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

Dr Wayne Thomas
Derriford Hospital, Plymouth
Why does it matter?

- Over 30% of the World's population are anaemic, around 2 billion people.
- Most due to iron deficiency.
- Globally the causes are different:
  - Low iron diet, hookworm, other infections
  - 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
Dietary iron (Fe^{3+})

GI tract

Enterocyte

Plasma

Hepatocyte

Macrophage

Macrophage

Bone marrow

Iron overload

Inflammation

Iron deficiency

Erythropoiesis

HepH

Dietary haem

Transferrin

Holo-transferrin

Erythrocyte

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 protein 1

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 protein 1

TfR1

Transferrin receptor 1

HEPCIDIN

Bone marrow

Mitochondrial haem exporter

FerR

Ferrireductase
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**

<table>
<thead>
<tr>
<th>Method</th>
<th>N</th>
<th>Mean</th>
<th>CV%</th>
<th>U_95</th>
<th>SDPA</th>
<th>ERC.</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Methods</td>
<td>1.864</td>
<td>110.297</td>
<td>9.45</td>
<td>79.48</td>
<td>9.45</td>
<td>79</td>
</tr>
<tr>
<td>Abbott Architect Chemiluminescence</td>
<td>3.333</td>
<td>125.354</td>
<td>4.43</td>
<td>3.39</td>
<td>13.75</td>
<td>30</td>
</tr>
</tbody>
</table>

- **Year Result:**
  - SDI: 0.25
  - TDIt: Too Few

- **Mean for Comparison:**
  - 125.354
  - SDI: Too Few

- **RDIV:**
  - 3.1
  - SDI: Too Few

Acceptable limits derived from Biological Variation:
- 14.96

Acceptable limits of performance for RRJAS:
- 14.198
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 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.
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
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

- It's not unique to Sysmex
  - Using Abbott Sapphire we found:

<table>
<thead>
<tr>
<th>Ferritin value</th>
<th>&lt;5</th>
<th>6 to 9</th>
<th>10 to 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average MCV</td>
<td>83.3</td>
<td>84.14</td>
<td>85.86</td>
</tr>
<tr>
<td>Average MCH</td>
<td>26.87</td>
<td>27.16</td>
<td>27.44</td>
</tr>
<tr>
<td>Average HB</td>
<td>114.39</td>
<td>116.7</td>
<td>118.86</td>
</tr>
<tr>
<td>% MCH &lt;28</td>
<td>73.81</td>
<td>56.1</td>
<td>48.7</td>
</tr>
<tr>
<td>% MCV &lt;80</td>
<td>50.8</td>
<td>32.06</td>
<td>26.7</td>
</tr>
</tbody>
</table>
- 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*
And for all the 429 patients:
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

Give
**Intravenous Iron**

SF ≤300 mcg/l but >30 mcg/l

Check MCHr

MCHr ≤27.5pg

Give
**Intravenous Iron**

MCHr >27.5pg

Intravenous iron less likely to be of benefit pre-op. Suggest repeat test post-op.

Not suitable for iron pathway. Discuss with Surgeon or Anaesthetist

SF >300 mcg/l
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

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

Early PAC; FBC; CRP; Ferritin

Hb normal, Ferritin normal

Refer back to GP to decide whether investigation warranted.
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/o w/o anaemia)
Normal FBC and Iron

Consider erythropoietin
Replace iron IV
No action

On admission check response:
FBC and reticulocyte count

Proceed to Surgery
(Audit transfusion requirement and post-operative Hb)

End
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

- Linear fit: $(-0.3955 + 1.064x)$
- 95% CI
- 95% Prediction interval
Difference (MCHr (pg) - MCHR) / Mean of All

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

Carl Sagan