Diagnostic Work-Up of Anemia in General Practice

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SBOH
voor artsen in opleiding

Provided by thesis specialist Ridderprint, ridderprint.nl

ISBN: 978-94-6416-886-0
Printing: Ridderprint
Layout and design: Marilou Maes, persoonlijkproefschrift.nl

Diagnostic Work-Up of Anemia in General Practice

Diagnostiek van anemie in de huisartsenpraktijk

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. A.L. Bredenoord

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 8 december 2021 om 10:30 uur

door

Annemarie Schop
geboren te Ridderkerk

Erasmus University Rotterdam

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General introduction

General practitioners regularly see patients with a low hemoglobin count. This condition is also known as anemia, and is a symptomatic diagnosis, which implies the presence of an underlying disease causing the anemia. Anemia in itself is associated with a variety of adverse outcomes, such as increased mortality, physical and cognitive decline, cardiovascular events and reduced quality of life [1, 2]. It is therefore important that patients with a low hemoglobin count receive a diagnostic work-up to identify the underlying disease. Then, the disease and the anemia itself can be treated to avoid adverse outcomes.

Etiologies of anemia

Additional blood tests can reveal the pathogenesis of the anemia. Various pathogeneses of anemia are known, which are referred to as the anemia etiologies. Four of these are common in the Western world [3 - 6]. The most common etiology is anemia of chronic disease (ACD), which is based on a functional iron deficiency as seen in infections and malignancies [7]. The second anemia etiology commonly seen is iron deficiency anemia (IDA), which is based on an absolute iron deficiency as seen in (gastro-intestinal) bleeding [8]. Thirdly, a decreased erythropoietin production seen in renal insufficiency causes renal anemia [9]. Fourth, a nutritional deficiency etiology is present in a low proportion of anemia patients, caused by a decreased vitamin B12 and/or folic acid status [10]. Besides these four common etiologies, some less prevalent anemia etiologies are present in the Western world, such as hemoglobinopathy, bone marrow diseases and hemolysis [11]. In addition, based on laboratory analysis, approximately a third of patients with anemia cannot be assigned to one of the anemia etiologies. These patients have anemia of uncertain etiology [3-6].

Approach of anemia

The diagnostic work-up for suspected anemia starts with determining the hemoglobin count in a blood test, which will confirm anemia if this is low. Secondly, the anemia etiology needs to be defined by analyzing a variety of blood values. As mentioned above, anemia etiologies exist long a broad continuum, and each requires a different set of blood values analysis and interpretation [11]. The third step of the diagnostic work-up is to match the laboratory results (i.e., the defined anemia etiology) with the clinical presentation of the patient. The result may give reasons to request one or more additional investigations, such as an endoscopy in case of anamnestic rectal bleeding and blood values indicative for IDA. The matching of the anemia etiology with clinical presentation and any additional investigations will most likely result in the final diagnosis. The final and last step of the diagnostic work-up is treatment – if possible – of the underlying disease that

caused the anemia) in order to prevent a relapse of the anemia or a declining clinical condition.

The additional blood analysis in patients with anemia

The broad spectrum of anemia etiologies and related diseases makes anemia a challenging symptom for general practitioners. A complete and correct diagnostic work-up is necessary to treat the patient and to avoid adverse outcomes. Several anemia guidelines worldwide provide direction towards the diagnostic work-up of patients with anemia [12-15]. However, these guidelines differ strongly with regard to the blood tests that should be performed. Especially a patient's iron status can be interpreted in several ways, since up to nine blood tests can be analyzed. Determining the iron status is crucial to distinguish between an IDA and ACD (figure 1). Since all blood tests must be adequately interpreted, the additional analysis might be very challenging.

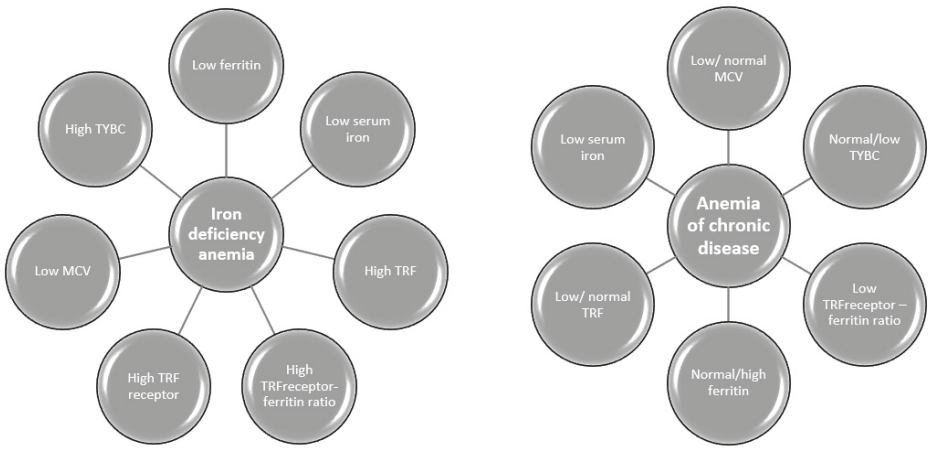

Figure 1. All blood tests which can be determined to distinguish between IDA and ACD.

Mean corpuscular volume

An intermediate step which might help to define the anemia etiology, is the classification of anemia based on the mean corpuscular volume (MCV). Wintrobe first described this classification algorithm in 1930 [16]. According to this algorithm, anemia can be classified as microcytic (MCV < 80 fl), normocytic (MCV 80 – 100 fl) and macrocytic (MCV > 100 fl). Microcytic anemia would be present in case of a production disorder of the red blood cells, resulting in small erythrocytes [17]. This category is commonly seen in IDA and thalassemia. Normocytic anemia

would be present in patients with acute blood loss resulting in a decreased amount of normal-sized erythrocytes (i.e., IDA) or in patients with reduced production of erythrocytes as the consequence of reduced erythropoietin production as seen in renal insufficiency [18]. Normocytic anemia as a result of reduced production of erythrocytes in the bone marrow is also commonly seen in patients with chronic diseases or acute inflammations. Lastly, macrocytic anemia would be present in case of larger erythrocytes than normal, which is usually seen in patients with vitamin deficiencies and bone marrow diseases [19].

Iron deficiency anemia

As mentioned above, iron deficiency anemia is one of the most common but also complex anemia etiology. Iron deficiency has long been considered one of the nutritional deficiencies causing anemia [20]. Nowadays, a lack of iron due to dietary-related factors is uncommon in the western world. However, iron deficiency is still one of the most prevalent etiologies of anemia and is often the result of (gastro-intestinal) blood loss. In patients with IDA and without explanatory blood loss (such as hypermenorrhea) additional investigations of the gastro-intestinal tract are necessary [20]. The most effective investigation of the gastro-intestinal tract is endoscopy. Colorectal cancer is one of the most concerning causes of IDA, with a prevalence of 5-10% among the elderly [21, 22]. Some studies have suggested that IDA-related colorectal cancer is more often located on the right side of the colon [23]. These right-sided tumors would often not be accompanied by GI-symptoms such as rectal bleeding. In such a case, a tumor might bleed unnoticed for a longer time, resulting in a diagnosis of IDA before the colorectal cancer is found. Perhaps the lack of symptoms of the GI-tract might be the reason why general practitioners request endoscopic evaluation in only relatively few patients with IDA. Indeed, the scarce literature from general practice on IDA patients showed that around one third of patients receive endoscopic evaluation after their IDA diagnosis [24, 25]. In contrast, most guidelines about IDA in general practice recommend endoscopic evaluation for all patients aged 50 years and older, unless hypermenorrhea is present [12, 20]. It is unknown on which basis GPs decide to request or decline endoscopic evaluation in IDA patients. Moreover, little is known about the long-term outcomes in IDA patients who did not undergo endoscopic evaluation.

Anemia of chronic disease

The second most common anemia etiology, is anemia of chronic disease. This etiology is defined as a functional iron deficiency, which implies that a good interpretation of the complex iron status of anemia patients is necessary. Although ACD is one of the most common etiologies, not much is known about the prevalence

of the underlying diseases in general practice. Furthermore, the functional iron deficiency seen in patients with ACD might confusingly suggest that oral iron supplementation would be helpful in these patients. However, the functional iron deficiency implies that, although sufficient iron is present in the body, the iron is not used [7]. Iron supplementation in patients with ACD might, therefore, lead to an iron overload and this might negatively affect patients' health. The awareness of this phenomenon among general practitioners is unknown.

Motivation of this thesis

The above introduction makes clear that there are still a number of challenges in the field of anemia diagnostics. Internationally, guidelines about anemia diagnostics differ and it is unknown to which extent these guidelines are used in daily practice and how effective they are. In line with this, the accuracy of the classification of anemia on the basis of MCV has not been well investigated, although this classification is commonly used and recommended in guidelines. Furthermore, the step-wise approach recommended in anemia guidelines seems to underestimate the occurrence of multifactorial anemia etiologies, while literature suggest that mainly the elderly anemia patients have anemia of multifactorial origin. Concerning the anemia etiology, iron deficiency anemia and anemia of chronic disease are commonly seen, and it appeared that there is a need for more research to optimize the care of these patients. The incidence of anemia in general practices in the Netherlands is 8.6 per 1000 patients each year [12]. Therefore, the challenges in anemia diagnostics are primarily applicable to patients in the general population, since the first presentation of anemia will be often in the general practice setting.

The PAGAS database

To improve the care of patients with anemia in general practice, a collaborative project was started in 2007 in the Albert Schweitzer hospital, Dordrecht, the Netherlands. This project was of a multidisciplinary nature, involving GPs, clinical chemists, internists, and gastroenterologists. The project was called PAGAS (Project of Anemia analysis from the General practitioner to the Albert Schweitzer hospital), and 81 out of the 150 GPs working in Dordrecht or the Dordrecht region decided to join the project. The project ran from February 2007 until November 2018. In this period, GPs had the opportunity to request a special laboratory analysis (i.e., the PAGAS package) in case of the suspicion of anemia in males aged ≥ 18 years and females aged ≥ 50 years. The PAGAS package consisted of an extended laboratory analysis which would be automatically run when anemia was detected. The analysis included hemoglobin (Hb), MCV, serum iron, transferrin, ferritin, iron saturation percentage, folic acid, vitamin B12, reticulocytes, erythrocytes, thrombocytes, leukocytes,

creatinine, c-reactive protein and lactate dehydrogenase. Anemia was defined as hemoglobin <13.7 g/dL and <12.1 g/dL for males and females, respectively. Since all research questions in this thesis are related to the diagnostic work-up of anemia and its etiologies, patients already known with anemia in the two years prior to the analysis were excluded from the PAGAS cohort. Thus, only newly-diagnosed anemia patients were included and investigated.

In this thesis, a set of 8 laboratory-orientated etiologies of anemia was defined, based on literature and the Dutch general practitioners guideline for anemia (**table 1)**. The use of these definitions ensures a uniform representation of the anemia etiologies and takes into account the possibility of multiple etiologies applicable in one patient. However, the uniform enforcement of the definitions excluded the combination of IDA and ACD. The general practitioners who requested the PAGAS package for a patient received all laboratory results along with an interpretation of these results based on the definitions described in table 1. The PAGAS collaboration project offered an additional component, which was the possibility of fast-track endoscopy referrals (i.e., gastroscopy and colonoscopy within 4 weeks for patients with IDA in whom hypermenorrhea was excluded). In the Netherlands, GPs are not able to request endoscopic evaluation directly, but need to refer their patients to a gastroenterologist or internist. This intermediate step in combination with a waiting list for endoscopy means an approximately two months of waiting between IDA diagnosis and endoscopy. To circumvent this throwback, this project offered GPs fast-track places for endoscopy.

Table 1. Predefined etiologies of anemia.

Anemia etiology	Definition
Anemia of chronic disease	Serum ferritin > 100 µg/L and at least one of the following: transferrin ≤ 3.60 g/L or iron < 14 (male)/ < 10 (female) µmol/L
Renal anemia	Estimated glomerular filtration rate < 45 mL/min/1.73m^2
Iron deficiency anemia	Serum ferritin < 25 (male) or < 20 (female) µg/L
Hemoglobinopathy	Hemoglobin electrophoresis was conducted in case of low MCV (< 80 fl) in combination with increased erythrocyte count (> 6.0 µl). If electrophoresis yielded suspected hemoglobinopathy, subsequent genetic testing was performed.
Suspected hemolysis	Lactate dehydrogenase > 241 U/L and reticulocytes > 2.5%.
Suspected bone marrow disease	Reticulocytes < 2.5% and leukocyte count < 4.3 or > 10 10^9/L and thrombocyte count < 150 / > 390 10^9/L
Vitamin B12 deficiency	Serum vitamin B12 < 130 pmol/L
Folic acid deficiency	Serum folic acid < 5 nmol/L
Multifactorial	Satisfied more than one of the above definitions
Uncertain	Satisfied none of the above definitions

Aim and outline of this thesis

The first part of this thesis focuses on the anemia etiologies and the related diagnostic work-up/laboratory analysis. In **chapter 2,** the distribution is described of the etiologies of anemia according to the predefined definitions of the PAGAS project. In this way, we were also able to evaluate the multiple aspect of etiologies among anemia patients. In addition, this chapter focus on markers that might predict the anemia etiology. In **chapter 3,** we evaluated the diagnostic accuracy of MCV as a screening test in establishing the anemia etiology.

The next part of the thesis focuses on the effectiveness of an extensive laboratory analysis (i.e., PAGAS project) in the diagnostic work-up of anemia patients in daily practice. This study was designed as an online survey study among general practitioners and is presented in **chapter 4.** In addition, in **chapter 5** the cost-effectiveness of this extensive laboratory analysis is shown.

The final part of the thesis focuses on two prevalent anemia etiologies, anemia of chronic disease and iron deficiency anemia. In **chapter 6** an overview is given of the additionally requested investigations and the final diagnoses made in patients with anemia of chronic disease. **Chapter 7** presents the endoscopic evaluations and final diagnosis of patients with iron deficiency anemia during a median follow-up of 4.6 years. This chapter also provides a survival analysis on patients with either an early or a delayed colorectal cancer diagnosis. The thesis is concluded with a discussion about the new study results presented in the above chapters and its clinical implications in **chapter 8**. Last, a summary of the main results is presented in **chapter 9** and the Dutch translation in **chapter 10**.

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2

A new diagnostic work-up for defining anemia etiologies: a cohort study in patients ≥50 years in general practices

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BMC Family Practice. 2020; 21(1):
DOI: 10.1186/s12875-020-01241-7

Abstract

Background. To study etiologies of anemia using an extensive laboratory analysis in general practices.

Method. An extensive laboratory analysis was performed in blood of newly diagnosed anemia patients aged ≥50 years from the general population in the city of Dordrecht area, the Netherlands. Eight laboratory-orientated etiologies of anemia were defined. Patients were assigned one or more of these etiologies on the basis of their test results.

Results. Blood of 4152 patients (median age 75 years; 49% male) was analyzed. The anemia etiology was unclear in 20%; a single etiology was established in 59%; and multiple etiologies in 22% of the patients. The most common etiologies were anemia of chronic disease (ACD) (54.5%), iron deficiency anemia (IDA) (19.1%) and renal anemia (13.8%). The most common single etiologies were IDA (82%) and ACD (68%), while the multiple etiologies most commonly included folic acid deficiency (94%) and suspected bone marrow disease (88%). Older age was associated with a lower incidence of IDA and a higher incidence of renal anemia. Mild anemia was more often associated with ACD and uncertain anemia, while severe anemia was mainly seen in patients with IDA.

Conclusion. Extensive laboratory analysis in anemic patients from the general population helped clarify the etiology of anemia and revealed many various combinations of etiologies in a significant proportion of patients. Age, sex and the severity of anemia are predictive of the underlying etiology.

Background

In clinical practice, anemia diagnostics is a four-step process, starting with the clinical suspicion of anemia and confirmation of a decreased hemoglobin concentration in a blood test. Secondly, the anemia can be further categorized into an underlying etiology based on additional laboratory analysis. The third step is to match the anemia etiology to the patient's clinical presentation, symptoms and comorbidities. If necessary, additional diagnostic tests can be performed (such as endoscopy) to find the cause of the anemia. The final step is to confirm the underlying condition that is causing anemia and to treat this to prevent relapse of the anemia or a decline in clinical condition [1, 2].

In the Western world, four common etiologies for anemia are anemia of chronic disease (ACD) (17–40%), iron deficiency anemia (IDA) (5–19%), renal anemia (2–15%) and vitamin B12 or folic acid deficiency (2–14%) [3–6]. For still 26–44% of patients, the etiology of anemia is uncertain. Anemia in relation to aging and sex has been studied previously, although but few studies distinguished between the various anemia etiologies [2, 3]. One study in a cohort of anemic women aged ≥65 years found a higher incidence of ACD and renal anemia in older age [4]. Another study in a general population found an increasing incidence of ACD and uncertain anemia etiology with higher age, while IDA was more common in females [5]. Studies concerning anemia etiologies in community dwelling patients usually present data obtained from either a limited or a step-wise laboratory analysis. In one study, the use of a limited laboratory analysis was compared to a more extensive laboratory analysis. They found that an extensive laboratory analysis leads more often to establishment of the anemia etiology [7]. Moreover, extensive laboratory analysis would prevent a delay in the initiation of treatment for the underlying disease and has shown to be cost-effective [8]. Another drawback of a limited or step-wise laboratory analysis is that this cannot assess the multifactorial role of etiologies causing anemia. In some previous studies, multiple causes of anemia were found in about 30–50% of included patients [9, 10].

We set out to systematically study common etiologies of anemia and the role of multiple etiologies of anemia on the basis of the results of extensive laboratory analysis in a population of newly diagnosed patients in general practices. Furthermore, we evaluated the severity of anemia and its relation with the underlying etiology or etiologies.

Methods

Study population

A cohort study was designed for our research purpose. Patient data were collected from a large database set up in the laboratory of the Albert Schweitzer Hospital, Dordrecht, the Netherlands. This project was of multidisciplinary origin; general practitioners (GPs), internal medicine physicians and clinical chemists participated. Eighty-one of the 150 GPs in the city of Dordrecht area, the Netherlands, agreed to participate in this project, which started on 1 February 2007. The department of clinical chemistry of the Albert Schweitzer Hospital is the referral laboratory for GPs in the area.

If a participating GP requested a blood test for a patient aged ≥50 years and this revealed a low hemoglobin concentration according to the references values of the participating laboratory (i.e. hemoglobin < 13.7 g/dL and < 12.1 g/dL for males and females, respectively), a further extensive laboratory assessment was performed. This assessment consisted of measuring hemoglobin, mean corpuscular volume (MCV), reticulocyte count, leukocyte count, thrombocyte count, lactate dehydrogenase, vitamin B12, folic acid, creatinine, ferritin, transferrin, and serum iron. These laboratory tests were chosen based on the Dutch guideline and tests regularly ordered by the participating internal medicine physicians [11]. The test results, together with the patient's sex and age were stored anonymously in the database. As we wished to study anemia etiologies during diagnostic work-up, we excluded data of patients already known to have anemia in the previous 2 years.

This study was approved by the institutional review board of the Albert Schweitzer Hospital and was conducted in accordance with the Declaration of Helsinki.

Definitions

We defined a set of 8 laboratory-orientated etiologies of anemia based on literature, the Dutch general practitioners' guideline of anemia and the references values of the participating laboratory (Table 1) [3–6, 11–14]. These eight etiologies were ACD, renal anemia, IDA, hemoglobinopathy, suspected hemolysis, suspected bone marrow disease, vitamin B12 deficiency and folic acid deficiency. The use of these definitions ensures a uniform representation of the laboratory etiologies and takes into account the possibility of multiple etiologies in a patient. The strict application of the definitions made the combination of IDA and ACD impossible. To be able to visualize and analyze trends between anemia etiology and patient characteristics, we clustered some etiologies that had a low incidence.

Thus, vitamin B12 deficiency and folic acid deficiency were taken together, and hemoglobinopathy, suspected hemolysis and suspected bone marrow disease were clustered as 'other etiologies'. In addition, hemoglobin values were clustered into three groups proportionally. Severe anemia was defined as hemoglobin ≤9.7 g/dL; moderate anemia as hemoglobin > 9.7 and ≤ 12.9 g/dL for males and > 9.7 and ≤ 11.3 g/dL for females. Mild anemia was defined as hemoglobin > 12.9 and < 13.7 g/dL for males and > 11.3 and < 12.1 g/dL for females.

Table 1. Laboratory-orientated etiologies of anemia

	Definition
Anemia of chronic disease	Serum ferritin > 100 µg/L and at least one of the following transferrin ≤ 3.60 g/L or iron < 14 (male) / < 10 (female) µmol/L
Renal anemia	Estimated glomerular filtration rate < 45 mL/min/1.73m^2
Iron deficiency anemia	Serum ferritin < 25 (male) or < 20 (female) µg/L
Hemoglobinopathy	Hemoglobin electrophoresis was conducted in case of low MCV (< 80 fl) in combination with increased erythrocyte count (> 6.2 (male) or > 5.4 (female) µl) (and followed by genetic testing).
Suspected hemolysis	Lactate dehydrogenase > 241 U/L and reticulocytes > 2.5%
Suspected bone marrow disease	Reticulocytes < 2.5% and leukocyte count < 4.3 or > 10 10^9/L and thrombocyte count < 150 / > 390 10^9/L
Vitamin B12 deficiency	Serum vitamin B12 < 130 pmol/L
Folic acid deficiency	Serum folic acid < 5 nmol/L
Multiple causes	Satisfied more than one of the above definitions
Uncertain	Satisfied none of the above definitions

Statistical analysis

Laboratory protocol violations had resulted in missing laboratory values (Table 1). To avoid selection bias as a consequence of the exclusion of patients with missing values, we applied single imputation using an expectationmaximization algorithm [15]. For nine laboratory values, the percentage of missing values was below one. For creatinine, however, it was 19.6%. This could be ascribed to the fact that GPs could follow two pathways when they requested laboratory analysis, one of which did not include creatinine. Still, this percentage was low enough to allow single imputation to be applied. Characteristics of the study population are described in terms of frequency, median and interquartile ranges. The distribution of underlying etiologies of anemia and the multiple aspect of the etiologies is described using counts and relative frequencies. We visualized the distribution of the etiologies of anemia by age, sex and level of anemia severity. To assess whether the etiology of anemia is associated with age, sex and/or the severity of anemia,

we performed a multinomial logistic regression analysis. The dependent variable was anemia etiology (ACD, renal, IDA, vitamin B12 or folic acid deficiency, other, multiple etiologies or uncertain). The independent variables were age (continuous variable), sex (male/female) and anemia severity (mild/moderate/severe). Model assumptions were tested and met. We used SPSS for Windows, version 24 (IBM Corp., Armonk, NY, USA), for data handling and analyses. All statistical tests were two-sided and we considered a p-value $p < 0.05$ statistically significant.

Results

Inclusion and general characteristics

From February 1, 2007 until February 1, 2017, a total of 5026 patients from the collaboration project were registered with anemia in the laboratory system. A total of 874 patients (17%) already known with a recent history of anemia 2 years prior to laboratory analysis were excluded. Thus, data of 4152 patients with a new diagnosis of anemia were included in the analyses. A consort diagram shows the process of the selection of the patients (Fig. 1). The median age of this population was 75 years (IQR 64–83 years) and 2036 (49%) were male. A total of 887 patients (21%) had one or more missing laboratory values (Table 2).

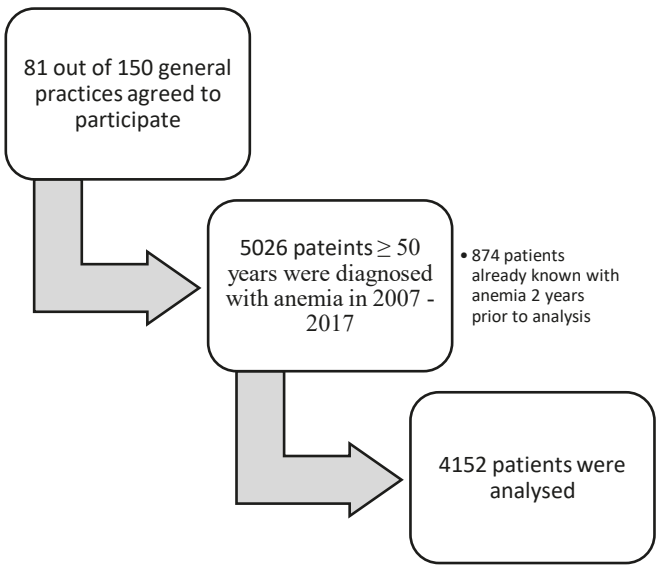

Figure 1. Consort diagram of the patient cohort.

Table 2. General characteristics of the study population (n = 4152).

	Median (interquartile ranges) / count (%)	Missing counts (%)
Age (per year)	75 (64 – 83)	
Male sex	2036 (49%)	
Hemoglobin (g/dL)		-
• **Male**	12.9 (12.1 – 13.4)	
• **Female**	11.4 (10.6 – 11.8)	
Reticulocytes (%)	1.0 (0.8 – 1.4)	16 (0.4)
Leukocyte count (10^9/L)	7.1 (5.7 – 9.0)	20 (0.5)
Thrombocyte count (10^9/L)	269 (216 – 344)	28 (0.7)
LDH (E/L)	306 (221 – 372)	11 (0.3)
eGFR (mL/min/1.73m²)	> 60 (54 - >60)	812 (19.6)
Ferritin (µg/L)		7 (0.2)
• **Male**	157 (60 – 321)	
• **Female**	81 (21 – 207)	
Transferrin (g/L)	2.38 (2.06 – 2.82)	10 (0.2)
Serum iron (µmol/L)		10 (0.2)
• **Male**	11.2 (6.5 – 15.6)	
• **Female**	8.8 (4.9 – 12.5)	
Vitamin B12 (pmol/L)	288 (209 – 430)	26 (0.6)
Folic acid (nmol/L)	16 (11 – 25)	38 (0.9)
Uncertain anemia etiology	819 (20)	
Single anemia etiology	2430 (59)	
Multiple anemia etiologies	903 (22)	
• **Two**	811 (90)	
• **Three**	88 (10)	
• **Four**	4 (0.4)	

Anemia etiology

According to the definitions of laboratory etiologies of anemia, a single etiology was found in 2430 patients (59%); multiple etiologies in 903 patients (22%); and an uncertain etiology in 819 patients (20%). Among the patients with multiple etiologies of anemia, two etiology categories were assigned to 811 patients (90%); three to 88 patients (10%); and four to 4 patients (0.4%).

The most common etiology was ACD (54.5% of patients), followed by IDA (19.1% of patients) and renal anemia (13.8% of patients) (Table 3). IDA and ACD were the most frequent single etiologies of anemia, i.e. 82 and 68% of diagnoses, respectively. In contrast, folic acid deficiency and suspected bone marrow disease most often

were part of multiple etiologies (94 and 88% of diagnoses, respectively) (Table 3). Among the 903 patients in whom multiple etiologies were found, the commonest combinations were ACD with renal anemia (n = 307, 34%), ACD with suspected bone marrow disease (n = 178, 20%) and ACD with vitamin B12 deficiency (n = 69, 8%).

Table 3. Frequency of underlying anemia etiologies.

Underlying etiology	Single* (n = 2430)	Multiple* (n = 903)	Total** (n = 4152)
Anemia of chronic disease	1536 (68)	728 (32)	**2264 (54.5)**
Renal anemia	130 (23)	441 (77)	**571 (13.8)**
Iron deficiency anemia	646 (82)	146 (18)	**792 (19.1)**
Hemoglobinopathy	8 (35)	15 (65)	**23 (0.6)**
Suspected hemolysis	20 (17)	101 (83)	**121 (2.9)**
Suspected bone marrow disease	38 (12)	274 (88)	**312 (7.5)**
Vitamin B12 deficiency	49 (24)	153 (76)	**202 (4.9)**
Folic acid deficiency	3 (6)	44 (94)	**47 (1.1)**

**Count (percentage of total count of etiology) **Count (percentage of total study cohort)*

Anemia etiology in subgroups

The distribution of anemia etiologies in relation to specific patient characteristics is visualized in Fig. 2. Concerning age, the number of IDA etiologies declines with age, whereas the number of renal anemia cases increases with age (Fig. 2a). Regarding the severity of anemia, mainly ACD and uncertain etiology are more common in patients with mild anemia. Severe anemia is predominantly seen in relation with IDA (Fig. 2b). Looking at the sex distribution, we find that ACD etiology is more common in men, while IDA etiology is more common in women (Fig. 2c).

Predictors of anemia etiology

A multinomial logistic regression was performed to assess whether the anemia etiology could be predicted from the patient's age, sex and/or the severity of anemia. Anemia of uncertain etiology was defined as reference group. The results are shown in Table 4. Most striking was the association of ACD with male sex (OR 2.18, 95% CI 1.83–2.60), while IDA (OR 0.72, 95% CI 0.57–0.90) and other etiologies (OR 0.51, 95% CI 0.29–0.90) were more associated with female sex. Severe anemia was significantly associated with IDA (OR 13.12, 95% CI 8.63–19.94), other etiologies (OR 8.95, 95% CI 4.16–19.25) and the presence of multiple etiologies (OR 7.95, 95% CI 5.28–11.96).

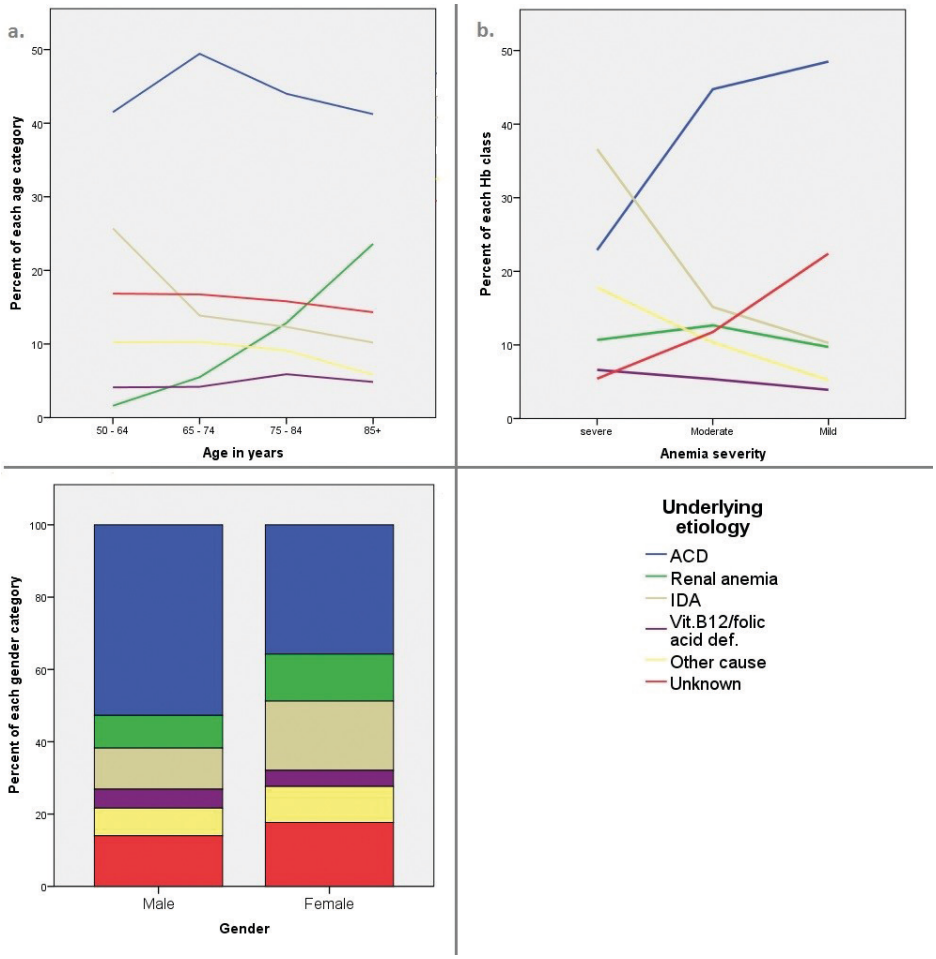

Figure 2. Distribution of patient characteristics among the anemia etiologies.

Table 4. Multinominal logistic regression of anemia etiology.

Anemia etiology	**Median (range) or count (%)**	**OR (95% CI)**	**P - value**
Unknown (n = 819)			
• Age (per year)	75 (50 – 99)		
• Male gender	348 (42.5)		
Anemia severity		Reference group	
• Mild	587 (71.7)		
• Moderate	200 (24.4)		
• Severe	32 (3.9)		
ACD (n = 1536)			
• Age (per year)	73 (50 – 101)	1.00 (0.99-1.01)	0.65
• Male gender	959 (62)	2.18 (1.83-2.60)	< 0.001
Anemia severity			
• Mild	879 (57)		*Ref*
• Moderate	608 (40)	1.32 (1.10-1.59)	0.003
• Severe	49 (3)	0.98 (0.62-1.55)	0.92
Renal (n = 130)			
• Age (per year)	86 (63 – 102)	1.10 (1.07-1.12)	< 0.001
• Male gender	40 (30.8)	0.87 (0.57-1.31)	0.50
Anemia severity			
• Mild	78 (60.0)		*Ref*
• Moderate	40 (30.8)	1.58 (1.06-2.36)	0.03
• Severe	12 (9.2)	2.61 (1.27-5.38)	0.010
IDA (n = 646)			
• Age (per year)	67 (50 – 103)	0.96 (0.95-0.97)	< 0.001
• Male gender	237 (36.7)	0.72 (0.57-0.90)	0.003
Anemia severity			
• Mild	257 (39.8)		*Ref*
• Moderate	232 (35.9)	2.90 (2.29-3.68)	< 0.001
• Severe	157 (24.3)	13.12 (8.63-19.94)	< 0.001
Vit B12 / FA def. (n = 52)			
• Age (per year)	79 (50 – 100)	1.02 (1.00-1.05)	0.07
• Male gender	23 (44.2)	1.12 (0.63-2.00)	0.69
Anemia severity			
• Mild	34 (65.4)		*Ref*
• Moderate	18 (34.6)	1.32 (0.74-2.35)	0.35
• Severe	-	NA	NA
Other etiologies (n = 66)			
• Age (per year)	72 (50 – 100)	0.98 (0.96-1.00)	0.09
• Male gender	19 (28.8)	0.51 (0.29-0.90)	0.02
Anemia severity			
• Mild	30 (45.5)		*Ref*
• Moderate	23 (34.8)	2.68 (1.52-4.71)	< 0.001
• Severe	13 (19.7)	8.95 (4.16-19.25)	< 0.001

Table 4. (Continued)

Anemia etiology	Median (range) or count (%)	OR (95% CI)	P - value
Multiple (n = 903)			
• Age (per year)	79 (50 – 103)	1.02 (1.01-1.03)	< 0.001
• Male gender	410 (45.4)	1.18 (0.97-1.44)	0.10
Anemia severity			
• Mild	356 (39.4)		*Ref*
• Moderate	397 (44.0)	2.94 (2.39-3.63)	< 0.001
• Severe	150 (16.6)	7.95 (5.28-11.96)	< 0.001

Discussion

Summary

Extensive laboratory analysis in the anemic patient from the general population enables to establish an etiology diagnosis more often. We found that 22% of patients have multiple etiologies defining their anemia. Moreover, many combinations of etiologies of anemia are possible. This study also estimated that age, sex and anemia severity are related to several anemia etiologies.

Comparison with existing literature

We found that ACD, IDA and renal anemia were the most common etiologies of anemia among the studied population, which is in line with previous studies [3–6]. The etiology of the anemia was uncertain for 20% of patients, which proportion is much lower than the 26–44% reported in previous studies [3–6]. An explanation could be our inclusion of suspected bone marrow disease, hemoglobinopathy and suspected hemolysis as anemia etiologies, which was not done in previous studies [3–5]. Another explanation could be a lower incidence of uncertain anemia when a more regular extensive laboratory analysis is performed. This hypothesis is supported by a previous study in which only 8% of hospitalized anemia patients had an uncertain anemia etiology, most likely due to a more extensive laboratory analysis [9, 10]. Nevertheless, for most patients an anemia etiology can be diagnosed based on the laboratory tests results. This information is of great relevance for the general practitioners as it provides insight in the additional diagnostic work-up and prevents unnecessarily (hematology) referral.

To date, data on multiple etiologies of anemia has been scarce. In our study, a multiple etiology was found in 22% of patients from the general population. Especially vitamin B12 deficiency and folic acid deficiency were often part of a

multiple anemia etiology. Therefore, extensive laboratory analysis might yield results that were not expected based on history and clinical presentation, but which may have treatment implications. Performing an extensive laboratory analysis in case of low hemoglobin concentrations is therefore recommended. This will reduce the number of missed causes of anemia and creates an optimal treatment framework. Furthermore, the laboratory protocol used for this study has shown to be effective at a minimal increase in costs [8]. In conclusion, an extensive laboratory analysis should be a standard first step during the diagnostic work-up of a newly diagnosed anemia patient, independently of the clinical presentation.

In this study, the distribution of anemia etiologies among specific patient characteristics showed a number of interesting trends. The peak of IDA etiology in the younger age group might be due to the arbitrary age cut-off of 50 years for inclusion. We offset this cut-off to exclude hypermenorrhea as predominant cause of IDA, but hypermenorrhea might still be present in some patients above 50 years. Furthermore, IDA is predominantly seen in patients with severe anemia, which might be explained by the more insidious course of anemia often seen in patients with IDA. As a consequence of the insidious course, the patient presents with symptoms at a later stage, resulting in a more severe anemia at moment of diagnosis. We also found that other etiologies presented more frequently with severe anemia. This finding highlights the relevance of additional tests (i.e. genetic screening or bone marrow biopsy) based on the clinical suspicion and family history. The mild anemia seen in patients with uncertain anemia etiology has been described in literature before [16]. Overall, Andres et al. noted no correlation between the severity of anemia and its underlying cause. We were able to demonstrate significant associations, which might be due to our large cohort resulting in a more precision of observed point estimates [10]. Regarding associations with the patients' sex, we observed a trend towards twice as many ACD etiologies among men compared with women. Previous studies showed a broad range in this ratio [3, 5]. The trend we observed might have resulted from the strict definition of ACD including ferritin > 100 µg/L. The median ferritin level in the women in our study was lower than that in men (Table 2: 81µg/L versus 157 µg/L, respectively). Therefore, women might be less likely to meet our definition of ACD.

Strengths and limitations

A major strength of our study design is that it permitted to establish the onset of anemia. This was achieved by excluding patients already known with anemia 2 years previously. Another strength is the large cohort, which increased the precision of observed point estimates. Eighty-one out of 151 GP practices registered at our laboratory system participated in the study. These 81 GP practices can be

considered reflective of all 151 GP practices in the area of Dordrecht. Therefore, we assume that there are no considerable differences between the distribution of anemia etiology among participating and nonparticipating GP practices.

As a limitation of our study, we did not know the indications for blood analysis; these are not registered. We, therefore, could not match a patient's clinical information with the results of the blood analysis. We used a laboratory-orientated uniform enforcement when defining the etiologies and acknowledge the limitation of the missing clinical information. Still, our approach means a solid step forward in the anemia diagnostic work-up and we encourage the use of clinical information during the diagnostic process. On top of that, we are aware of the fact that the gold standard for anemia etiology diagnosis would be a bone marrow biopsy. However, this examination would be non-ethical to apply on each patient and can't be performed in general practice setting. We are aware of the fact that the WHO uses slightly different cut-off values of hemoglobin for anemia and not all laboratories have the same reference values. However, we decided to maintain the reference values according to the participating laboratory. A limitation is a gap in the analysis of ferritin values between 20/25– 100 µg/L and vitamin B12 levels between 130 and 200 pmol/L, which is a consequence of our strict definitions. In the current study design, it was not possible to add additional parameters such as serum transferrin receptor, methylmalonic acid and homocysteine as additional parameters for further interpretation. This might have resulted in an underestimation of IDA, ACD and vitamin B12 deficiencies. Although we defined a standard extensive laboratory protocol to be performed in each patient, data were missing for a proportion of patients. We applied single imputation to avoid selection bias and thus could include data of all patients in the analysis.

Conclusions

In clinical practice, the extensive laboratory analysis during the diagnostic work-up of anemia patients seems to be valuable. Not only because more patients can be assigned an etiology, but also because the possible multiple aspect of etiologies can be effectively analyzed. Moreover, a patient's age, sex and the severity of anemia may serve as markers that can guide the diagnostic work-up, resulting in a faster diagnosis and treatment initiation of the underling disease causing anemia.

Acknowledgements

The authors thank the participating general practitioners for their contributions to this study.

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3

The accuracy of mean corpuscular volume guided anaemia classification in primary care

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Fam Pract. 2021 Aug 4; cmab034
DOI: 10.1093/fampra/cmab034

Abstract

Background. Anaemia can be categorized into micro-, normo- or macrocytic anaemia based on the mean corpuscular volume (MCV). This categorization might help to define the aetiology of anaemia.

Methods. The cohort consisted of patients newly diagnosed with anaemia in primary care. Seven aetiologies of anaemia were defined, based on an extensive laboratory protocol. Two assumptions were tested: (i) MCV < 80 fl (microcytic) excludes vitamin B12 deficiency, folic acid deficiency, suspected haemolysis and suspected bone marrow disease as anaemia aetiology. (ii) MCV >100 fl (macrocytic) excludes iron deficiency anaemia, anaemia of chronic disease and renal anaemia as anaemia aetiology.

Results. Data of 4129 patients were analysed. One anaemia aetiology could be assigned to 2422 (59%) patients, more than one anaemia aetiology to 888 (22%) patients and uncertainty regarding the aetiology remained in 819 (20%) patients. MCV values were within the normal range in 3505 patients (85%). In 59 of 365 microcytic patients (16%), the anaemia aetiology was not in accordance with the first assumption. In 233 of 259 macrocytic patients (90%), the anaemia aetiology was not in accordance with the second assumption.

Conclusions. Anaemia aetiologies might be ruled out incorrectly if MCV guided classification is used as a first step in the diagnostic work-up of anaemia. We recommend using a broader set of laboratory tests, independent of MCV.

Introduction

A widely used algorithm in the diagnostic work-up of anaemia is the classification based on mean corpuscular volume (MCV) as first described by Wintrobe (1). This algorithm uses the MCV to categorize the anaemia into either microcytic (MCV 100 fl), normocytic (MCV 80 – 100 fl) or macrocytic (MCV < 100 fl). Each of these categories is presumed to have its own anaemia aetiology or aetiologies, based on the pathophysiologic mechanism. For instance, iron deficiency anaemia (IDA) may underlie microcytic anaemia, and vitamin B12 deficiency may underlie macrocytic anaemia. Most guidelines recommend this classification system as a first step in the diagnostic work-up of anaemic patients (2–5).

In the past few years, however, several reports have pointed out limitations of a MCV guided anaemia classification algorithm (6,7). For one thing, the MCV represents a mean value, which still might be within the normal range—especially in the early stage of a disease. Furthermore, the MCV outcome might also be within the normal range when multiple aetiologies occur simultaneously in a patient (6). Although the usefulness of the MCV classification system in clinical practice has been questioned, very few of these reports mentioned specific numbers or analysis on the usefulness and/or limitations of MCV in this setting. In a study in hospitalized patients with anaemia, only 7% of patients with vitamin B12 or folic acid deficiency had macrocytic anaemia (8). Furthermore, Seward et al. concluded that MCV was not a useful first criterion for the selection of follow-up laboratory tests in the diagnostic work-up of anaemia in hospitalized patients (9). This conclusion was based on the fact that over half of the patients did not have the anaemia aetiology as would be expected based on MCV results. This study also showed low sensitivities and specificities for MCV to identify the anaemia aetiologies. These both studies that showed the limitation of MCV were performed in clinical settings.

Little is known on the predictive value of MCV in general practices. Therefore, we set out to study the predictive value of MCV as a first step in the diagnostic work-up of microcytic and macrocytic anaemia patients by systematically screening for a variety of aetiologies in a cohort of newly diagnosed patients with anaemia in general practices.

Methods

Study population

The original cohort study was designed by general practitioners, clinical chemists and internists (10). Patient data were selected from a database with patients from general practice. This database holds data of individuals from the general population, aged ≥ 50 years and newly diagnosed with anaemia (i.e. no anaemia 2 years previously). GPs selected the patients by requesting one of the two available laboratory panels when anaemia was suspected. Both panels consisted of an extensive laboratory work-up for all patients at the time of anaemia diagnosis; i.e. measurement of haemoglobin, MCV, reticulocyte count, thrombocyte count, leucocyte count, lactate dehydrogenase, vitamin B12, folic acid, ferritin, transferrin, serum iron and creatinine (sidenote: creatinine was only included in one of two panels). More detailed information about the study population can be found in a previously published study (10). The project operated from 1 February 2007 until 1 February 2017.

The present study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board of the Albert Schweitzer Hospital

Definitions

Seven aetiologies for anaemia were defined most often occurring in primary care based on literature (2,4). These seven aetiologies were anaemia of chronic disease (ACD), renal anaemia, IDA, suspected haemolysis, suspected bone marrow disease, folic acid deficiency and vitamin B12 deficiency. For each aetiology, a definition was drawn up based on the extensive laboratory work-up. Each definition was based on literature and the Dutch general practitioners' guideline of anaemia (2,3,11–14). The definitions are added as Supplemental Data 1 (10). The definitions were strictly applied, which made it possible to have multiple aetiologies in one patient. To avoid incorporation bias, the MCV was not included in the definitions of the seven aetiologies. The laboratory system used in this study automatically conducted an electrophoresis in case of low MCV (6.2 (male) or >5.4 (female) µl]. Therefore, we excluded in retrospect patients with a haemoglobinopathy.

Based on various MCV guided anaemia classification algorithms and as indicated in several reports (6,15), two assumptions were designed: (i) a MCV < 80 fl excludes vitamin B12 deficiency, folic acid deficiency, suspected haemolysis and suspected bone marrow disease as anaemia aetiology; and (ii) a MCV >100 fl excludes IDA, ACD and renal anaemia as anaemia aetiology.

Statistical analysis

Missing laboratory values ranged from 0.0% to 0.9% for all parameters, except for creatinine (19.6%) (Table 2). We employed single imputation using an expectation–maximization algorithm (10,16). The relatively large amount of missing creatinine values could be ascribed to the fact that general practitioners were allowed to follow either one of two pathways when they requested laboratory analysis, one of which did not include creatinine. Single imputation was allowed in view of the large cohort size. Relative frequency was used to analyse the assumptions and the mismatches between the predefined anaemia aetiologies and the MCV values. A sensitivity analysis was conducted using the World Health Organization definitions for anaemia. This included anaemia defined as haemoglobin < 12.9 g/dl (males) or < 11.9 g/dl (females). A second analysis was performed with exclusion of data of patients with multiple aetiologies and uncertain anaemia. Data were analyzed using SPSS for Windows, version 24 (IBM Corp., Armonk, NY, USA).

Results

Inclusion and characteristics

A total of 4152 patients with newly diagnosed anaemia were included. Data of 23 patients (0.6%) were excluded from further analyses because a haemoglobinopathy was confirmed by genetic testing. Thus, data of 4129 were analysed in this study. The median age of the study population was 75 years (interquartile range 64–84 years) and 2028 patients (49%) were male. Laboratory characteristics of the study population are shown in Table 1.

Table 1. Characteristics of 4129 anaemia patients in primary care (2007-17).

	Median (IQR) / Count (%)
Age (per year)	75 (64 – 84)
Male sex	2028 (49)
Haemoglobin (g/dl)	
• **Male**	12.9 (12.1 – 13.4)
• **Female**	11.4 (10.6 – 11.8)
MCV (fl)	91 (86 – 94)
Reticulocytes (%)	1.0 (0.8 – 1.4)
Leukocyte count (10^9/L)	7.1 (5.7 – 9.0)
Thrombocyte count (10^9/L)	269 (216 – 345)
LDH (E/L)	306 (221 – 373)
eGFR (mL/min/1.73m²)	68.8 (53.7 – 83.6)

Table 1. (Continued)

	Median (IQR) / Count (%)
Ferritin (µg/L)	
• **Male**	156 (60 – 321)
• **Female**	81 (21 – 207)
Transferrin (g/L)	2.38 (2.05 – 2.82)
Serum iron (µmol/L)	
• **Male**	11.2 (6.5 – 15.6)
• **Female**	8.8 (4.9 – 12.4)
Vitamin B12 (pmol/L)	288 (209 – 430)
Folic acid (nmol/L)	16 (11 – 25)

eFGR, estimated flomerular filtration rate; IQR, interquartile ranges; LDH, lactate dehydrogenase.

MCV as a first step in anaemia diagnostics

One anaemia aetiology could be assigned in 2422 (59%) cases, and more than one anaemia aetiology in 888 (22%) cases. A total of 819 (20%) patients did not meet any of the predefined criteria for anaemia aetiologies and therefore the aetiology of the anaemia remained unclear. Table 2 shows the frequencies of micro-, normo- and macrocytic anaemia for each aetiology. MCV values were within the normal range in the vast majority of patients [n = 3505 (85%)]. The range of MCV values for each anaemia aetiology is visualized in Figure 1.

If MCV is used as a first step, an MCV < 80 fl should exclude patients with vitamin B12 deficiency, folic acid deficiency, suspected haemolysis and suspected bone marrow disease as aetiology. However, this assumption did not apply to 59 of 365 microcytic patients (16%), in whom one or more of these aetiologies of anaemia were diagnosed. In line with this, an MCV > 100 fl should exclude patients with IDA, ACD and renal anaemia as anaemia aetiology. However, 233 of 259 macrocytic patients (90%) did demonstrate one or more of these aetiologies of anaemia.

A sensitivity analysis maintaining the WHO definitions for anaemia resulted in exclusion of 1310 patients. Of the 2842 patients included, 347 were microcytic (12%), 2305 were normocytic (81%) and 190 were macrocytic (7%). In total, 57 out of 347 microcytic patients (16%) and 172 out of 190 macrocytic patients (91%) demonstrated an anaemia aetiology contradictory to what would be expected. More details concerning this subgroup can be found in Supplemental Data 2.

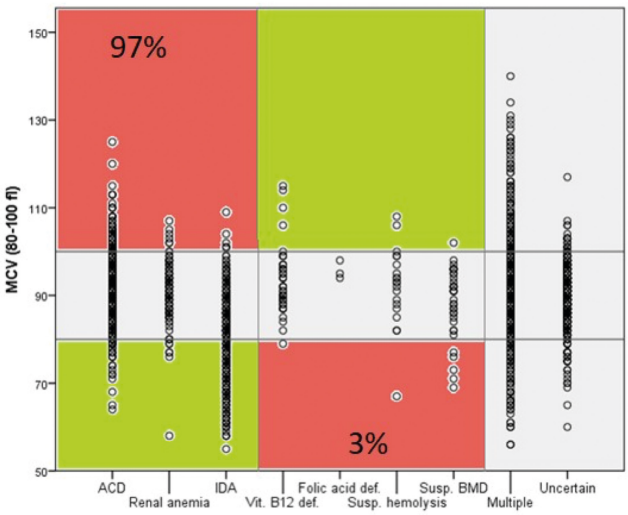

Figure 1. Scatter plot visualizes the anaemia aetiology versus the MCV value in 4129 anaemia patients in primary care.
The aetiology of the anaemia is plotted against the patient's MCV value. Each dot represents a case. The cases that fall in the green squares correlate correctly with the MCV guided anaemia classification algorithm. The cases in the red squares would not have been found using the MCV guided anaemia classification system. This represents 3% of the microcytic and 97% of the macrocytic cases.
BMD, bone marrow disease; def., deficiency; susp., suspected; vit., vitamin.

Table 2. The anaemia aetiologies found in 4129 anaemia patients in primary care (2007-17) plotted against the MCV classification.

Anaemia aetiology	Count (%)	**Microcytic** count (%)	**Normocytic** count (%)	**Macrocytic** count (%)
ACD	1536 (37)	26 (2)	1409 (92)	101 (7)
Renal anaemia	130 (3)	6 (5)	118 (91)	6 (5)
IDA	646 (16)	230 (36)	410 (63)	6 (1)
Suspected haemolysis	20 (0.5)	1 (5)	17 (85)	2 (10)
Suspected bone marrow disease	38 (0.9)	5 (13)	32 (84)	1 (3)
Vitamin B12 deficiency	49 (1)	1 (2)	44 (90)	4 (8)
Folic acid deficiency	3 (0.1)	-	3 (100)	-
Multiple etiologies	888 (22)	70 (8)	695 (78)	123 (14)
• Combination of IDA, ACD and/or renal anaemia	363 (41)	18	319	26
• Combination of vit. B12 def., folic acid def., susp. BMD and/or susp. haemolysis	7 (0.8)	1	3	3
• Combination of both of the above options	518 (58)	51	373	94
Uncertain anaemia	819 (20)	26 (3)	777 (95)	16 (2)
Total	4129 (100)	365 (9)	3505 (85)	259 (6)

MCV assumptions analysis in restricted aetiologies

The above assumptions were tested in a second analysis excluding patients with multiple anaemia aetiologies and those with uncertain anaemia. First, 7 out of 269 microcytic patients (3%) demonstrated an anaemia aetiology contradictory to what would be expected. Second, 113 out of 120 macrocytic patients (94%) demonstrated an anaemia aetiology contradictory to what would be expected.

Conclusions

Principal findings

Our study results implicate that an MCV guided anaemia diagnostic work-up would lead to a suboptimal diagnostic work-up in microcytic and macrocytic anaemia, which might result in inappropriate treatment for most anaemic patients. The a priori probability of an abnormal MCV value is low, since the majority of anaemic patients are normocytic (85%). For these patients, the MCV is not useful as a first step (6). Our analysis showed that anaemia aetiologies are not restricted to any MCV guided anaemia classification algorithm. In our cohort, 90% of macrocytic- and 16% of microcytic patients demonstrated an anaemia aetiology contradictory to what would be expected based on a MCV guided anaemia classification algorithm. In addition, almost one quarter (22%) turned out to have multiple aetiologies with various combinations, and in 20% of patients the diagnosis of anaemia remained uncertain. For these patients, a MCV guided anaemia classification system is not applicable, although they cannot be singled out during first clinical presentation. Additional analyses excluding this group still violated a MCV guided anaemia classification algorithm in 3% of microcytic and 94% of macrocytic patients.

Strengths and limitations of this study

The cohort studied has some strong features. First, they had all been newly diagnosed with anaemia in general practices, and thus had not yet received additional investigations or treatment for anaemia. Furthermore, the participating general practices represent a typical area of residents in the Netherlands. In addition, in the Netherlands every resident is registered at a general practice. Therefore, the study population is a representation of the general population. Second, the cohort included a large number of patients, which increases the precision of observed point estimates. Furthermore, in all patients an extensive systematic laboratory work-up was conducted at the moment of anaemia diagnosis. In this way, we were able to diagnose or exclude the most common anaemia aetiologies for every patient.

In this study, we used different cut-off values of haemoglobin levels for anaemia compared with the World Health Organization definition (17). The cohort is part of the Dutch population and care is based on the reference values of the participating laboratory. For this reason, we maintained the reference values of the participating laboratory. Nevertheless, we increased the robustness of the study results by adding a sensitivity analysis using the WHO defined cut-off values of haemoglobin levels for anaemia. The sensitivity analysis showed no difference in results outcome. Hence, it can be concluded that the findings of this study have a high external validity. It is important to realize that this study employed a laboratory-orientated approach to define the anaemia aetiology, and that clinical information is lacking. The diagnosed aetiologies give guidance to further diagnostic work-up, the outcome of which should be matched with the clinical presentation to pursue further investigations and/or treatment.

Implications for clinicians

As it appeared that a large majority of patients from primary care had normocytic anaemia, any MCV guided anaemia classification algorithm is not applicable in most anaemia patients. Furthermore, a MCV guided anaemia classification algorithm seems to have no added value for patients with a micro- or macrocytic anaemia. On top of that, multiple aetiologies of anaemia, in this cohort present in 22% of cases, cannot be diagnosed with this algorithm. Application of a MCV guided anaemia classification based algorithm would lead to a suboptimal diagnostic work-up and might result in an initially inappropriate treatment for most anaemic patients. On top of that, since almost a quarter of anaemic patients have more than one anaemia aetiology, a broad laboratory work-up should be considered in every newly diagnosed anaemia patient.

Acknowledgments

The authors thank the participating general practitioners for their contributions to this study.

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Supplemental data 1. Definitions of the anaemia aetiologies.

	Definition
Anaemia of chronic disease	Serum ferritin > 100 µg/L and at least one of the following transferrin ≤ 3.60 g/L or iron < 14 (male) / < 10 (female) µmol/L
Renal anaemia	Estimated glomerular filtration rate < 45 ml/min/1.73m^2
Iron deficiency anaemia	Serum ferritin < 25 (male) / 20 (female) µg/L
Suspected haemolysis	Lactate dehydrogenase > 241 U/L and reticulocytes > 2.5%
Suspected bone marrow disease	Reticulocytes < 2.5% and leukocyte count < 4.3 or > 10 10^9/L and thrombocyte count < 150 / > 390 10^9/L
Vitamin B12 deficiency	Serum vitamin B12 < 130 pmol/L
Folic acid deficiency	Serum folic acid < 5 nmol/L
Multiple aetiologies	Satisfied more than one of the above definitions
Uncertain	Satisfied none of the above definitions

4

The effectiveness of a routine versus an extensive laboratory analysis in the diagnosis of anaemia in general practice

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Annals of clinical biochemistry, 2017; 0(0):
DOI: 10.1177/0004563217748680

Abstract

Background. We investigated the percentage of patients diagnosed with the correct underlying cause of anaemia by general practitioners when using an extensive versus a routine laboratory work-up.

Methods. An online survey was distributed among 836 general practitioners. The survey consisted of six cases, selected from an existing cohort of anaemia patients (n = 3325). In three cases, general practitioners were asked to select the laboratory tests for further diagnostic examination from a list of 14 parameters (i.e. routine work-up). In the other three cases, general practitioners were presented with all 14 laboratory test results available (i.e. extensive work-up). General practitioners were asked to determine the underlying cause of anaemia in all six cases based on the test results, and these answers were compared with the answers of an expert panel.

Results. A total of 139 general practitioners (partly) responded to the survey (17%). The general practitioners were able to determine the underlying cause of anaemia in 53% of cases based on the routine work-up, whereas 62% of cases could be diagnosed using an extensive work-up (P = 0.007). In addition, the probability of a correct diagnosis decreased with the patient's age and was also affected by the underlying cause itself, with anaemia of chronic disease being hardest to diagnose (P = 0.003).

Conclusion. The use of an extensive laboratory work-up in patients with newly diagnosed anaemia is expected to increase the percentage of correct underlying causes established by general practitioners. Since the underlying cause can still not be established in 31.3% of anaemia patients, further research is necessary.

Introduction

Anaemia (i.e. a lowered concentration of haemoglobin) is a common finding among elderly patients (aged 65 years and older) in general practice. Besides the sign of an underlying condition, it has long been considered a benign consequence of aging. However, during the last decade, many studies have been published detailing the relevance of anaemia, such as the associations between anaemia and increased mortality, physical and cognitive decline, cardiovascular events and reduced quality of life have been found [1, 2]. The only way to manage anaemia is through treatment of the underlying cause, which requires an additional diagnostic work-up. The most common underlying causes of anaemia in general practice are iron deficiency anaemia (IDA) (16.3%–19.0%), anaemia of chronic disease (ACD) (19.7%–31.4%) and renal anaemia (8.2%–12.9%). In addition, a considerable proportion of anaemia cases have no clear cause and are classified as unknown anaemia (31.3%–44.0%) (see literature, [3–6] own data). Different guidelines are published to help diagnosing the underlying cause of anaemia. In most of them, mean corpuscular volume (MCV) occupies a central position [7, 8]. However, Oosterhuis et al [9] demonstrated that when assigning a major role to ferritin concentration instead of MCV, leads to an increase of the percentage of patients diagnosed with an underlying cause from 48% to 71%. In addition, another study has shown that the percentage of patients diagnosed with an underlying cause of anaemia increases from14% when general practitioners (GPs) personally order laboratory analysis to 53% when a standard set of 14 laboratory parameters was offered [10].

The high prevalence of anaemia, as well as the need for establishing the underlying aetiology prior to initiating treatment, demands an optimized diagnostic approach. We used an online survey among GPs to establish whether a routine or extensive laboratory approach is more effective in diagnosing the underlying cause of anaemia in general practice.

Materials and methods

Study design

The percentage of correct diagnoses of the underlying cause of anaemia when using an extensive versus a routine laboratory work-up was investigated through an online survey using LimeSurvey [11]. The survey was distributed among 836 GPs, operating in different parts of the Netherlands and was available online for a period of one month (January 2016). The cases used in this survey were selected

from a large database of GP patients, included between the 1 February 2007 and 1 February 2015 (n = 3325) [12]. This prospective cohort study was approved by the internal ethics committee of the Albert Schweitzer Hospital. The database consisted of patients aged 50 years and older (in order to exclude a predominance of iron deficiency due to hypermenorrhoea), newly diagnosed with anaemia (i.e. no established diagnosis of anaemia in the previous two years). Anaemia was defined as haemoglobin below 13.7 g/dL (8.5 mmol/L) for males and below 12.1 g/dL (7.5 mmol/L) for females. An extensive laboratory work-up was performed in all included patients, consisting of haemoglobin (Hb), MCV, C-reactive protein (CRP) and/or erythrocyte sedimentation rate (ESR), vitamin B12, creatinine, ferritin, folic acid, lactate dehydrogenase (LDH), transferrin, reticulocytes, leukocytes, thrombocytes and serum iron. According to the current guidelines about anaemia diagnostics in general practice, these 14 parameters cover all underlying causes of anaemia that can be diagnosed in general practice [7].

An expert panel, consisting of an experienced GP, internist and clinical chemist, determined the underlying cause of anaemia for each patient based on 10 predefined causes (i.e. IDA, anaemia of chronic disease (ACD), renal anaemia, possible bone marrow disease, possible haemolysis, haemoglobinopathy, vitamin B12 deficiency, folic acid deficiency, other and unknown) (supplemental data 1). Patients with multiple aetiologies were excluded from the study (n = 293), since those cases were considered too complex for the design of this study. In addition, patients with missing laboratory values were also excluded (n = 643). The remaining patients from the prospective database (n = 2389) were divided into four subgroups: IDA (n = 389, 16.3%), ACD (n = 751, 31.4%), renal anaemia (n = 307, 12.9%) and other (including anaemia with an unknown cause) (n = 942, 39.4%). Based on the prevalence of each of the underlying causes of anaemia, 201 cases were selected from the database and used in the online survey. By selecting 201 cases, three equalsized pools (each consisting of 67 cases) could be used for randomization. For the subgroups IDA, ACD and renal anaemia, stratified randomization was used for selection of cases to ensure that the percentage of included cases of each of those underlying causes was representative of its occurrence in the database [13]. In the fourth subgroup (i.e. other causes including unknown causes), cases of anaemia with an unknown cause were also selected through stratified randomization. The remaining other causes were selected manually by maximum variation sampling (based on patients' age, gender and the underlying cause of anaemia), which was necessary due to the small numbers of cases per cause [14]. The distribution of underlying causes of anaemia within the four subgroups (i.e. IDA, ACD, renal anaemia and other), before and after randomization is shown in Table 1.

Table 1. Distribution of underlying cause of anaemia.

Underlying cause of anaemia	Number of cases before randomization (%)	Number of cases after randomization (%)
Iron deficiency anaemia	389 (16.3%)	33 (16.4%)
Anaemia of chronic disease	751 (31.4%)	63 (31.3%)
Renal anaemia	307 (12.9%)	26 (12.9%)
Other	942 (39.4%)	79 (39.3%)
• **Unknown**	• 748 (79.4%)	• 63 (79.7%)
• **Haemoglobinopathy**	• 16 (1.7%)	• 1 (1.3%)
• **Haemolysis**	• 9 (1.0%)	• 1 (1.3%)
• **Possible bone marrow disease**	• 40 (4.2%)	• 3 (3.8%)
• **Vitamin B12 deficiency**	• 64 (6.8%)	• 5 (6.3%)
• **Folic acid deficiency**	• 8 (0.8%)	• 1 (1.3%)
• **Other**	• 57 (6.1%)	• 5 (6.3%)
Total	**2389**	**201**

Note: the distribution of the underlying causes of anaemia in the larger data-set of newly diagnosed anaemia patients was used to randomly select 201 cases with a similar distribution.

Structure of online survey

For each participating GP, the survey included six patient cases which were randomly selected by LimeSurvey from the set of 201 available cases. For each case, GPs were provided with the age and gender of the patient and were informed that the patient presented with suspicion of anaemia. While filling in the survey, respondents were invited to use guidelines or other tools they also use in daily practice. For the first three cases, respondents were asked to choose the laboratory tests they considered necessary from the predefined list of 14 parameters. This was referred to as the 'routine work-up'. The respondents were then presented with the results of the selected laboratory tests and were invited to determine the underlying cause of anaemia when possible. In cases where they responded that additional information was required, they were given one opportunity to request additional tests from the same list of 14 tests. No limit was set on the number of laboratory tests that could be selected per analysis, but the same test could not be ordered twice. For the second set of three cases, respondents were presented with the results of all 14 tests, and they did not have the ability to request additional tests. This was referred to as the 'extensive work-up'. Again, respondents were asked to determine the underlying cause of anaemia. The survey ended with several personal questions regarding the GPs' gender, age, years active as GP, zip code, daily use of guidelines for the diagnosis/treatment of anaemia patients and whether the GP had any special affinity with the subject of anaemia. The diagnoses of the respondents were compared with the diagnoses of the expert

panel. For the cases with IDA, ACD and renal anaemia as underlying cause, the diagnoses had to be in accordance with the diagnoses of the expert panel. For all other underlying causes (i.e. vitamin B12 deficiency, unknown cause, et cetera), the diagnosis was considered correct if the option IDA, ACD or renal anaemia had not been chosen by the respondent. This classification is a commonly used method in literature and was considered appropriate as diagnosing or excluding of the three most prevalent underlying causes is clinically the most relevant [3 – 6].

A sample of the survey is shown in supplemental data 2. The percentage of correct underlying causes of anaemia as established by GPs was defined as 'effectiveness' and is referred to as such throughout this manuscript.

Statistical analysis

The patient population was described by standard descriptive statistics. An additional analysis was performed to confirm that the choice of laboratory test(s) requested by the GP was in accordance with the diagnosis established by the same GP. The effects of the characteristics of both the patient case and the responding GP on the probability of a correct diagnosis were analysed using generalized linear mixed models with a logistic link function and a binomial error distribution (i.e. logistic regression analyses with random effects) [15]. These models take into account the correlations between observations due to repeated measurements for both cases and respondents and can handle data with missing observations in the outcome. In the analysis of the effectiveness of the routine versus the extensive laboratory work-up, the dependent variable was a dichotomous variable, indicating whether the case had been diagnosed correctly, and the independent variables were type of work-up (i.e. routine or extensive), age and gender of the case and the cause of anaemia as established by the expert panel. This model was also used for the analysis of the effect of GP characteristics on correct diagnoses, but with the following variables added as independent variables: number of years of working experience as GP, use of guidelines, and whether the respondent had specific affinity with anaemia. GPs with missing information on any of these GP characteristics were excluded from the latter analysis (n = 16, 11.5%). To take into account correlations due to repeated measurements, the relevance of including random effects of both cases and respondents was determined with the Akaike information criterion. Based on this criterion, only random effects of cases were included in the final model. The data were analysed using IBM SPSS Statistics, version 24 and R version 3.3.1 with the NLME package [16, 17]. All statistical tests were two-sided, and P-values lower than 0.05 were considered significant.

Results

Respondents' online survey

A total of 125 respondents (15%) completed the survey, resulting in 375 complete cases for both the routine and extensive work-up. In addition, 14 respondents (1.7%) filled in only part of the survey, resulting in an additional 29 cases in the routine work-up (total 404 cases) and three cases in the extensive work-up (total 378 cases) (Figure 1). Of all 139 respondents, 123 (89.5%) answered the questions regarding personal characteristics. According to the NIVEL institute (Netherlands Institute for Health Services Research), the proportion of males participating in this survey as compared with the overall population of GPs (55.6% versus 55.0%), as well as the median age (48 years versus a median age category of 45–49 years) did not show major differences, suggesting that the respondents are a representative sample of the overall GP population in the Netherlands [18].

Effect of diagnostic work-up on correctly diagnosing anaemia

Of the 404 cases assessed with a routine work-up, laboratory tests were ordered once in 216 cases (53.5%) and twice in 183 cases (45.3%). No tests were ordered in the remaining five cases (1.2%). The median number of tests ordered during the first and second laboratory analyses was five (IQR: 3–8) and four (IQR: 2–6), respectively. A detailed overview of the frequency at which each laboratory analysis was requested by GPs in the routine work-up is shown in Table 2.

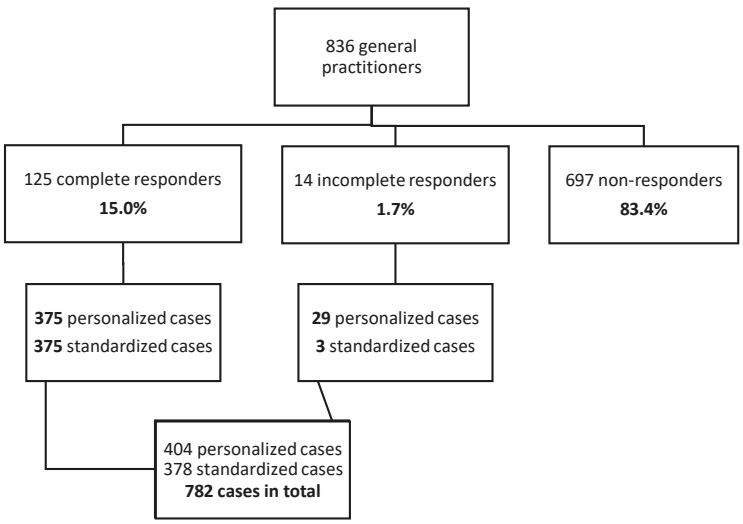

Figure 1. A flow diagram showing the general practitioners who responded to the survey.

Table 2. Routine requested laboratory analysis of GPs.

Parameter	1st round (N = 399)	2nd round (N = 183)	Total (N = 404)
Haemoglobin	394 (98%)	0 (0%)	394 (98%)
MCV	370 (92%)	4 (1%)	374 (93%)
Reticulocytes	85 (21%)	65 (16%)	150 (37%)
Leukocytes	160 (40%)	57 (14%)	217 (54%)
Thrombocytes	133 (33%)	48 (12%)	181 (45%)
CRP and/or BSE	267 (66%)	65 (16%)	332 (82%)
Creatinine	235 (58%)	68 (17%)	303 (75%)
Ferritin	184 (46%)	72 (18%)	256 (63%)
Vitamin B12	126 (31%)	74 (18%)	200 (50%)
Folic acid	110 (27%)	67 (17%)	177 (44%)
LDH	47 (12%)	63 (16%)	110 (27%)
Transferrin	50 (12%)	70 (17%)	120 (30%)
Serum iron	70 (17%)	75 (19%)	145 (36%)
No laboratory analysis	5 (1%)	221 (55%)	5 (1%)

Note: of the 404 cases assessed with a routine work-up, laboratory tests were ordered once in 216 cases (53.5%) and twice in 183 cases (45.3%). The requested tests are shown as number (percentage). LDH: lactate dehydrogenase; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; MCV: mean corpuscular volume; GP: general practitioner

When using a routine work-up, the correct underlying cause of anaemia was established in 214 of 404 cases (53.0%), and with the extensive laboratory workup, 234 of 378 cases (61.9%) were correctly diagnosed (Table 3). The increase in probability of a successful diagnosis due the extensive laboratory work-up was statistically significant in the multivariate analysis (OR: 1.56 [95% CI: 1.12–2.17]) (Table 4). In addition, patients aged 65 years or older seemed more difficult to diagnose correctly compared with patients aged 50–64 years (65–74 years, OR: 0.48 [95% CI: 0.25–0.90], 75–84 years, OR: 0.45 [95% CI: 0.25–0.83], 85þ years, OR: 0.47 [95% CI: 0.25–0.87]). Finally, the underlying cause itself affects the probability of a correct diagnosis, with ACD being the most difficult to diagnose compared to IDA, OR: 0.37 (95% CI: 0.19–0.73). When running the analysis again including the GP characteristics (i.e. excluding GPs with missing data in their characteristics), none of the analysed GP characteristics showed a significant association with the probability of diagnosing the correct underlying cause of anaemia (Table 5).

Table 3. Diagnosis of underlying cause of anaemia using a routine or extensive work-up.

	Diagnosis expert panel			
Diagnosis GP	**IDA**	**ACD**	**Renal anaemia**	**Other (including unknown)**
Routine				
• IDA	**45 (69.2)**	13 (10.3)	2 (3.8)	14 (8.8)
• ACD	5 (7.7)	**56 (44.4)**	17 (32.1)	51 (31.9)
• Renal anaemia	0 (0)	4 (3.2)	**28 (52.8)**	10 (6.3)
• Other (including unknown)	15 (23.1)	53 (42.1)	6 (11.3)	**85 (53.1)**
• Total	**65**	**126**	**53**	**160**
Extensive				
• IDA	**47 (70.1)**	19 (14.4)	0 (0)	11 (8.3)
• ACD	6 (9.0)	**72 (54.5)**	10 (21.7)	28 (21.1)
• Renal anaemia	4 (6.0)	8 (6.1)	**30 (65.2)**	9 (6.8)
• Other (including unknown)	10 (14.9)	33 (25.0)	6 (13.0)	**85 (63.9)**
• Total	**67**	**132**	**46**	**133**

Note: the diagnosis set by the GPs are showed against the diagnosis set by the expert panel for both routine and extensive work-up. The bold values are the correct diagnoses. GP: general practitioner; ACD: anaemia of chronic disease; IDA: iron deficiency anaemia.

Table 4. Multivariate analysis of the efficacy of extensive versus routine laboratory work-up.

	Odds ratio (95% CI)	**P-value**
Extensive work-up	1.56 (1.12-2.17)	0.007
Age of patient		
• **50 - 64 years**	Reference category	
• **65 - 74 years**	0.48 (0.25-0.90)	0.022
• **75 - 84 years**	0.45 (0.25-0.83)	0.010
• **85+ years**	0.47 (0.25-0.83)	0.016
Gender of patient (female)	1.28 (0.83-1.97)	0.258
Underlying cause		
• **IDA**	Reference category	
• **ACD**	0.37 (0.19-0.72)	0.003
• **Renal anemia**	0.69 (0.31-1.56)	0.376
• **Other incl. unknown**	0.58 (0.31-1.10)	0.097

Note: multivariate analysis using a generalized linear mixed model showed a significant influence of the laboratory work-up, age of patient and the underlying cause of anaemia itself on the correct diagnosis of the underlying cause of anaemia.

Table 5. Multivariate analysis of the effect of GPs characteristics on the correct diagnosis of the underlying cause of anaemia.

	Value	Odds ratio (95% CI)	P-value
Work experience in years (median (IQR))	16 (9-25)	1.01 (0.99-1.02)	0.512
Use national GP guideline	58.5% regular 41.5% never	1.21 (0.84-1.74) Reference category	0.306
Affinity with anaemia	25.2% yes 74.8% no	1.18 (0.78-1.80) Reference category	0.437

Note: results based on a generalized linear mixed model that also included work-up type (i.e. routine or extensive), age and gender of the case, and the cause of anaemia as established by the expert panel as independent variables. Of all 139 respondents, 123 (89.5%) answered the questions regarding personal characteristics. None of the analyzed characteristics showed a significant effect on the probability of diagnosing the correct underlying cause of anaemia.

Discussion

When diagnosing the underlying aetiology of anaemia, laboratory tests can be selected by the GP (i.e. routine work-up) or a standard set of tests may be offered by the laboratory (i.e. an extensive work-up). To determine which work-up would be more effective in supporting the GP to diagnose the correct underlying cause of anaemia, we compared both approaches using an online survey among GPs. Our study included 14 widely available laboratory tests, which are all recommended by the current Dutch anaemia guideline. This extensive laboratory work-up was shown to be more effective than a routine work-up, in which GPs could order laboratory tests twice (from this predefined set of 14 tests). Patient characteristics (aged 65 years and older) and the underlying cause itself (i.e. ACD) may negatively affect the probability of a correct diagnosis.

For many years, anaemia diagnosis was directed by MCV, which divided cases into microcytic, normocytic and macrocytic, with each category including a set list of causes. In recent years, several studies have shown that MCV should not be granted such a central role. (Relevance of mean corpuscular volume for the evaluation of anaemia in general practice, manuscript submitted for publication) [19, 20]. However, in this study, MCV was still the second most often requested test during the first round of the routine work-up (93%), suggesting that GPs still follow the old classification system. This approach may lead to missed causes since, for example, a microcytic anaemia will lead to the exclusion of vitamin B12 deficiency as a possible cause according to the old classification system, while in fact, this deficiency may still be present and contributing to the anaemia [19]. In addition

to MCV, the most requested tests during the first round of the routine work-up are CRP/ESR (67%), creatinine (59%) and ferritin (46%). These three tests allow for the exclusion of the three most common causes of anaemia, namely ACD (CRP and ferritin), renal anaemia (creatinine) and IDA (ferritin). This suggests that GPs, when ordering tests, first aim to diagnose or exclude the three most common causes of anaemia.

This study also demonstrated that increased age (65 years and older), decreases the likelihood of establishing the correct underlying cause of anaemia independently from the laboratory work-up. This may be due to the fact that older patients more often display slightly increased infection parameters [21, 22]. This abnormality in the laboratory results, in combination with

consensus-based definitions of underlying causes, might confuse GPs when determining the underlying cause of anaemia. In addition, it is important to realize that the elderly often have multiple diseases or co-morbidities and each of those may (individually) contribute to anaemia. Especially in these patients, the laboratory results might be of less relevance and the clinical presentation (i.e. health condition) would be the guiding principle for treatment. This may have created complications for GPs while attempting to establish the cause in this study, since the survey relied entirely on laboratory results and did not include health condition or co-morbidities of the patient. However, there was no increase in the percentage of cases that were diagnosed as 'unknown' by GPs among the elderly (data not shown). Finally, since the elderly often have multiple diseases or co-morbidities, GPs may be reluctant to commit to an extensive diagnostic process and therefore only perform limited laboratory analyses in this group of patients.

The actual underlying cause of anaemia may also affect the probability of a correct diagnosis by GPs, ith ACD being the most difficult to diagnose. The interpretation of laboratory values can be challenging when ACD is present, especially in multiple causes, for example when an ACD co-exists with an iron deficiency. This combination was not present in the cases selected in our study. However, the cases in this study were classified by an expert panel according to pre-determined definitions of underlying causes of anaemia. These definitions were based both on existing guidelines and on the opinion of this expert panel [23]. As a result, we used a strict cut-off value of ferritin (i.e. >100µg/L) in the diagnosis of ACD. A participating GP may have used a different cut-off value or a different definition of ACD, based on his or her own experience. For the purpose of this study, the answer of these GPs may thus have been classified as incorrect and the number of correct diagnoses

would, in fact, be higher. Furthermore, some causes have a very low prevalence in general practice. Being less familiar with these causes and their current definitions may make diagnosing them more challenging.

This study showed that the percentage of correct diagnoses of the underlying cause of anaemia increased from 53.0% when using a routine laboratory work-up to 61.9% when using an extensive laboratory work-up. Taking into consideration that annually 57,000 patients aged 550 years present with a new anaemia in Dutch general practice, this modest absolute difference of almost 9% will benefit a large number of patients (approximately 5130 patients) in whom the underlying aetiology of anaemia can be established [24, 25]. Moreover, the extensive laboratory work-up is also expected to be cost-effective compared with the routine work-up, as is shown by the accompanying article by our group, published elsewhere in this journal.

Strengths and limitations

This study aimed to investigate which type of diagnostic work-up (i.e. extensive versus routine) results in the most correct diagnoses of the underlying cause of anaemia. To achieve this, we used real-life patient data from a large transmural project. The participating GPs showed no differences in age and gender compared with the overall population of GPs in the Netherlands, suggesting that the participating GPs are an appropriate representative sample of the Dutch GP population.

A possible limitation of this study is the lack of clinical information in the cases of the survey, besides age, gender and the suspicion of anaemia. This clinical information may be of considerable importance in the diagnosis of the underlying cause of anaemia. Addition of this information may have allowed for a higher percentage of correct diagnosis. However, neither approach (routine and extensive work-up) contained this information and thereby this drawback did not affect the difference in percentage of correct diagnoses between both approaches. In addition, the cases were randomly assigned to the GPs, which mean that there was a small chance that the same cause could have been the result for all six of the cases presented to a GP. This may cause the GP to perform better or worse, as this study has shown that the underlying cause itself has an effect on correctly diagnosing. However, since this scenario is also possible in daily practice, the survey reflects a real-word situation. In addition, five cases in whom the GP requested no laboratory test(s) were included in the final analysis because it was deemed a conscious choice of the GPs. In this study, we excluded anaemia with multiple

aetiologies, since this is not addressed in the Dutch anaemia guideline and in order to avoid statistical complexity (i.e. more power would be needed against lower prevalence). In the routine laboratory work-up, multiple aetiologies might be missed if additional laboratory tests are withheld once an underlying cause is established. Therefore, it is to be expected that the extensive laboratory workup performs better in the diagnosis of multiple aetiologies than the routine work-up, since all additional laboratory are simultaneously performed, making multiple aetiologies immediately apparent. Finally, each survey started with three cases for the routine workup followed with three cases for the extensive workup. This might have led to an overestimation of the effectiveness of the extensive work-up, since GPs have practiced and possibly learned from the previous cases and apply this to the following cases. However, to ensure that GPs chose the routine laboratory analysis based on their own experience, it was not possible to first show the cases of the extensive laboratory workup, as this would have led to a larger bias.

Conclusion

An extensive laboratory work-up in patients with newly diagnosed anaemia is more effective in finding the underlying aetiology than a routine laboratory workup selected by GPs. Nevertheless, the percentage of incorrect diagnoses remains significant, which should take into account the fact that stand alone laboratory diagnostics is not the gold standard for anaemia diagnostics. Further studies should focus on an extensive laboratory work-up and the added value of multidisciplinary diagnostic approaches in patients with anaemia.

Acknowledgments

We thank the participating general practitioners for their generous contribution. In addition, the authors thank Dr Rebecca Buis for critical reading of the manuscript. This paper was previously presented at the Science day, Albert Schweitzer Hospital, on June 2017.

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Supplemental data 1. Definitions of underlying causes of anaemia.

Underlying cause of anaemia	Definition
Anaemia	Haemoglobin <13.7 g/dL (8.5 mmol/L) for males and <12.1 g/dL (7.5 mmol/L) for females
Iron deficiency anaemia	Ferritin <25 µg/L (males), <20 µg/L (females)
Anaemia of chronic disease	Ferritin >100 µg/L, transferrin <2.0 g/L and/or serum iron <14 µmol/L (males), <10 µmol/L (females)
Renal anaemia	Estimated creatinine clearance (MDRD) ≤45 mL/min/1.73m^2
Possible bone marrow disease	Abnormal number of leukocytes and platelets, reticulocytes <2.5%
Possible haemolysis	Raised LDH, reticulocytes >2.5% and bilirubin > 17 µmol/L
Vitamin B12 deficiency	Serum vitamin B12 <130 pmol/L
Folic acid deficiency	Serum folic acid <5 nmol/L
Other	Reported by the treating physician

Supplemental data 2. Structure online survey.

I) Based on the information provided, which of the 14 laboratory tests below would you like to request?
   a. Multiple choice: erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), ferritin, folic acid, hemoglobin, creatinine & MDRD, lactate dehydrogenase (LDH), leukocytes, mean corpuscular volume (MCV), reticulocytes, serum iron, transferrin, thrombocytes, vitamin B12.

II) Based on the laboratory analysis provided, which type of anemia does this patient most likely have?
   a. Multiple choice: iron deficiency anemia, anemia of chronic disease, renal anemia, other type, unknown and I wish more laboratory analysis or unknown but I don't wish more laboratory analysis.

III) In case 'other type' was selected in the previous question: what type of anemia do you suspect in this patient?
   a. Open question

IV) Given your chosen answer '_____' as the type of anaemia for this patient: what would be your next step in the treatment of this patient?
   a. Multiple choice (multiple answers possible): end consultation, referral to medical specialist, prescription of medication and/or follow-up, other (open field).

V) Based on your previous answer to refer the patient to a medical specialist, to which specialist would you refer the patient?
   a. Open question

VI) Based on your previous answer to prescribe medication and/or follow-up, which medication would you prescribe and would you like to follow up the patient?
   a. Multiple choice: oral iron supplementation, vitamin B12, folic acid, I would like to follow up this patient, other (open field)

VII) Based on your previous answer to follow up the patient, after how many weeks you decide to make a follow up appointment?
   a. Open question

5

Assessing the cost-effectiveness of a routine versus an extensive laboratory work-up in the diagnosis of anaemia in Dutch general practice

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Annals of clinical biochemistry, 2017; 0(0):
DOI: 10.1177/0004563217748984

Abstract

Background. Establishing the underlying cause of anaemia in general practice is a diagnostic challenge. Currently, general practitioners individually determine which laboratory tests to request (routine work-up) in order to diagnose the underlying cause. However, an extensive work-up (consisting of 14 tests) increases the proportion of patients correctly diagnosed. This study investigates the cost-effectiveness of this extensive work-up.

Methods. A decision-analytic model was developed, incorporating all societal costs from the moment a patient presents to a general practitioner with symptoms suggestive of anaemia (aged550 years), until the patient was (correctly) diagnosed and treated in primary care, or referred to (and diagnosed in) secondary care. Model inputs were derived from an online survey among general practitioners, expert estimates and published data. The primary outcome measure was expressed as incremental cost per additional patient diagnosed with the correct underlying cause of anaemia in either work-up.

Results. The probability of general practitioners diagnosing the correct underlying cause increased from 49.6% (95% CI: 44.8% to 54.5%) in the routine work-up to 56.0% (95% CI: 51.2% to 60.8%) in the extensive work-up (i.e. +6.4% [95% CI: -0.6% to 13.1%]). Costs are expected to increase slightly from €842/patient (95% CI: €704 to €994) to €845/patient (95% CI: €711 to €994), i.e. +€E3/patient (95% CI: €-35 to €40) in the extensive work-up, indicating incremental costs of €43 per additional patient correctly diagnosed.

Conclusions. The extensive laboratory work-up is more effective for diagnosing the underlying cause of anaemia by general practitioners, at a minimal increase in costs. As accompanying benefits in terms of quality of life and reduced productivity losses could not be captured in this analysis, the extensive work-up is likely cost-effective.

Background

Anaemia is a common medical problem that carries substantial costs to the healthcare system and can be a burden on the health and quality of life of many individuals [1-10]. Therefore, adequate diagnosis and early initiation of correct treatment are essential [11]. However, anaemia is not a disease in itself, but is considered a sign of an underlying condition. Consequently, diagnosing the underlying cause of anaemia is often complex [12-14]. More specifically, previous research estimated that in 14–33% of older persons with anaemia, the underlying cause is unknown [1, 12]. In addition, anaemia is often under-diagnosed and under-treated, as it is often considered a consequence of aging and not as a specific symptom of disease [12, 15].

The three most common underlying causes are: iron deficiency anaemia (IDA), anaemia of chronic disease (ACD) and renal anaemia [16]. Anaemia may also have a variety of other causes, including bone marrow diseases or vitamin deficiencies, such as B12 and/or folic acid. Besides anamnesis and physical examination, laboratory analyses are required to identify the different underlying causes of anaemia. However, depending on the laboratory protocol used, the proportion of patients for whom no underlying cause of anaemia can be identified based on their laboratory analyses ranges from 28% to 52% [17]. In order to enhance the effectiveness of laboratory analyses, a guideline has been developed by the Dutch College of General Practitioners (DCGP) [18]. Previous research evaluated all laboratory tests of patients (women>50 years and men ≥18 years) with anaemia newly diagnosed by general practitioners (GPs) within a two-year time period. Unfortunately, 83.9% of those patients could not be diagnosed when applying the DCGP-guideline, because at least one of the required laboratory tests had not been performed [11].

In a recently performed study, we investigated whether an extensive laboratory work-up (consisting of a set of 14 tests) could increase the probability that patients are diagnosed with the correct underlying cause of anaemia in Dutch general practice [19]. Although this study has shown that this work-up likely improves the probability that patients are correctly diagnosed with the underlying cause of anaemia, it is unknown whether this approach is cost-effective. In addition, even though most routine laboratory tests for diagnosing anaemia are relatively inexpensive, these test results will likely impact subsequent patient management decisions. As this may involve more expensive diagnostic testing and the referral of patients to secondary care, it is crucial to quantify the impact of such an extensive

laboratory work-up further downstream the patient management pathway. Therefore, the current study aims to estimate the cost-effectiveness of this extensive laboratory work-up as compared with the current situation, the routine work-up, in which GPs decide for themselves which tests to request in patients presenting with symptoms of anaemia.

Methods

Survey

The effectiveness of diagnosing the underlying cause of anaemia in general practice, using either the extensive or a routine work-up, was investigated through an online survey using LimeSurvey [20]. A full description of this survey is provided elsewhere [19]. Details on how this survey was distributed are provided in Supplemental Data 1. In the survey, all participating GPs (139 out of 836, i.e. 16.6%) received six real-world cases of patients presenting with a new anaemia in general practice. In all six cases, the participating GPs were only provided with the age and gender of the patient and were informed that the patient was suspected of anaemia. In the first three cases, GPs were able to choose freely which tests they would request based on a predefined list of 14 common tests (haemoglobin, mean corpuscular volume [MCV], C-reactive protein [CRP] and/or erythrocyte sedimentation rate [ESR], vitamin B12, creatinine, ferritin, folic acid, lactate dehydrogenase [LDH], transferrin, reticulocytes, leukocytes, thrombocytes and serum iron) and were only given the results of the tests they selected. In the second set of three cases, GPs received the results of all 14 tests. In all six cases, the GPs were asked to choose between IDA, ACD, renal anaemia and 'other'. In cases where the GPs chose 'other', they were asked to specify the underlying cause of anaemia. GPs could also state that the cause was 'unknown', if they did not consider it possible to determine the underlying cause based on the laboratory results provided. Besides, the GPs were also asked which of the subsequent actions they would take in each case: close the consultation (i.e. do nothing), refer the patient to secondary care, prescribe medication (which involved prescribing iron, vitamin B12, folic acid or antibiotics), or see the patient again in a few weeks (follow-up). During the survey, the GPs were encouraged to use guidelines (e.g. the DCGP guideline) or other tools they use in daily practice.

Database used in the survey

A detailed explanation regarding how the cases used in the survey were obtained from this database has been described previously [19].

Health economic model

As health economic models are typically complex, a description of the main aspects regarding model structure and model inputs is provided below. A more extensive description, including the assumptions used, is provided in Supplemental Data 1.

A decision tree was developed to estimate the cost-effectiveness of an extensive laboratory work-up compared with a routine work-up in diagnosing the underlying cause of anaemia in patients presenting with anaemia, aged ≥50 years, in Dutch general practice. A simplified version is shown in Figure 1. The correct underlying cause of anaemia was determined according to an expert panel, composed of an internist, a GP and a clinical chemist. Incremental effect was defined as the difference in the percentage of patients for whom the underlying cause of anaemia was correctly determined using a routine laboratory work-up (i.e. current practice) as compared with the extensive work-up. According to Dutch health economic guidelines, a societal perspective was taken in the cost-effectiveness analysis [21]. This means that all costs were included from the moment such a patient presents at the GP, until the patient is (correctly) diagnosed and treated in primary care, or referred to (and diagnosed in) secondary care. As the treatment of anaemia in secondary care strongly varies depending on its underlying cause, quantifying the impact of either work-up on patients' quality of life would require extensive individual patient-level data, which were not available. As such, this was considered outside the scope of this analysis. The time horizon in this study was therefore estimated to be, at most, 200 days. Incremental cost was defined as the difference in average costs per patient for whom the underlying cause of anaemia was determined using the extensive work-up as compared with the routine work-up. Costs were expressed in 2016 Euros. The model outcome was expressed as an incremental cost-effectiveness ratio (ICER), representing the incremental costs per additional patient diagnosed with the correct underlying cause of anaemia. To obtain further insight as to testing for which underlying causes of anaemia (i.e. IDA, ACD, renal anaemia and 'other') potentially has the most room for improvement in terms of cost-effectiveness, subgroup analyses were performed.

Model inputs

The incidence of the different underlying causes of anaemia in Dutch patients aged ≥50 years, was based on the above mentioned database [16]. The results of the survey were used to calculate the probability that the right underlying cause of anaemia (according to the expert panel) was established within each of those patient categories, for both work-ups. The likelihoods that GPs chose a certain type of treatment based on the different underlying causes of anaemia

(i.e. prescribe medication (including the type of medication), refer the patient to secondary care, perform follow-up of the patient or to close the consultation without performing additional actions) were also derived from this survey. In cases where the GP decided to hold a follow-up appointment with the patient or to close the consultation, the probability of spontaneous recovery of a patient's anaemia was taken into account for each of the different underlying causes. A detailed overview of the model structure, input parameters and the assumptions used is provided in Supplemental Data 1. Values for input parameters that could not be obtained from literature, such as the duration of oral iron supplementation, were derived from expert elicitations with two internist-haematologists (Supplemental Data 1).

Probabilistic sensitivity analyses

Random samples were simultaneously drawn for all input parameters based on predefined parameter distributions. Distributions were parameterized based on the observed parameter mean and on the observed or assumed standard error (Supplemental Data 1). To determine the effect of joint uncertainty in all input parameters on model outcomes, a probabilistic sensitivity analysis (PSA) was performed based on a Monte Carlo simulation with 10,000 samples.

One-way sensitivity analysis

To identify which individual parameters substantially influence model outcomes, a one-way deterministic sensitivity analysis was conducted. For each parameter, the impact on total costs per patient resulting from a change from the base case value to the lower and to the upper limit for the corresponding 95% confidence interval was analysed. A detailed overview of the inputs used in the one-way sensitivity analysis is provided in Supplemental Data 1.

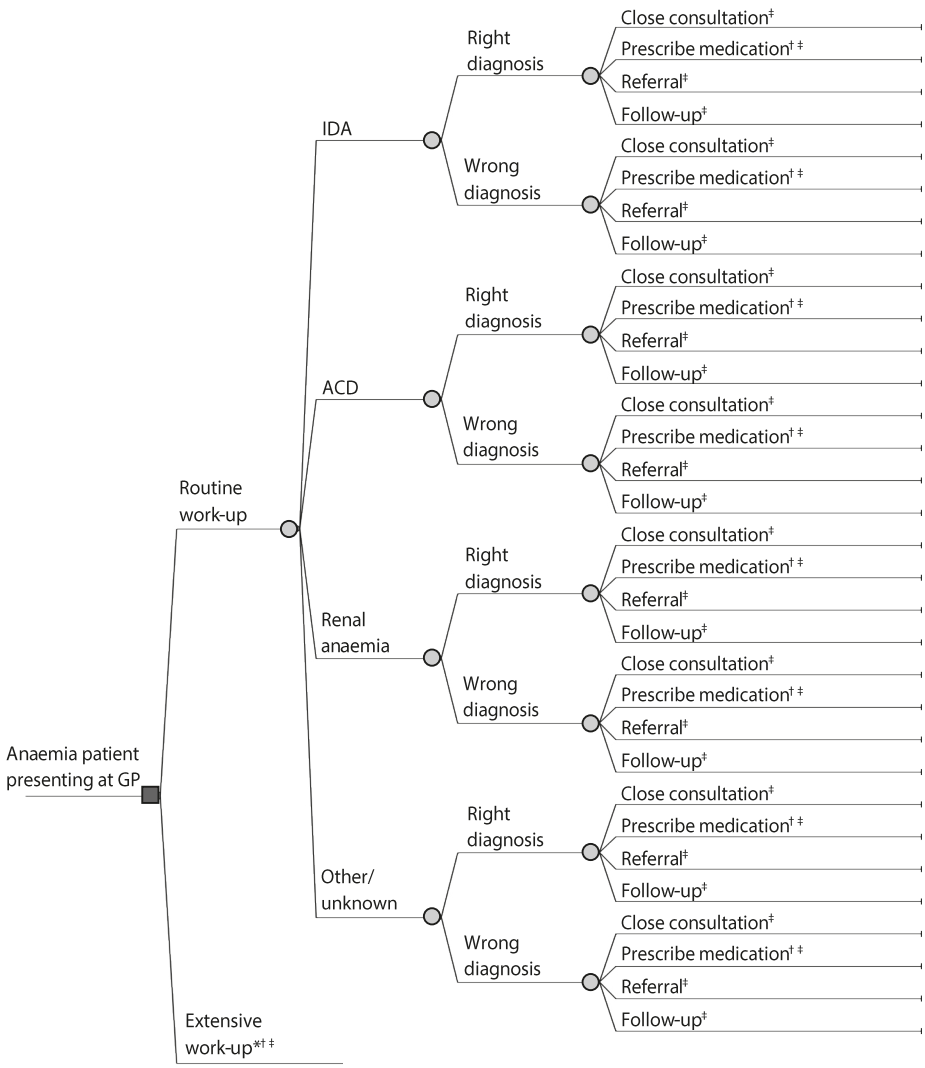

Figure 1. Simplified decision tree demonstrating both laboratory work-ups (routine and extensive) of patients presenting with new anaemia in general practices.

*The structure of this decision tree is identical to the routine work-up, but differs in the probabilities that are used. The structure of the entire decision tree could not be shown due to lack of space.
†In patients prescribed medication, the GPs chose to prescribe iron, vitamin B12, folic acid, or antibiotics.
‡Patients whom initially received a treatment that is ineffective (according to the expert panel), either have recover spontaneously, or are assumed to present at the GP again within a few weeks, and undergo a second round of diagnostics and treatment. In this second round, it is assumed that GPs will only make management decisions that are considered effective (medication or referral, depending on the underlying cause of anaemia).

Table 1. Detailed overview of costs and effects of both the routine and the extensive laboratory work-up, for the different underlying causes of anaemia.

	% of patients	Work-up	Probability right cause (95% CI)	Costs initial lab (95% CI)	Costs referral (95% CI)	Costs medication (95% CI)	Costs other lab (95% CI)	Total costs (95% CI)
IDA	19%	Routine work-up	69.26% (57.51% to 80.03%)	€94.51 (€70.70 to €123.36)	€613.52 (€457.00 to €798.41)	€37.64 (€8.16 to €82.85)	€91.28 (€72.84 to €112.21)	€836.95 (€664.11 to €1035.74)
		Extensive work-up	70.13% (58.64% to 80.28%)	€93.63 (€75.88 to €114.36)	€643.31 (€479.02 to €830.42)	€33.28 (€7.26 to €73.48)	€94.75 (€75.95 to €116.15)	€864.98 (€689.65 to €1063.49)
		Effect	0.87% (-14.82% to 16.42%)	-€0.88 (-€10.56 to €7.49)	€29.79 (-€54.22 to €114.20)	-€4.36 (-€12.40 to -€0.11)	€3.47 (-€7.06 to €15.76)	€28.03 (-€61.85 to €119.65)
ACD	32%	Routine work-up	44.48% (35.92% to 53.11%)	€94.51 (€70.70 to €123.36)	€496.59 (€374.31 to €645.54)	€21.93 (€8.02 to €43.18)	€174.65 (€135.57 to €220.56)	€787.67 (€636.73 to €963.29)
		Extensive work-up	54.56% (46.20% to 62.96%)	€93.63 (€75.88 to €114.36)	€543.67 (€420.41 to €691.52)	€14.14 (€3.23 to €30.96)	€170.23 (€134.13 to €213.60)	€821.68 (€676.97 to €989.12)
		Effect	10.08% (-2.01% to 22.12%)	-€0.88 (-€10.56 to €7.49)	€47.08 (€2.75 to €90.54)	-€7.79 (-€14.98 to -€3.15)	-€4.42 (-€15.44 to €5.29)	€34.00 (-€24.82 to €89.92)
Renal anaemia	11%	Routine work-up	52.82% (39.64% to 66.27%)	€94.51 (€70.70 to €123.36)	€664.56 (€492.23 to €873.25)	€0.00 (€0.00 to €0.00)	€173.22 (€137.18 to €216.25)	€932.29 (€736.12 to €1158.71)
		Extensive work-up	65.21% (51.06% to 78.12%)	€93.63 (€75.88 to €114.36)	€606.01 (€441.41 to €801.95)	€0.00 (€0.00 to €0.00)	€150.71 (€119.42 to €187.93)	€850.35 (€668.07 to €1062.52)
		Effect	12.39% (-06.82% to 31.15%)	-€0.88 (-€10.56 to €7.49)	-€58.56 (-€147.27 to €28.53)	€0.00 (€0.00 to €0.00)	-€22.51 (-€45.63 to -€0.60)	-€81.94 (-€192.83 to €26.24)

Table 1. (Continued).

	% of patients	Work-up	Probability right cause (95% CI)	Costs initial lab (95% CI)	Costs referral (95% CI)	Costs medication (95% CI)	Costs other lab (95% CI)	Total costs (95% CI)
Other	39%	Routine work-up	43.50% (36.21% to 51.05%)	€94.51 (€70.70 to €123.36)	€561.78 (€437.78 to €706.01)	€23.53 (€8.03 to €47.14)	€184.25 (€147.26 to €227.85)	€864.06 (€716.99 to €1026.68)
		Extensive work-up	47.80% (39.63% to 55.74%)	€93.63 (€75.88 to €114.36)	€549.54 (€429.11 to €692.74)	€24.84 (€10.98 to €45.13)	€184.66 (€147.42 to €229.00)	€852.67 (€711.57 to €1012.88)
		Effect	4.30% (-6.84% to 15.12%)	-€0.88 (-€10.56 to €7.49)	-€12.24 (-€58.69 to €34.80)	€1.32 (-€3.43 to €5.79)	€0.41 (-€12.13 to €13.34)	-€11.39 (-€70.81 to €48.56)
Overall	100%	Routine work-up	49.64% (44.76% to 54.46%)	€94.51 (€70.70 to €123.36)	€561.92 (€445.11 to €694.93)	€23.10 (€7.39 to €47.22)	€162.62 (€131.77 to €198.46)	€842.15 (€704.35 to €993.96)
		Extensive work-up	56.01% (51.20% to 60.75%)	€93.63 (€75.88 to €114.36)	€571.37 (€454.80 to €706.01)	€20.32 (€6.91 to €40.69)	€159.58 (€130.05 to €194.17)	€844.90 (€710.65 to €993.89)
		Effect	6.37% (-0.56% to 13.10%)	-€0.88 (-€10.56 to €7.49)	€9.44 (-€20.03 to €39.19)	-€2.78 (-€7.15 to -€0.06)	-€3.04 (-€9.96 to €3.80)	€2.75 (-€34.72 to €39.86)

Note: in addition, costs of initial lab (at GP presentation, maximum of two times), costs of referrals, costs of medication and costs of other laboratory tests (performed during follow-up, to monitor medication, or during referral) are presented. Percentages may not add up to exactly 100% due to rounding. ACD; anaemia of chronic disease, CI; confidence interval, IDA; iron deficiency anaemia.

Results

Overall cost-effectiveness

As shown in Table 1, the routine laboratory work-up costs €842 (95% CI: €704 to €994) per patient, as compared with €845 (95% CI: €711 to €994) for the extensive laboratory work-up (an increase of €3 per patient [95% CI: €+35 to E40], i.e. -0.3%). Compared with the routine work-up, the extensive work-up showed a trend of an increase in the percentage of patients diagnosed with the correct underlying condition of anaemia from 49.6% (95% CI: 44.8% to 54.5%) to 56.0% (95% CI: 51.2% to 60.8%), i.e. +6.4% (95% CI: -0.6% to 13.1%). This resulted in an ICER of €43 per additional patient diagnosed with the correct underlying cause of anaemia. Estimating that 57,000 patients aged ≥50 years present with a new anaemia in Dutch general practices annually (i.e. no anaemia in preceding two years) [22, 23], this can result in an increase of around 3600 patients who are diagnosed both earlier and with the correct underlying cause of anaemia, at an additional cost of €156,000/year.

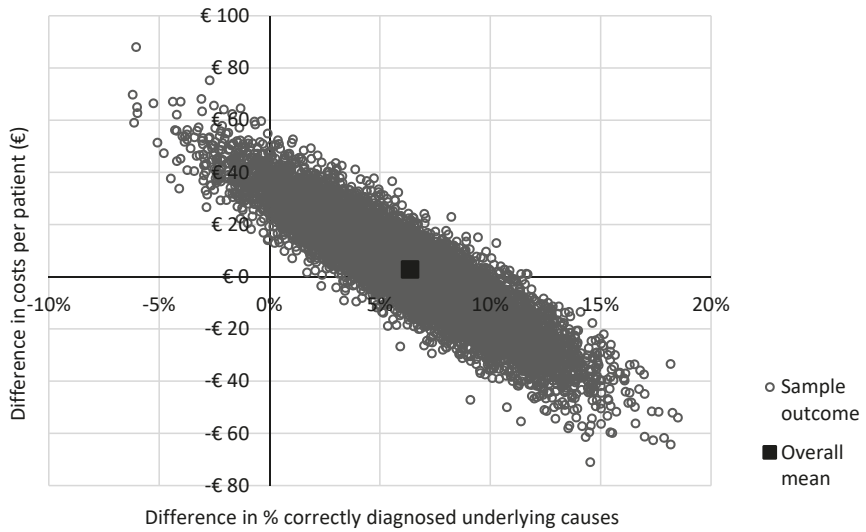

Figure 2. Incremental cost-effectiveness plan showing the impact of the extensive laboratory work-up as compared with the routine laboratory work-up on the difference in the percentage of correctly diagnosed underlying causes of anaemia, as well as the difference in costs per patient, for 10.000 model simulations.

Diagnosis-specific cost-effectiveness

The results of subgroup analyses indicated that average per patient costs were expected to increase by €28 (95% CI: €-62 to €120) among IDA patients and by €34 (95% CI: €-25 to €90) among ACD patients. In contrast, among renal anaemia patients and patients with 'other' underlying causes of anaemia, those costs are expected to decrease by €82 (95% CI: €-193 to €26) and by €11 (95% CI: €-71 to €49), respectively. The percentage of patients for whom the correct underlying cause is established is expected to increase by 0.9% (95% CI: -14.8% to 16.4%) among IDA patients, by 10.1% (95% CI: -2.0% to 22.1%) among ACD patients, by 12.4% (95% CI: -6.8% to 31.2%) among renal anaemia patients and by 4.3% (95% CI: -6.8% to 15.1%) among patients with 'other' underlying causes. Table 1 shows a detailed overview of all model outcomes, sorted by underlying cause of anaemia. The incremental cost-effectiveness plane demonstrating the overall result of 10,000 model simulations is shown in Figure 2. This figure indicates that 44.2% of the model simulations resulted in lower total costs of the extensive work-up as compared with the routine work-up. The incremental costeffectiveness plane for the subgroups of anaemia patients (i.e. IDA, ACD, renal anaemia and 'other') is shown in Supplemental Data 1. The impact of separately inserting the inputs of the internist-haematologists on model outcomes as opposed to using their averaged estimates (as in the base case analysis) was considered negligible (Supplemental Data 1). In addition, 56.2% of the model simulations indicated lower costs in the extensive work-up (data not shown), when only considering costs of the initial GP consultation and accompanying phlebotomy and laboratory analyses (with a maximum of requesting laboratory tests twice in the routine work-up).

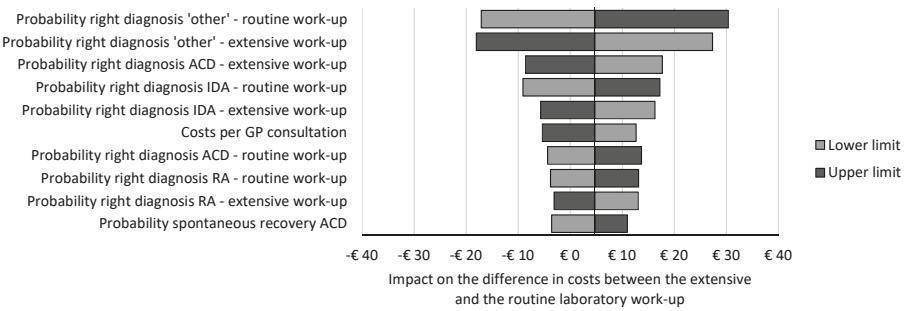

Figure 3. Tornado diagram showing the impact of changes in the most relevant input parameters on the difference in costs.

One-way sensitivity analysis

In Figure 3, the results of one-way sensitivity analyses are shown for the 10 parameters with the highest impact on the difference in costs. The difference in costs between both strategies was found to be most sensitive to changes in the frequency with which GPs correctly diagnose the underlying cause of anaemia, in both the extensive and the routine work-ups. The costs per GP consultation and the probability of spontaneous recovery of ACD have the highest impact on the difference in costs between both work-ups.

Conclusion and discussion

The use of an extensive laboratory work-up likely increases the percentage of patients diagnosed with the correct underlying cause of anaemia as compared with the routine work-up. Simultaneously, a very minor increase in costs of ~€3/patient (+0.3%) was expected. Whereas the improvement in diagnosis was quite likely (chance of an increase in correct diagnosis equals 6.4%), the exact effect of this extensive workup on the changes in costs was quite uncertain (chance that the extensive work-up would actually save costs equals 44.2%). Although higher costs were expected among IDA and ACD patients, most cost savings were expected to be achieved among patients with renal and 'other' types of anaemia.

Despite the probable increase of 6.4% in correct diagnoses, the results varied considerably between the different underlying causes of anaemia. The largest improvements in this probability were expected in ACD and renal anaemia patients (i.e. +10.1% and +12.4%, respectively). However, costs of referrals were expected to increase by €47/patient among ACD patients, whereas those costs decreased by €59/patient among renal anaemia patients. The increase in costs among ACD patients was explained by the higher immediate referral rate to secondary care (i.e. referral following the initial GP consultation) after the extensive work-up rather than after the routine work-up (38% vs. 26%), while the probability of follow-up was lower (48% vs. 60%). As the probability of spontaneous recovery among ACD patients was estimated to be relatively high (i.e. 38%), higher referral rates lead to higher costs in the extensive work-up in this patient category. Combined with the relatively high frequency of ACD (32% of all newly diagnosed anaemia patients in general practice), this may strongly impact the overall costs. Therefore, effort should be spent on deciding which patient management strategy (i.e. referral or follow-up) results in most improvements in the patient's quality of life, at the lowest costs. For renal anaemia patients, the results indicate that 42% and 48% of

those patients were immediately referred to secondary care in the routine versus the extensive work-up, which was considered the only appropriate management decision according to the expert panel (Supplemental Data 1). Thus, besides expected improvements in diagnosing renal anaemia due to the extensive work-up, this work-up also increased the number of patients who would immediately receive an effective management strategy, which likely decreases costs further downstream the care pathway.

In IDA patients, the probability of a correct diagnosis remained almost unchanged in the extensive as compared with the routine work-up (i.e. +0.9%), while costs increased with €28/patient. When considering the management decisions made in those patients, it was found that this increase in costs was mostly attributable to an expected 6% increase in immediate referrals (43% vs. 37%). However, the DCGP guideline recommends a colonoscopy and/or gastroscopy in IDA patients aged >50 years, to exclude a gastrointestinal malignancy [24]. As such malignancies can be detected in 6–15% of IDA patients, this increase in immediate referrals may increase the probability that a gastrointestinal malignancy is diagnosed [25-30]. Thereby, this increase in costs will likely enhance rapid initiation of adequate treatment, potentially improving treatment effectiveness. However, as such a referral decision should be based on a patient's clinical signs and symptoms (which were unknown in the current analysis), the real-life impact of this extensive work-up on the diagnosis and treatment of gastrointestinal malignancies remains to be investigated.

Although the previously performed effectiveness analysis distinguished only four underlying causes of anaemia (IDA, ACD, renal anaemia and 'other'), the current study further divided the 'other' category into suspected bone marrow disease, vitamin B12 or folic acid deficiency and unknown causes [19]. Subsequently, only the remaining patients were categorized as 'other'. Although those additional subgroups were too small for any demonstrable results, this subdivision allowed for a more precise calculation on the costs of these diagnoses. However, it is reasonable to assume that GPs are unable to diagnose these less common causes of anaemia based solely on laboratory test results: they often require further diagnostic testing or referral to secondary care to determine these causes. Therefore, in order to allow an accurate cost estimation, the approach taken to analyse the effectiveness in the current study differed slightly from the approach taken in the previously published article. Consequently, the results from the current study indicate a slightly lower percentage of correctly diagnosed underlying causes when compared with the previous study [19].

Strengths

The results are expected to provide a good representation of the Dutch population aged ≥50 years with newly diagnosed anaemia in primary care, because the cases in the survey were based on real-life patient data, the incidence of the various causes of anaemia in the survey correspond with their occurrence in daily practice, and because the participating GPs were representative of the GP population in the Netherlands [19]. As 96.4% of the 10,000 model simulations indicated that the extensive laboratory work-up would increase the percentage of patients correctly diagnosed, this result was robust to uncertainty in input parameters. Of those simulations, 44.2% indicated lower total costs with the use of an extensive work-up, although there was an average increase of ~€3 per patient, overall, indicating that the exact impact on costs is likely very limited but remains uncertain. However, the average number of tests performed in the extensive work-up doubled those performed in the routine work-up (14 vs. 7). That the increase in costs remains so small can easily be explained as the costs of additional diagnostic tests are offset by performing all tests during one GP visit and one phlebotomy, thereby preventing repeated blood sampling (involving additional costs of GP visits, the order tariff for requesting laboratory tests, and lost productivity among patients). This is also confirmed by the results, as 55.4% of 10,000 model simulations indicated that the costs of diagnostic testing at the GP were actually lower in the extensive work-up.

Limitations

This study has certain limitations. First, as described previously, the GPs were not provided with the patient's anamnesis, medical history and physical examination [19]. It is therefore likely that the accuracy of the diagnoses of the responding GPs may be higher in real-life. Although this limitation was present in all cases within the study, their potential effect on the differences between the two analysis methods is most likely limited, although it cannot be excluded that the abovementioned patient characteristics may have affected the tests that would have been requested by the GPs. Furthermore, it is uncertain to what extent GPs' diagnostic and treatment decisions in the survey may differ from real life.

Secondly, as mentioned previously, the costs of treating anaemia in secondary care have not been included in the model because of large differences in treatments for the different underlying causes and the lack of patientlevel data. However, a delayed correct diagnosis likely delays the initiation of adequate treatment, negatively affects quality of life and potentially increases treatment costs owing to an increased severity of anaemia [3]. Therefore, it was conservatively assumed that costs after establishing the correct diagnosis will not differ between patients.

Consequently, current results are likely an underestimation of the potential additional benefits provided by the extensive laboratory work-up.

Third, as information for some model input parameters could not be obtained from literature, expert estimates had to be used. Although the number of experts was limited (n = 2), the results of probabilistic (Supplemental Data 1) and one-way sensitivity analyses indicated that the impact of changes in model parameters (as based on those expert elicitations) on model outcomes was limited. Fourth, in the Netherlands, 64% of the clinical chemistry laboratories offer reflex testing [31]. In reflex testing, GPs do not decide themselves which laboratory tests to perform in suspected anaemia patients, but instead request 'anaemia analysis'. The laboratory will then perform a predefined set of tests, and sequentially perform additional tests if the initial tests indicate the presence of anaemia [31]. In addition, 27% of these laboratories provide an interpretative comment along with the test result [31]. As this reflex testing could not be incorporated in the current study, the proportion of patients correctly diagnosed in current clinical practice (reflected by the routine work-up) may be underestimated. However, the set of tests performed in reflex testing differs strongly between laboratories and often involves fewer diagnostic tests compared with the extensive work-up presented in the current study [31]. In addition, although the DCGP -guidelines recommend reflex testing in patients with newly diagnosed anaemia, it is not yet offered by all laboratories and can therefore not yet be considered common practice among GPs [24]. Thus, although the added benefit of the extensive work-up may be slightly overestimated in the current analysis, it likely still increases the number of patients for whom the underlying cause is correctly diagnosed by the GP. Furthermore, the insights obtained from this study are likely of added value to decide upon the optimal combination of tests to be used for reflex testing, which may increase similarity in diagnostic work-ups between laboratories.

Implications for practice

As subgroup analyses revealed that the added value of the extensive laboratory work-up depends on the underlying cause of anaemia, further research into this variation is recommended. Which tests contribute most to establishing the correct underlying cause should be investigated, in order to select a laboratory work-up that can correctly diagnose the majority of patients with minimal inconvenience (i.e. multiple venipunctures) and at minimal costs. Transferability of these results to other countries is not straightforward, as the work-up regarding the diagnosis of anaemia patients and the attributed costs may vary greatly between countries.

Populating the model with country-specific data would support reliable country-specific estimations of the cost-effectiveness of both work-ups.

In conclusion, although the extensive laboratory work-up is usually more effective for diagnosing the underlying cause of anaemia by GPs, it is not always more cost-effective than the routine work-up. Nevertheless, the impact on costs was found to be minimal. Given that the extensive work-up results in additional benefits which could not be captured in the current analysis (i.e. in terms of a faster diagnosis which may improve a patient's quality of life, and reduced productivity losses among anaemia patients and their caregivers), using an extensive laboratory work-up is expected to be cost-effective.

Acknowledgments
We thank all of the GPs for their participation in the survey, and the internist haematologist for participating in the expert elicitation. In addition, we thank Dr. Rebecca Buis for critical reading of the manuscript.

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6

Diagnostics in anaemia of chronic disease in general practice: a real-world retrospective cohort study

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British journal of general practice open access, 2017:
DOI: 10.3399/bjgpopen18X101597

Abstract

Background. Limited research has been performed that focused on the diagnosis of the underlying cause of anaemia of chronic disease (ACD) in general practice or on prevalence data of the underlying causes of ACD in general practice, although this is one of the most common types of anaemia.

Aim. To clarify the diagnostic strategies of GPs in patients newly diagnosed with ACD and to determine the most common underlying causes.

Design & setting. Retrospective cohort study.

Method. Patients newly diagnosed with ACD were selected based on laboratory criteria. ACD was defined as confirmed anaemia and ferritin levels above 100 mg/l combined with decreased iron and/or reduced transferrin. Additional medical information on patients was obtained from the electronic medical files of the GP and/or the referral hospital.

Results. Of the 267 analysed patients with ACD, additional investigations were performed in 205 patients (77%); in 31 patients (12%) the cause was apparent at the time of diagnosis, and for 31 patients (12%) no additional investigations were requested. In 210 (79%) of the 267 patients, an underlying cause was established, with infection (n = 68, 32%), autoimmune disease (n = 51, 24%) and malignancy (n = 48, 23%) as the most frequently observed etiologies. In 35 (13%) of the ACD patients, oral iron supplementation was prescribed by the GP. This was mainly done in patients with severe anaemia or less enhanced ferritin levels.

Conclusion. For most patients with newly diagnosed ACD, the GP undertakes additional investigations to establish underlying causes. However, the cause of ACD remains unknown in a small proportion of patients. The use of oral iron supplementation in these patients requires caution.

Introduction

Anaemia is a common finding in general practice and is associated with increased mortality, physical and cognitive decline, collapse, fractures, frailty, cardiovascular events, and reduced quality of life [1, 2]. ACD is one of the most common types of anaemia [3 – 6]. This type of anaemia can be caused by a variety of conditions, including acute and chronic infections, chronic diseases, autoimmune disorders, acute trauma, surgical interventions, renal failure, heart failure, and malignancies [7 – 13]. ACD is described as a functional iron deficiency caused by elevated hepcidin levels, which implies that oral iron supplementation is unnecessary [14 – 16]. Patients with ACD aged ≥50 years have a relative risk for mortality of 1.48 compared to adults without anaemia [17]. To ensure proper treatment of ACD, the underlying cause needs to be elucidated and treated. If the cause is not clear, additional investigations are required [2, 5, 15, 16, 18, 19]. These steps require active participation of the GPs involved with patients with ACD in general practice.

The National Institute for Health and Care Excellence (NICE) published guidelines on the management and treatment of anaemia in patients with chronic kidney disease, which is an underlying cause often observed in ACD patients [20]. Besides these guidelines, there is not much research available focusing specifically on the diagnosis of the underlying cause of ACD in general practice, or on prevalence data of the underlying causes of ACD in general practice. Therefore, this study aims to clarify the diagnostic strategies employed by GPs for their patients with ACD and provides an overview of the underlying causes as established by GPs and/or medical specialists.

Method

Patient inclusion

Patients were recruited as part of a transmural project aimed at anaemic patients aged ≥50 years. The main goal of the project is to improve the care for anaemic patients, and it has been running since 2007 [21]. In patients presenting to one of the 63 participating GPs with symptoms indicative of anaemia, an extensive laboratory protocol was performed that consisted of haemoglobin, mean corpuscular volume, C-reactive protein and/or erythrocyte sedimentation rate, vitamin B12, creatinine, ferritin, folic acid, lactate dehydrogenase, transferrin, reticulocytes, leukocytes, thrombocytes, and serum iron [18]. Anaemia was defined as haemoglobin ≤13.7 g/dL (8.5 mmol/L) for males and ≤12.1 g/dL (7.5 mmol/L) for females. ACD was defined as confirmed anaemia and ferritin ≥100 mg/l combined with decreased serum iron (<14 mmol/L for males and <10 mmol/L for females) and/or reduced transferrin (below the lower limit of the reference interval) [21 22]. As part of the transmural project, a comment was added to the laboratory results alerting GPs to the most likely cause of anaemia. For the present study, patients diagnosed with ACD were retrospectively selected from the large database that was compiled between 1 February 2007 and 1 February 2013.

Data collection

Clinical data was extracted from the electronic medical files of the GPs and referral hospital. Data could only be collected on the basis of the willingness of the GP to participate in the study and the availability of the data in electronic documentation. If these conditions could not be met, the patient was excluded from the study (n = 564) (Figure 1). Since patients were recruited over a 6-year period, the length of follow-up varied considerably. The end of follow-up was recorded as the date of data collection, or the date of the last available documented data (due to patients changing their GP in case of relocation or migration). If the patient had died before the moment of data collection, the date of death was recorded as the end of follow-up.

Collected clinical information consisted of all diagnostic investigations ordered by GPs, including those requested in consultation with a medical specialist, from the moment of discovery of ACD until the diagnosis of the underlying cause or the first outpatient visit to the referral hospital. These investigations were defined as 'additional investigations'. In addition, all underlying causes of ACD (those diagnosed by the GP and those diagnosed by the referral hospital's medical specialist) were registered. If the patient was known to suffer from diabetes, chronic

lung disease, or heart failure, and no other underlying cause of ACD could be established, these diseases were assigned as the underlying cause of ACD [7 – 13]. Any prescription of oral iron supplementation, as well as the time (in weeks) from the confirmation of ACD to the establishment of the underlying cause were also recorded.

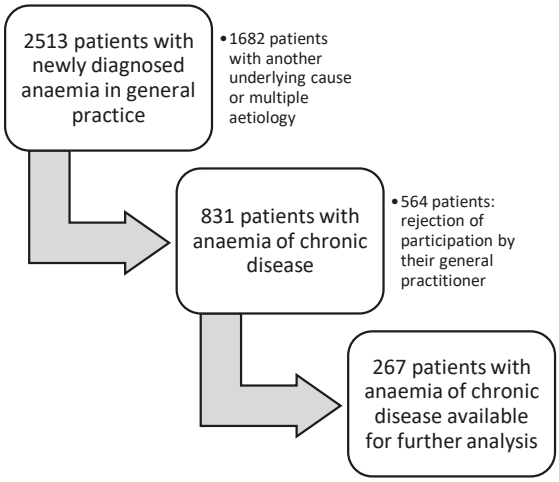

Figure 1. Flow diagram of the selection of study patients.

Definitions

Guidelines published by the Dutch College of General Practitioners in 2003 (revised in 2014) recommend handling newly diagnosed ACD in general practice as follows:

1. further diagnostic investigations to clarify the underlying illness (unless an underlying cause is already known); and
2. no oral iron supplementation [16].

In the present study this was defined as 'current recommendations'.

Statistical analysis

The study population was characterised by standard descriptive statistics. Comparisons of the included and excluded patients were performed using the chi-2 test and independent samples t-test (as appropriate). Differences in mean haemoglobin levels between patients referred and not referred to a medical specialist after establishment of ACD were tested using an independent samples t-test. Factors associated with the prescription of oral iron supplementation were analysed using the chi-2 test, independent samples t-test, and multivariable logistic

regression analysis. The independent variables in the logistic regression were age, sex, haemoglobin, and ferritin. Haemoglobin and ferritin were dichotomised according to the median of those variables. In addition, the same univariable and multivariable analyses were used to investigate factors associated with the recommended diagnostic and therapeutic strategy of patients with ACD. A two-sided P-value ≤0.05 was considered statistically significant. Data were analysed using the SPSS for Windows (version 24).

Results

Patient characteristics

Between 1 February 2007 and 1 February 2013, patients with newly diagnosed anaemia were included in a large transmural project.21 In 831 (33%) of these 2513 patients, the anaemia was defined as ACD based on predefined laboratory criteria and evaluated by experienced clinical chemists. All tests were performed in the clinical chemistry laboratory of the Albert Schweitzer Hospital. Because of lack of consent of the GP due to time restraints and loss of follow-up, a total of 267 patients (32%) with newly diagnosed ACD were finally available for the analysis (Figure 1). Characteristics of the study group are presented in Table 1.

Table 1. Characteristics of the study population (n = 267).

	Mean ± S.D.	Reference value
Gender	148 male 119 female	
Age (years):	74.3 ± 10.5	
• Male	72.3 ± 10.7	
• Female	76.7 ± 9.7	
Haemoglobin (g/dl):	11.8 ± 1.3	
• Male	12.4 ± 1.1	13.7 – 17.7
• Female	11.0 ± 1.0	12.1 – 16.1
Transferrin (g/l)	1.9 ± 0.4	2.0 – 3.6
Ferritin (µg/l):		
• Male	445 ± 325	25 – 250
• Female	379 ± 373	20 – 150
Serum iron (µmol/l):		
• Male	7.1 ± 3.7	14 – 28
• Female	5.9 ± 3.4	10 - 25

No significant difference was found between the included patients and the non-participants regarding sex (P = 0.11), but a small difference in age was found (mean age of included patients 74.3 years versus 73.5 years in non-participants, P = 0.05). For both males and females, no significant differences in haemoglobin, ferritin, or transferrin levels were found between the included patients and non-participants (data not shown). In addition, the included patients demonstrated a significantly lower serum iron level compared with the non-participants (P = 0.003 for males, P = 0.01 for females).

Additional investigations by GPs

In 205 (77%) of the 267 included patients with newly established ACD, additional investigations were requested by GPs to clarify the underlying illness (Table 2). Moreover, in 78 patients multiple investigations were performed, resulting in a total of 311 investigations. The most frequently requested additional investigations were chest X-ray (24%), referral to an internist (21%), and referral to the emergency room (10%). The cause was already apparent at the time of diagnosing the ACD in 31 patients (12%), mostly due to diabetes mellitus (39%) and autoimmune diseases (32%). For 31 patients (12%), no additional investigations were requested, even though the cause of ACD was not clear. Patients who were referred to a medical specialist (n = 145, 54%), either at initial diagnosis or after additional investigations by the GP, had a mean haemoglobin level of 11.7 (range 8.1–13.5) g/dl compared with 11.8 (range 7.9–13.5) g/dl in patients who were not referred (P = 0.378).

Underlying causes of ACD

In 210 (79%) patients an underlying cause of ACD was determined, most frequently infection (n = 68, 32%), autoimmune disease (n = 51, 24%), and malignancy (n = 48, 23%) (Table 3). In 154 patients (58%), the median time from presentation with anaemia to clarification of the underlying cause could be collected; this was 2 weeks (range 0–48). Of these 154 patients, the underlying cause was established within 1 week for 66 (43%), and within 4 weeks after presentation for 103 patients (67%). Further information is available from the authors on request.

Current guideline recommendations

In 205 (77%) patients, the GP acted according to the current Dutch guidelines (additional investigations to clarify the underlying illness causing ACD [unless an underlying cause was already known], and no oral iron supplementation prescribed). These recommendations were adhered to more frequently in patients with a high ferritin level, that is >324 mg/l (OR 2.46; 95% confidence interval [CI] = 1.33 to 4.52), as shown in Table 4. In addition, less frequent implementation of

the recommendations in older patients (OR 0.97, 95% CI = 0.94 to 1.00) and patients with more severe anaemia, that is haemoglobin <11.8 g/dl (OR 0.47, 95% CI = 0.2 to 1.03) was detected, but these associations were not statistically significant.

Oral iron supplementation

Of all 267 patients, 35 (13%) received oral iron supplementation despite being diagnosed with ACD and demonstrating ferritin levels ≥100 mg/l. Severe anaemia (haemoglobin <11.8 g/dl) and less enhanced ferritin levels (<324 mg/l) were associated with a higher probability of oral iron supplementation (OR 4.97; 95% CI = 1.82 to 13.62 and OR 2.30; 95% CI = 1.06 to 4.99, respectively), as shown in Table 4.

Table 2. Data on established cause and recorded investigations.

	Frequency (%)
Physical examination[a]	17 (5.5)
X-ray	
• **Thorax**	75 (24)
• **Abdomen**	11 (3.5)
• **Joint**	9 (2.9)
• **Sinus**	2 (0.6)
Ultrasound	
• **Abdomen**	28 (9.0)
CT scan	
• **Abdomen/thorax**[b]	12 (3.9)
Endoscopy	
• **Full endoscopy**	2 (0.6)
• **Gastroscopy**	5 (1.6)
• **Colonoscopy**	5 (1.6)
Referral	
• **Internist**	66 (21.2)
• **Emergency room**	32 (10.3)
• **Pulmonologist**	12 (3.9)
• **Geriatrician**	11 (3.5)
• **Rheumatologist**	8 (2.6)
• **Other**	16 (5.1)
Total	311

[a] Only physical examinations directly related to a diagnosis of the underlying cause were noted. [b] CT scan was always requested in consultation with a medical specialist. CT= computed tomography.

Table 3. Underlying causes of anaemia of chronic disease.

	Additional investigated (n=205 (77%))	Underlying disease already apparent (n=31 (12%))	Not additional investigated, no cause apparent (n=31 (12%))	Total (n = 267 (100%))
Underlying cause established	179 (87)	31 (100)	0 (0)	210 (79)
• **Autoimmune disease**	41 (23)	10 (32)	-	51 (24)
• **Infection**	68 (38)	-	-	68 (32)
• **Renal failure**	4 (2)	-	-	4 (2)
• **Recent operation**	2 (1)	1 (3)	-	3 (1)
• **Malignancy**	44 (25)	4 (13)	-	48 (23)
• **Diabetes**	7 (4)	12 (39)	-	19 (9)
• **Heart failure**	5 (3)	2 (5)	-	7 (3)
• **Chronic lung disease**	4 (2)	1 (5)	-	5 (2)
• **Other causes** *	4 (2)	1 (3)	-	5 (2)
No cause established	26 (13)	0 (0)	31 (100)	57 (21)

Percentage of underlying causes is calculated from the total number of causes established in each subgroup.
[a] Such as liver cirrhosis, haematoma, and alcohol abuse.

Table 4. Factors associated with implementation of the current recommendations and prescription of oral iron supplementation.

	Univariate analysis	Multivariate analysis
	Percentage or mean difference (95% CI)	Odds ratio (95% CI)
Recommendations		
Age, years	3.63 (0.65 to 6.60)	0.97 (0.94 to 1.00)
Female	84.0	Reference category
Male	91.9	0.82 (0.38 to 1.75)
Haemoglobin ≥11.8 g/dl	90.4	Reference category
Haemoglobin <11.8 g/dl	86.6	0.47 (0.21 to 1.03)
Ferritin >324 µg/l	92.5	Reference category
Ferritin ≤324 µg/l	84.3	2.46 (1.33 to 4.52)
Oral iron supplementation		
Age, years	-1.29 (-5.05 to 2.47)	1.00 (0.97 to 1.04)
Female	15.1	Reference category
Male	11.5	2.00 (0.83 to 4.83)
Haemoglobin ≥11.8 g/dl	6.4	Reference category
Haemoglobin <11.8 g/dl	19.0	4.97 (1.82 to 13.62)
Ferritin >324 µg/l	8.3	Reference category
Ferritin ≤324 µg/l	17.9	2.30 (1.06 to 4.99)

Discussion

Summary

This study provides a representative overview of the diagnostic strategies employed by GPs for their patients with ACD and an overview of the underlying causes as established by GPs and/or medical specialists. Additional investigations were requested in 77% of the analysed 267 ACD patients. The most frequently requested additional investigations were chest x-ray (24%), referral to an internist (21%), and referral to the emergency room (10%). The underlying cause of ACD was established in 79% of these patients, with the most common causes being infection (in 32%), autoimmune disease (in 24%), and malignancy (in 23%). The median time from presentation with anaemia to clarification of the underlying cause of ACD was 2 weeks. The prescription of oral iron supplementation in patients with ACD is not recommended and requires caution [14 – 16].

Strengths and limitations

A possible limitation of this study is related to the recruitment of patients. Of all GPs participating in the project, about one-third declined participation (mainly due to time constraints). However, the group of newly diagnosed ACD patients not included in this study presented no significant difference in sex, or haemoglobin, ferritin, or transferrin levels compared with those included in the analyses. In addition, because a difference in age and in serum iron levels was observed between the groups, all analyses were corrected for these two variables. Another limitation is the retrospective design of the study, which can lead to differences in the completeness of individual GPs' medical reports and, perhaps, to loss of patient information, such as the reason to decline additional investigations after establishment of ACD.

Strengths of the study include a large sample size of representative patients providing a realistic overview of patients with ACD in general practice. In addition, patients were selected based on well-defined laboratory criteria, making these results generalisable to other countries. Finally, the content of the Dutch guidelines used for handling newly diagnosed patients with ACD is also widely accepted in other guidelines worldwide [2, 20 23].

Comparison with existing literature

This study demonstrated that 77% of the ACD patients received additional investigations after establishment of the ACD, with the most common strategy being referral to a medical specialist (47%). This relatively large proportion of referrals

might be because the diagnosis or treatment of the underlying cause of ACD might be beyond the scope of GPs' focus, for example, the diagnosis or treatment of malignancies. In addition, for 12% of patients without a clear cause of ACD, no additional investigations were requested. For most of these patients, no reason was documented for disregarding further investigations. However, GPs may have considered these patients too vulnerable for extensive tests due to, for example, advanced age and/or comorbidities, or the lack of consequences in these frail patients [24].

Comparable studies analysing the underlying causes of ACD have investigated hospitalised populations, whereas no data are available for community-dwelling adults with ACD. In this study, the most common underlying causes of ACD were infections, autoimmune inflammation, and malignancies; this is in line with data from hospitalised patients with ACD [4, 25, 26]. However, compared with the present study population, a markedly higher prevalence of renal insufficiency was reported among hospitalised patients with ACD [3, 25]. This might be due to selection bias in the present study population; the transmural project separated renal anaemia as a distinct category of anemia, resulting in less renal insufficiency in this ACD patient group. In addition, since only patients with newlydiagnosed anaemia (that is, no anaemia in the preceding 2 years) were included, this probably excluded patients frequently hospitalised and those already being treated by, for instance, a nephrologist.

In this study, the diagnostic strategy of GPs was in accordance with the current Dutch recommendations in 77% of the patients with newly diagnosed ACD. Patients presenting with high ferritin levels (>324 mg/l) were more often treated according to these recommendations. Since increased ferritin is a diagnostic parameter for ACD, elevated levels of ferritin might be an alarm symptom for GPs, prompting them to more frequently perform additional investigations. In addition, more increased ferritin levels might exclude iron deficiency more clearly, thereby preventing unnecessary oral iron supplementation. A trend was observed towards less frequent implementation of the recommendations in older patients and patients with more severe anaemia. In older patients, multiple diseases and/or comorbidities often determine the clinical presentation (that is, health status). Therefore, especially in these patients, GPs might decide to forgo additional investigations. Patients with severe anaemia may be rapidly treated with oral iron supplementation to elevate haemoglobin levels. However, this is not recommended in patients with ACD.

Nevertheless, about 13% of the patients in this study still received oral iron supplementation as part of their treatment, even though no benefit is expected due to enhanced levels of hepcidin and, therefore, reduced oral iron absorption [2, 14 – 16]. In this study, severe anaemia and minimally reduced ferritin levels (i.e. <324 mg/l) are associated with increased oral iron prescription by GPs. The association of minimally reduced ferritin levels and the more frequent prescription of oral iron supplementation might be caused by the absence of a definite ferritin cut-off value defining iron deficiency [16, 21]. The effect of oral iron supplementation in patients with ACD might cause unnecessary side effects and drug–drug interactions, and can lead to unnecessary (although small) costs [27, 28].

Implications for practice

To the authors' knowledge, this is the first study to explore and describe additional investigations to establish underlying causes in patients with newly diagnosed ACD in primary care. In these patients, infections, autoimmune diseases, and malignancies were the most frequently occurring underlying causes. Oral iron supplementation, which is not indicated in ACD patients, needs to be abandoned in primary care to prevent unnecessary side effects and costs, as well as possible drug–drug interactions. The information emerging from this study and data on the prevalence of the underlying causes in primary care may lead to more targeted investigations and, potentially, to a more targeted diagnosis and more appropriate treatment.

Acknowledgements

The authors thank the participating GPs for their generous contribution to this study.

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7

Long-term outcomes in patients newly diagnosed with iron deficiency anaemia in general practice: a retrospective cohort study

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BMJ open. 2019; 9(11):
DOI: 10.1136/bmjopen-2019-032930

Abstract

Objectives. To describe all iron deficiency anaemia (IDA)–related causes during follow-up of patients newly diagnosed with IDA and to assess whether a delayed colorectal cancer (CRC) diagnosis influences survival.

Design and setting. Retrospective cohort study of patients from general practices in the Dordrecht area, the Netherlands.

Participants. Men and women aged ≥50 years with a new diagnosis of IDA (ie, no anaemia 2 years previously).

Method. From February 2007 to February 2018, all relevant data were collected from the files of the referral hospital. Early IDA-related cause was defined as established within 18 weeks after IDA diagnosis. Cox proportional-hazards regression was used to analyse survival of patients with CRC diagnosis.

Results. 587 patients with IDA were included with a median follow-up of 4.6 years. Early and late IDA-related causes could be established in 32% and 8% of patients, respectively. Early and late CRC was found in 8% and 2% of patients, respectively, and were located mainly right sided. After adjustment for age, gender and TNM classification, mortality risk was lower in patients with IDA with early CRC diagnosis, but not significantly (HR 0.30, 95% CI 0.09 to 1.02).

Conclusion. Even with extended follow-up, the cause of IDA remains elusive in the majority of patients with IDA in general practice. However, patients with IDA are at increased risk for in particular right-sided CRC and a late diagnosis of CRC appears to have a detrimental effect on survival in patients with IDA.

Introduction

Around 5%–10% of patients with iron deficiency anaemia (IDA) have colorectal cancer (CRC) and, therefore, endoscopic evaluation is advised [1 – 5]. However, most studies reporting on IDA and CRC only included patients already referred for endoscopic evaluation. Information on general practice patients diagnosed with IDA and *not* referred for endoscopic evaluation is scarce. So far, only two cohort studies have included patients from the moment of IDA diagnosis in general practice [6, 7]. These studies reported that a third of patients with IDA diagnosed in general practice receive an endoscopic evaluation within several weeks after IDA diagnosis. Of these patients with IDA, the incidence of CRC was 4.6% and 5.9%, respectively [6, 7]. During follow-up of patients with IDA, including those who were not endoscopically evaluated, an additional 3.2% (follow-up of 3 years) and 2.4% (follow-up of 1 year) of patients were diagnosed with CRC [6, 7]. Limited data are available for the group of patients who do not receive an endoscopic evaluation and for whom other underlying causes related to IDA might be diagnosed later on. Furthermore, it is unknown whether the 3% of general practice patients with IDA who have a delayed CRC diagnosis have a poorer overall survival.

This study investigated a cohort of general practice patients with a new laboratory diagnosis of IDA. For the entire cohort, all IDA-related diagnoses made in-hospital are described during extended follow-up. Furthermore, we analysed the characteristics of patients with a CRC diagnosis, and the association between a delayed CRC diagnosis and overall survival.

Methods

Patient and public involvement

This study has been retrospectively conducted in a cohort of patients formed by general practitioners (GPs) participating in a large project on anaemia evaluation improvement [8]. Therefore, the patients' involvement has been limited. Patients were informed about this project by their treating GP. Data about these patients were processed anonymously. The participating GPs were informed about the study results and encouraged to give feedback.

Study population

The laboratory system of the Albert Schweitzer Hospital started a project to improve anaemia evaluation in general practice on 1 February 2007, in which 81 of

the invited 150 GPs (in the Dordrecht area, the Netherlands) agreed to participate. Every time the participating GPs requested a blood test for their patients aged ≥50 years and this revealed a low haemoglobin concentration, a comprehensive laboratory assessment was performed. This laboratory protocol consisted of measurement of haemoglobin, mean corpuscular volume (MCV), C-reactive protein and/or erythrocyte sedimentation rate, vitamin B12, creatinine, ferritin, folic acid, lactate dehydrogenase, transferrin, reticulocytes, leucocytes, thrombocytes and serum iron. IDA was defined as haemoglobin <13.7 g/dL for men and <12.1 g/dL for women in combination with ferritin <25 µg/L for men and <20 µg/L for women [9]. The participating GPs were provided with the laboratory results for each patient, including an interpretation of the laboratory diagnosis of IDA, and they were advised to request endoscopic evaluation for their patient. Fast-track endoscopic evaluation places (ie, within 4 weeks) were available for these patients.

Retrospectively, we selected a cohort of patients from the anaemia improvement project who presented with a new laboratory diagnosis of IDA during the period 1 February 2007 until 1 February 2016 and collected additional clinical information. We excluded patients who were already known with anaemia in the previous 2 years.

Data collection

Clinical data were extracted (if available) from the electronic medical files of the Albert Schweitzer Hospital. Collected clinical information consisted of endoscopic evaluations performed within 18 weeks after IDA diagnosis and all IDA-related causes established in-hospital including the date of the IDA-related diagnosis. Other diagnostic investigations to evaluate IDA and/or endoscopic evaluations performed 18 weeks after IDA diagnosis were not collected. CRC and other IDA-related malignancies were checked for registration in the Netherlands Comprehensive Cancer Organization [10]. The location of CRC was verified using pathology and operation reports and divided into either right (ie, colon ascendens, caecum and transversum) or left (ie, sigmoid, colon descendens and rectum). The stage of CRC was registered using the TNM 5 classification [11]. The length of follow-up was variable since patients were selected over a 9-year period (2007–2016). The end of follow-up Was defined as the date of death or the last noted hospital record before 1 February 2018 to ensure a follow-up time of at least 2 years.

Definitions

Early endoscopic evaluation was defined as all full endoscopies (ie, gastroscopy and colonoscopy) and colonoscopies performed within 18 weeks after establishment of

IDA. Gastroduodenoscopy alone was only counted as early endoscopic evaluation if an IDA-related Diagnosis according to the Dutch anaemia guideline was found [9]. Early IDA-related cause was defined as all in-hospital registered causes within 18 weeks after the establishment of IDA. Late IDA-related cause was defined as all in-hospital registered causes defined 19 weeks or later after the establishment of IDA.

The IDA-related causes were divided into four categories. The first category was defined as all gastrointestinal (GI) malignancies and those with a strong suspicion of GI malignancy but no pathological proof. The second category was defined as other malignancies causing IDA (ie, urothelial or endometria carcinoma) and patients with a GI metastasis of a non-GI primary tumour. The third category was defined as benign GI causes of IDA, which included angiodysplasia, gastritis/erosions, ulcers, haemorrhoids, polyps ≥1 cm, coeliac disease, Crohn's disease and radiation proctitis. Finally, the fourth category was defined as all other non-GI causes of IDA that included in-hospital registration of repetitive blood donation, hypermenorrhoea, low-iron diet and severe epistaxis as cause of IDA.

Statistical analysis

All patients with IDA were divided into either early or no (early) endoscopic evaluation and the data were characterized with standard descriptive statistics. Chi-square tests and Mann-Whitney U tests were used to compare patients receiving endoscopic evaluation and patients without endoscopic evaluation. Univariable and multivariable logistic regression analysis was performed with CRC diagnosis (yes/no) as dependent variable. In these logistic regression analyses, the independent variables were age, haemoglobin, ferritin, gender (male/female) and MCV (microcytic or normocytic/macrocytic anaemia). Interaction effects between gender and haemoglobin and between gender and ferritin were tested, but not included in the final model as they were not statistically significant. The goodness-of-fit of the multivariable logistic regression was assessed using the Hosmer-Lemeshow test. A Cox proportional-hazards regression analysis was performed including only those patients who received a CRC diagnosis during follow-up. In the Cox regression, the time at risk was the time from CRC diagnosis until death, or to the end of follow-up. In the Cox regression, the independent variables were age, gender (male/female), early or late CRC diagnosis, and stage of CRC (I, II, III, IV or unknown stage). The proportional-hazards assumption was tested by including interactions of independent variables and follow-up time in a Cox regression with time-dependent covariates. The location of CRC was compared between patients with an early or late diagnosis using a $\chi 2$ test. Data were analysed using SPSS for Windows, V.24. All statistical tests were two-sided and a p value <0.05 was considered statistically significant.

Results

Cohort description

Between 1 February 2007 and 1 February 2016, a total of 587 patients aged ≥50 years with a new laboratory diagnosis of IDA were included, consisting of 212 (36%) men and 375 (64%) women. The median haemoglobin concentration was 12.3 (range, 5.3–13.5) g/dL and 10.8 (range, 4.0–11.9) g/dL and the median ferritin level was 10 (range, 2–24) µg/L and 7 (range, 1–19) µg/L for men and women, respectively. Basic characteristics (age, gender and laboratory values at point of IDA diagnosis) are presented in table 1.

Early endoscopic evaluation

Of all 587 general practice patients diagnosed with IDA, 211 (36%) had an early endoscopic evaluation, consisting of 45 (21%) colonoscopies, 154 (73%) full endoscopies and 12 (6%) gastroscopies. The median time for early endoscopic evaluation was 5 weeks (IQR 2–7 weeks). Patients receiving endoscopic evaluation versus those who did not receive early endoscopy were significantly different in terms of laboratory values and gender, but not in age (table 1). In 115 (55%) of the 211 patients, who received an early endoscopic evaluation, a GI-related diagnosis could be made.

IDA-related causes

Median time of follow-up for the entire cohort was 4.6 (IQR 2.6–7.0) years, during which an IDA-related cause could be made in 234 (40%) patients, consisting of 190 (32%) early causes and 44 (8%) late causes. However, in 353 patients (60%), no IDA-related cause was found during the entire follow-up period (figure 1). In 18 patients, more than one IDA-related cause was established resulting in 254 in-hospital registered causes of IDA (table 2). Other causes (34%) and benign GI causes (35%) were the most common categories. An early malignant cause for IDA was found in 59 patients (10%), of whom 49 had CRC. An additional 21 patients (4%), of whom four received an early endoscopic evaluation without abnormalities, were diagnosed with a malignant cause for IDA later on, consisting of 12 CRC.

Table 1. Baseline characteristics of the 587 patients in general practice with iron deficiency anaemia (IDA).

	IDA cohort n = 587 (100%)	Early endoscopic evaluation n = 211 (36%)	No early endoscopic evaluation n = 376 (64%)	p-value	Reference value
Age in years	68 (50 – 101)	69 (50 – 95)	66 (50 – 101)	0.207	
Gender				**<0.001**	
• Male	212 (36%)	100 (47%)	112 (30%)		
• Female	375 (64%)	111 (53%)	264 (70%)		
Hemoglobin in g/dL					
• Male	12.3 (5.3 – 13.5)	10.6 (5.3 – 13.5)	12.8 (5.8 – 13.5)	**<0.001**	13.7 – 17.7
• Female	10.8 (4.0 – 11.9)	10.2 (4.5 – 11.9)	11.1 (4.0 – 11.9)	**<0.001**	12.1 – 16.1
MCV in fl	82 (55 – 102)	79 (55 – 96)	83 (58 – 102)	**<0.001**	82 – 98
ESR in mm/h[a]	20 (2 – 120)	23 (4 – 118)	20 (2 – 120)	**0.015**	< 35
C-reactive protein in mg/L[b]	<5 (<5 – 144)	<5 (<5 – 71)	<5 (<5 – 144)	**<0.001**	< 10
Ferritin in µg/L					
• Male	10 (2 – 24)	9 (2 – 24)	11 (2 – 23)	**<0.001**	25 – 250
• Female	7 (1 – 19)	7 (1 – 19)	8 (1 – 19)	0.055	20 – 250
Iron saturation %[c]	7 (2 – 55)	6 (2 – 22)	7 (2 – 55)	**<0.001**	20 – 60
Serum iron in µmol/L					
• Male	6.2 (1.9 – 22.1)	4.5 (1.9 – 22.1)	7.4 (2.0 – 21.0)	**<0.001**	14 – 28
• Female	5.0 (1.6 – 42.5)	3.9 (1.9 – 17.6)	5.3 (1.6 – 42.5)	**0.001**	10 – 25
Transferrin in g/L	3.36 (1.97 – 4.74)	3.38 (2.38 – 4.74)	3.34 (1.97 – 4.74)	**0.043**	2.00 – 3.60

*Missing values n=157. †Missing values n=3. ‡Missing values n=38. Values are median (range). Measured laboratory values are at point of IDA diagnosis. The p value compares the baseline characteristic of patients with and those without an early endoscopic evaluation.
ESR, erythrocyte sedimentation rate; MCV, mean corpuscular volume.

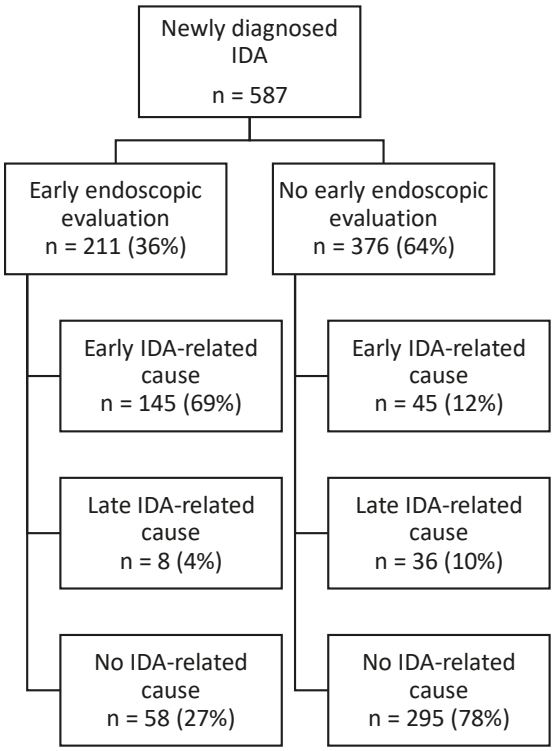

Figure 1. Flowchart of the cohort with iron deficiency anaemia (IDA).

Colorectal cancer

Of the 587 patients with IDA, 61 (10%) received a diagnosis of CRC during the study period (ie, 49 early and 12 late CRC diagnoses). In four patients, two separate tumours were diagnosed in the colon, and one patient presented with three different colon tumour locations. The CRC was right sided in 41 of 49 patients (84%) with an early diagnosis and in 8 of 12 patients (67%) with a late CRC diagnosis (p=0.184). Older age (OR 1.03; 95% CI 1.00 to 1.05), male gender (OR 2.12; 95% CI 1.18 to 3.79), lower haemoglobin concentrations (OR 1.27; 95% CI 1.08 to 2.49) and microcytic anaemia (OR 2.63; 95% CI 1.30 to 5.31) showed a significant association with CRC diagnosis (table 3).

The 61 patients with CRC diagnosis had a median follow-up of 4 years (range, 5 weeks to 10.7 years) from the moment of CRC diagnosis; during this period, 19 patients (31%) died. No significant violations of the proportional-hazards assumption of the Cox regression were detected in patients with CRC diagnosis. After correction

for age, gender and the TNM classification at the moment of CRC diagnosis, mortality risk was lower, but not significant, in the group of patients with an early CRC diagnosis (HR 0.30; 95% CI 0.09 to 1.02) (table 4).

Table 2. Iron deficiency anaemia (IDA)-related causes.

	Early endoscopic evaluation		No early endoscopic evaluation		
	Early cause	Late cause	Early cause	Late cause	Total
GI malignancies					**74 (29%)**
Colon	48	1	1*	11	
Gastric	3			1	
Oesophagus	2				
Small bowel	1				
Strong suspicion of GI malignancy		1	1	4	
Other malignancies					**6 (2%)**
Urothelial		1	1	1	
GI metastasis of a non-GI primary tumour	2	1			
Other causes†	**36**	**3**	**43**	**4**	**86 (34%)**
Benign GI causes					**88 (35%)**
Angiodysplasia	16	2		5	
Gastritis/erosions	19	1		4	
Ulcers‡	10				
Haemorrhoids	4		2		
Polyps >1 cm	13	1		3	
Coeliac/Crohn/ulverative colitis	4			3	
Radiation proctitis	1				
Total no. of IDA-related causes	**159**	**11**	**48**	**36**	**254 (100%)**

*Diagnosis made on the basis of surgery for colon perforation. †Other causes included repetitive blood donation, hypermenorrhoea, low-iron diet and severe epistaxis. ‡Including gastric ulcers as a result of *Helicobacter pylori* infection. GI, gastrointestinal.

Table 3. Logistic regression analysis comparing patients with colorectal cancer (CR) to those without CRC.

	CRC diagnosis n = 61	No CRC diagnosis n = 526	Univariable analysis OR (95% CI)	P value	Multivariable analysis OR (95% CI)	P value
Age, median (range)	75 (50-89)	66 (50-101)	1.03 (1.01 to 1.03)	**0.002**	1.03 (1.00 to 1.05)	**0.027**
Gender						
Male	28 (5%)	184 (31%)	*Reference group*		2.12 (1.18 to 3.79)	**0.012**
Female	33 (6%)	342 (58%)	0.63 (0.37 to 1.08)	0.095	*Reference group*	
Haemoglobin (g/dl)*, median (range)	9.3 (4.5-12.9)	11.3 (4.0-13.5)	1.41 (1.23 to 1.58)	**<0.001**	1.27 (1.08 to 1.49)	**0.007**
Ferritin (µg/l)*, median (range)	7 (1-22)	9 (1-24)	0.94 (0.89 to 1.00)	0.052	1.01 (0.93 to 1.09)	0.844
MCV category						
Normocytic/macrocytic† (MCV 80-102 fl)	20 (3%)	349 (60%)	*Reference group*		*Reference group*	
Microcytic (MCV <80 fl)	41 (7%)	177 (30%)	4.04 (2.30 to 7.11)		2.63 (1.30 to 5.31)	**0.007**

* Analysed as continuous variables. ORs should be interpreted as the effect of 1-unit decrease in concentration for haemoglobin and 1-unit increase in concentration of ferritin.
† Three patients had an MCV value >100 fl at the time of IDA diagnosis. IDA, iron deficiency anaemia; MCV, mean corpuscular volume.

Table 4. Cox proportional-hazards survival analysis for patients diagnosed with colorectal cancer.

	Deceased n = 19 (31%)	Alive n = 42 (69%)	Univariable analysis HR (95% CI)	P value	Multivariable analysis HR (95% CI)	P value
Age, median (range)	78 (50-87)	73 (52-89)	1.06 (1.00 to 1.12)	0.063	1.10 (1.02 to 1.19)	**0.017**
Gender						
Male	8 (13%)	20 (33%)	0.77 (0.31 to 1.93)	0.582	1.58 (0.57 to 4.36)	0.377
Female	11 (18%)	22 (36%)	*Reference group*		*Reference group*	
Time to diagnosis						
Early	13 (21%)	36 (59%)	0.32 (0.12 to 0.87)	**0.026**	0.30 (0.09 to 1.02)	0.053
Late	6 (10%)	6 (10%)	*Reference group*		*Reference group*	
TNM stage						
I	1 (2%)	11 (18%)	*Reference group*		*Referece group*	
II	3 (5%)	13 (21%)	3.23 (0.32 to 32.97)	0.323	3.47 (0.33 to 36.75)	0.302
III	5 (8%)	12 (20%)	4.16 (0.48 to 35.97)	0.195	5.35 (0.57 to 49.94)	0.141
IV	10 (16%)	5 (8%)	24.31 (2.78 to 212.41)	**0.004**	80.15 (7.47 to 859.67)	**<0.001**
Unknown	-	1 (2%)	NA	NA	NA	NA

NA, not applicable.
*Analysed as continuous variables. ORs should be interpreted as the effect of 1-unit decrease in concentration for haemoglobin and 1-unit increase in concentration of ferritin.
†Three patients had an MCV value >100 fl at the time of IDA diagnosis. IDA, iron deficiency anaemia; MCV, mean corpuscular volume.

Discussion

Principal findings

In this large retrospective cohort study, an early endoscopic evaluation was done in a third of newly diagnosed patients with IDA, and these patients were more often male gender and had more severe laboratory abnormalities. The most common category if IDA-related causes established in this cohort were benign GI causes (35%). However, in the majority of patients (60%), no in-hospital IDA-related cause could be found during the median follow-up of 4.6 years. In addition, there were 8% early and 2% late CRC diagnoses. A CRC diagnosis was associated with older age, male gender, lower haemoglobin concentrations and microcytic anaemia. In addition, some evidence was found for increased survival in patients with IDA with an early CRC diagnosis adjusted for age, gender and TNM classification, but this association was not statistically significant.

Strengths and limitations of this study

Inclusion of patients in our cohort occurred after an anaemia diagnosis was established in the laboratory. The GPs' reason for laboratory testing was not known. It could have been either specific symptoms in a patient or routine testing for other indications. Still, a diagnostic work-up should start after an IDA is established according to the Dutch GP guideline. This is a well-defined moment in practice.

The participating GPs usually referred their patients to the associated hospital; however, some patients may have preferred referral to a different hospital resulting in an underestimation of the amount of endoscopic evaluations and IDA-related causes. However, since almost all patients in our cohort had an electronic record in the associated hospital, this limits the possibility of missed cases. Furthermore, the two nearest hospitals, both outside the Dordrecht region, had a longer waiting time for endoscopic evaluation compared with the fast-track places we offered the participating GPs. The fast-track places that were offered to the participating GPs during the study period might have led to an increase in early endoscopic evaluations and thereby an increased early IDA-related diagnoses. However, the rate of early endoscopic evaluation was comparable with existing literature and therefore the fast-track places very likely did not affect the study results [6, 7].

Another aspect that we were unable to monitor was the possibility of consciously refraining from endoscopic evaluation by the GP and/or the patient. One study found that endoscopic evaluation in patients aged ≥85 years is safe and enables a high rate of diagnoses and therapeutic modifications [12]. Nevertheless,

comorbidities, age and limited life expectancy may still be reasons to refrain from endoscopic evaluation. We did not collect data on other diagnostic investigations and/or endoscopic evaluations performed more than 18 weeks after IDA diagnosis. However, we did document all IDA-related diagnoses made in-hospital during follow-up and some diagnoses, especially non-GI related, were made by means of other investigations than endoscopic evaluation.

It is important to realise that, in the present study, all diagnoses made during follow-up were registered in-hospital. A limitation is that the IDA-related causes found by the GP are not registered and, therefore, might be underestimated. However, the most relevant IDA-related causes, mainly GI-tract related, require in-hospital investigations, which makes the incidence of these causes found in our cohort reliable. In addition, the most worrying outcome is IDA-related malignancy; therefore, we registered these diagnoses from the Netherlands Comprehensive Cancer Organization. This organisation covers the entire Dutch population regarding cancer registrations, and therefore this ensures full representation of IDA-related malignancies. Finally, the survival analysis provided some evidence for an association between early CRC diagnosis and decreased mortality; however, lead-time bias may still be present. A more detailed analysis with a larger cohort is required to confirm our hypothesis of increased overall survival in patients with early CRC diagnosis.

Comparison with existing literature

In the present study, a third of newly diagnosed patients with IDA in general practice received early endoscopic evaluation; this is in line with others [6, 7]. Patients with IDA receiving early endoscopic evaluation are more often men. In the Netherlands, CRC has a higher incidence among men compared with women [13]. This might have stimulated GPs to perform more endoscopies in men. In addition, if women presented with hypermenorrhea, GPs may not have made an initial endoscopic evaluation or a hospital referral. Furthermore, patients with IDA with early endoscopic evaluation had more severe laboratory abnormalities. Some GPs will have a different policy in case of marginal anaemia, that is, these patients might first be treated with iron supplementation before GPs decide to refer for endoscopic evaluation.

This study shows, for the first time, that a small proportion of the IDA-related causes is made by means of investigations other than endoscopy. These diagnoses were mainly non-GI causes, including blood donation and hypermenorrhoea (despite applying a 50-year cut-off to prevent hypermenorrhoea as predominant cause of

IDA). Most of these IDA-related diagnoses were made in the subgroup of patients who received early endoscopic evaluation. The majority of patients who did not receive early endoscopic evaluation did not have an IDA-related cause defined during the extended follow-up. To our knowledge, this has not been described in detail before. Although the majority of patients with IDA without endoscopic evaluation have no IDA-related diagnosis during follow-up, a third of the patients with a late IDA-related cause were diagnosed with a GI malignancy, and an additional 11% had a strong suspicion of GI malignancy. These diagnoses might represent missed opportunities at the time of IDA diagnosis and should alert GPs in their aim to unravel the underlying cause of IDA. Based on our study results, omitting early endoscopic evaluation in patients with IDA is not safe. As also has been described in several guidelines before, endoscopic evaluation should be performed in all patients aged 50 years and older with a new diagnosis of IDA [9, 11].

We observed 8% of CRC diagnoses within 18 weeks of finding IDA in our cohort, whereas 2% of patients received this diagnosis during follow-up. Comparison of these results with previous data on patients with IDA in general practice revealed a slight increase in early diagnoses and a decrease in delayed diagnoses [6, 7]. This might be due to the comment that was added to the laboratory results alerting GPs to an endoscopic evaluation in patients with IDA. Furthermore, we offered participating GPs fast-track places for endoscopic evaluation, which may have led to earlier endoscopic evaluations. Although the association between early versus late CRC diagnosis and mortality was not statistically significant in our data, the point estimate and its 95% CI were in line with previous studies in which early colonoscopy significantly increased overall survival in patients with CRC diagnosis [14, 15]. The estimated association was adjusted for the TNM classification of the tumour at the time of diagnosis. Apparently, early-stage disease is important for better survival rates, and early CRC diagnosis, regardless of TNM classification, is too. This might be due to a better clinical condition at the moment of CRC diagnosis and start of treatment.

Finally, it was observed that in patients with IDA most early CRC diagnoses were right sided (84%) possibly caused by the absence of GI complaints such as rectal bleeding [16]. This lack of complaints may allow tumours to bleed unnoticed for a longer time, resulting in a diagnosis of IDA before the CRC is found. During our study period, 34% of all CRC diagnoses in the whole Dutch population were right sided according to the Netherlands Comprehensive Cancer Organization (data not

shown) [10]. Our results support the suspicion that right-sided tumours are more frequently encountered in patients with IDA [9, 16].

Implications for clinicians

The majority of patients with IDA in general practice do not have an IDA-related cause defined during extended follow-up. However, endoscopic evaluation for all patients with IDA is justified since these patients are at increased risk for, in particular, right-sided CRC. Importantly, patients with IDA in general practice with a delayed diagnosis of CRC might risk decreased overall survival.

Acknowledgements

The authors thank the participating GPs for their contribution to this study. Furthermore, the authors thank the registration team of the Netherlands Comprehensive Cancer Organization (IKNL) for the collection of data for the Netherlands Cancer Registry as well as the IKNL staff for scientific advice.

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8

General discussion

Diagnosing anemia is not the completion of a diagnostic process. Anemia is rather a symptom or laboratory finding after which a search is started for the underlying cause of the reduced hemoglobin. A variety of diseases can be manifested through anemia, such as (gastro-intestinal) malignancies, chronic diseases, acute infections, renal failure and hemoglobinopathies. The management of patients with anemia might be challenging for general practitioners (GPs). Nevertheless, it is widely recognized that anemia needs to be further evaluated, because it has been associated with reduced quality of life, a negative impact on possible comorbidities and increased mortality [1]. In fact, monitoring anemia, defining the etiology, and adequate treatment should all be part of adequate patient care [1, 2]**.**

Main results of the studies

The PAGAS cohort

This thesis presents studies that all have been conducted with data of a large cohort of anemia patients. This cohort was initiated through a comprehensive project called PAGAS (Project of Anemia analysis from the General practitioner to the Albert Schweitzer hospital). The project was started on the 1st of February 2007 and ran until February 2017. The aim of the project was to improve the care of anemia patients in general practices in the vicinity of the Albert Schweitzer hospital, Dordrecht, the Netherlands. A multidisciplinary team consisting of clinical chemists, internists, gastroenterologists and GPs set up the initial design of the cohort. A total of 81 out of 150 general practices in the region of Dordrecht decided to participate. The project was designed as follows: I) participating GPs were able to request a 'PAGAS laboratory work-up' if they suspected anemia in males ≥ 18 years and females ≥ 50 years (the age limit for female was set to exclude an abundance of hypermenorrhoe as leading cause of anemia). II) An extensive set of blood test was conducted for patients who actually had a decreased hemoglobin count, and a decreased hemoglobin had not been registered in the previous two year. III) The extensive laboratory work-up was evaluated by a clinical chemist, and the results together with an interpretation were sent to the GP. IV) In case of an iron deficiency anemia, GPs were permitted to refer the patient for a fast-track endoscopic evaluation of the gastrointestinal tract (i.e., within 4 weeks).

The study results

This thesis starts with an introduction describing the relevance of the conducted studies (**chapter 1**). Anemia is commonly seen in general practice, and a wide range of diseases might be the underlying cause of the anemia. Therefore, it is important

to have available a diagnostic work-up which is feasible for GPs, cost-effective for society, and time-effective for patients and clinicians. In the presented studies, therefore, attention has been paid to three themes. The first theme includes defining the anemia etiology based on blood test results. The second theme focuses on the effectiveness and cost-effectiveness of the diagnostic work-up to establish the anemia etiology. The third theme describes the further diagnostics and care of patients diagnosed with either of two common etiologies, iron deficiency anemia (IDA) and anemia of chronic disease (ACD).

The first study, described in **chapter 2**, explores the first step after anemia has been established, focused on finding the etiology of the anemia. The use of an extensive laboratory work-up in 4152 patients, newly diagnosed with anemia, resulted in a single etiology in 59% of patients. In a substantial proportion (22%) of patients, multiple etiologies were found, but an anemia etiology could not be established in 20% of patients. The most frequent single etiologies were IDA and ACD, whereas folic acid deficiency and suspected bone marrow disease most often were part of multiple etiologies. The analysis of patient characteristics showed that older age was more often seen in renal anemia, patients with IDA were more often younger and ACD was more often established in men. While ACD and uncertain etiology presented more often as mild anemia, IDA was more often underlying severe anemia. A multinomial logistics regression showed that age, sex, and severity of anemia might serve as predictive markers for finding an anemia etiology.

In the study presented in **chapter 3,** we analyzed the accuracy of mean corpuscular volume (MCV) as a screening test in establishing the anemia etiology. In our study population, the majority of patients (85%) had MCV values within the normal range. For these normocytic anemia patients, the MCV appeared not to be useful as a screening test to find the anemia etiology. On top of that, anemia etiologies were not restricted to a MCV-guided anemia classification algorithm in 90% of macrocytic patients and 16% of microcytic patients. Almost one quarter (22%) of anemic patients turned out to have multiple etiologies with various combinations, and another 20% of patients remained without an etiology established. For these subgroups of patients, MCV-guided anemia classification algorithm would not be predictive. In conclusion, MCV-guided anemia classification should not be used in the diagnostic work-up of anemia.

In the second part of this thesis, the applicability of an extensive laboratory work-up in newly diagnosed anemia patients in general practice was evaluated. First, the effectiveness of an extensive laboratory work-up versus a routine laboratory

work-up was studied in **chapter 4**. General practitioners were able to establish the correct anemia etiology in 53.0% of patients if using a routine work-up, compared to 61.9% of correct etiologies if using an extensive work-up (OR = 1.56 [95% CI: 1.12 – 2.17]. Moreover, establishing the anemia etiology appeared to be more difficult in older patients. In addition, the study results showed that the etiology itself affects the probability of a correct establishment, with ACD being the most difficult to correctly establish for GPs. Finally, the analysis of GPs' characteristics (i.e., number of years of working experience, use of guidelines and whether the GP had specific affinity with anemia) showed no significant associations with the probability of finding the correct anemia etiology. We concluded that an extensive laboratory work-up helps the GP in a more adequate management of patients diagnosed with an anemia.

The cost-effectiveness of an extensive laboratory work-up in anemia diagnostics was investigated in **chapter 5**. The results of this study showed that an extensive laboratory work-up was €3 per patient more expensive than a routine work-up. On the other hand, the amount of correct anemia etiologies established increased with 6.4%, resulting in an ICER of €43 per additional patient with a correct anemia etiology. Extrapolation of these results to the overall population showed that an extensive laboratory work-up can result in an increase of around 3600 patients with both an earlier and correct etiology. The implementation of an extensive laboratory work-up for all GPs in the Netherlands would imply an additional cost of €156.000/ year. From our analysis we conclude that an extensive laboratory work-up is cost-effective and that its implementation should be considered.

In the last part of this thesis, the most common anemia etiologies seen in general practice, ACD and IDA, were discussed. The evaluation in **chapter 6** showed that 77% of the 267 patients with a newly diagnosed ACD had received additional diagnostic tests to clarify the underlying illness. Most frequently, x-ray (24%), referral to an internist (21%) and referral to the emergency room (10%) had been requested upon an ACD establishment. In 12% of patients, the underlying cause of ACD was apparent at the time of diagnosis. Twelve percent of patients did not receive additional diagnostic tests and neither an underlying cause was noted by their GP. This evaluation also showed that a cause of ACD was diagnosed in 79% of patients. The most common causes were infection (32%), autoimmune disease (24%) and malignancy (23%). Despite that oral iron supplementation is not advised for patients with ACD, the evaluation showed that 13% of patients with ACD received oral iron supplementation prescribed by the GP. This supplementation was more often prescribed for patients with severe anemia and less enhanced ferritin levels. In conclusion, for most patients with ACD an underlying disease could be

diagnosed. The prescription practice of oral iron supplementation was relatively high given the contra-indication in ACD patients.

The final study, described in **chapter 7**, concerned patients with IDA. We found that during a median follow-up time of 4.6 years, an IDA-related diagnosis was made in 40% of the 587 patients. The most common category of IDA-related diagnoses consisted of benign gastro-intestinal diseases (35%). Colorectal cancer was found in 61 patients, of whom 20% had a delayed diagnosis (i.e., more than 18 weeks after the IDA diagnosis). The colorectal cancer was predominantly located right-sided (80%). Patients with colorectal cancer as IDA-related diagnosis were more often older in age, were more often male gender, and had lower hemoglobin and MCV serum levels than IDA patients without a colorectal cancer diagnosis. The mortality risk among patients with colorectal cancer was lower in the group of patients with an early diagnosis of colorectal cancer than in patients with a delayed diagnosis. In conclusion, after a long follow-up period, the underlying cause of IDA remains elusive in the majority of patients with IDA in general practice. Given the increased risk for in particular right-sided colorectal cancer, additional investigations should be considered for each patient with IDA.

Wider implications of the combined results

An extensive laboratory approach

As described in **chapter 1**, the anemia etiologies found in these studies are based on an extensive laboratory approach. Currently, such an extensive set of laboratory tests is not common in general practices. Most anemia guidelines recommend ordering laboratory tests based on which clinical etiology is suspected. Another common recommendation in anemia guidelines is to first determine MCV and/or ferritin. Based on the results of these tests, clinicians may request a further diagnostic process. In addition, some laboratories nowadays use reflex testing whereby a set of tests is automatically obtained when indicated by the initial blood test results [3, 4]. An extensive laboratory approach, as conducted in the studies of this thesis, provides unique information about the anemia etiologies. It does not only permit establishing more etiologies, but also makes that almost a quarter of patients with anemia demonstrate more than one etiology.

The implementation of an extensive laboratory panel in patients with anemia might be challenging, but is a crucial step towards improvement of the healthcare. A good example of the value of an extensive laboratory panel is provided by a

study of patients with possible curative colorectal cancer [5]. In this study, patients with normocytic anemia demonstrated a significantly poorer 5-year cancer-specific survival (82%) than those with microcytic anemia (90%). According to the authors of this study, the reason would be the association of normocytic anemia with systemic inflammation. The valuable information of an iron status indicating an iron deficiency combined with systemic inflammation in these patients might be immediately clear if an extensive laboratory approach is used. By assessing ferritin, transferrin and serum iron levels, this approach enables to find an IDA combined with an ACD. In this way, the co-occurrence of IDA and ACD can be simultaneously established. Another important issue that can be overcome by an extensive laboratory panel, is that the clinical presentation might be misleading in patients with more than one anemia etiology. For instance, a patient with IDA might also have a folic acid deficiency, although this can clinically be less obvious. In case of reflex testing or a stepwise approach of laboratory analysis, blood test results of a particular etiology might be masked by another etiology [6]. This could lead to a delayed or missed etiology. The impact of a delayed etiology establishment depends on the nature of the etiology itself. A delayed gastro-intestinal malignancy underlying an IDA has more impact on survival than a delayed folic acid deficiency. For each patient, however, anemia is associated with reduced quality of life and possibly premature death [1]. Additionally, the one-stop-anemia-analysis as executed in the framework of an extensive laboratory approach, may be more patient-friendly than a stepwise approach, which necessitates repetitive blood draws. Thus, an extensive laboratory approach in itself might be of benefit to the quality of care of anemia patients.

The implementation, however, of an extensive laboratory work-up might be challenging. For one thing, it provides more information which requires adequate interpretation. Especially the interpretation of a patient's iron status, as already indicated in the introduction chapter, might be demanding for GPs. The interpretation of the laboratory results is part of the post-post analytic phase of laboratory testing in primary care [7]. This phase is prone to errors (5%) related to cognitive factors such as the interpreter's intelligence and the quality of data. The risk of interpretation errors can be reduced through a closer collaboration between GPs and clinical chemists. For instance, clinical chemists can provide an interpretation of the laboratory results purely based on the probability of the presence of an underlying etiology or etiologies. In our PAGAS cohort, clinical chemists interpreted each laboratory request and added an interpretative comment. For instance, "*the laboratory results are indicative for an iron deficiency anemia*". However, when a clinical chemist interpret the laboratory results there

is a lack of clinical information, which hampers an adequate interpretation of the laboratory results. For example, if a clinical suspicion of vitamin B12 deficiency persists, it is recommended to determine homocysteine if vitamin B12 test results are on the border of normal[8]. Adding clinical information to laboratory requests might allow clinical chemists to make a better interpretation of the results [7]. In a previous study dealing with the interpretation of test results, a computerized prompt added to the laboratory results of patients with IDA resulted in increased iron supplementation prescription [9]. In our PAGAS cohort, GPs also received an interpretative comment, as well as an advice to perform endoscopic evaluation in case of IDA. This resulted in immediate endoscopic evaluations in 36% of cases of IDA (**chapter 7**). Although not well investigated, this proportion is higher than the approximately 19% of endoscopic evaluations described in literature [10].

The incidence of anemia in general practice is 8.6 per 1000 patients each year, which implies a significant investment of time if interpretation of laboratory results by a clinical chemist would be standardized care [8]. An interesting alternative would be to only provide an interpretation analysis by a clinical chemist in a subgroup of patients. For example, patients in whom multiple blood test abnormalities were found. The effectiveness of this strategy deserves to be investigated.

Overall, an extensive laboratory approach may be conducive to higher quality of care and possibly higher quality of life of anemia patients. The additional information gained by this approach optimizes the further diagnostic work-up of anemia. For patients, the simultaneous extra blood sample is hardly an effort and provides a higher yield of goal-directed care, although the up-scaled diagnostic process is a possible downside. Additional education programs and extension of guidelines should support interpretation by GPs.

MCV in anemia diagnostics

In 1929, Wintrobe introduced the MCV-based approach, considered useful in clarifying the etiology of anemias [11]. An MCV-guided anemia classification algorithm includes the division of anemia into three categories, namely micro, normo-, and macrocytic anemia. However, for three reasons this algorithm hampers adequate care for patients with anemia.

Firstly, the strict division of anemia into three MCV categories limits the possibility to establish multiple etiologies of anemia. The multiple etiology aspect appears to be relevant in nearly a quarter of anemia patients, as appeared from our study presented in **chapter 2**. Secondly, according to the algorithm, any etiology might

be possible in normocytic anemia, which means that the algorithm is not helpful in clarifying the etiology of anemia. Normocytic anemia was the major type in our study population(85%), consistent with the 82.4% reported by McCartney et al. in a cohort of elderly persons (>65 years) from primary care [12]. The authors of that study commented that the use of MCV alone to categorize anemic patients would likely miss many cases of IDA. Lastly, even if application of the algorithm would be limited to the patients with a micro- or macrocytic anemia and only one etiology, it still would be ineffective. We demonstrated that 16% of microcytic and 90% of macrocytic patients in primary care were not limited to the etiologies according to the MCV-guided anemia classification algorithm (**chapter 3**). Correspondingly, Bach et al. found in hospitalized patients aged ≥64 years only a significant association for microcytic anemia and iron deficiency, but no other correlations with MCV could be established [13].

The MCV is a value automatically generated by contemporary cell tellers. It represents the mean volume of erythrocytes and is calculated by dividing the hematocrit count by the concentration of red blood cells [14]. Macrocytosis, in general, is caused by a derangement in DNA synthesis which leads to postponed cell division and unintended ongoing maturation of erythrocytes [15]. The underlying mechanism of deranged DNA synthesis is not completely unraveled but may be due to impaired thymidine synthesis or the synthesis of predominantly arginine-rich histones. Various diseases can induce a derangement in DNA synthesis, such as vitamin B12 deficiency and/or folate acid deficiency. But also other factors affect the DNA synthesis, such as smoking, a number of medications and alcohol abuse [16, 17]. If such a co-factor is present in a patient with an IDA, both a macrocytosis and a microcytosis process are present. In that case, the calculated MCV value could theoretically lead to establishing a macrocytic IDA. In our study population, six of all 120 macrocytic anemic patients with one underlying etiology were classified as macrocytic IDA with a low ferritin count (**chapter 3**).

In conclusion, a MCV-guided anemia classification algorithm is too restrictive to use as a basis for an anemia diagnostic work-up. The Dutch guideline for the management of anemia encourages a broad strategy to start the anemia diagnostic work-up [8]. However, MCV is still mentioned as one of the criteria for etiologies and is still common practice for many GPs. An extensive laboratory approach would gain more valuable information on the anemia etiology and should be considered in every newly diagnosed anemia patient.

Cost-effectiveness of anemia diagnostics

It is important to consider all anemia etiologies at the start of a newly discovered anemia. This approach would prevent tunnel vision and enables a targeted care approach. What is more, an integrated care approach is rather needed because almost a quarter of anemia patients are found to have more than one etiology. To avoid a missed anemia etiology, a broad diagnostic work-up seems unavoidable. The current Dutch anemia guideline recommends the GP to request additional blood tests based on the suspicion of an etiology from the application of the guideline flowchart [8]. In this way, GPs have a fairly free choice to request blood tests for patients with anemia. We showed a large variation in the laboratory tests requested by GPs for anemic patients, and that a second blood sample was needed in 45% of cases (**chapter 4**). Besides the free choice of blood tests, most laboratories already offer reflex testing in case of an anemia (as discussed above). The current practice of anemia diagnostics appears to be suboptimal and raises the question whether it should be renewed. A new standard care approach might include the implementation of an extensive laboratory work-up at the start of the diagnostic process of a newly diagnosed anemia However, this raises the question whether too many diagnostic tests may be conducted in individual cases. The application of a large number of blood tests at the same time increases the risk of false positive test results and is also accompanied with additional health care costs. Therefore, we set out to evaluate if the additional costs of such an extensive laboratory work-up would be compensated for improvements in health outcomes and cost savings further downstream the diagnostic and treatment pathway, as discussed in **chapter 5**. This method to assess the value of a new technology is called Health Technology Assessment [18, 19].

A potential drawback of the implementation of an extensive laboratory protocol is the increased chance of false positive (abnormal) test results. Taking into consideration the confidence interval, on average 5% of test results are outside their reference ranges [20]. GPs should be aware of this when they routinely order an extensive laboratory work-up in patients with anemia. Research into cascade testing has shown that GPs often do not request additional investigations for patients with slightly abnormal laboratory test results [21]. This might suggest that GPs consider slightly abnormal blood test results to be of no clinical relevance. This observation was also established in the study presented in **chapter 7**. In this study, patients with iron deficiency anemia who received early endoscopic evaluation had significantly more severe laboratory abnormalities compared to patients who had not received endoscopic evaluation (**chapter 7**). Besides the confidence interval of test results, more elements of the diagnostic process deserve attention. The anemia

itself, the patient's clinical presentation and symptoms, the outcome of the physical examination and the possible occurrence of co-morbidity are all elements that make one etiology more or less likely than another etiology and, in addition, point to the clinical relevance of additional testing [22]. Combining these elements, a clinical algorithm arises in which it can be decided if additional investigation and/or treatment is indicated. GPs should be encouraged to review all elements of the diagnostic process, especially for patients with slightly abnormal test results. For this group of patients, GPs may want to follow-up of the blood test results instead of immediately requesting additional investigations or starting treatment.

It is important to realize that our PAGAS cohort included patients at the start of a new anemia diagnosis, so there is no over-diagnoses. However, the study presented in **chapter 4** of this thesis made clear that an extensive laboratory work-up would reveal more etiologies than otherwise apparent. The establishment of more anemia etiologies most likely would increase the quality of patient care. As an example, the treatments of an IDA, vitamin B12 anemia or ACD differ considerably [8, 23, 24]. Identification of the etiology is essential to start proper treatment of the anemia. Furthermore, the additional information which is gained from the identification of an (extra) etiology might help the GP to decide whether to request additional investigations [22]. For example, after the establishment of an IDA, endoscopic evaluation might be postponed or even be dispensed with based on the condition and/or wishes of a patient. If then extensive laboratory work-up reveals a vitamin B12 deficiency anemia, this could easily be treated without the need of additional investigations. Treatment of vitamin B12 deficiency might increase a patient's quality of life. Further research is needed to establish the clinical relevance of the increase in anemia etiologies established with an extensive laboratory work-up approach in patients in general practice.

Diagnostic testing accounts for more than 10% of all healthcare costs, with a large contribution of laboratory tests [22]. To investigate whether standardization of an extensive laboratory work-up in anemia care is necessary, we performed a cost-effectiveness analysis in **chapter 4**. To this aim, we compared an extensive laboratory work-up with the GPs own choice of blood testing (i.e. standard care). We found an increase of 8.9% of correct anemia etiology with the use of an extensive laboratory protocol. Performing an extensive laboratory work-up led to an incremental cost-effectiveness ratio (ICER) of €43 per additional patient correctly diagnosed and treated. Despite that the ICER could not be expressed in QALYs, the additional costs of an extensive laboratory work-up seem very minor in view of all the costs involved in these patients [25]. The extra costs associated with

a second round of laboratory tests (i.e. €12.28 order rate + costs additional tests), consultation time (i.e. €10.51) and societal costs (i.e. travel costs and productivity losses), quickly equal the ICER of €43 [26, 27].

The start of the diagnostic process after an anemia diagnosis is very broad due to the great variation in symptoms associated with anemia. In addition, a wide variety of additional blood tests can be requested. These factors taken together may lower the probability that a GP chooses the appropriate diagnostic pathway for an individual patient. Considering this, the implementation of an extensive, evidence-based laboratory work-up protocol has great potential to improve anemia care. The risks of false positive results and more etiologies establishment seem minor. Moreover, there is no potential harm for patients and results always are interpreted in the context of a broader clinical diagnostic process.

Importantly, we looked at two extreme strategies, namely a free choice of laboratory tests and the immediate application of an extensive test panel. More recently, we established that the amount of laboratory tests of the extensive work-up could be safely reduced from 15 to 9 [28]. Although this strategy would gain relatively small cost savings, it deserves to be further investigated. For instance, the PAGAS cohort of this study was limited to patients with a single etiology of anemia. Therefore, it did not take into consideration that almost a quarter of patients have more than one anemia etiology. Reducing the laboratory test panel would likely increase the probability of missing a second etiology.

In short, implementation of an extensive laboratory work-up protocol does not only lead to establishing more possible etiologies, but is a cost-effective way of taking care of patients with anemia.

Anemia of chronic disease

ACD is one of the most common etiologies of anemia. Nevertheless, it is regularly incorrectly established, notably by GPs (45.5% of all ACD cases) as shown in **chapter 4** of this thesis. The latter observation may be explained by the complexity of interpreting the patient's iron status.

ACD is a characterized by functional iron deficiency. In other words, the total iron storage appears to be adequate but the production of hemoglobin is hampered due to limited access of iron stores – resulting in less availability of soluble iron, which is one of the essential elements of hemoglobin [29, 30]. Iron is stored as ferritin predominantly in macrophages and in the liver [29]. Enhancing the level of

circulating free serum iron, ferroportin is expressed on macrophages and intestinal cells, leading to a flux of iron atoms from macrophages and the intestinal cavity to the blood [29]. This influx of free iron atoms to the blood is negatively regulated by a chain protein produced in the hepatic parenchymal cells, called hepcidin [29, 31].

In case of (chronic) infection, immune activation or malignancy, several acute phase response cytokines are produced [29]. One of these is interleukin-6, which stimulates the hepcidin production by the liver. Hepcidin, in turn, binds to ferroportin, thereby leading to degradation of ferroportin in the cell walls of macrophages and intestinal cells. As a consequence, the number of circulating free iron atoms is reduced, and iron is locked as ferritin in macrophages or is excreted with the feces. It is presumed that this mechanism protects the body from proliferation of cancer cells and bacterial pathogens due to the essential role of iron for the development of cancer and bacterial cells. It also means, however, that iron is not available for the production of hemoglobin. Another acute phase protein is TNF-α, which is secreted by neutrophils, macrophages, T cells, and natural killer cells [29]. TNF- α stimulates ferritin synthesis. As a result of this acute phase response, low serum iron and normal/high ferritin levels can be expected.

The most useful parameter to differentiate between IDA and ACD is ferritin. A ferritin level <25 μg/ml is distinctive for an absolute iron deficit, as is the case in IDA. Patients with ACD typically have ferritin levels >100 μg/ml, due to the stimulation of ferritin synthesis by TNF- α [29, 32]. However, a grey area of ferritin levels between 25-100 μg/ml exists, for which additional interpretation of the iron status is essential, such as transferrin or the iron saturation. Transferrin is normal or slightly elevated in IDA, due to the fact that non-iron bound transferrin is measured, which is more frequent in case of less soluble iron states. However, in the case of an ACD, transferrin values will be reduced due to circulating cytokines inhibiting transferrin production by the liver [33]. Although ACD and IDA can be differentiated from each other, the complexity of interpreting the iron status is made even more difficult if an IDA or other etiology co-exists with ACD (**chapter 2**). Several previous studies have focused on the differentiation of IDA and ACD, for instance by using additional parameters to interpret the iron status [34]. These parameters, such as soluble transferrin receptor and transferrin/log(ferritin) ratio, are unfamiliar to most GPs [35]. The interpretation can be difficult, therefore, and missing an IDA or ACD diagnosis might also influence the quality of care. Considering all this together, GPs are recommended to consult an internist, hematologist or clinical chemist if the interpretation of the iron status is not straightforward.

Because of the central role of hepcidin in the pathophysiology of ACD, determination of the hepcidin level in blood might be an important diagnostic step forwards [36]. Research is currently focusing on the implementation of a hepcidin assay in the clinical setting. However, there are a number of limitations [37]. For one thing, hepcidin levels fluctuate throughout the day. The concentration of hepcidin increases during the day and is influenced by the food intake [38]. In addition, the interpretation of the hepcidin value should be related to the clinical setting. Especially elderly anemia patients often have (inflammatory) comorbidities, which may independently increase the hepcidin concentration [33]. Research has shown that while patients with IDA and an inflammatory co-factor have slightly elevated hepcidin levels, patients with exclusively ACD have more elevated hepcidin levels [39]. Currently, the diagnostic value of hepcidin in the clinical setting is not well known. Therefore, the implementation of hepcidin tests in general practice seems too early.

Despite the complexity of establishing an ACD, an underlying disease can be diagnosed in the majority of ACD patients, which may be of high importance for the patient. In the study described in **chapter 6** of this thesis, the most common underlying diseases were acute infection (32%), auto immune disease (24%) and malignancy (23%). Besides, in the past few years a wide variety of diseases have been suggested as a possible underlying disease or condition leading to ACD, such as obesity [33].

An underlying disease could not be identified in a small group of patients with ACD. About half of this group had received additional investigations, which may have been too limited. Another explanation for not identifying an underlying disease could be that the GPs lacked knowledge about the conditions and diseases that can explain an ACD. Close monitoring of the hemoglobin status and performing additional investigations could possibly lead to more insight in the underlying condition causing an ACD.

Because ACD leads to high hepcidin levels, which implicates loss of ferroportin on intestinal wall cells, the efficacy of oral iron suppletion is blunted. Treatment with oral iron supplementation would then only lead to preventable side effects such as diarrhea and nausea [40]. Nevertheless, in our study population, 13% of ACD patients received oral iron supplementation from their GPs (**chapter 6**). An extensive iron status was known for each patient, and the GP was even informed of the presence of an ACD. Apparently, GPs are still inclined to start oral iron supplementation in some patients with ACD. A possible explanation might be that this group of

ACD patients have mild laboratory abnormalities without an evident underlying condition. For this group, GPs might decide to try oral iron supplementation first before conducting extensive additional investigations. Another explanation might be that GPs have insufficient insight into the pathophysiology of ACD and the contra-indication of oral iron supplementation. This awareness may be improved by adapting the Dutch guideline of anemia, mentioning oral iron supplementation as a contra-indication for ACD. Instituting more learning opportunities for GPs on anemia is recommended.

In conclusion, an extensive laboratory protocol is necessary to interpret the iron status and may lead to better recognition of anemia of chronic disease. In the majority of patients the clinical setting provides clues to the underlying condition. Oral iron supplementation in this group of patients should be avoided. The clinical condition and laboratory values of patients without an underlying condition explaining ACD and who show mild abnormalities should be closely monitored.

Iron deficiency anemia

IDA is in most cases related to gastro-intestinal blood loss when hypermenorrhoea in females is excluded. Therefore, endoscopic evaluation is indicated in each patient newly diagnosed with IDA and no symptoms of hypermenorrhoea. Several previous studies have described that approximately one third of IDA patients underwent an endoscopic evaluation [10, 41]. The question that arises is how patients without endoscopic evaluation are managed. In the study presented in **chapter 7** of this thesis, a diagnosis of a condition or disease causing the IDA had not been made in 60% of the patients during a follow-up period of 4.6 years (**chapter 7**). The majority of this group of patients had not undergone endoscopic evaluation in case of an IDA etiology. Apparently, GPs are well able to select those patients for whom endoscopic evaluation is indicated. Although we did not investigate this, a patient's clinical presentation seems to have an important decisional aspect. Another observation which might explain the selection of patients for endoscopic evaluation by the GP is the association between early endoscopic evaluation and more severe laboratory abnormalities (**chapter 7**). Patient with more severe anemia received more often immediate endoscopic evaluation and also had significantly more often colorectal cancer compared to patients without direct endoscopic evaluation. Apparently, association between the severity of anemia and gastro-intestinal malignancy motivates the GP to select the right patients for endoscopic evaluation.

Although the initial selection of IDA patients for endoscopic evaluation seems adequate, 20% of patients have a delayed diagnosis of colorectal cancer (**chapter 7**). In another study, IDA patients with a negative endoscopy result received a second-look endoscopy, which in 64% of cases led to changes in treatment [42]. In addition, a final underlying cause of IDA was definitely established in 27% of these patients, which cause was significantly associated with persistence of the anemia [42]. Additionally, the persistence of anemia was shown to be an important alarm symptom in the diagnostic process.

From the above, endoscopic evaluation is justified in every newly diagnosed IDA patient to prevent a delayed gastro-intestinal malignancy diagnosis, provided that hypermenorrhoea is excluded as a leading cause. Patients who do not receive endoscopic evaluation or for whom the result of endoscopic evaluation is negative should be closely monitored. Endoscopic evaluation should be (re)considered in case of persistent anemia.

In our PAGAS cohort, patients were diagnosed with IDA, which might be indicative for a gastro-intestinal malignancy. Another test to early detect gastro-intestinal malignancy is the Fecal Immunochemical Test (i.e. FIT) [43]. In the Netherlands, the FIT, which identifies the presence of blood in feces, is used in a national colorectal cancer screening program for males and females aged 55 to 75 years [43]. In 2019, the screening test was positive in 4.3% of the participants [45]. Colorectal cancer was diagnosed in 5% of the patients who underwent endoscopic evaluation. As a comparison, we found that10% of patients with a new diagnosis of IDA had colorectal cancer (**chapter 7**). The FIT test is no replacement for blood tests in patients with symptoms indicative for anemia and/or GI pathology [8]. Neither should the FIT test be used as a supportive indication for endoscopic evaluation. A recently published review concluded that 42% of patients with an underlying cause for IDA through endoscopic evaluation would have a negative FIT [46]. FIT tests should only be used as a screening tool in patients without any symptoms and/or anemia. Therefore, the FIT test has no diagnostic value in general practice, especially not for patients with IDA. The higher percentage of colorectal cancer among IDA patients in comparison with that identified in the screening program emphasizes the importance of endoscopy in both groups of patients.

Implications for practice and further research

This thesis ends with a short interpretation of the discussed results for the various stakeholders, namely general practitioners, patients with anemia, and clinical chemists. Lastly, recommendations for further research are given.

General practitioners

This thesis focuses on the care of anemia patients provided by GPs. In the Netherlands, almost the whole population is registered in general practices. Therefore, the GP is often the one who diagnoses anemia. The Dutch College of General Practitioners (NHG) has published a guideline for anemia which represents the main guidance in Dutch GP care [8]. Based on the findings of the studies presented in this thesis, some adaptations of this guideline can be considered which would improve the quality of anemic patient care. First, an extensive laboratory panel testing in case of a reduced hemoglobin count should be advised. Not only will this provide more insight into the presence of multiple etiologies, but also forms the starting point for further goal-directed diagnosis and treatment. Possible disadvantages such as interpretation errors seem to be minor. In this respect, close collaboration between GPs, internists, hematologists and clinical chemists, as well as interpretation of a broad clinical diagnostic process is advised. Second, an extensive laboratory approach disable a MCV guided anemia classification algorithm. In this thesis, we have shown that GPs should no longer be guided by MCV in the diagnostic work-up of anemia. Last, the use of an extensive laboratory panel provides more insight into a patient's iron status. Not only it permits clearly establishing an ACD, it also enables the combination of IDA and ACD in one patient. Because interpretation of a patient's iron status might be complex, additional educational programs and extension of the guideline should facilitate GPs.

The implementation of an extensive laboratory panel might also benefit the further care of patients with ACD and IDA. More insight into a patient's iron status can prevent unnecessary iron supplementation. In addition, this insight also reflects the importance of endoscopic evaluation in case of an IDA.

Patients with anemia

The main goal of this thesis has been to find factors that may improve the quality of care for patients with anemia. The relevance of optimal care for these patients has been described in previous literature. The essence lies mainly in the reduced quality of life and increased mortality rates in patients with anemia [1]. The first step, i.e. defining the anemia etiology, is not only essential in light of targeted additional

investigations but is also critical for the treatment of the anemia itself. The gold standard to define the anemia etiology is performing a bone marrow biopsy. However, it would neither be feasible nor ethical to perform such a biopsy in each patient with a newly diagnosed anemia. A bone marrow biopsy should be reserved for those patients who are critically ill without explanatory insights regarding the anemia and those in whom a bone marrow illness is suspected. Although a bone marrow biopsy is not available for each patient, the extensive laboratory approach, as conducted in this thesis, has been proven to be a cost-effective approach for anemic patients. The cost-effectiveness also implies beneficial effects on the social costs for patients. In addition, the simultaneous extra blood sample is hardly an effort and provides a higher yield of goal-directed care.

Not only is the care considering the work-up of anemia improved, the positive effect of an extensive laboratory panel is also reflected in the further treatment of, among other things, two common etiologies of anemia, namely IDA and ACD. Patients are more protected from unnecessary iron supplementation, including any side effects. In addition, the importance of endoscopy would be more apparent.

Clinical chemists

The results of this thesis have no great impact on a clinical chemist' field of work. Once the extensive laboratory panel is part of standard care, it would likely increase the amount of blood tests performed in the laboratory. Most practical, the extensive laboratory panel should be automatically performed when a reduced hemoglobin count is detected. The standardization of an extensive laboratory panel could, however, imply the necessity of closer collaboration between GPs and clinical chemists. The way in which to shape this concept requires further investigations, and is discussed in the next section.

Further research

Throughout the general discussion, various aspects have been addressed requiring further research. One of these concerns the quality of patient care. It should be investigated whether the identification of more anemia etiologies leads to faster and more targeted investigation(s), faster treatment of the anemia and, as a result, improvement in the quality of life of anemia patients. On top of that, more research should focus on the improvement of the quality of interpretation of laboratory test results related to anemia. Especially the interpretation of a patient's iron status requires a well-considered approach. Additional education programs and extension of guidelines could be one of the possibilities. But also more cooperation might be needed between GPs and clinical chemists. The greatest potential here

is standardized interpretation analysis by a clinical chemist in anemic patients with multiple blood test abnormalities. This concept needs to be investigated.

Another aspect which requires further investigation is the effectiveness of an extensive laboratory work-up in patients with multiple etiologies. This category of patients has not been investigated in **chapters 4 and 5** of this thesis. In line with this, a more compact laboratory panel can be investigated as an alternative. Here, too, it is important to conduct further research into patients with multiple etiologies. After all, at the start of the diagnostic work-up it is still unknown whether a patient presents with one or more etiologies.

More research should also focus on anemic patients with slightly abnormal blood test results. Results of this thesis might suggest that close monitoring can be safely considered in this group of patients. The validity of this suggestion needs to be investigated. In line with this, the combination of clinical information and the extensive laboratory work-up should also be investigated. Finally, if current research focusing on hepcidin blood test leads to the implementation of this test in general practice, this should be investigated in patients with anemia.

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8

9

Summary

A frequently encountered condition in adults in general practice is anemia. The reduced red blood cell count is often the result of an underlying disease. For this reason, anemia should be considered a symptom. A first step, in order to treat the anemia, is to establish the anemia etiology (i.e. origin). In most cases, this can be done through additional blood tests. For example, an anemia can originate from a shortness of iron, an important component of red blood cells, which is called iron deficiency anemia. After defining the anemia etiology, goal-directed investigations can be requested to diagnose the underlying illness.

This thesis focuses on the laboratory part of the diagnostic process of anemia. The main objective of this thesis is to optimize the diagnostic pathways and thus the care of anemia patients in general practice. For all studies, a large group of patients (cohort) is used in whom anemia has been established in general practice. The cohort consist of males and females aged 50 years and older with a new anemia (i.e. no anemia confirmed in 2 years previously).

Specific laboratory tests can be requested to determine an anemia etiology. General practitioners often select tests based on complaints and symptoms of their patients. In **chapter 2** of this thesis, we investigated how often an anemia etiology can be established when an extensive panel of laboratory tests is standard performed in each patient with a new anemia. In our cohort of 4152 patients, the anemia etiology can be found in 80% of patients based on the extensive panel of laboratory tests. In addition, in almost a quarter of these patients more than one anemia etiology can be established. This substantial proportion of patients with more than one anemia etiology in general practice has not been previously describe in literature. In our cohort, an anemia etiology could not be established, based on the extensive panel of laboratory tests, in 20% of patients. Which points to a reasonable efficacy of the used extensive laboratory panel, because literature describes 26-44% of unknown etiologies in patients with anemia. We concluded that the extensive panel of laboratory tests is of great importance in order to execute a goal-directed anemia analysis. Not only the amount of patients without an anemia etiology decreased, it also revealed that a substantial proportion of patients had more than one anemia etiology.

One laboratory test, the mean corpuscular volume (MCV) is often requested by general practitioners when an anemia has been found. A decreased MCV is referred to as microcytic anemia. A normal MCV value refers to a normocytic anemia and an elevated MCV value is called a macrocytic anemia. Each of these categories should give direction to which etiologies might be present. For example,

an iron deficiency anemia is associated with a microcytic anemia, while a vitamin B12 deficiency leads to a macrocytic anemia. In various anemia guidelines, it is advised to use MCV as (one of) the first screening test to determine the anemia etiology. Therefore, we investigated the accuracy of MCV as the first screening test for determining the anemia etiology in **chapter 3**. In this study, it appeared that the MCV had a normal value (normocytic anemia) in 85% of the 4152 patients. In these cases, the MCV demonstrated no added value because a normocytic anemia does not discriminate between one of the etiologies of anemia. In case of a microcytic anemia (365 patients), 16% of patients turned out to have another anemia etiology than should be expected based on the decreased MCV. In case of a macrocytic anemia (259 patients), 90% of patients turned out to have another anemia etiology than should be expected based on the increased MCV. In addition, 20% of the patients in the anemia cohort had more than one anemia etiology. As the screening test of MCV does not take into consideration multiple aspects, this group of patients could not be analyzed at all by MCV. For these patients, it is even likely that an etiology can be missed if the MCV is used as the first screening test. We concluded that the use of MCV as a screening test in anemia diagnostic should be discouraged.

In **chapter 4** we investigated the effectiveness of an extensive panel of laboratory test in anemia diagnostic in general practice. For this research purpose, we offered a panel of general practitioners multiple virtual cases in which they could request laboratory tests themselves and cases in which immediately an extensive panel of laboratory tests was presented. It appeared that if general practitioners requested laboratory tests themselves, a correct anemia etiology was found in 53% of cases. In contrast, if an extensive panel of laboratory test was immediately available, general practitioners were able to find the correct anemia etiology in 62% of cases. This implicate that the effectiveness of anemia diagnostics increased by 9% if the results of an extensive panel of laboratory tests was available.

Although the effectiveness increased when using an extensive panel of laboratory tests, there was some variation observed in individual cases. It turned out that finding the correct etiology was more difficult with increasing age of the patient. In addition, anemia of chronic diseases was the most difficult etiology to diagnose correctly for general practitioners in this study. We concluded that the use of an extensive panel of laboratory tests in anemia diagnosis improved the care of patients with anemia. We discussed the possible need to provide more education for general practitioners and to encourage multidisciplinary consults in case of complex interpretation of laboratory results.

In **chapter 5** we continued the previous study by investigating whether the extensive panel of laboratory tests is also cost-effective compared to the free choice of general practitioners in requesting laboratory tests. In this study, we demonstrated that the extensive panel of laboratory tests was associated with an additional cost of €3 per patient. If we extrapolated these costs for the whole Dutch population, this will amount to €156.000 per year of additional costs for health care, based on the incidence of anemia. These additional costs will render an expected increase of 6.4% extra patients in whom directly an anemia etiology can be found. We concluded that our cost-effectiveness model contains great uncertainty, mainly explained by the high variability in costs, complexity and further diagnostic- and treatment process of each etiology. However, the absolute increase of costs which was accompanied by an extensive panel of laboratory tests is very limited given the gains achieved very early in the diagnostic work-up of anemia and may prevent unnecessary and burdensome diagnostic interventions.

In **chapter 6** we investigated the course of general practitioners after a patient has been diagnosed with a new anemia of chronic disease. We found that 77% of the 267 investigated patients received additional investigations to find the underlying cause of the anemia of chronic disease. In an additional 12% of patients an underlying disease was already diagnosed at the time of blood collection. The most common underlying diseases found, were infection (32%), auto-immune disease (24%) and malignancy (cancer) (23%). In patients with anemia of chronic disease, treatment with iron tablets is not indicated. Despite this, 13% of the 267 patients received iron tablets. This was done more often in patients with severe anemia and with a modest increase in ferritin. A possible explanation might be that general practitioners cannot exclude iron deficiency and therefore treat these patients accordingly or they have a lack of knowledge regarding anemia of chronic disease due to the complexity in interpretation of the iron status. We concluded that an underlying cause was diagnosed in a significant proportion of patients with anemia of chronic disease. It is important to realize that iron tablets are not indicated in this subgroup of patients and can potentially cause unnecessary side effects.

Patients with iron deficiency anemia often have an absolute iron shortness based on blood loss of the gastrointestinal tract. This is seen, for example, in intestinal tumors that chronically and unnoticed bleed gently. In **chapter 7** we investigated how often colorectal cancer was found and we looked in particular at the time frame from finding anemia to diagnosis of colorectal cancer. We used the following definitions throughout this study. Colon cancer was diagnosed early if the diagnosis

was made within 18 weeks of the anemia found. Delayed colorectal cancer was defined when diagnosed more than 18 weeks after anemia has been found. In our study, 587 patients were followed for a median duration of 4.6 years. Colon cancer was found in 61 patients. Of these patients, 20% had a delayed colorectal cancer diagnosis. In addition, we found that the risk of death, i.e. the mortality risk, tended to be higher in patients with a delayed colorectal cancer diagnosis compared to patients with an early colorectal cancer diagnosis. We concluded that general practitioners should be aware that in some patients an unnecessary delay in the diagnosis of colorectal cancer was present in the case of a new iron deficiency anemia. This was emphasized by the observation of a potentially increase mortality risk in this group of patients.

This thesis ended with a number of points for discussion in **chapter 8**. In particular, the pros and cons of implementing an extensive panel of laboratory tests as standard care was pointed out. Finally, a number of recommendations were made for general practitioners, patients with anemia and clinical chemist and suggestions were made for further research.

10

Nederlandse samenvatting

Anemie (bloedarmoede) komt veelvuldig voor bij volwassenen in de huisartsenpraktijk. Dit tekort aan rode bloedcellen is vaak het gevolg van een onderliggende ziekte of aandoening. Om deze reden wordt anemie ook wel een symptoom genoemd. Om de onderliggende ziekte of aandoening te vinden is het zinvol om als eerste stap door middel van aanvullende bloedtesten te kijken naar de oorzaak, ook wel etiologie genoemd, van de anemie. Op deze manier kan er bijvoorbeeld worden vastgesteld dat er een te kort is aan ijzer, een belangrijk bestandsdeel van rode bloedcellen, waardoor er dus sprake is van ijzergebreksanemie als etiologie.

De anemie etiologie kan in de meeste gevallen worden vastgesteld door middel van aanvullend laboratorium onderzoek. Dit proefschrift richt zich op dit onderdeel van het diagnostisch proces na het constateren van een anemie. De hoofddoelstelling van dit proefschrift was het optimaliseren van de diagnostiek en daarmee de zorg voor patiënten met anemie in de huisartsenpraktijk. Voor alle onderzoeken is gebruik gemaakt van een grote groep patiënten (cohort) waarbij een anemie werd gevonden in de huisartsenpraktijk. De patiënten bestonden uit mannen en vrouwen van 50 jaar of ouder met een nieuwe anemie (d.w.z. geen anemie in de 2 jaar hier aan voorafgaand).

Om een anemie etiologie vast te stellen kan de huisarts zelf gericht laboratoriumtesten aanvragen. Vaak gebeurt dit op basis van de klachten en symptomen die de patiënt heeft. In **hoofdstuk 2** van het proefschrift onderzochten wij hoe vaak een anemie etiologie kan worden vastgesteld wanneer er een uitgebreid panel aan laboratoriumtesten standaard wordt bepaald bij elke patiënt met een nieuwe anemie. Dit dus in tegenstelling tot de (klacht-gestuurde) vrije keuze van de huisarts om testen aan te vragen. Deze manier van aanpak leidde vaker tot het vaststellen van een anemie etiologie vergeleken met cijfers in de huidige literatuur. In het cohort van 4152 patiënten kon een anemie etiologie worden gevonden bij 80% van de patiënten. Bovendien bleek bij een kwart van deze patiënten meer dan één anemie etiologie aanwezig te zijn. De grote rol van meer dan één anemie etiologie in een patiënt is nog niet eerder beschreven in literatuur. In het cohort kon uiteindelijk bij 20% van de patiënten, ondanks het uitgebreide panel aan laboratoriumtesten, geen anemie etiologie in het laboratorium worden vastgesteld. Dit in tegenstelling tot de 26-44% onbekende oorzaken beschreven in de literatuur. Concluderend, in dit hoofdstuk lieten wij het belang zien van een uitgebreid panel versus een klacht gestuurde keuze aan laboratoriumtesten om de anemie etiologie vast te stellen. Het bleek namelijk dat het uitgebreide panel leidt tot meer patiënten waarbij een etiologie kan worden vastgesteld. Daarnaast

kwam ook aan het licht dat een grote groep patiënten meer dan één etiologie van de anemie heeft.

Bij het vaststellen van de anemie etiologie door middel van laboratoriumtesten wordt vaak door huisartsen de test 'mean corpuscular volume' (MCV) aangevraagd. Het MCV zou namelijk richting kunnen geven aan welke etiologie wel of niet gedacht dient te worden. Bij een verlaagd MCV wordt gesproken over een microcytaire anemie. Een normale MCV waarde is een normocytaire anemie en een verhoogde MCV waarde is een macrocytaire anemie. Deze categorieën zouden richtinggevend zijn voor de anemie etiologie. Bijvoorbeeld, een ijzergebreksanemie zou gepaard gaan met een microcytaire anemie, terwijl een vitamine B12 deficiëntie leidt tot een macrocytaire anemie. Om deze reden is de bepaling van het MCV in diverse richtlijnen opgenomen als eerste screenende test na het vaststellen van een anemie. In **hoofdstuk 3** onderzochten wij de nauwkeurigheid van MCV als eerste screenende test voor het vaststellen van de anemie etiologie. Uit dit onderzoek bleek dat het MCV bij 85% van de 4152 patiënten een normale waarde had (normocytair). In het geval van deze normocytaire anemie, heeft de test geen toegevoegde waarde in het vinden van de etiologie. Bij de patiënten met een verlaagd MCV (microcytair) bleek de test in 16% van de patiënten de verkeerde richting op te sturen en werd er dus een andere etiologie gevonden dan zou worden verwacht gebaseerd op het verlaagde MCV. Bij de patiënten met een verhoogd MCV (macrocytair) bleek de test de verkeerde richting op te sturen in maar liefst 90% van de patiënten. Bovendien, zoals reeds eerder besproken heeft 20% van de patiënten meer dan één etiologie van de anemie. Bij deze groep patiënten is het aannemelijk dat een etiologie wordt gemist wanneer er bij het vinden van de etiologie als eerste test op het MCV wordt gestuurd. Wij concludeerden dat het gebruik van MCV als eerste screenende test in anemie diagnostiek moet worden afgeraden.

In **hoofdstuk 4** onderzochten we de effectiviteit van een uitgebreid panel aan laboratoriumtesten bij de diagnostiek van anemie. Dit deden wij door huisartsen virtueel casussen aan te bieden waarbij ze enerzijds zelf laboratoriumtesten mochten aanvragen en anderzijds direct een uitgebreid panel aan laboratoriumtesten kregen aangeboden. Hieruit bleek dat wanneer huisartsen zelf laboratoriumtesten mochten aanvragen in 53% van de casussen de correcte anemie etiologie werd gevonden. In tegenstelling, wanneer direct een uitgebreid panel aan laboratoriumtesten werd gegeven, konden huisartsen in 62% van de casussen de correcte anemie etiologie diagnosticeren. Dit houdt in dat de effectiviteit van anemie diagnostiek stijgt met 9% wanneer een uitgebreid panel

aan laboratoriumtesten wordt aangevraagd in plaats van een vrije keuze aan testen. Gelet op de effectiviteit van een uitgebreid panel aan laboratoriumtesten bij de anemie diagnostiek was er variatie te zien in de individuele casussen. Het bleek dat het vinden van de correcte etiologie moeilijker is naarmate de patiënt een hogere leeftijd heeft. Daarnaast bleek ook dat de uiteindelijke etiologie van invloed is, waarbij anemie als gevolg van een chronische ziekte het meest moeilijk is om correct vast te stellen voor de huisartsen in dit onderzoek. Wij concludeerden dat het gebruik van een uitgebreid panel aan laboratoriumtesten bij anemie diagnostiek leidt tot verbetering van de zorg van anemie patiënten. Hierbij bediscussieerde wij de mogelijke noodzaak om huisartsen meer educatie te geven en multidisciplinair te werken aangezien de laboratorium uitslagen in het uitgebreide panel complex te interpreteren kunnen zijn.

In **hoofdstuk 5** vervolgden we ons effectiviteitsonderzoek door te onderzoeken of het uitgebreide panel aan laboratoriumtesten ook kosteneffectief is in vergelijking tot de vrije keuze in het aanvragen van laboratoriumtesten. In dit onderzoek toonden we aan dat het uitgebreide panel aan laboratoriumtesten gepaard gaat met €3 extra kosten per patiënt. Voor heel Nederland, gelet op de anemie incidentie, zal dit neerkomen op €156.000 per jaar extra kosten voor de gezondheidszorg. Daar tegenover staat een te verwachten stijging van 6.4% in het direct vinden van de correcte etiologie van anemie. Wij concludeerden dat het kosteneffectiviteitsmodel veel onzekerheden bevat, voornamelijk verklaard door een hoge variabiliteit in kosten, complexiteit en vervolgbeleid van elke anemie etiologie. Echter, de absolute extra kosten die gepaard gaan met het uitgebreide set aan laboratoriumtesten is gering gegeven het feit dat veel winst wordt behaald aan de vroege start van het diagnostische traject bij anemie.

In **hoofdstuk 6** onderzochten we het verdere beloop nadat een anemie als gevolg van een chronische ziekte, een veel voorkomende anemie etiologie, werd vastgesteld. Wij stelde vast dat bij 77% van de 267 onderzochte patiënten aanvullend onderzoek werd ingezet om chronische ziekte te vinden die de anemie verklaart. Bovendien, bleek bij 12% van de patiënten al een verklarende aandoening aanwezig te zijn op het moment van bloedafname, veelal een chronische aandoening. De meest voorkomende aandoeningen die werden gevonden als oorzaak van de anemie als gevolg van een chronische ziekte waren infectie (32%), auto-immuun ziekte (24%) en maligniteit (kanker) (23%). Bij patiënten met een anemie als gevolg van een chronische ziekte is behandeling met ijzertabletten niet geïndiceerd. Desondanks kreeg 13% van de patiënten wel deze behandeling. Dit werd vaker gedaan bij patiënten met een ernstige anemie en bij een minder

sterke stijging van de laboratoriumtest ferritine. Een mogelijke verklaring hiervoor is dat huisartsen bij een ernstige anemie hopen dat de rode bloedcellen alsnog stijgen door ijzertabletten en dit afwegen tegen de eventuele bijwerkingen van ijzertabletten. Het geven van ijzertabletten bij een minder sterke stijging van het ferritine kan suggereren dat huisartsen meer educatie nodig hebben omtrent de interpretatie van de ijzerstatus van een patiënt. Wij concludeerden dat bij een aanzienlijk deel van de patiënten met een anemie van chronische ziekte een achterliggende oorzaak kan worden vastgesteld. Hierbij is het belangrijk om te realiseren dat ijzertabletten niet geïndiceerd zijn en wel potentieel bijwerkingen kunnen geven.

Patiënten met een ijzergebreksanemie hebben vaak een ijzertekort door bloedverlies in het maag-darm kanaal. Dit wordt onder andere gezien bij darmtumoren die chronisch ongemerkt zachtjes bloedden. In **hoofdstuk 7** onderzochten wij hoe vaak er darmkanker wordt gevonden en keken we met name naar het tijdspad vanaf het vinden van een anemie tot het vaststellen van de darmkanker. Hierbij hanteerde we de volgende definities. Er is sprake van een vroege darmkanker diagnose als de diagnose wordt gesteld binnen 18 weken na het vaststellen van de anemie. Er is sprake van een verlate darmkanker diagnose, wanneer deze wordt gediagnosticeerd meer dan 18 weken na het vaststellen van de anemie. Tijdens deze studie werden 587 patiënten gevolgd voor een mediane duur van 4.6 jaar. In totaal werd er bij 61 patiënten darmkanker gevonden. Van deze patiënten had 20% een verlate darmkanker diagnose. Bovendien bleek dat het risico op overlijden, dat wil zeggen het mortaliteitsrisico, bij patiënten met een verlate darmkanker diagnose hoger was dan bij een vroege diagnose van darmkanker. Wij concludeerden dat bij het vaststellen van een nieuwe ijzergebreksanemie bij een deel van de patiënten een onnodige vertraging optreedt in het stellen van de diagnose darmkanker. Dit kan potentieel het mortaliteitsrisico verhogen.

In **hoofdstuk 8** werd ter afsluiting een aantal discussiepunten aan de orde gesteld. Hierbij werd met name gekeken naar de voor- en nadelen van implementatie van een uitgebreid panel aan laboratoriumtesten als standaard diagnostiek bij anemie. Tenslotte werden een aantal aanbevelingen gedaan voor huisartsen, patiënten met anemie en klinisch chemici en werden er suggesties gedaan voor verder onderzoek.

A

Addendum

List of publications

Curriculum vitae

Dankwoord

PhD portfolio

List of publications

2018

Annemarie Schop, Karlijn Stouten, Ron J van Houten, Jurgen A Riedl, Joost van Rosmalen, Patrick J.E Bindels and Mark-David Levin. Diagnostics in anaemia of chronic disease in general practice: a real-world retrospective cohort study. BJGP Open. 2018 Jul 25; 2(3).

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2021

Annemarie Schop, Karlijn Stouten, Jurgen A Riedl, Ron J van Houten, Maarten J.G Leening, Patrick J.E Bindels and Mark-David Levin. The accuracy of mean corpuscular volume guided anemia classification in primary care. Fam Pract. 2021 Aug 4; cmab034.

Curriculum vitae

Annemarie Schop werd geboren op 5 januari 1992 in Ridderkerk. In 2010 voltooide zij het Voortgezet Wetenschappelijk Onderwijs aan de Passie te Rotterdam. Zij startte vervolgens met de studie biomedische wetenschappen aan het Leids Universitair Medisch Centrum. Na het behalen van haar bachelor heeft ze nog een jaar de master Biomedical Science met research specialisatie gevolgd aan het Leids Universitair Medisch Centrum. In 2014 werd zij toegelaten tot het zij-instroom programma geneeskunde aan het Leids Universitair Medisch Centrum, waarbij zij het artsexamen aflegde in april 2018. Het onderzoek wat uiteindelijk heeft geleid tot dit proefschrift is gestart in November 2013 als onderdeel van haar master Research stage. Na het afleggen van het artsexamen is zij gestart met het promotiewerk onder begeleiding van dr. M-D. Levin (afdeling interne geneeskunde, Albert Schweitzer ziekenhuis) en prof. dr. P.J.E. Bindels (afdeling huisartsgeneeskunde, Erasmus MC). Daarnaast is zij in maart 2019 gestart als basisarts in de huisartsenpraktijk van drs. J. Hartman te Dordrecht. Vanaf 1 september 2020 is zij in opleiding tot huisarts aan het Erasmus MC te Rotterdam.

Annemarie Schop was born on January 5th, 1992 in Ridderkerk, the Netherlands. She graduated frow secondary school in 2010. Thereafter, she started her bachelor Biomedical science at Leiden University Medical Center. After obtaining her bachelor's degree, she followed the master Biomedical Science with a research specialization for another year. In 2014 she was admitted to the "side-entry program" for the study Medicine at Leiden University Medical Center. She obtained her medical degree in April 2018. The research that ultimately led to this thesis started in November 2013 as part of her master Research internship. After obtaining her medical degree, she started her PhD work under supervision of dr. M-D. Levin (department of internal medicine, Albert Schweitzer hospital) and prof. dr. P.J.E. Bindels (department of general practice, Erasmus MC). In March 2019 she started as a medical doctor in the general practice of drs. J. Hartman in Dordrecht. From September 2020 and onwards, she is in training as a general practitioner at Erasmus MC in Rotterdam.

Dankwoord

Mijn promotietraject en dit proefschrift waren nooit tot stand gekomen zonder de hulp en begeleiding van de mensen om mij heen. Ik wil het proefschrift dan ook afsluiten door een aantal mensen expliciet te benoemen die mij tot steun zijn geweest de afgelopen jaren in zowel het promotietraject als ontwikkeling naar (huis)arts.

Allereerst wil ik mijn promotor, Patrick Bindels, bedanken. Het "promotie leven" is een hele nieuwe wereld en vaak genoeg had ik jou hulp nodig om deze te begrijpen. Je jarenlange ervaring en open vriendelijke houding maakte dat ik mij altijd welkom voelde en alles kon vragen. Bedankt voor de tijd en energie die je erin hebt gestoken.

Mark-David Levin, mijn co-promoter, 8 jaar geleden startte ik onder jouw supervisie een master stage. Jouw aanmoediging om door te gaan heeft uiteindelijk geleid tot dit promotietraject. Wellicht was ik ietwat naïef toen je mij vertelde dat promoveren bestond uit "artikelen met een kaftje erom". Ook al waren we het niet altijd met elkaar eens en maakte ik keer op keer dezelfde spellingsfouten, ik heb nooit het gevoel gekregen dat je twijfelde aan mijn kunnen, bleef je mij aanmoedigen om door te gaan en gaf je mij de veiligheid die ik nodig had. Ontzettend bedankt voor al je tijd en energie die je hebt gestoken in mijn werk maar ook in mijn zelfontwikkeling.

Leden van de kleine commissie, prof. dr. M.J. Bruno, prof. dr. J. Cals en prof. dr. Y.B. de Rijke, hartelijk dank voor jullie bereidheid om mijn proefschrift te beoordelen.

Het uitvoeren van onderzoek als promovendus doe je gelukkig niet alleen! De input van mede-promovenda, artsen en statisticus zijn onmisbaar. Maar ook de kletspraatjes, wandelingen, borrels en BBQ waren een welkome afwisseling! Peter, Hanah, Inge, Nathalie, Sara, Lina, Martine en Joost bedankt! Karlijn, vanaf het moment dat ik stage kwam lopen was jij mijn grote voorbeeld! Bedankt dat je mij vanaf dag 1 aan de hand hebt genomen en naast mij bent blijven staan, letterlijk tot aan de plechtigheid!

Inmiddels ben ik in opleiding tot huisarts en ook in dit traject ben ik een aantal mensen in het bijzonder dankbaar voor de tijd die ze hebben besteed om mij te helpen in zelfontwikkeling maar ook de tijd die ze mij gaven om deze promotie naast de opleiding af te ronden.

Jasper, je gaf mij de unieke kans om als basisarts werkzaam te zijn in jouw huisartsenpraktijk. Ik snapte vaak niks van je en dat gaf een hoop frustratie. Maar juist die frustraties zette mij aan het denken waardoor ik zelf tot nieuwe inzichten kwam!

Alex, bij jou leerde ik een andere manier van werken. Je liet mij zien hoe waardevol aandacht voor de patiënt is. Dit gecombineerd met continue zorgvuldigheid maakt dat tijd een minder grote rol speelt voor arts maar ook patiënt.

Femke en Annemarie, wat een warm welkom bij jullie in de praktijk. Door jullie open houding en directe manier van communiceren was het voor mij een hele verademing om te zien dat we allemaal mens zijn!

Tot slot was dit proefschrift er niet geweest zonder mijn gezin en thuis.

Mijn lieve Jasmijn Yara, prachtig meisje met je mooie krullen. Nog maar 3 jaar oud maar je hebt mij al zoveel geleerd. Verliezers bestaan niet, we zijn allemaal winnaars! Als ik aan jou vraag wie je het allerliefste vindt, zeg je vol overtuiging "mama, moeder, Jenthe, de baby en ik!" En zo is het lieverd, wij zitten alle vijf in elkaars hart en vergeet nooit om vooral jezelf te blijven noemen!

Lieve Jenthe Suze, mijn poppedein, mijn duimelot. Wat ben jij een dapper meisje met een sterke eigen wil. Ik weet zeker dat deze twee eigenschappen jou ver gaan brengen!

Lief kleintje, Isa Fenna, nog maar kort in ons leven maar zo perfect in het plaatje. We zullen de ijzersterke band met je zussen altijd stimuleren want jullie drie kunnen de hele wereld aan!

Tot slot wil ik mijn mooie vrouw Miranda bedanken. Het is niet makkelijk voor je geweest. Met iemand naast je die gemiddeld 50 uur per week werkt, veel tijd verloren ging aan files en treinstoringen en vaak tussendoor "eventjes" verder gewerkt werd.

Desondanks wist jij voor ons een thuis te creëren, een veilige basis, waar je mij elke dag trouw bleef opwachten. De afgelopen jaren heb ik regelmatig een schop onder mijn kont gehad omdat ik op het punt stond van opgeven of weer eens vergat om fatsoenlijk te eten. Zonder jou had ik het nooit gered!

Nu deze periode is afgerond ben ik vastberaden hetzelfde voor jou te doen. Er komt nu tijd vrij waarin jij verder mag ontwikkelen in je carrière naast de perfecte moeder en vrouw die je in mijn ogen bent!

PhD portfolio

Name PhD student: Annemarie Schop
Institution: Albert Schweitzer hospital and Erasmus MC
Period: April 2018 – June 2021
Promoter: prof. dr. P.J.E. Bindels
Co-promoter: dr. M-D. Levin

	Workload	
	Year	ECTS
PhD training		
General courses		
Good clinical practice	2018	0.5
Effective scientific writing in English	2018	1.0
Advanced medical writing and editing	2018	0.7
Research integrity	2018	0.3
Specific courses		
Safe microbiological techniques	2011	0.7
Stralingsbescherming deskundigheidsniveau 5B	2012	0.7
Clinical research in practice	2013	4
Reflection course: scientific conduct	2013	1
Developmental cardiovascular biology	2014	6
Cardiovascular disease and metabolic syndrome	2014	4
LIMSC	2017	0.3
NIHES: causal inference	2018	1.4
NIHES: markers and prediction research	2018	0.7
NIHES: principles of research in medicine and epidemiology	2019	0.7
NIHES: advances in clinical epidemiology	2019	0.7
Seminars and workshops		
RCP critical appraisal	2019	0.3
Presentations		
3 posters ASz science day	2017 - 2019	3.0
Poster ASz experience day	2018	1.0
Presentation ASz science day	2019	1.0
Teaching		
Teaching residents internal medicine, subject: anemia	2020	3.0
Other		
Peer reviews for international journals	2019 - 2021	0.8
Total		**31.8**

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