Laboratory diagnosis of iron deficiency: The interpretation of automated counting parameters.

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Why does it matter?

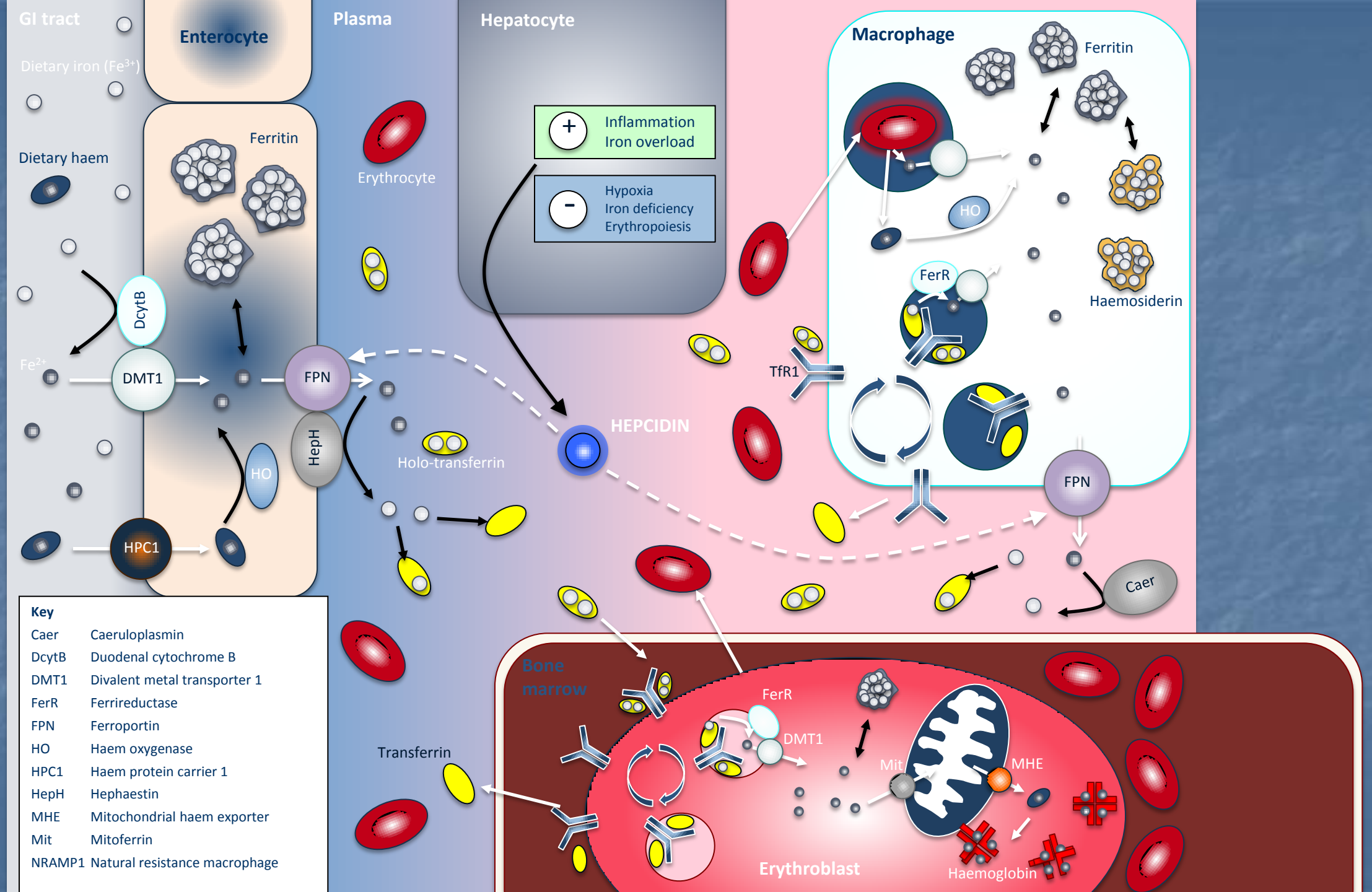
- Over 30% of the Worlds population are anaemic, around 2 billion people
- Most due to iron deficiency
- Globally the causes are different
  - Low iron diet, hookworm, other infections
    - vs
  - The anaemia of blood loss

Bowel cancer UK

- Bowel cancer is the fourth most common cancer in the UK (2014)
- 11% of all new cases.
- Third most common cancer in both males (12% of the male total) and females (10%) separately
- In 2014, there were 41,265 new cases of bowel cancer in the UK: 55% were male
- The crude incidence rate shows that there are 72 new bowel cancer cases for every 100,000 males in the UK and 56 for every 100,000 females.

Morbidity of iron deficiency

- Developing world, poorer pregnancy outcomes
- Reduced physical and cognitive functioning
- Exacerbates underlying medical condition
  - CKD
  - Heart Failure
  - IBD
  - Other inflammatory disorders



Key

Caer	Caeruloplasmin
DcytB	Duodenal cytochrome B
DMT1	Divalent metal transporter 1
FerR	Ferrireductase
FPN	Ferroportin
HO	Haem oxygenase
HPC1	Haem protein carrier 1
HepH	Hephaestin
MHE	Mitochondrial haem exporter
Mit	Mitoferrin
NRAMP1	Natural resistance macrophage

So how sure are we that we're getting it right?

- What tests can be used?
- Are they readily available?
- What is their clinical utility?
- Are there any quality control issues?

Tests

- Hb
- Serum ferritin
- MCH
- MCV
- Retic count
- %hypo or %HRC
- CHr, Ret-He, MCHr, LHD%
- Bone marrow
- ZPP
- STfR
- Serum iron, TIBC, transferrin saturation
- Serum Epo
- Hepcidin
- Response to iron

So what would we like to achieve?

- Diagnose iron deficiency before IDA develops and intervene
- Diagnose IDA and find out the cause
- Predict and pre-emptively treat those that may develop IDA (eg: around surgery)
- Predict and pre-emptively treat those that may develop FID (eg: Anaemia with CKD)

Serum Ferritin

- Is the hallmark test for iron deficiency
- 1mcg/l of SF is equivalent to approximately 8mg of storage iron (Walters et.al 1973)
- Should be compared against 3rd International standard for ferritin (NIBSC Code 94/572)

But what is ferritin?

- 450Kd protein found in all cells.
- Spheroidal structure, that can contain up to 4000 Fe^{3+} iron atoms
- L and H subunits. Nearly all SF is made up of the L subunits
- The mechanism of passage into the serum is poorly understood, but well recognised that infection and inflammation increase its release
  - SF no longer correlates with stores

Quality Control

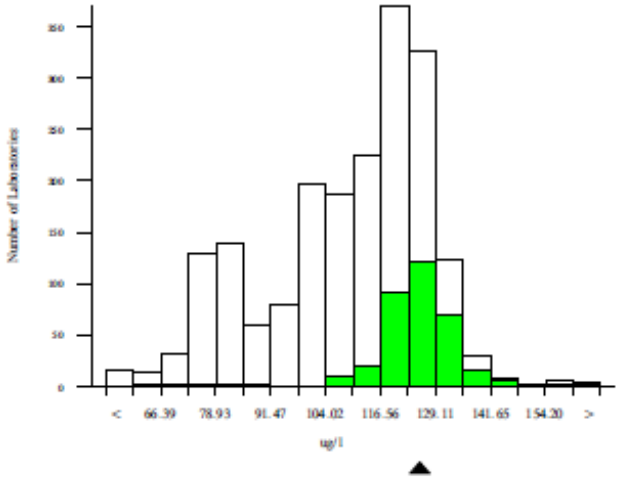
- If you are using SF to base your diagnostic assumptions upon, the QA needs to assess those values that have clinical utility

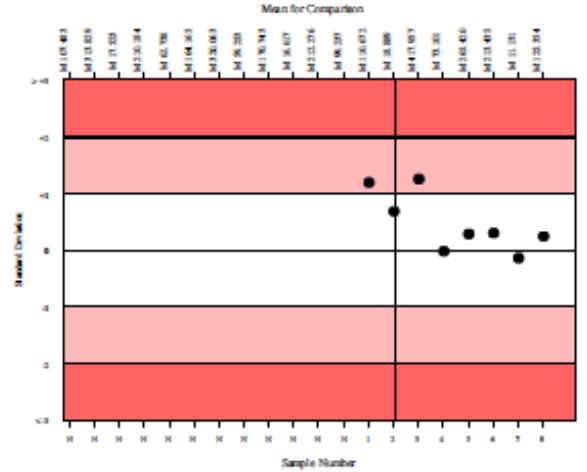
Ferritin, ug/l

	N	Mean	CV%	U_{in}	SDPA	Exc.
All Methods	1864	110.297	15.2	0.48	9.45	79
Abbott Architect Chemiluminescence	310	125.354	4.4	0.39	10.75	30

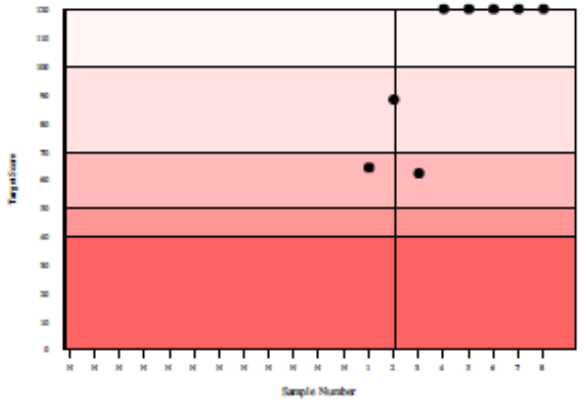
Your Result	128.000	SDI	0.25
		RMSDI	Too Few
Mean for Comparison	125.354	TS	120
		RMTS	Too Few
		%DEV	2.1
		RM %DEV	Too Few

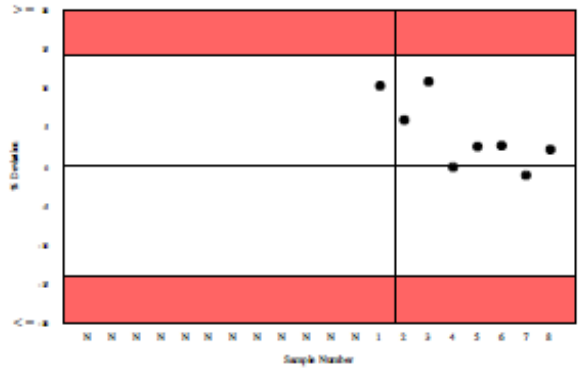
Acceptable limits derived from Biological Variation	16.9%
Acceptable limits of performance for RIQAS	14.10%





TDPA = 14.1%





Standard Deviation
<-3
-2
-1
0
+1
+2
>+3
N
N
N
N
N
N
N
N
N
N
N
N
1
2
3
4
5
6
7
8
M 107.483
M 313.829
M 17.523
M 210.184
M 62.738
M 164.165
M 320.063
M 59.253
M 170.743
M 16.617
M 212.276
M 99.237
M 110.672
M 18.889
M 417.957
M 73.101
M 265.420
M 213.435
M 11.131
M 125.354
Mean for Comparison

Normal range?!?

- Sensitivity vs. specificity
- Abbott Architect:
  - 60 females, 95% normal range down to 5mcg/l
  - Males 22mcg/l
- So…do we use 10mcg/l, 12mcg/l or 15mcg/l?
- Does it actually matter?
- Surely it depends on the other tests too?

Case

- 71 yr old lady. No weight loss. Tired. Some lower abdo ache.
- Hb 98g/l, MCV 83.6fl, Retics 47x10*9/l for Hct of 0.32 (i.e: normal). U&E, WCC and Plat normal
- Who would add haematinics?

- MCH 26pg, MCHC 311g/l, Ret-He 25.8pg,
- SF 6mcg/l, CRP 2

- Had colonoscopy:
  - Colorectal Cancer

Some food for thought?

- How good is the FBC at suggesting IDA?
- What should we base our SF normal range on?

The NEW ENGLAND JOURNAL of MEDICINE

REVIEW ARTICLE

Edward W. Campion, M.D., *Editor*

Microcytic Anemia

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THE MICROCYTIC ANEMIAS ARE THOSE CHARACTERIZED BY THE PRODUCtion of red cells that are smaller than normal. The small size of these cells is due to decreased production of hemoglobin, the predominant constituent of red cells (Fig. 1). The causes of microcytic anemia are a lack of globin product (thalassemia), restricted iron delivery to the heme group of hemoglobin (anemia of inflammation), a lack of iron delivery to the heme group (iron-deficiency anemia), and defects in the synthesis of the heme group (sideroblastic anemias). This review highlights new aspects of the most common microcytic anemias: thalassemia, anemia of inflammation, and iron-deficiency anemia.

THALASSEMIA

How do we diagnose iron deficiency?

- Suspect with a microcytic anaemia if you read the books
- But in real life….
- We looked at 500 patients FBC with SF between 10 and 12 mcg/l and this is what we found

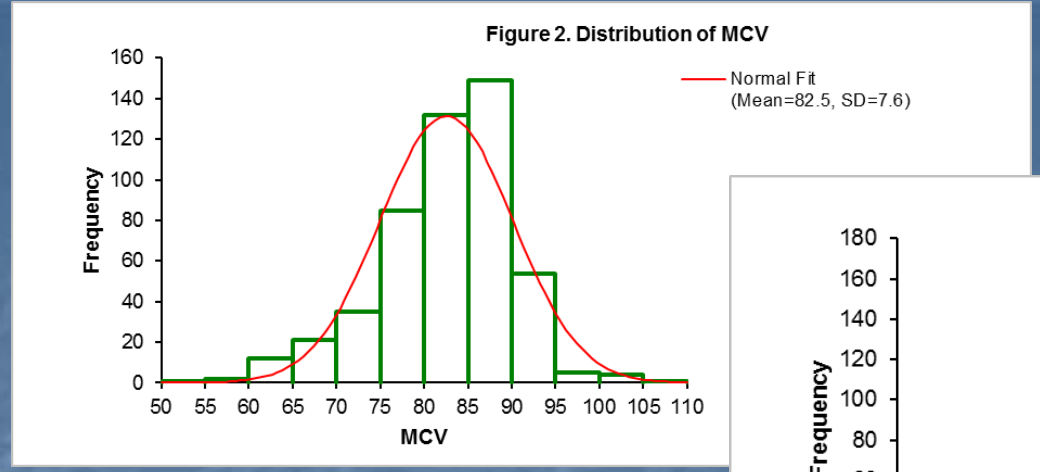


Figure 2. Distribution of MCV
Normal Fit
(Mean=82.5, SD=7.6)
Frequency
MCV
0
20
40
60
80
100
120
140
160
50 55 60 65 70 75 80 85 90 95 100 105 110

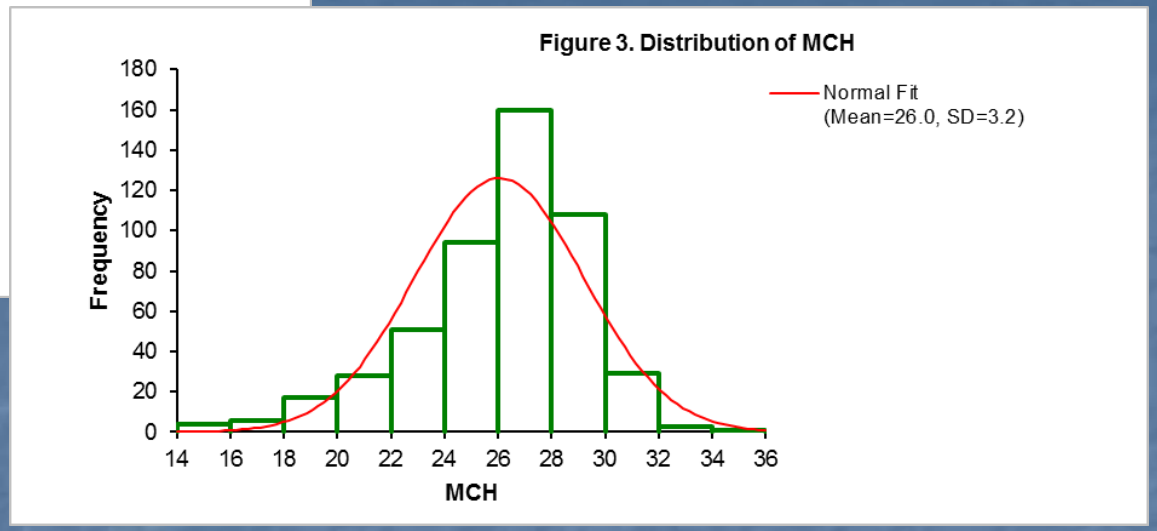


Figure 3. Distribution of MCH
Normal Fit
(Mean=26.0, SD=3.2)
Frequency
MCH
0
20
40
60
80
100
120
140
160
180
14 16 18 20 22 24 26 28 30 32 34 36

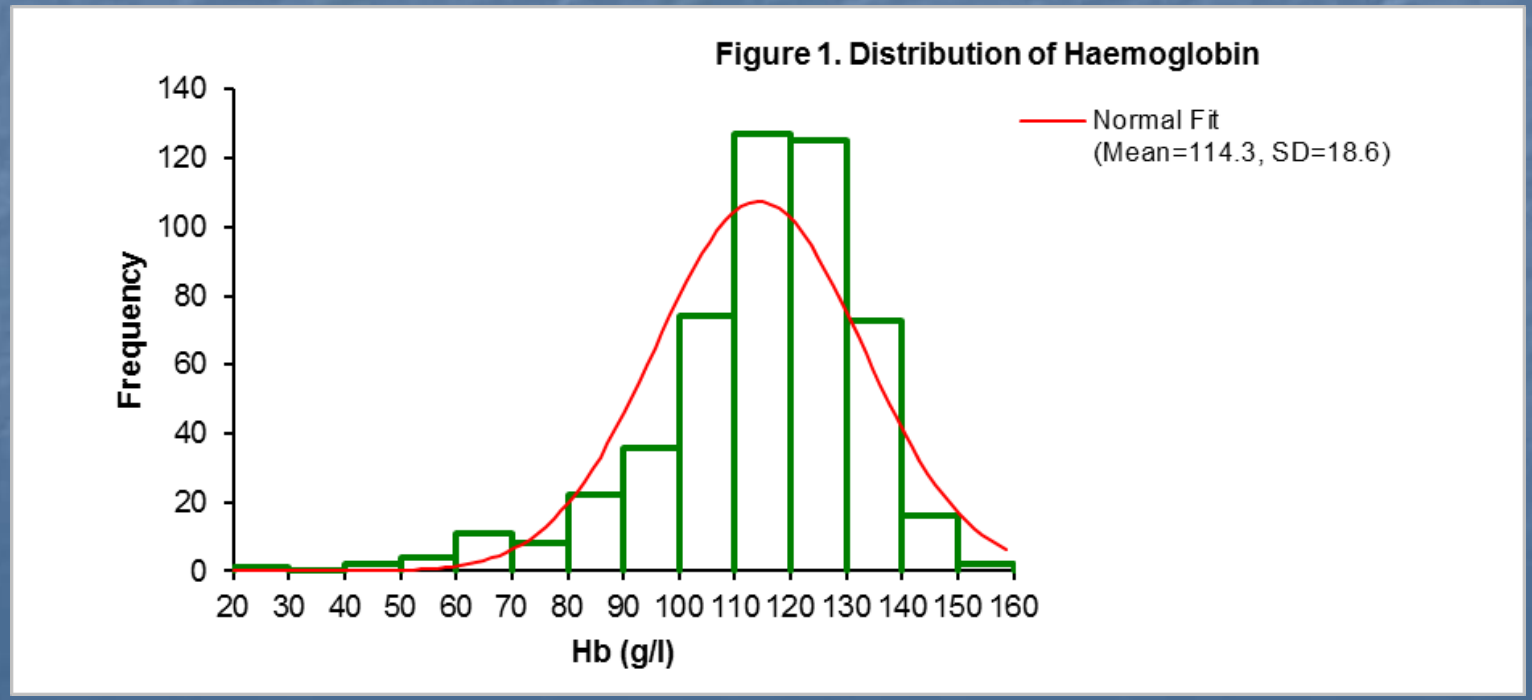


Figure 1. Distribution of Haemoglobin
Normal Fit
(Mean=114.3, SD=18.6)
Frequency
Hb (g/l)
0
20
40
60
80
100
120
140
20 30 40 50 60 70 80 90 100 110 120 130 140 150 160

Bottom line

- Around 2/3 of MCV normal with SF just below the lower limit of normal, yet ~2/3 have a low MCH
- These results are from Sysmex XE2100 analyser. Sysmex use is around 70% UK FBC lab market share

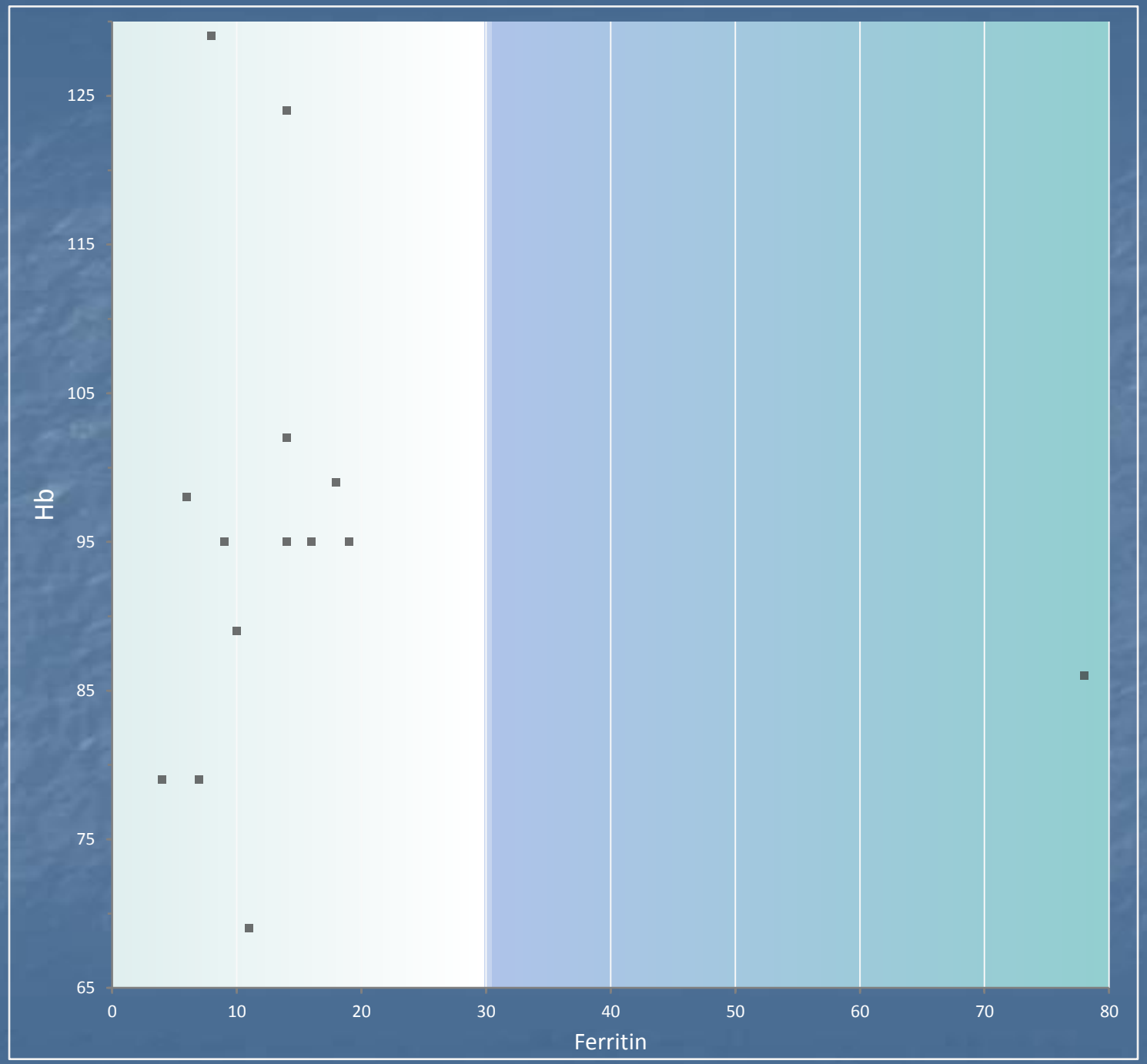
- Its not unique to Sysmex
  - Using Abbott Sapphire we found:

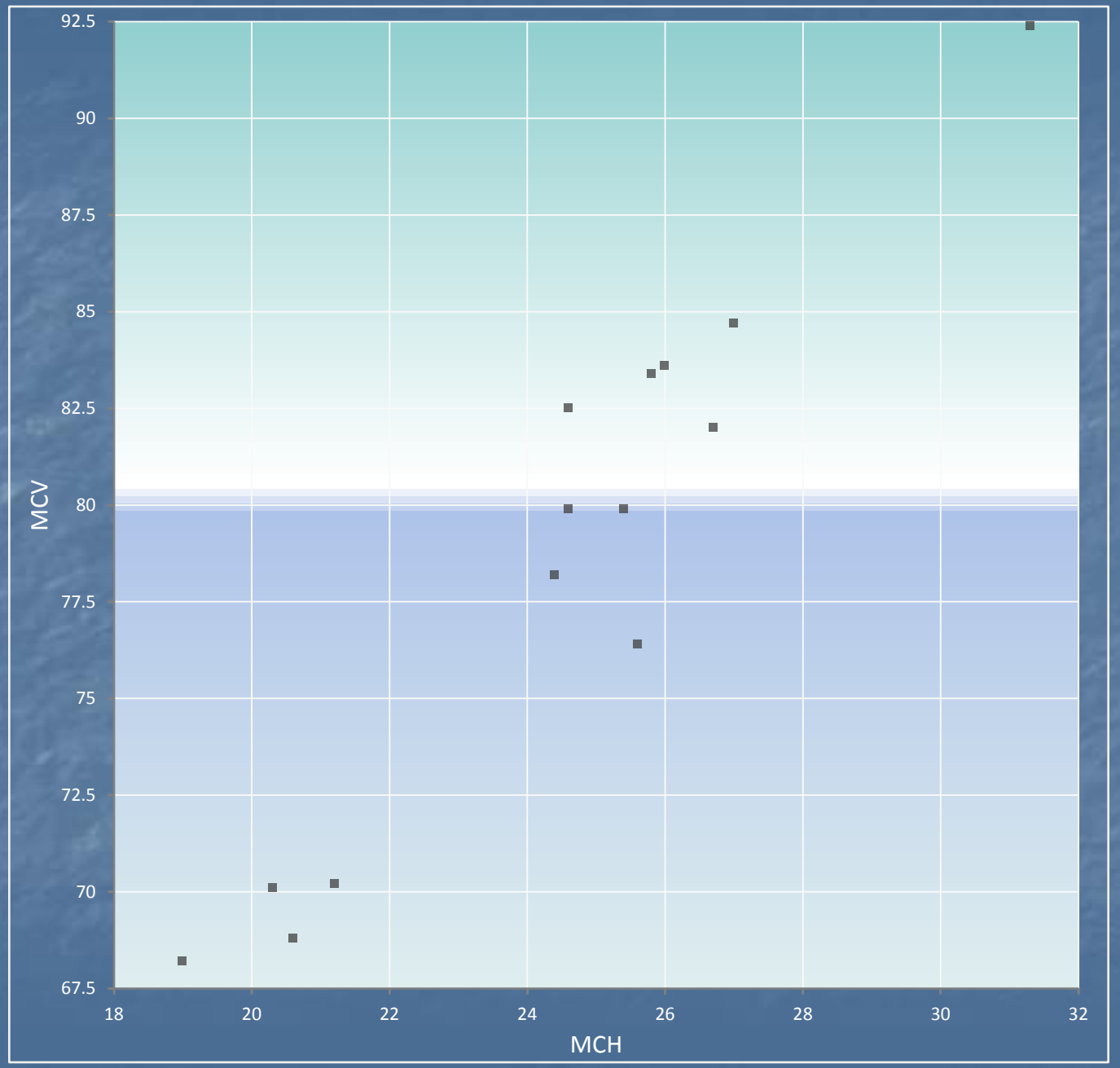
Ferritin value	<5	6 to 9	10 to 13
Average MCV	83.3	84.14	85.86
Average MCH	26.87	27.16	27.44
Average HB	114.39	116.7	118.86
% MHC <28	73.81	56.1	48.7
% MCV <80	50.8	32.06	26.7

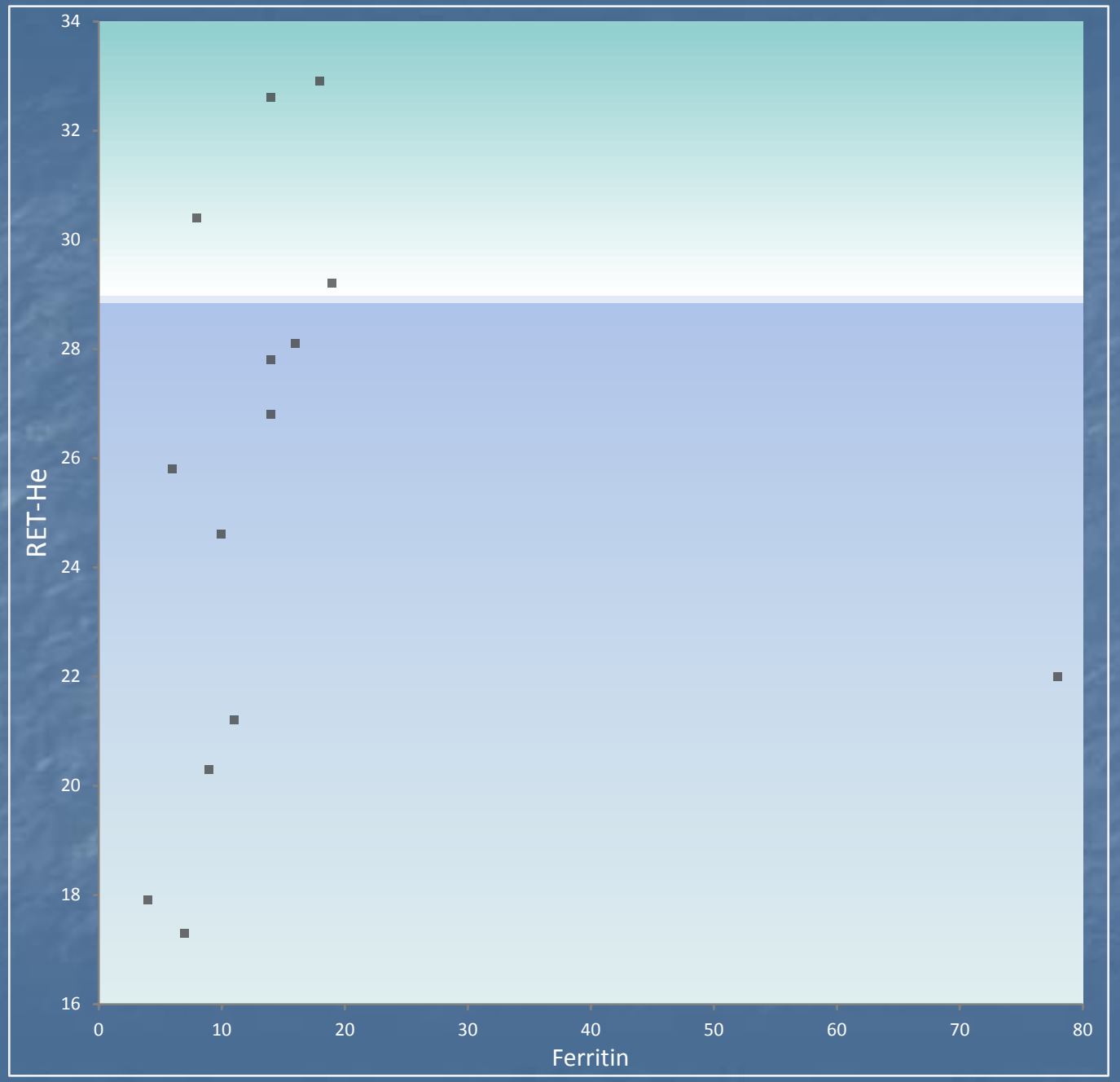
- To diagnose IDA we first have to suspect it
- The most sinister cause of IDA in the UK is colon cancer
- Looking at patients referred via the IDA pathway (from GP's)

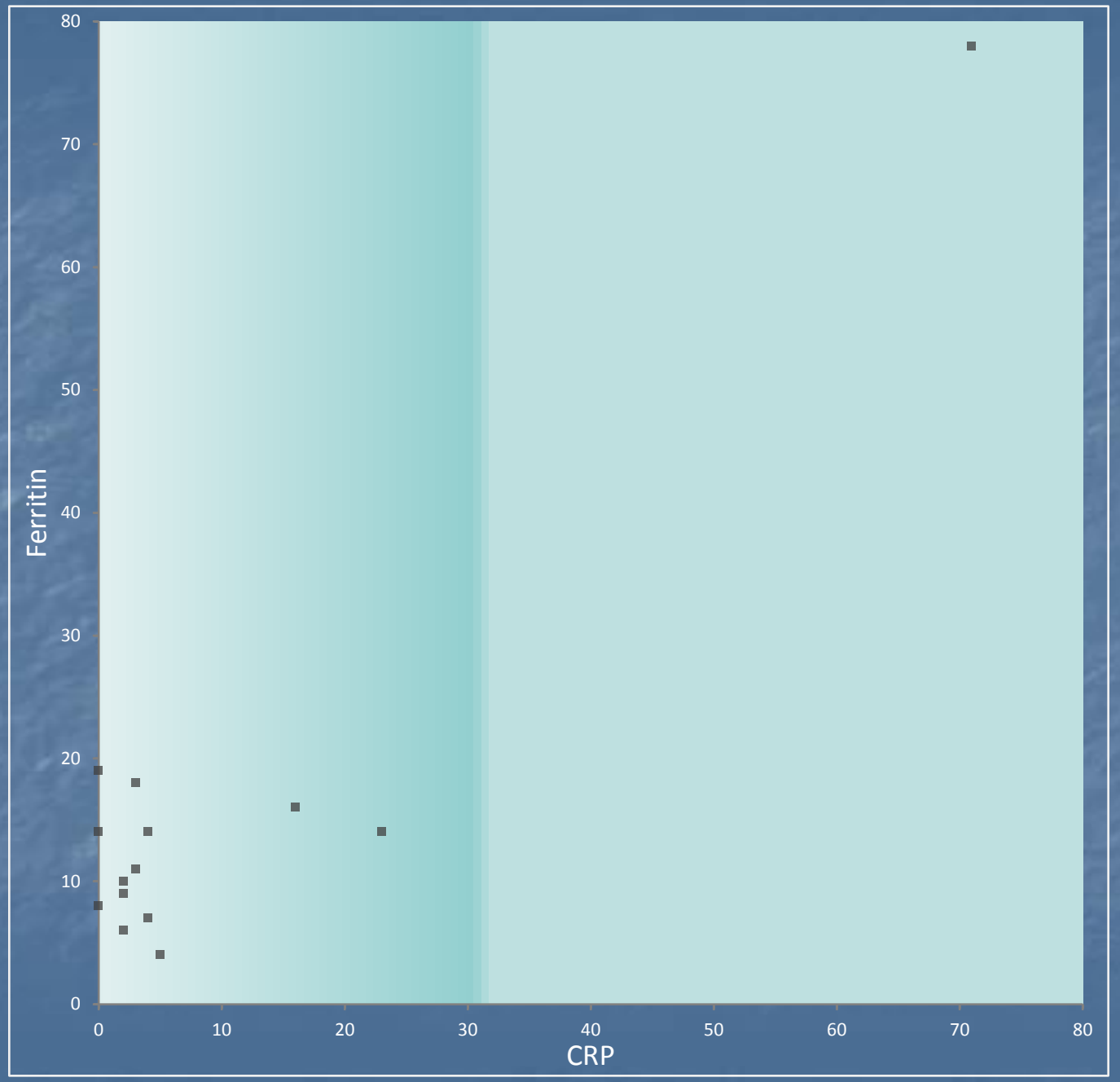
- Of 429 patients, 14 had colonic Ca
- What do we see in this group?

  - *Data kindly provided by Dr Stephen Lewis, Plymouth Hospitals NHS Trust*

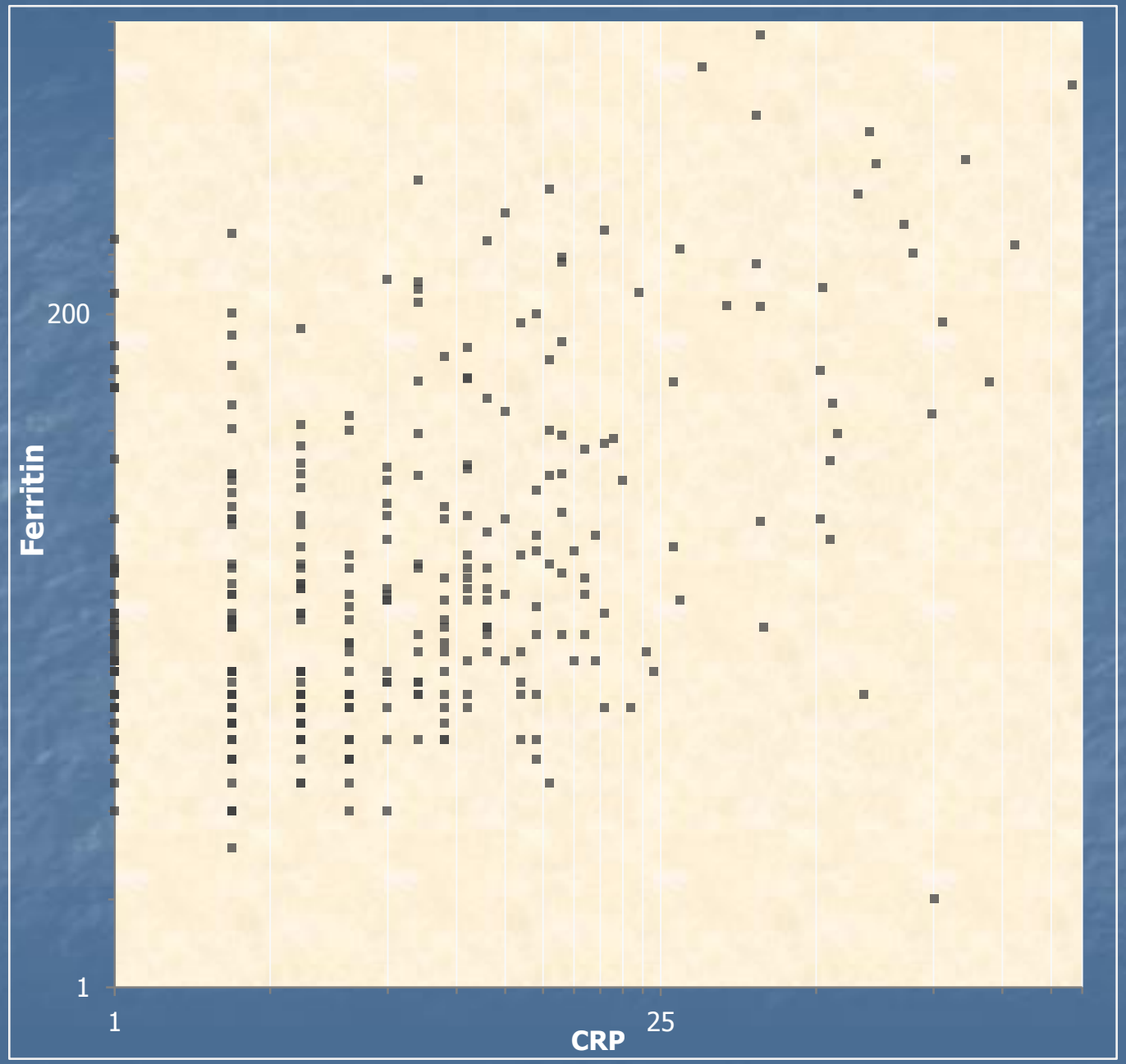

Hb
125
115
105
95
85
75
65
0
10
20
30
40
50
60
70
80
Ferritin

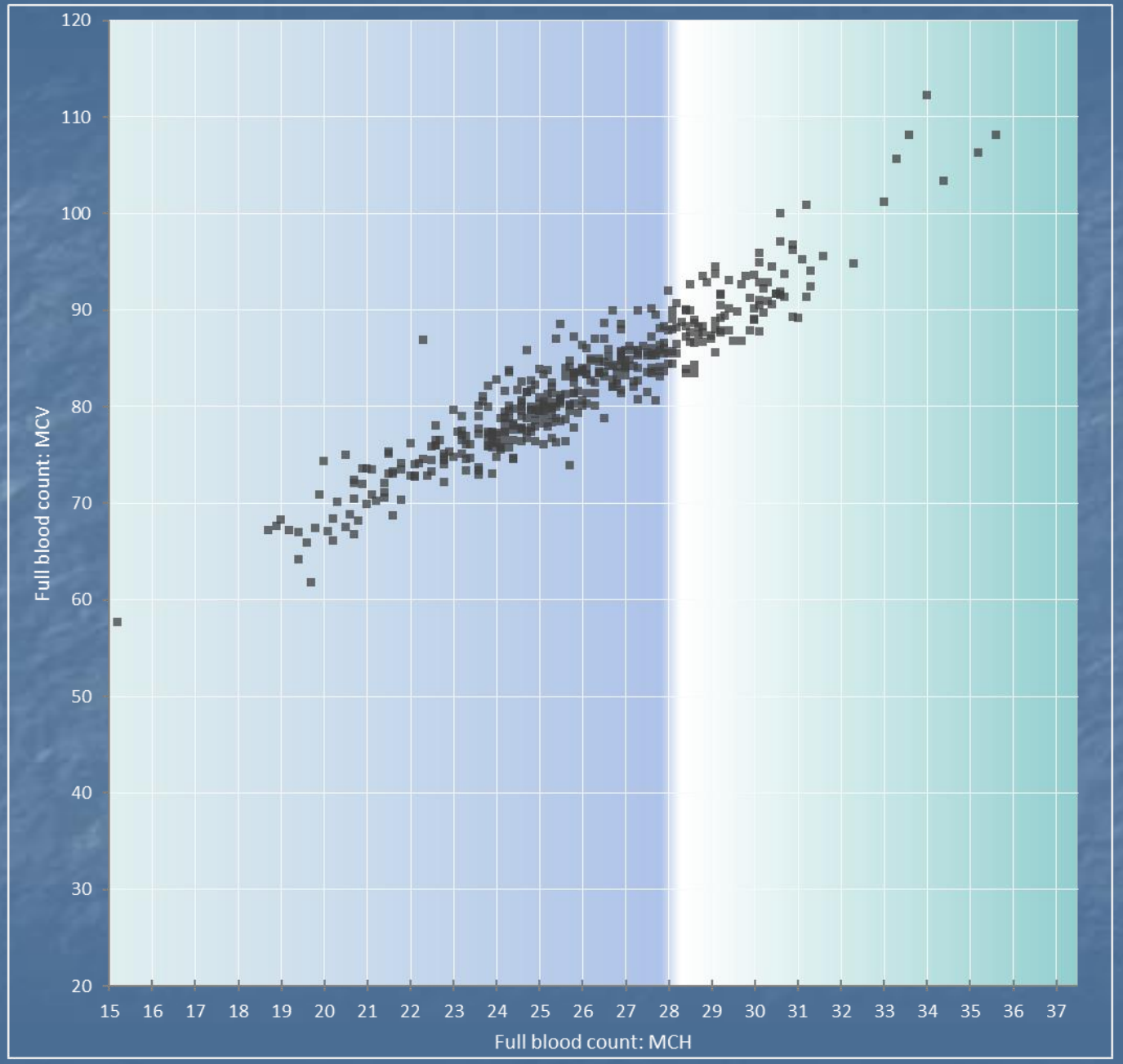

92.5
90
87.5
85
82.5
80
77.5
75
72.5
70
67.5
MCV
18
20
22
24
26
28
30
32
MCH

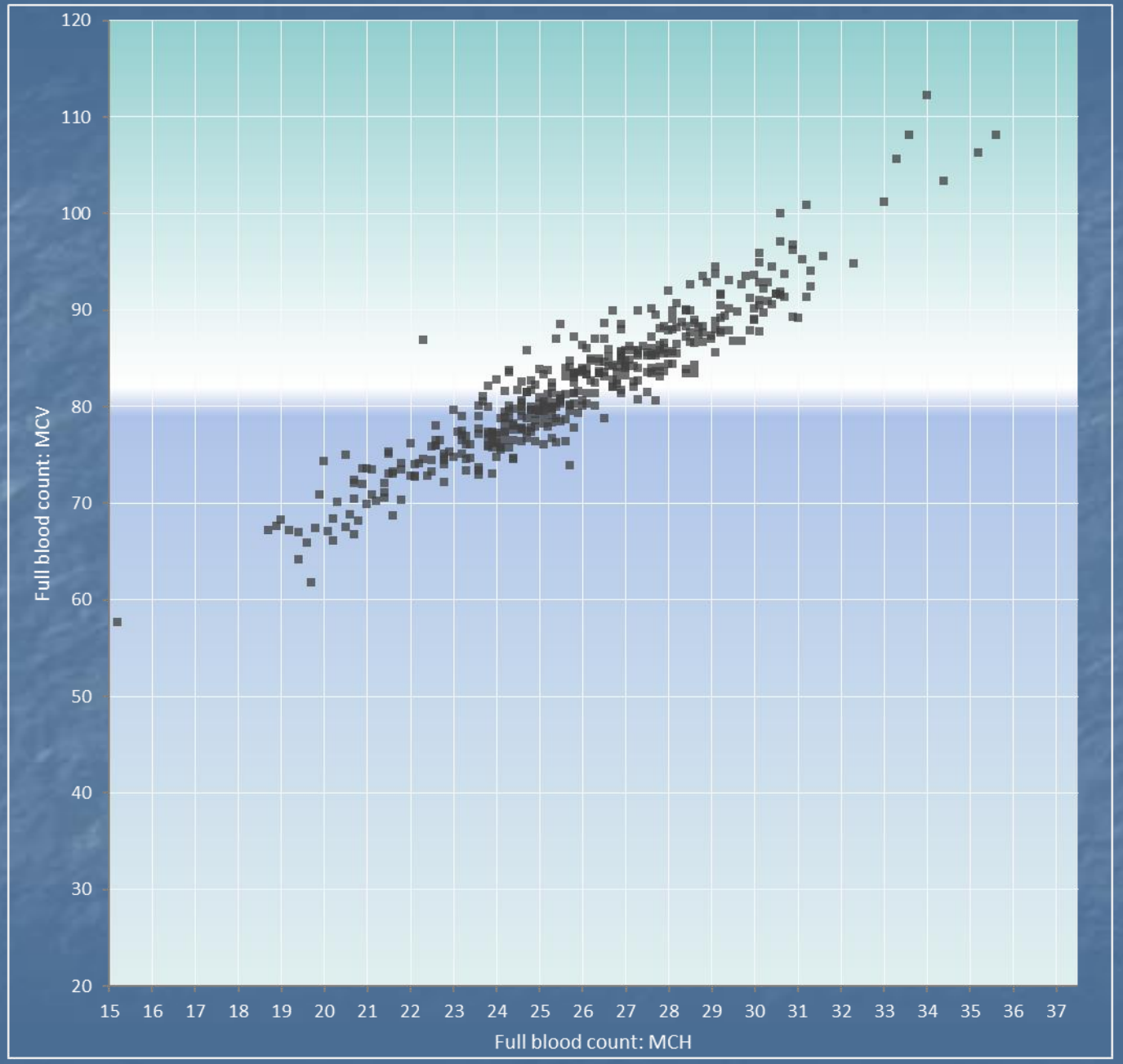

34
32
30
28
26
24
22
20
18
16
RET-He
0
10
20
30
40
50
60
70
80
Ferritin


80
70
60
50
40
30
20
10
0
Ferritin
0
10
20
30
40
50
60
70
80
CRP

- And for all the 429 patients:


200
1
Ferritin
1
25
CRP

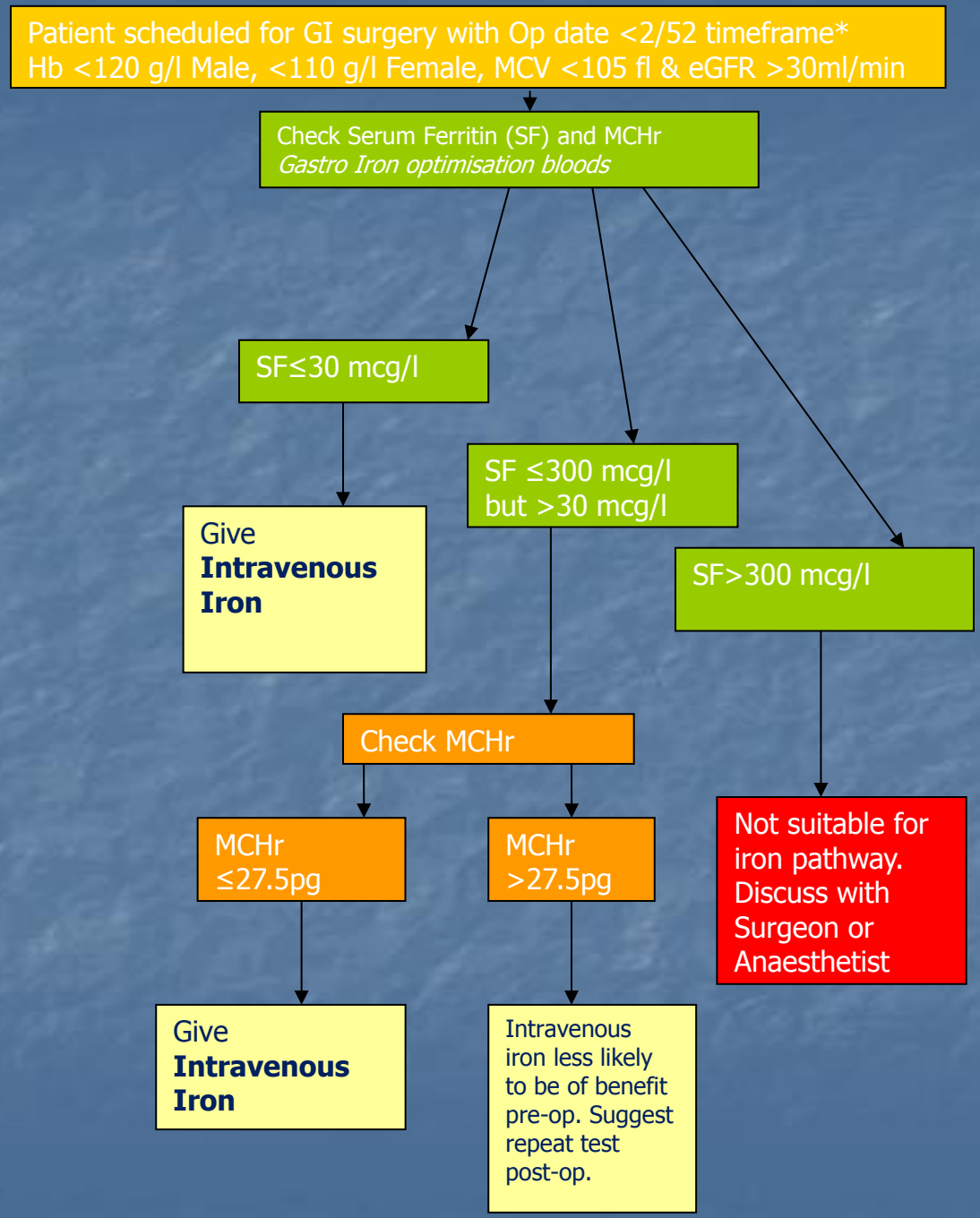

120
110
100
90
80
70
60
50
40
30
20
Full blood count: MCV
15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37
Full blood count: MCH


120
110
100
90
80
70
60
50
40
30
20
Full blood count: MCV
15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37
Full blood count: MCH

What does this suggest?

- All anaemic patients should have SF done!
- MCV compared to MCH has the worst predictive value
- Changing the normal range doesn't help. Looking at our own population the LLN is still 80fl
- CRP can help clarify if SF erroneously raised
- Ca patients: SF nearly always <30mcg/l
  - Those that do not, either the CRP may be raised, or their Retic Hb may be low.

- Essentially suggesting we need smarter requesting algorithms?
- Algorithms already in place of course
- Here are two examples:


Patient scheduled for GI surgery with Op date <2/52 timeframe*
Hb <120 g/l Male, <110 g/l Female, MCV <105 fl & eGFR >30ml/min
Check Serum Ferritin (SF) and MCHr
Gastro Iron optimisation bloods
SF≤30 mcg/l
SF ≤300 mcg/l but >30 mcg/l
SF>300 mcg/l
Give Intravenous Iron
Check MCHr
MCHr ≤27.5pg
MCHr >27.5pg
Not suitable for iron pathway. Discuss with Surgeon or Anaesthetist
Give Intravenous Iron
Intravenous iron less likely to be of benefit pre-op. Suggest repeat test post-op.

One + all | we care

Royal Cornwall Hospitals NHS
NHS Trust

Appendix 3. Pre-operative haemoglobin optimisation for elective surgery

Click here for the full guideline

Key: General Notes | GP/SWASFT | ED/MAU/SRU/Acute GP/Amb-Care | In-patient wards

Start

GP refers for Elective Surgery

Patient accepted onto elective surgery list

GP informed

Early PAC; FBC; CRP; Ferritin

Iron deficient, with or without anaemia, as defined by:
Ferritin <30 with CRP < 20
OR
Ferritin <70 with CRP > 20

Hb normal, Ferritin normal

Refer back to GP to decide whether investigation merited.

Ask GP to start oral iron replacement

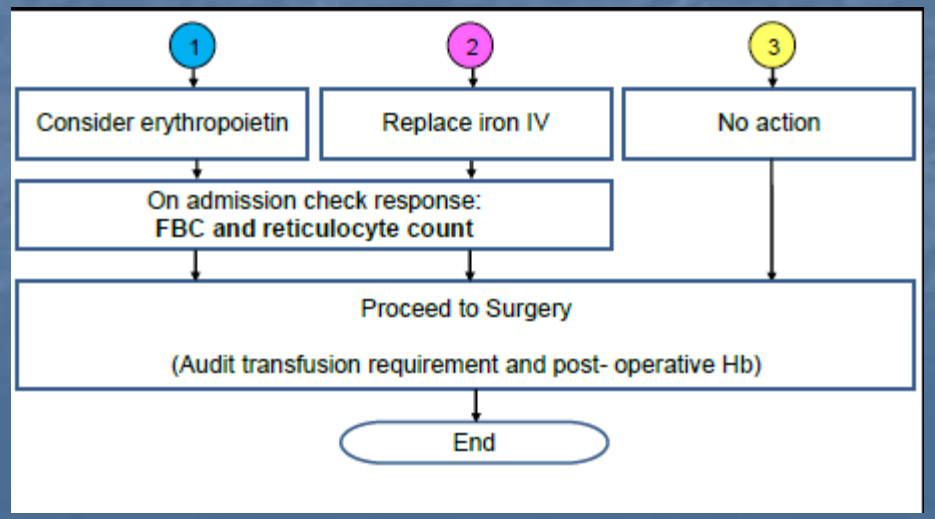
Pre-Admission Clinic 3-4 weeks before surgery
Recheck FBC, reticulocyte count and iron status

Hb< 12g/dl but not iron deficient

Iron deficient (w or w/o anaemia)

Normal FBC and Iron

1 2 3



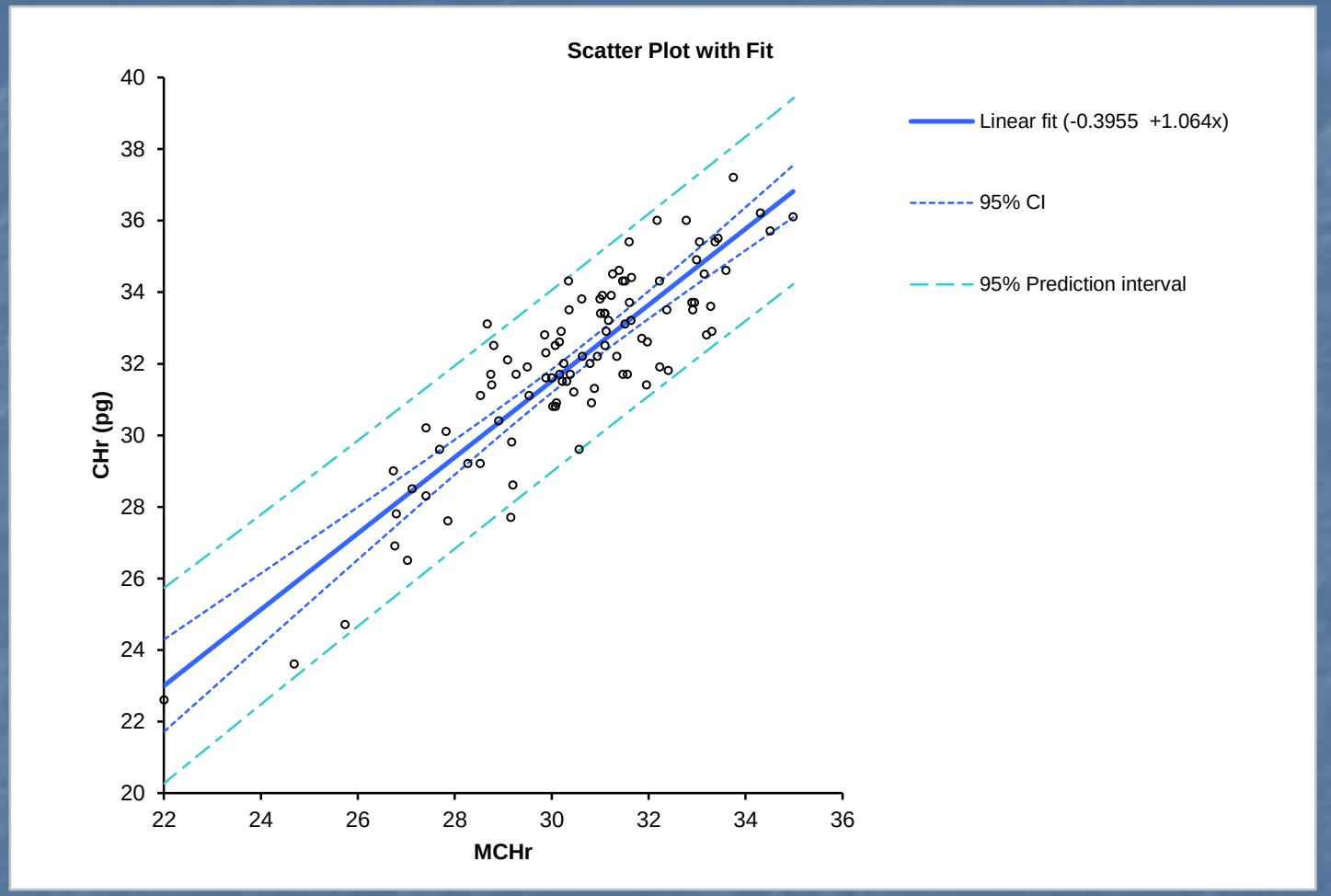
So, what about Reticulocyte Hb?

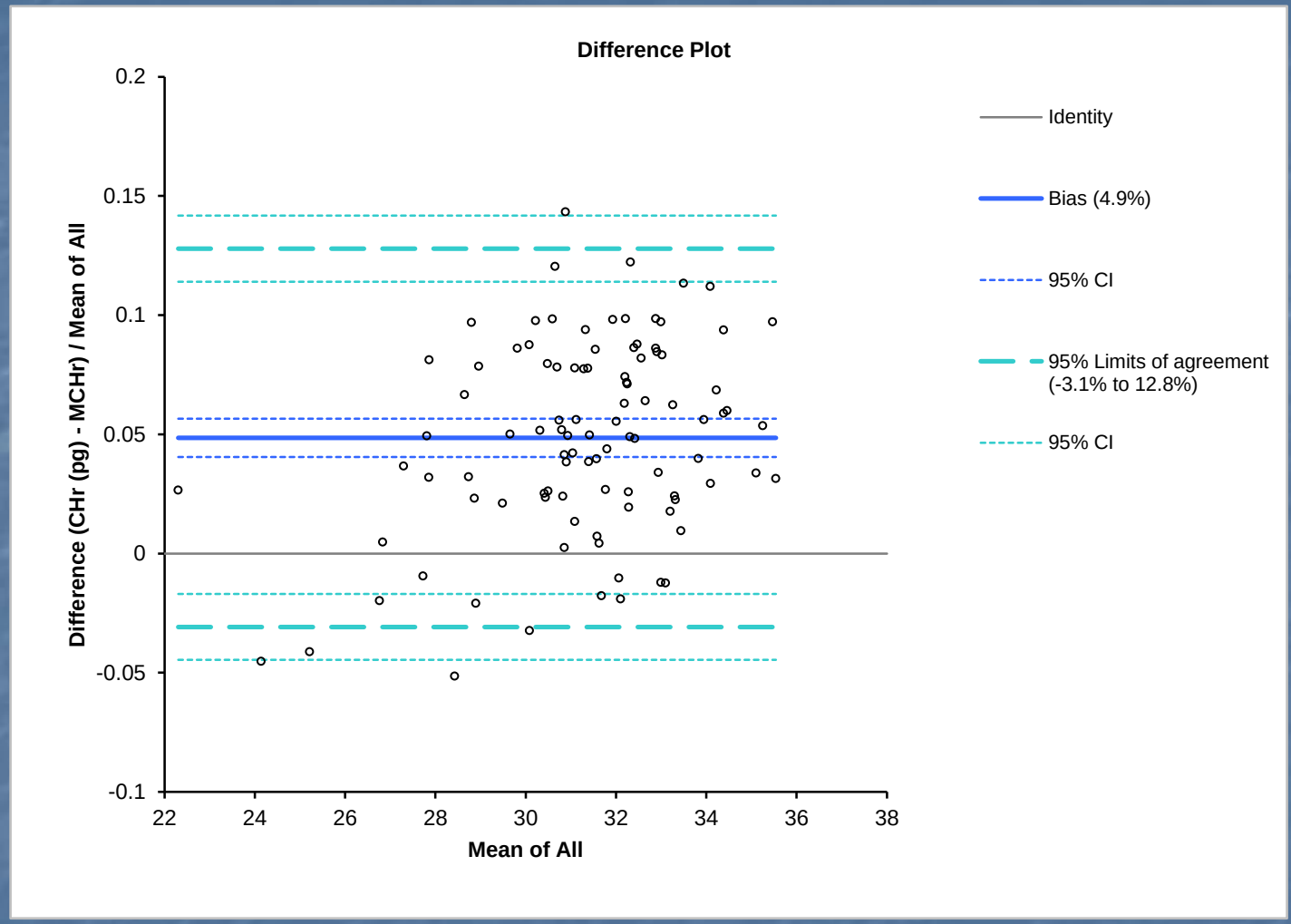
- Evidence suggests from NICE guideline NG8, Anaemia in patients with Chronic Kidney Disease
  - That in persons who do not have or carry Thalassaemia, CHr (or equivalent) is superior to either SF or %Tsat in addressing FID
  - SF not longer becomes a trigger value, but a ceiling value
    - For patients on HD, give iv iron up to SF of 800mcg/l

If you are:

- A Sysmex user, you can use Ret-He
- A Siemens user you can use %HRC or CHr
- An Abbott user, you can use MCHr

- And much of the evidence comes from CHr
- And you can use these tests for your pre-op optimisations too…why not?

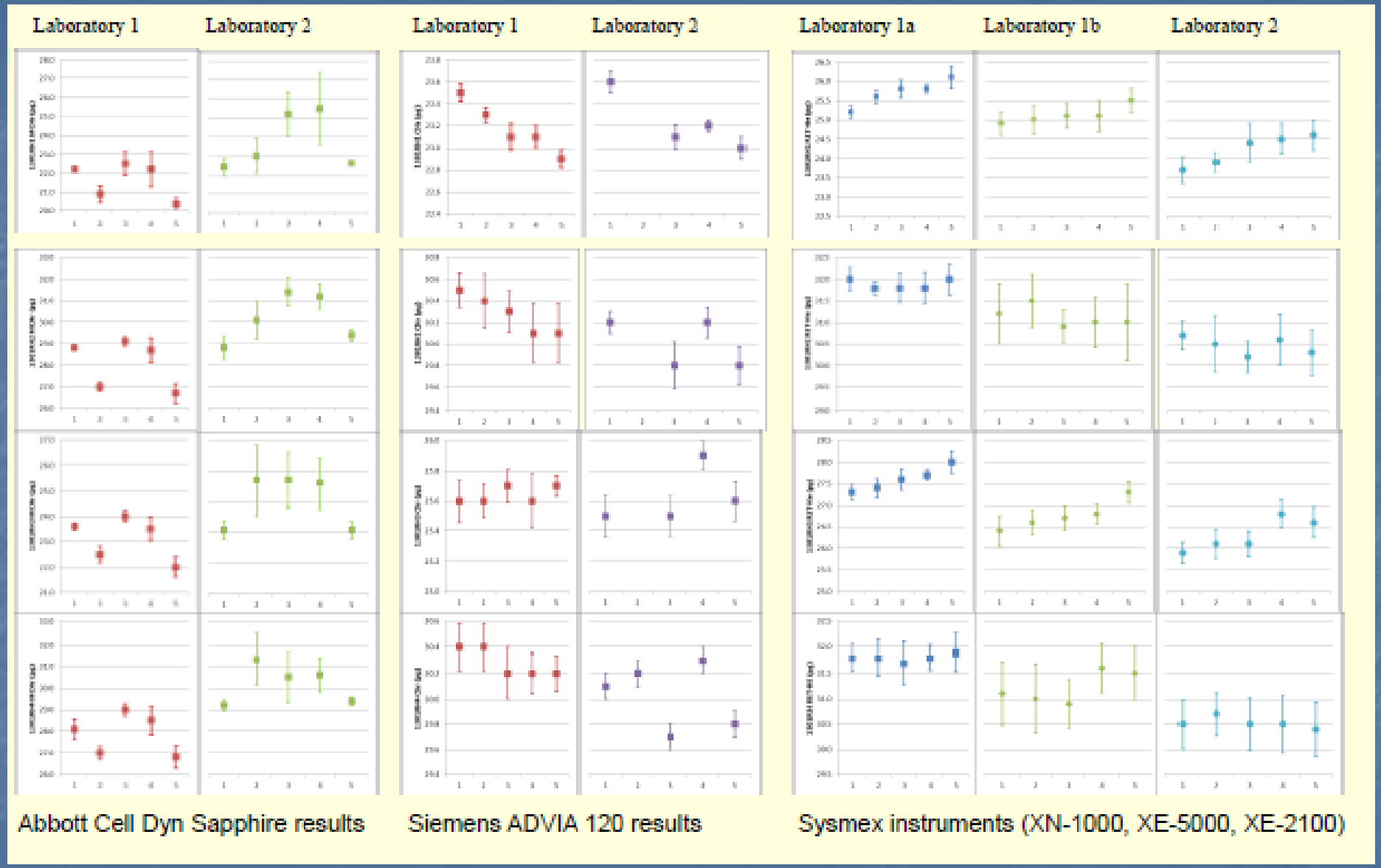

Scatter Plot with Fit
CHr (pg)
MCHr
40
38
36
34
32
30
28
26
24
22
20
22
24
26
28
30
32
34
36
Linear fit (-0.3955 +1.064x)
95% CI
95% Prediction interval


Difference Plot
Difference (CHr (pg) - MCHr) / Mean of All
Mean of All
0.2
0.15
0.1
0.05
0
-0.05
-0.1
22
24
26
28
30
32
34
36
38
Identity
Bias (4.9%)
95% CI
95% Limits of agreement
(-3.1% to 12.8%)
95% CI

The bottom line

- If there is hypochromia…why?
- If the Ret-He is low…why?
- You need to explain and explore the reasons

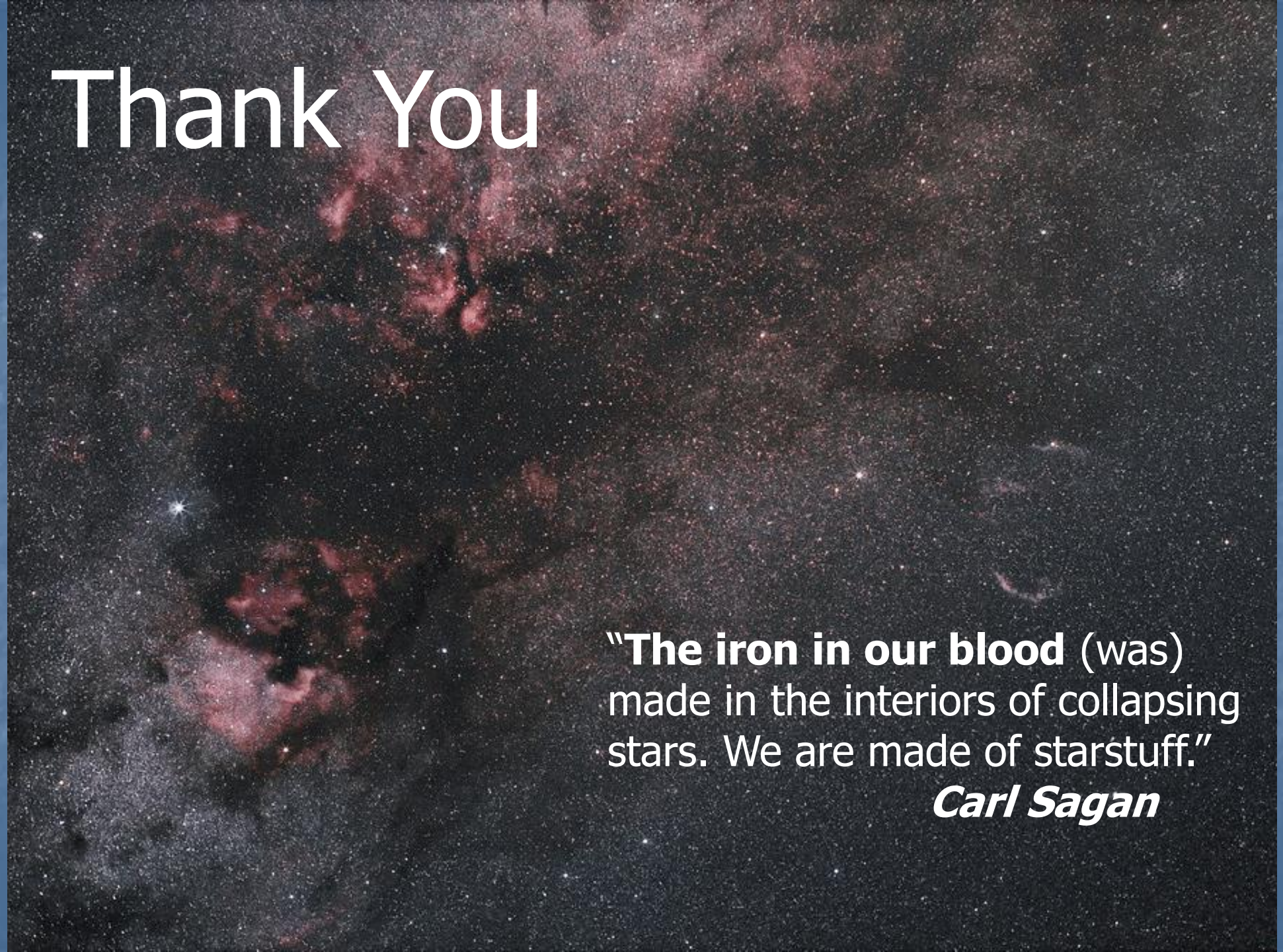
- We still though need to provide quality control around the Retic-Hb measurement


Laboratory 1
Laboratory 2
Laboratory 1
Laboratory 2
Laboratory 1a
Laboratory 1b
Laboratory 2
Abbott Cell Dyn Sapphire results
Siemens ADVIA 120 results
Sysmex instruments (XN-1000, XE-5000, XE-2100)

Future directions

- Combined analyte usage to help better define those who have absolute ID
- QA for our ID analytes
- Assess utility of hepcidin measurement
- Create a Best Practice guideline on laboratory testing for ID in adults and children

Thank You



"**The iron in our blood** (was) made in the interiors of collapsing stars. We are made of starstuff."

Carl Sagan