Original article:

Regression correction equation to adjust serum iron and ferritin concentrations based on C-reactive protein and albumin in patients receiving primary and secondary care.

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Running title: Adjusting ferritin by CRP and albumin

Abbreviations: AGP α-1-acid glycoprotein, Alb_{obs} observed albumin, Alb_{ref} Albumin reference value, ASA American Society of Anesthesiology, BRINDA Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia, CRP C-reactive protein, CRP_{obs} observed CRP, CRP_{ref} CRP reference value, EQA External Quality Assessment, Fer_{adj} adjusted ferritin, Fer_{obs} observed ferritin, Hb haemoglobin, IL interleukin, IQC Internal Quality Control, Ir_{adj} adjusted serum iron, Ir_{obs} observed serum iron, IQR interquartile range, MCV mean corpuscular volume, mGPS modified Glasgow Prognostic Score, nCRT neoadjuvant chemoradiotherapy, NEQAS National External Quality Assessment Services, NHS National Health Service, sTFR soluble transferrin receptor, TNF Tumor Necrosis Factor, TNM Tumor nodes metastasis stage, UICC Union for International Cancer Control, WHO World Health Organization,

Abstract

Background:

Systemic inflammation, even at low levels, is associated with significant derangement of measures of iron status, making diagnosis of iron deficiency difficult. The objective of the present study was to create linear regression correction equations to adjust serum ferritin and iron using the acute phase proteins C-reactive protein (CRP) and albumin.

Methods:

Data from a cohort (1) of patients (n=7,226) in primary and secondary care who had serum ferritin, iron, CRP and albumin measured at the same time point were examined. Linear regression coefficients calculated for CRP and albumin with serum iron and ferritin as the outcome variables. Patients were categorised as iron deficient (ferritin <15µg/L, or serum iron <10 µmol/L). The equation was then applied to a cohort (2) of patients with colorectal cancer who had ferritin and iron measured preoperatively (n=356).

Results:

In cohort 1 there was a significant difference in the proportions of patients with serum ferritin <15µg/L and serum iron <10µmol/L respectively when the unadjusted (7% and 55%), adjusted using CRP alone (13% and 26%), adjusted using albumin alone (11% and 37%), and using both CRP and albumin (24% and 15%) values were compared (both $p<0.001$). In cohort 2 there was a significant difference in the proportions of patients with serum ferritin <15µg/L and serum iron <10µmol/L respectively when the unadjusted (28% and 66%),

adjusted using CRP alone (39% and 57%), adjusted using albumin alone (39% and 59%), and using both CRP and albumin (46% and 44%) values were compared ($p<0.001$ and $p<0.004$).

Conclusions:

In both cohorts the greatest increase in the proportion of patients meeting definitions for iron deficiency was found when adjustment was made for both CRP and albumin together. Even low levels of inflammation had a significant effect on serum iron and ferritin values.

Keywords

Systemic inflammation, ferritin, iron deficiency, C-reactive protein, albumin, colorectal cancer

Introduction

Iron deficiency remains a significant burden of disease and in both developing and developed countries [1]. Total serum iron can be used to assess iron status, however, in clinical practice, ferritin is a more robust measure of iron status. Currently the World Health Organization (WHO) define adults with ferritin <15 μg/L as iron deficient [2]. However, ferritin is a positive acute phase reactant and therefore serum concentrations rise in the presence of systemic inflammation, leading to the possibility that patients may be truly iron deficient despite a serum ferritin >15 μg/L.

Compounding matters, inflammation is associated with lower serum total iron and transferrin saturation, due to the action of hepcidin, itself driven by circulating IL 6 [3]. This often leads to a difficult to interpret picture of serum measures of iron status which may mask functional iron deficiency [4], thereby making it difficult to assess the need for iron replacement, and to monitor the effectiveness of iron supplementation.

Therefore, attempts have been made to correct measures of iron status for the presence systemic inflammation, using exclusion criteria [5], correction factors [6], and novel markers of iron status [7]. Recently, the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) groups have used the acute phase markers C-reactive protein (CRP) and α-1-acid glycoprotein (AGP) to create linear correction regression equations to adjust serum ferritin by inflammation [8]. They argue that the significant effects of even low level inflammation make this the best method of adjustment [9]. However, as AGP is not widely clinical available at present, and compared to albumin is more expensive [10].

The effect on serum iron and ferritin of the combination of CRP and the widely clinically available negative acute phase protein albumin has also been quantified, having been shown to stratify serum iron and ferritin [11]. CRP is an opsonin and member of the pentraxin family synthesised by hepatocytes in response to stimulation by the proinflammatory cytokine IL 6, whilst the plasma transport protein albumin is redistributed due to changes in vascular permeability, and its synthesis is supressed by TNF and IL 1 [12]. To date, no regression correction equation to adjust serum iron or ferritin by these markers of systemic inflammation has been created.

Therefore, the aim of the present study was to derive linear correction regression equations to adjust serum iron and ferritin by CRP and albumin, and then to apply this to a cohort of patients with colorectal cancer.

Patients and Methods

Patients:

The present study included two cohorts of patients. The first (*n*=7,226), in which the regression correction equation was formulated, has been described previously [11].

Briefly, details of all requests for serum iron or ferritin were obtained from the laboratory information systems of 4 Glasgow hospitals respectively for the period 1st August 2006 to 31st July 2011. These requests were made from secondary inpatient, secondary outpatient and primary care healthcare providers. Iron and ferritin results were matched to CRP and albumin results obtained on the same calendar day using electronic laboratory patient identifiers. Any requests which were not accompanied by a CRP and albumin request were not included in the study. Where an individual had repeat measurements, only the first was included in the study to prevent the need for repeated measures analysis in the subgroup of included patients.

The second (*n*=356) were patients with colorectal cancer undergoing potentially curative treatment at a single surgical centre in Glasgow, between 1st January 2008 and 30th June 2017 who had a serum iron or ferritin measured in the preoperative period. Patients who had not had either a serum iron or ferritin request were not included in the study.

Ferritin was analysed using 2 step chemiluminescent microparticle immunoassay within the routine haematology laboratories. Serum total iron (chemically using ferene), serum CRP (by the immunoturbidimetric method) and albumin (chemically using Bromocresol purple) were measured using an automated analyser (Architect, Abbot Diagnosis, Maidenhead, UK) in the routine biochemistry laboratories. All sites used the same analytic materials and automated

platforms. There were no sustained concerns regarding IQC performance requiring investigation into the performance of the assays. The A, B and C scores were within the EQA (NEQAS) targets during the study period.

The audit was conducted with the intent of developing local guidelines and to aid in the interpretation of serum measurements of iron status and was approved by the health board (NHS Greater Glasgow and Clyde).

Methods:

The formulation of the regression correction equation was based on a modification of that used by the BRINDA group to correct ferritin for inflammation using CRP and AGP in asymptomatic populations [8, 13]. The equation used linear regression to adjust ferritin concentrations by the CRP and albumin concentrations on a continuous scale.

The observed ferritin ($Fer_{obs,}$ normal range 41-400 µg/L), serum iron (Ir_{obs}, normal range 10-30 µmol/L), CRP ($CRP_{obs,}$ normal range <10 mg/L) and albumin ($Alb_{obs,}$ normal range 35-50g/L) values were ln transformed to approximate a normal distribution. Bivariate linear regression coefficients were calculated with ferritin as the outcome variable and CRP (β1) and albumin (β2) as each of the explanatory variables. Multivariate linear regression was then used to calculate the variance inflation factor (VIF) as a measure of collinearity, with a value <5 allowing both variables to be included in the final equation.

Internal reference values for CRP (CRP_{ref}) and albumin (Alb_{ref}) were derived similarly to that used in the BRINDA methodology to prevent overcorrection for very low levels of inflammation. ln CRP_{ref} was calculated as the lowest decile of ln CRP_{obs} in the cohort as CRP is a positive acute phase reactant, and ln Alb_{ref} was calculated as the highest decile of ln Alb_{obs} in the cohort as albumin is a negative acute phase reactant. These reference values

were subtracted from the observed CRP and albumin values within the equation, and the equation only applied to those patients with observed CRP above the internal reference cut off, and / or observed albumin below the internal reference cut off.

The final ferritin correction equation is as follows:

$$\ln Fer_{adj} = \ln Fer_{obs} - (\beta 1(\ln CRP_{obs} - \ln CRP_{ref})) - (\beta 2(\ln Alb_{obs} - \ln Alb_{ref}))$$

The final serum iron correction equation is as follows:

$$\ln Ir_{adj} = \ln Ir_{obs} - (\beta 1(\ln CRP_{obs} - \ln CRP_{ref})) - (\beta 2(\ln Alb_{obs} - \ln Alb_{ref}))$$

The calculated adjusted ferritin ($\ln Fer_{adj}$) and serum iron ($\ln Ir_{obs}$) was then back transformed to obtain an adjusted estimate for ferritin (Fer_{adj}) and serum iron (Ir_{adj}) respectively.

Statistical analysis:

In both cohorts. Continuous data were presented as medians and interquartile range (IQR). The median unadjusted serum iron and ferritin were compared to the median serum iron and ferritin using adjustment by CRP alone, albumin alone, and both CRP and albumin using the related samples Friedmann's two way analysis of variance by ranks. The proportion of patients defined as iron deficient (ferritin <15µg/L, or serum iron <10µmol/L) was calculated for unadjusted serum iron and ferritin and using adjustment for both CRP and albumin and compared using McNemar's test.

In the second cohort patients were classified as having anemia using haemoglobin (Hb) based on WHO guidelines for males; <130 g/L and females; <120 g/L [13]. Furthermore, anemic patients were classified as having microcytic anemia with mean corpuscular volume (MCV) <80 f/L, or normocytic anemia with MCV 80-100 f/L.

All analyses were performed using SPSS software (SPSS version 24, Chicago, Illinois, USA). Two sided *P* values <0.05 were considered statistically significant.

Results

Internal CRP and albumin reference values:

The ln CRP_{ref} was calculated as lowest decile of ln CRP_{obs} which was 0.262 (back transformed corresponding CRP_{obs} = 1.3mg/L). The ln Alb_{ref} was calculated as the highest decile of ln Alb_{obs} which was 3.71 (back transformed corresponding Alb_{obs} = 40.9 g/L).

Ferritin correction regression equation:

Bivariate linear regression with ln Fer_{obs} as outcome variable and ln CRP_{obs} as the explanatory variable resulted in a regression coefficient (β1) of 0.331 (95% CI: 0.313, 0.348) and an intercept of 4.112 (95% CI: 4.057, 4.167). Bivariate linear regression with ln Fer_{obs} as outcome variable and ln Alb_{obs} as the explanatory variable resulted in a regression coefficient (β2) of -2.087 (95% CI: -2.212, -1.962) and an intercept of 12.157 (95% CI: 11.728, 12.586). At multivariate analysis there was no evidence of significant collinearity between ln CRP_{obs} and ln Alb_{obs} (VIF = 1.5).

The final series of regression correction equation was therefore as follows:

$$\ln Fer_{adj} = \ln Fer_{obs} - (0.331(\ln CRP_{obs} - 0.262)) - (-2.087(\ln Alb_{obs} - 3.71))$$

IF ln CRP_{obs} >0.262 AND ln Alb_{obs} <3.71

$$Fer_{adj} = EXP\ (\ln Fer_{adj})$$

Serum iron correction regression equation:

Bivariate linear regression with ln Ir_{obs} as outcome variable and ln CRP_{obs} as the explanatory variable resulted in a regression coefficient (β1) of -0.261 (95% CI: -0.270, -0.251), and an intercept of 2.747 (95% CI: 2.716, 2.778). Bivariate linear regression with ln Ir_{obs} as outcome variable and ln Alb_{obs} as the explanatory variable resulted in a regression coefficient (β2) of 1.176 (95% CI: 1.100, 1.252), and an intercept of -1.949 (95% CI: -2.211, -1.687). At multivariate analysis there was no evidence of significant collinearity between ln CRP_{obs} and ln Alb_{obs} (VIF = 1.5).

The final series of regression correction equation was therefore as follows:

$$\ln Ir_{adj} = \ln Ir_{obs} - (-0.261(\ln CRP_{obs} - 0.262)) - (1.176(\ln Alb_{obs} - 3.71))$$

IF $\ln CRP_{obs} > 0.262$ AND $\ln Alb_{obs} < 3.71$

$$Ir_{adj} = EXP(\ln Ir_{adj})$$

Ferritin and serum iron adjustment in primary and secondary care patients:

The primary and secondary care cohort included 7,226 patients of whom the majority 3,958 (55%) were female and 3,260 (45%) were male, with a median age of 68 years (range: 16-103 y). Within the cohort the median CRP_{obs} was 15.0 mg/L (range: 0.15-476 mg/L), and Alb_{obs} 32 g/L (range: 7-76 g/L).

There was a significant decrease (Table 1) in the median serum ferritin ($P<0.001$) and a significant increase in the proportions of patients with serum ferritin <15 µg/L ($P<0.001$) when the unadjusted, adjusted using CRP alone, adjusted using albumin alone, and using both CRP and albumin values were compared.

There was a significant increase (Table 1) in the median serum iron (P<0.001) and a significant decrease in the proportions of patients with serum iron <10 μmol/L (P<0.001) when the unadjusted, adjusted using CRP alone, adjusted using albumin alone, and using both CRP and albumin values were compared.

Concordance between serum ferritin and serum iron definitions of iron deficiency following adjustment:

The proportion of patients in the primary and secondary care cohort found to not meet definitions of iron deficiency with both serum ferritin and serum iron (i.e. negative result agreement / true negative) increased from 43% using the unadjusted values to 66% when adjustment was made by both CRP and albumin (Table 2). The proportion of patients found to meet definitions of iron deficiency with both serum ferritin and serum iron (i.e. positive result agreement / true positive) remained unchanged at 5% following the same adjustment.

Ferritin and serum iron adjustment in patients with colorectal cancer:

There were 356 patients with UICC stage I-IV colorectal cancer who underwent surgery with curative intent at a single centre and had a preoperative serum ferritin or measured, of which 67 had serum iron measured, 307 had CRP measured, and 236 had both CRP and albumin measured (Table 2). Within the cohort there were 161 females (45%) and 195 males (55%), with a median age of 71 years (range: 27-90 y), and the majority having colonic (n=259, 73%) and node negative (n=209, 59%) disease.

There was a significant decrease (Table 3) in the median serum ferritin (P<0.001) and a significant increase in the proportions of patients with serum ferritin <15 μg/L (P<0.001)

when the unadjusted, adjusted using CRP alone, adjusted using albumin alone, and using both CRP and albumin values were compared.

There was a significant increase (Table 3) in the median serum iron (P<0.001) and a significant decrease in the proportions of patients with serum iron <10 μmol/L (P<0.001) when the unadjusted, adjusted using CRP alone, adjusted using albumin alone, and using both CRP and albumin values were compared.

Association between adjusted iron deficiency and anaemia in patients with colorectal cancer:

Of the cohort of patients with colorectal cancer, 354 had serum ferritin, Hb and MCV measured allowing them to be grouped as having no anemia (n=174, 50%), microcytic anemia (n=41, 11%) or normocytic anemia (n=139, 39%) (Table 4). There was a significant association between the anemia groups and age (P<0.001), American Society of Anesthesiology (ASA) grade (P<0.001), tumor site (P<0.001), TNM stage (P=0.010), tumor differentiation (P<0.001), and modified Glasgow Prognostic Score (mGPS) (P<0.004). The proportion of patients found to have serum ferritin <15 μg/L was significantly different amongst the no anemia, microcytic anemia and normocytic anemia groups respectively whether unadjusted (P<0.001), adjusted by CRP alone (P<0.001), adjusted by albumin alone (P<0.001) or adjusted by both CRP and albumin (P<0.001) values were considered. The proportion of patients found to have serum iron <10 μmol/L was significantly different amongst the no anaemia, microcytic anemia and normocytic anemia groups using the unadjusted values only (P=0.007)

Discussion

The results of the present study report linear regression based correction equations to adjust observed serum iron and ferritin concentrations based on CRP and albumin in a cohort of primary and secondary care patients, and patients with colorectal cancer.

The BRINDA Working Group have previously reported the use of a linear regression based correction equation for ferritin adjusted by CRP and AGP in populations across multiple countries [8]. Unlike serum albumin, AGP is used to define the "phase" of inflammation, rather than its magnitude making estimation both dependent on their concentrations and the phase in which the patient is found [15]. In addition, unlike albumin, AGP is not at present widely used in clinical practice, especially in the developing countries from which many of the BRINDA cohorts were derived [13].

Although prior studies have suggested the use of correction factors based cut off values of acute phase proteins [6], or changing the ferritin concentration threshold for the diagnosis of iron deficiency to <30 μg/L to account for inflammation [14], the BRINDA group show that serum ferritin concentrations are significantly affected even at low levels of inflammation, below those cut off values used previously [9]. The same is true for serum total iron [11], which in addition is highly variable from individual to individual, within individuals, and is also significantly affected by dietary intake [3].

These findings are in keeping with the results of the present study and the previously reported results from this cohort in which patients were grouped by CRP and albumin [11]. In it, 13% of patients with CRP <10mg/L and albumin >35g/L (therefore thought to be minimally inflamed), were still found to have ferritin <15 μg/L. Although the use of such groupings to account for inflammatory status is useful, the BRINDA group suggest that regression based

253 correction, as in the present paper, provides greater accuracy. Particularly, as with CRP and
254 AGP in the BRINDA papers, because even low and "sub-clinical" levels of systemic
255 inflammation can significantly alter serum measures of iron status [16]. This study differs
256 from our previously reported work by using continuous regression equation adjustment rather
257 than grouping in attempt to account for the aforementioned "sub-clinical" inflammation.
258 Indeed, in the present study the CRP and albumin reference values used to prevent over-
259 correction for low level inflammation were 1.3 mg/L and 40.9 g/L respectively, much
260 different from "minimally inflamed" group threhsolds used previously.

261 The greatest difference in the proportion of iron deficient patients was found when
262 adjustment was made for both CRP and albumin together. It may be that this relates to the
263 different half-lives of CRP and albumin, with CRP peaking around 48 to 72 hours following
264 an inflammatory insult and then ending to decrease rapidly, with albumin taking much longer
265 to return to reference range concentrations [17]. It could be considered in this context that
266 albumin is perhaps a longer term index of inflammation and CRP a short term marker, hence
267 the additive effect with regard to ferritin and iron correction.

268 The present study reports the application of CRP and albumin based regression correction to
269 a small cohort of patients with colorectal cancer. A high proportion of patients with
270 normocytic anaemia (57%) and without anaemia (29%) were found to be apparently iron
271 deficient. This may represent patients who have functional iron deficiency related to the
272 presence of a host systemic inflammatory response to cancer. At present there is little data to
273 suggest whether iron replacement therapy will be of use in those patients with inflammation
274 who meet definitions of iron deficiency following mathematical adjustment compared to
275 those who do not. This is indeed an area requiring prospective investigation, and hopefully
276 the use of such correction strategies might allow patient stratification in such studies.

The present study has a number of limitations. The lack of clinical data regarding reasons for the investigation of iron status in the first cohort means that there were no data relating to the presence of underlying autoimmune disease or liver disease or markers of synthetic liver function associated with treated systemic inflammation or altered iron homeostasis. Within the second cohort, the selection of patients for whom ferritin had been requested may introduce selection bias, although the prevalence of anaemia, and its subtypes is in keeping with recently published reports [18].

In summary the present study reports a regression correction equation to adjust serum iron and ferritin concentrations based on CRP and albumin in a cohort of patients in primary and secondary care, and in a cohort of patients with colorectal cancer. In both cohorts the greatest difference in the proportion of iron deficient patients was found when adjustment was made for both CRP and albumin together. Even very low levels of inflammation were associated with significant perturbment of ferritin. Although the presented regression equations do not produce perfect agreement amongst different measures of iron status, their use did improve concordance and suggest that with ongoing refinement further similar techniques may prove useful in the absence of novel markers of iron status.

Disclosure

None of the authors have any conflicts of interest.

Contributions

SM, IJ, DM and DT designed research. AT and IJ conducted research. AT, SM and IJ analyzed data. SM, IJ, DM and DT wrote the paper, SM had primary responsibility for final content. All authors read and approved the final manuscript.

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Tables and Footnotes

Table 1 Estimated prevalence of iron deficiency; serum ferritin <15µg/L or serum iron <10 µmol/L, based on regression correction using serum C-reactive protein (CRP) and albumin in an unselected cohort of primary and secondary care patients.[1]

Iron measure	Unadjusted	CRP only	Albumin only	CRP and albumin	*P*
Serum Ferritin					
n	7226	7219	6406	6406	-
Ferritin, µg/L,	162 (57-385)	69 (28-161)	89 (35-203)	38 (15-92)	<0.001[2]
Ferritin <15 µg/L, *n* (%)	479 (7)	939 (13)	679 (11)	1557 (24)	<0.001[3]
Serum Iron					
n	7182	7182	6371	6371	-
Iron, µmol/L,	8 (4-15)	15 (10-23)	12 (7-19)	20 (13-32)	<0.001[2]
Iron <10 µmol/L, *n* (%)	4027 (55)	1877 (26)	2705 (37)	925 (15)	<0.001[3]

[1]Continuous values are presented as median and IQR, [2]Medians of all four adjustment methods compared using related samples Friedmann's two way analysis of variance by ranks, [3]Proportions of "Unadjusted" and "CRP and albumin adjusted" methods compared using McNemar's test . CRP, C-reactive protein; IQR, interquartile range.

Table 2 Concordance between serum ferritin and serum iron definitions of iron deficiency before and after adjustment by serum C-reactive protein (CRP) and albumin:

Unadjusted			
		Iron <10μmol/L	
		No, *n* (%)	Yes, *n* (%)
Ferritin <15μg/L	No, n(%)	3059 (43)	3644 (51)
	Yes, n(%)	96 (1)	383 (5)
Adjusted by CRP and albumin			
		Iron <10μmol/L	
		No, *n* (%)	Yes, *n* (%)
Ferritin <15μg/L	No, n(%)	4253 (66)	630 (10)
	Yes, n(%)	1241 (19)	294 (5)

CRP C-reactive protein

Table 3 Estimated prevalence of iron deficiency; serum ferritin <15µg/L, or serum iron <10µmol/L; based on regression correction using serum C-reactive protein (CRP) and albumin in patients undergoing surgery for colorectal cancer.[1]

Iron measure	Unadjusted	CRP only	Albumin only	CRP and albumin	*P*
Serum Ferritin					
n	356	307	236	236	-
Ferritin, µg/L	38 (13-93)	24 (8-66)	24 (9-70)	18 (6-52)	<0.001[2]
Ferritin <15 µg/L, *n* (%)	100 (28)	120 (39)	91 (39)	107 (46)	<0.001[3]
Serum Iron					
n	67	58	46	46	-
Iron, µmol/L	5 (3-13)	8 (3-19)	6 (4-17)	11 (5-26)	<0.001[2]
Iron <10 µmol/L, *n* (%)	44 (66)	33 (57)	27 (59)	20 (44)	0.004[3]

[1]Continuous values are presented as median and IQR. [2]Medians of all four adjustment methods compared using related samples Friedmann's two way analysis of variance by ranks, [3]Proportions of "Unadjusted" and "CRP and albumin adjusted" methods compared using McNemar's test . CRP, C-reactive protein; IQR, interquartile range.

Table 4 Association between anemia[1] and estimated prevalence of iron deficiency (ferritin <15µg/L) based on regression correction using serum C-reactive protein (CRP) and albumin in patients undergoing surgery for colorectal cancer.

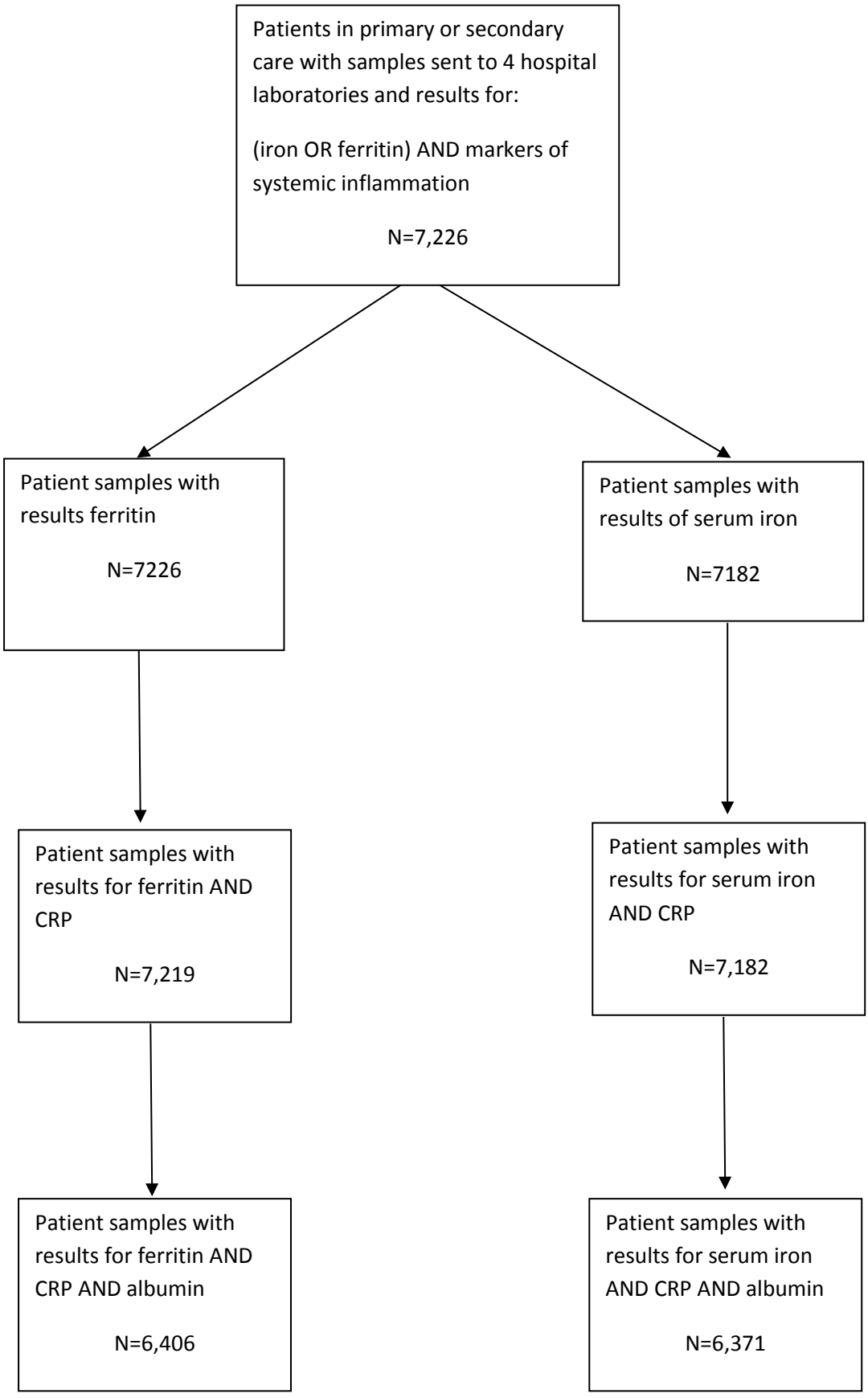
Anemia[1]		**None**	**Microcytic**[2]	**Normocytic**[3]	***P***
n		174 (50)	41 (11)	139 (39)	-
Demographic					
Sex, *n* (%)					0.398[4]
	Male	89 (51)	24 (59)	81 (58)	
	Female	85 (49)	17 (41)	58 (42)	
Age, *n* (%)					<0.001[5]
	<65	66 (38)	16 (39)	24 (17)	
	65-70	73 (42)	12 (29)	51 (37)	
	>70	35 (20)	13 (32)	64 (46)	
ASA, *n* (%)					<0.001[5]
	1	42 (28)	6 (16)	12 (11)	
	2	63 (43)	17 (45)	50 (45)	
	3	39 (26)	14 (37)	45 (40)	
	4	4 (3)	1 (2)	5 (5)	
nCRT, *n* (%)					0.739[4]
	Yes	8 (5)	2 (5)	9 (7)	
	No	165 (95)	38 (95)	127 (93)	
Pathological					
Tumor site, *n* (%)					<0.001[4]
	Colon	106 (61)	39 (95)	113 (82)	
	Rectum	67 (39)	2 (5)	25 (18)	
TNM stage, *n* (%)					0.010[5]
	0/1	44 (26)	1 (3)	15 (11)	
	2	66 (38)	18 (45)	64 (47)	
	3	60 (34)	18 (45)	51 (38)	
	4	3 (2)	3 (7)	6 (4)	
Differentiation, *n* (%)					<0.001[4]
	Well / Mod	166 (97)	29 (74)	118 (88)	
	Poor	5 (3)	10 (26)	16 (12)	
Venous invasion, *n* (%)					1.000[4]
	Present	111 (64)	25 (64)	88 (64)	
	Absent	62 (36)	14 (36)	49 (36)	
Systemic inflammation					
mGPS					0.004[5]
	0	114 (80)	25 (76)	76 (72)	
	1	18 (13)	3 (9)	5 (5)	
	2	10 (7)	5 (15)	24 (23)	
Serum Ferritin					
Unadjusted, *n* (%) (*n*=354)					<0.001[4]
	<15µg/L	26 (15)	28 (68)	46 (33)	
	≥15µg/L	148 (85)	13 (32)	93 (67)	
CRP only, *n* (%)(*n*=305)					<0.001[4]
	<15µg/L	33 (22)	31 (80)	56 (48)	
	≥15µg/L	117 (78)	8 (21)	60 (52)	
Albumin only, *n* (%) (*n*=234)					<0.001[4]
	<15µg/L	25 (21)	23 (85)	43 (50)	
	≥15µg/L	96 (79)	4 (15)	43 (50)	
CRP and albumin, *n* (%)(*n*=230)					<0.001[4]
	<15µg/L	34 (29)	24 (89)	48 (57)	
	≥15µg/L	84 (71)	3 (11)	37 (44)	

Serum Iron					
Unadjusted, *n* (%) (*n*=66)					0.007[4]
	<10µmol/L	5 (36)	12 (92)	27 (69)	
	≥10µmol/L	9 (64)	1 (8)	12 (31)	
CRP only, *n* (%) (*n*=57)					0.129[4]
	<10µmol/L	5 (36)	8 (73)	20 (63)	
	≥10µmol/L	9 (64)	3 (27)	12 (37)	
Albumin only, *n* (%) (*n*=45)					0.055[4]
	<10µmol/L	5 (36)	6 (86)	16 (67)	
	≥10µmol/L	9 (64)	1 (14)	8 (33)	
CRP and albumin, *n* (%) (*n*=45)					0.173[4]
	<10µmol/L	4 (29)	5 (71)	11 (46)	
	≥10µmol/L	10 (71)	2 (29)	13 (54)	

[1] Males = Hb <130g/L, females <120g/L, [2]Anemia and MCV <80fL, [3]Anemia and MCV 80-100fL. [4]Chi square test, [5]Chi square test for linear association. ASA, American Society of Anesthesiology; mGPS, modified Glasgow Prognostic Score; MCV, mean corpuscular volume; nCRT, neoadjuvant chemoradiotherapy; TNM, tumor nodes metastasis stage.

Supplementary data

Supplementary data 1: Patient Flow Chart – Cohort 1



Supplementary data

Supplementary data 2: Patient Flow Chart – Cohort 2