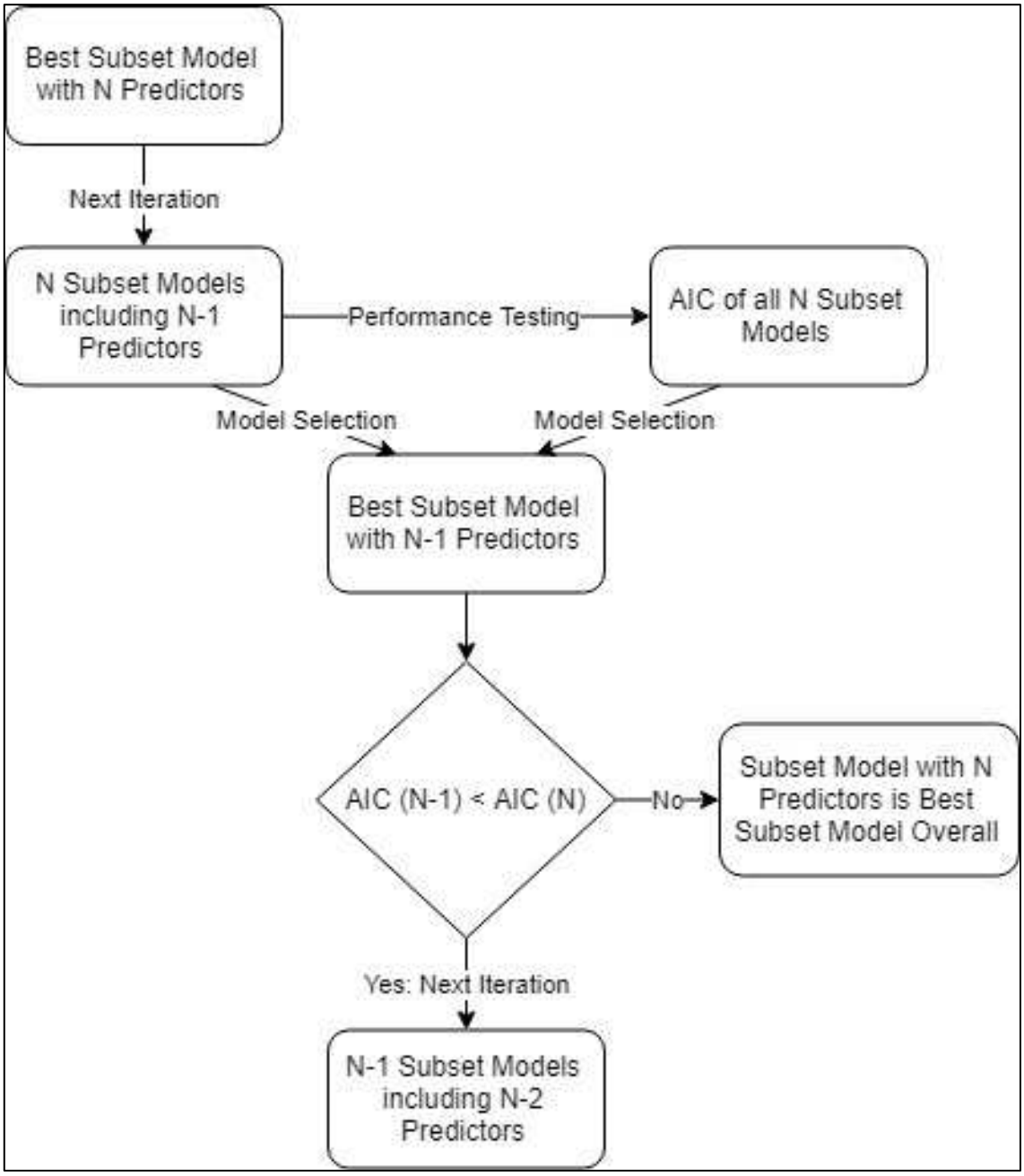


*Figure 1 Description of an iteration in the best subset selection process.*

Appendix II

| Selection Process | | |
|---|---|---|
| Subset Size | AIC Model | Predictor Eliminated |
| 17 | 814.39 | - |
| 16 | 801.83 | Transferrin |
| 15 | 790.15 | MCV |
| 14 | 783.84 | Thrombocytes |
| 13 | 779.12 | Creatinine |
| 12 | 775.40 | Vitamin B12 |
| 11 | 772.42 | LDH |
| 10 | 770.93 | Gender |
| Most Efficient Subset | | |
| Subset Size | AIC Model | Resulting Predictors |
| 10 | 770.93 | Ferritin<br>CRP<br>Reticulocytes<br>Serum Iron<br>ESR<br>MDRD<br>Haemoglobin<br>Leukocytes<br>Folic Acid<br>Age |

*Table 4 Selection of the best subset of laboratory tests for diagnosing an underlying cause of anaemia.*

Appendix III

| Selection Process | | |
|---|---|---|
| Subset Size | AIC Model | Predictor Eliminated |
| 17 | 497.65 | - |
| 16 | 495.70 | Gender |
| 15 | 493.82 | Thrombocytes |
| 14 | 492.05 | ESR |
| 13 | 490.32 | Leukocytes |
| 12 | 488.67 | Reticulocytes |
| 11 | 487.24 | Vitamin B12 |
| 10 | 485.88 | LDH |
| 9 | 484.71 | Haemoglobin |
| 8 | 483.25 | Serum Iron |
| 7 | 482.47 | Creatinine |
| 6 | 481.86 | MDRD |
| Most Efficient Subset | | |
| Subset Size | AIC Model | Resulting Predictors |
| 6 | 481.86 | Ferritin<br>CRP<br>MCV<br>Transferrin<br>Folic Acid<br>Age |

*Table 5 Selection of the best subset of laboratory tests for diagnosing the correct underlying cause of anaemia.*

Appendix IV

| Variance Inflation Factor – Resulting subset for diagnosing an underlying cause of anaemia | |
|---|---|
| Folic Acid | 1.14 |
| Leukocytes | 1.16 |
| Reticulocytes | 1.21 |
| MDRD | 1.30 |
| Age | 1.34 |
| Haemoglobin | 1.66 |
| Ferritin | 1.96 |
| Serum Iron | 2.19 |
| CRP | 2.44 |
| ESR | 2.58 |
| Variance Inflation Factor – Resulting subset for diagnosing the correct underlying cause of anaemia | |
| Folic Acid | 1.03 |
| Age | 1.14 |
| CRP | 1.46 |
| MCV | 2.07 |
| Ferritin | 2.66 |
| Transferrin | 2.99 |

*Table 6 Variance inflation factors determined for assessing multicollinearity.*