Investigating normocytic normochromic anaemia in adults

In this series, we present authoritative advice on the investigation of a common clinical problem, specially commissioned for family doctors by the Board of Continuing Medical Education of the Royal Australasian College of Physicians.

MEAGHAN WALL
MB BS, FRACP, FRCPA

ALISON STREET
MB BS, FRACP, FRCPA

Dr Wall is a Graduate Student, Research Division, Peter MacCallum Cancer Centre, East Melbourne, and Department of Medicine, Melbourne University, Melbourne.
Dr Street is Head, Haemostasis Thrombosis Unit, Alfred Hospital, Melbourne, Vic.

Series Editor
CHRISTOPHER S. POKORNY
MB BS, FRACP

Dr Pokorny is a member of the Board of Continuing Education, Royal Australasian College of Physicians, and a Gastroenterologist in private practice, Sydney, NSW.

In most cases of normocytic anaemia, a combination of clinical acumen, logic and a few well considered investigations will establish the diagnosis. Sometimes phone consultation with the laboratory haematologist is needed. Rarely, formal haematological referral of the patient and, possibly, bone marrow examination will also be needed before the cause of the anaemia can be determined.

Does my patient have normocytic anaemia?

Anaemia essentially refers to a reduction in red cell mass (the product of the number of circulating red blood cells and their size). The haemoglobin concentration, haematocrit and red cell count will all be low in an anaemic individual, but the most practical working definition of anaemia is probably a haemoglobin level below the lower limit of normal.[1] Defining a normal haemoglobin range depends on the use of appropriate statistical methodologies to identify and sample the normal population – a process that rarely impacts on clinical practice. However, it is worth remembering that the reference ranges quoted by the pathology service performing the assay are:

- statistically derived from a normal distribution, so that 2.5% of haemoglobin values from ostensibly healthy individuals will lie below the normal range defined by 95% confidence intervals
- age and sex dependent, so that incorrect or incomplete demographic data may lead to the result being compared to an inappropriate reference range and a 'wrong call' made about the presence or absence of anaemia.

Once anaemia is defined, many diagnostic algorithms then classify it as microcytic, normocytic or macrocytic according to the mean cell volume. This review focuses on the common causes for a normocytic anaemia in the adult population.

Starting the investigation – the total reticulocyte count

Today, sophisticated multiparameter automated

IN SUMMARY

- **Normocytic normochromic anaemia is common and has many possible causes.**
- **Its presence in a patient should alert the clinician to the possibility of organic disease.**
- **In most cases the cause can be elucidated on the basis of the clinical history and examination, together with a few simple laboratory tests.**
- **In more complicated cases, communication between the clinician and the laboratory haematologist is vitally important.**
- **In carefully selected cases, referral to a haematologist and/or bone marrow examination may hold the key to the diagnosis.**

haematology analysers are used to determine reticulocyte counts, overcoming some of the technical challenges and difficulties in the interpretation of previously used methods. These analysers identify reticulocytes on the basis of the binding of fluorescent dyes to their residual RNA and provide accurate and precise total reticulocyte counts.[2] By providing a reliable measure of the activity of the erythroid compartment within the bone marrow, the reticulocyte counts form the cornerstone of the diagnostic approach to normocytic anaemias.

The causes of normocytic anaemia can be divided into two groups according to whether the total reticulocyte count is:

- high (for example, >147.5 x 10^9/L),[3] with blood loss and haemolysis being the most likely culprits, or
- normal or low, with a more diverse range of causes being implicated (Table 1).

Helpfully, reticulocyte counts can be determined retrospectively on the same sample of blood that has already been collected for the full blood examination, provided the laboratory is notified within 72 hours or so of sample collection. Thus, when anaemia is an unexpected finding, repeat sample collection need not slow down the diagnostic process.

Conditions with a high reticulocyte count

Acute blood loss

The diagnosis of anaemia due to blood loss is usually readily apparent. In the unlikely event that blood loss is masked, clues to the aetiology on the blood film are the normal red cell morphology, together with polychromasia, and acute reactive changes of neutrophilia and/or thrombocytosis. The haemolytic screen is negative, prompting the clinician to explore in more detail the possibility of covert bleeding.

Haemolytic anaemias

Conditions that cause haemolysis are numerous and varied. Successful management hinges on the ability to recognise that a haemolytic process is the cause of the anaemia, leading to an appropriate and timely referral of patients for specialist review. The appearance of the blood film in many of these conditions is distinctive (Figure 1), so the laboratory comment on the blood film will often suggest the diagnosis or contain a list of differential diagnoses. Indeed, this category of anaemias includes some conditions with an alarming looking peripheral blood appearance (e.g. the microangiopathic anaemia thrombotic thrombocytopenic purpura [TTP; see Figure 1]), and the requesting doctor may receive an urgent phone call from the pathology laboratory when examination of the blood film raises the possibility of a haemolytic process.

Clinically, anaemia (which is usually symptomatic) and scleral icterus are common to all haemolytic conditions. (Scleral icterus due to haemolysis is known as acholuric jaundice because the water insoluble unconjugated bilirubin is not renally excreted, leading to a negative urinary dipstick result for bilirubin.) The laboratory diagnosis of haemolysis cannot be made on the basis of a single test (including a positive direct Coombs' test in isolation) but relies on the results of a constellation of investigations – the so called haemolytic screen (Table 2).

Due to the diversity of conditions that cause haemolysis, the key to diagnosis is interpreting parameters of haemolysis in light of the clinical context, as shown in the following examples.

- A young man with a fever, sore throat and cervical lymphadenopathy together with a blood film that shows red cell agglutination and a Coombs' test that is positive for complement, most likely has cold agglutinin disease associated with Epstein Barr virus infection.
- An anaemic elderly woman with spherocytes on the blood film, a Coombs' test that is positive for IgG, and a past history of chronic lymphocytic leukaemia probably has warm autoimmune haemolytic

Table 1. Causes of normocytic normochromic anaemia

Increased reticulocyte count

- Acute blood loss
- Haemolytic anaemia*

Normal or reduced reticulocyte count

- Anaemia of chronic disease†
- Renal anaemia
- Hypersplenism
- Endocrine disease
  - hypothyroidism*
  - hyperthyroidism†
  - hypoadrenalism
  - hyperparathyroidism
  - hypopituitarism
  - hypogonadism
- Primary bone marrow diseases
  - aplastic anaemia or pure red cell aplasia
  - bone marrow infiltration or replacement by haematological malignancy, nonhaematological malignancy or 'benign' conditions
- Myelosuppression due to drugs or alcohol
- Physiological anaemia of pregnancy

Conditions that are usually microcytic hypochromic, but may be normocytic normochromic (or normocytic hypochromic)

- Iron deficiency

Conditions that usually cause macrocytic anaemia but may be normocytic normochromic

- Masked megaloblastic anaemia (e.g. vitamin B_{12} or folate deficiency plus co-existing iron deficiency or thalassaemia)
- Myelodysplastic syndromes

* May also be macrocytic; † may also be microcytic.

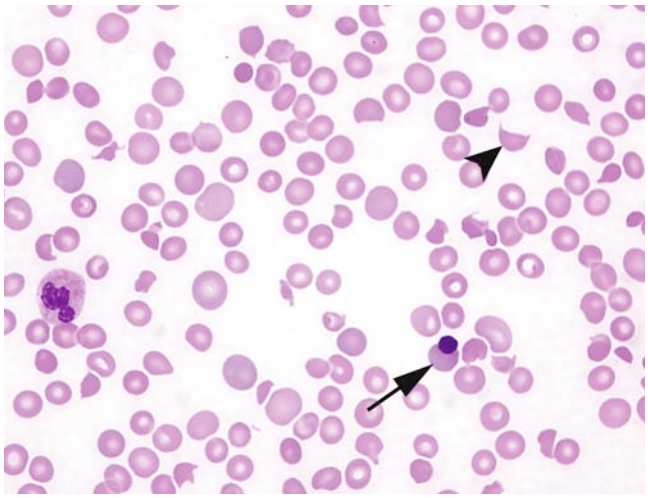


Figure 1. Peripheral blood film of a patient with thrombotic thrombocytopenic purpura showing red cell fragmentation (arrowhead), polychromasia and a circulating nucleated red blood cell (arrow). Platelets are virtually absent.

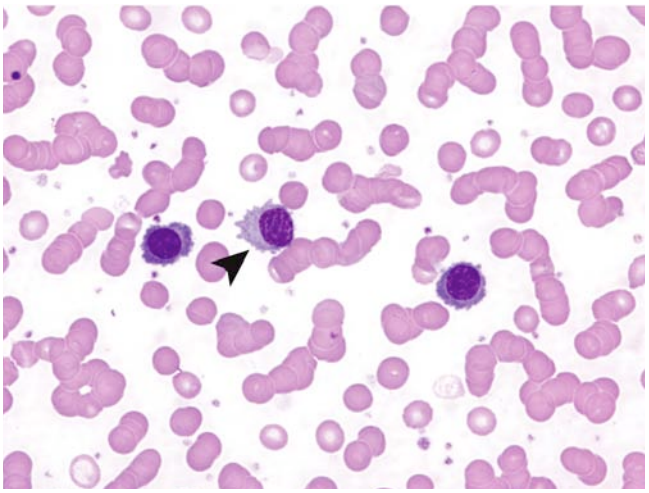


Figure 2. Peripheral blood of a patient with myeloma showing increased rouleaux formation and circulating plasma cells (arrowhead).

anaemia secondary to lymphoproliferative disease.
- A middle aged woman with splenomegaly, spherocytic anaemia, a negative Coombs' test and a family history of anaemia probably has hereditary spherocytosis.
- A woman who recently started taking dapsone to treat rheumatoid arthritis and has a blood film that shows bite and blister cells is likely to have a drug-induced oxidative haemolysis.
- A man of Mediterranean background with severe anaemia after eating broad beans and the same peripheral blood appearance as the woman described above probably has glucose-6-phosphate dehydrogenase deficiency.

It is vital to have a high index of clinical suspicion for the rare microangiopathic disorder TTP with its classical presenting pentad of fever, neurological symptoms, renal impairment, haemolytic anaemia and thrombocytopenia. TTP can progress rapidly and may be fatal without the prompt institution of therapy, so urgent referral for hospital admission and specialist evaluation is warranted when a patient has microangiopathic anaemia and a low platelet count.

Conditions with a decreased or normal reticulocyte count

Anaemia of chronic disease

Anaemia of chronic disease (ACD) stems from the effects of proinflammatory cytokines on iron metabolism, leading to defective recycling of iron from intramedullary histiocytes to red cell precursors and decreased red cell production. The response is adaptive in so far as sequestering iron in histiocytes means that it is not available to aid the replication of pathogenic micro-organisms or tumour cells. In keeping with an effective host defence strategy, ACD is rarely profound.

Generally, expect the haemoglobin to be 70 g/L or more in uncomplicated cases.

The anaemia may be seen in the context of established disease or may be the presenting symptom that prompts a search for a disorder such as an autoimmune disease, chronic infection, inflammation or neoplasia. It can be normocytic or microcytic and occur with a raised platelet count and lymphopenia. These features, together with rouleaux formation on the blood film, an increased erythrocyte sedimentation rate or C-reactive protein level and a polyclonal hypergammaglobulinaemia are highly suggestive of ACD. Iron studies classically show low serum iron, low serum transferrin and elevated serum ferritin levels.

Table 2. Markers of haemolysis

Biochemical markers
- Serum bilirubin: increased unconjugated bilirubin, usually <100 mmol/L
- Lactate dehydrogenase (LDH): increased
- Haptoglobin: usually decreased, may be undetectable in marked intravascular haemolysis

Haematological markers
- Blood film: often provides a vital clue to the likely cause of haemolysis
- Reticulocyte count: usually raised
- Direct Coombs' test (DCT): usually positive in haemolytic anaemias that have an immune basis

Before you ascribe any anaemia to chronic disease, consider the key role that inflammatory cytokines have in the pathogenesis of the disease. Normocytic anaemia in a patient with any chronic disease that lacks an identifiable inflammatory component should trigger a search for an alternative diagnosis.

Renal anaemia

Renal anaemia is primarily due to reduced erythropoietin (EPO) secretion by the failing kidney. Anaemia usually becomes apparent when the creatinine clearance falls below about 50 mL/min but may occur earlier in patients with diabetic renal disease.[4,5] Sometimes, iron deficiency, chronic disease or ongoing red cell losses due to haemodialysis may compound the anaemia. A calculated creatinine clearance consistent with the above level of renal impairment is usually sufficient to make the diagnosis.

The successful introduction of recombinant EPO therapy for renal anaemia has transformed the management of this condition over the last decade or so. However, its widespread use has also been associated with an increase in the number of cases of pure red cell aplasia (PRCA) secondary to the development of anti-EPO antibodies.[6] Recent changes in policy and procedures relating to the formulation, handling and administration of these products have seen rates of PRCA subside; however, because of the serious nature of this complication, we recommend recombinant EPO be initiated only in consultation with a renal physician.

Hypersplenism

Moderate degrees of splenomegaly are often associated with mild pancytopenia. The circulating blood volume is expanded and has a dilutional effect on the haemoglobin concentration, and a greater proportion of blood cells are sequestered in the spleen and not sampled by venepuncture. Where the aetiology of the splenomegaly is infectious, inflammatory or haematological, anaemia is often multifactorial. The clinical and laboratory features of the primary condition are likely to predominate in such cases, guiding the diagnostic strategy. Occasionally, the incidental discovery of mild anaemia (which is almost invariably accompanied by thrombocytopenia) may herald the discovery of hitherto unrecognised liver disease with portal hypertension.

Endocrine disease

Endocrinopathy is often overlooked as a cause of anaemia. Paradoxically, it is the bland nature of the haematological presentation that points to endocrine dysfunction as a likely aetiological factor. Typically, the anaemia is isolated and mild to moderate in degree, and the blood film is usually nondiagnostic. Clinically it may be well compensated or asymptomatic. In fact, anaemia may even be 'appropriate' in that metabolism is slowed because reduced oxygenation (and therefore haemoglobin requirements) is a consequence of the endocrine dysfunction.[7] In the face of such an underwhelming haematological picture, it is worth performing a thorough clinical and biochemical assessment of endocrine function (Table 1).

Primary bone marrow disease

Bone marrow disorders are usually distinguished by:

- moderate to marked pancytopenia
- a combination of anaemia plus a strikingly abnormal white cell count or platelet count
- a suggestive or diagnostic blood film (e.g. the leucoerythroblastic blood film of marrow fibrosis or the circulating blasts of marrow infiltration or leukaemia)
- a low total reticulocyte count.

Formal diagnosis of conditions included under this diagnostic umbrella almost always requires specialist review and bone marrow examination. While a seemingly inexhaustible number of conditions may be responsible for this type of presentation, those listed in Table 1 offer a concise classification.

One noteworthy presentation in this category is the middle aged or elderly patient with myeloma presenting with a combination of anaemia, bone pain, hypercalcaemia and renal impairment. Red blood cell rouleaux are often marked, and careful inspection of the blood film may reveal circulating morphologically atypical plasma cells (see Figure 2). The diagnosis is confirmed by the detection of:

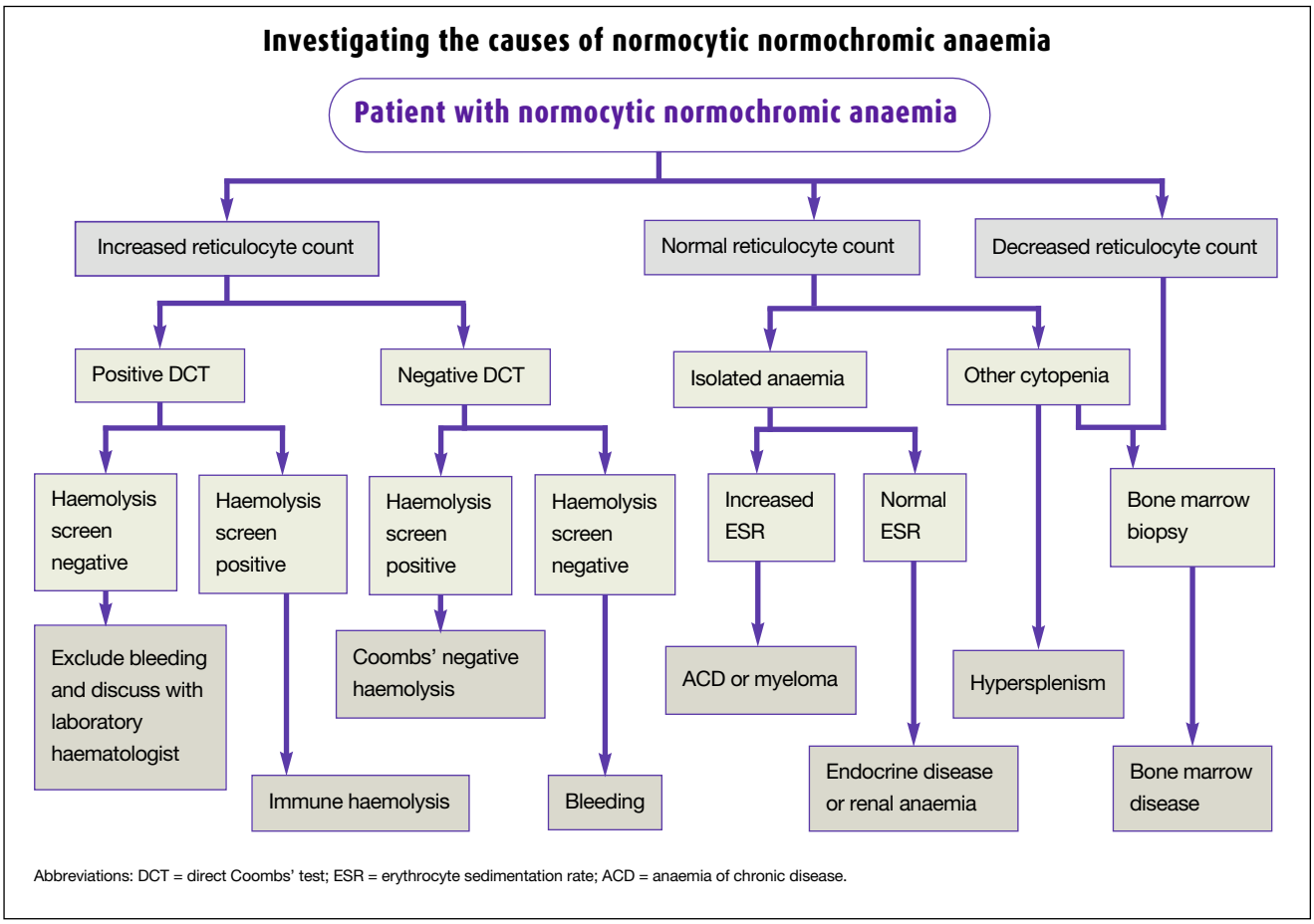
- a monoclonal gammopathy by immunoelectropheresis of blood and urine
- lytic lesions on the skeletal survey
- increased numbers of malignant plasma cells in the bone marrow.[8]

Myelodysplasia may be among the more subtle but common presentations in this category of disorders. Refractory anaemia, for example, may manifest itself as a mild to moderate persistent anaemia of unclear aetiology. This means that bone marrow examination is a reasonable diagnostic and prognostic strategy in an elderly person with a stable or slowly progressive anaemia in whom all other causes have been excluded by less invasive means.

A comprehensive drug history is essential when bone marrow dysfunction (or haemolysis) is suspected. When the blood film is unremarkable and there is pancytopenia or leucopenia/thrombocytopenia, medications and alcohol are often implicated.

Physiological anaemia of pregnancy

Anaemia is a consequence of a greater expansion in plasma volume than red cell mass during pregnancy, leading to a haemodilution. An observable reduction in haemoglobin from a baseline normal female adult range (e.g. 115 to 160 g/L) is apparent from around weeks 6 to 8 of pregnancy and continues until a plateau is reached around weeks 16 to 20, at an average level of about 110 g/L.[7] Coexisting,



easily treatable nutritional deficiencies (especially iron deficiency) are not uncommon in pregnancy, and pregnancy is no guarantee against developing organic disease manifesting as normocytic anaemia. Thus anaemia that deviates significantly from the typical pattern for physiological anaemia in a pregnant woman in terms of timing or severity should prompt clinical review and investigations designed to detect pathological anaemias.

Anaemia in the elderly

Epidemiological studies of the prevalence of anaemia in the elderly have led to ongoing controversy about the significance of mild, unexplained anaemia in people aged over 65. Even allowing for disagreement over the place of age-adjusted reference ranges, evidence is mounting to suggest that the anaemic elderly have higher mortality and poorer functional outcomes than matched non-anaemic cohorts. Thus anaemia is probably not a physiological consequence of ageing; however, as yet, it is not known if correction of mild unexplained anaemia (by transfusion or other means) will translate into improved patient outcomes.[9]

Investigation and management of anaemia in the elderly may be one of the most difficult challenges in clinical medicine. The diagnostic flowchart on this page needs to be prudently applied on a case by case basis to maximise the yield of correctable anaemias while minimising the risk to frail patients from invasive procedures or ineffective treatments.

For the adult population as a whole, there is likely to be a subset of patients in whom mild anaemia remains unexplained after investigation. It may be that a diagnosis will become apparent only after ongoing clinical and haematological monitoring.

Conclusion

As you and your patient proceed down the diagnostic algorithm for investigating these anaemias, remember that you are not alone. The laboratory haematologist is as interested in finding an explanation for the anaemia as you are, makes a valuable ally and is only a phone call away. MT

A list of references is available on request to the editorial office.

DECLARATION OF INTEREST: None.

Medicine Today 2006; 7(3): 43-49

Investigating normocytic normochromic anaemia in adults

MEAGHAN WALL MB BS, FRACP, FRCPA **ALISON STREET** MB BS, FRACP, FRCPA

References

1. Lee, GR. Anaemia: general aspects. In: Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM, eds. Wintrobe's clinical haematology. Baltimore: Williams and Wilkins; 1999: 897-907.
2. D'Onofrio G, Zina, G, Rowan RM. Reticulocyte counting: methods and clinical applications. In: Rowan RM, van Assendelft OW, Preston FE, eds. Advanced laboratory methods in haematology. London: Arnold; 2002; 78-126.
3. Perkins SL. Appendix A. Normal blood and bone marrow values in humans. In: Lee GR, Foerster J, Lukens J, et al, eds. Wintrobe's clinical haematology. Baltimore: Williams and Wilkins; 1999: 2738-2748.
4. Astor BC, Muntner P, Leven A, Eustace JA, Coresh J. Association of kidney function with anaemia: the Third National Health and Nutrition Examination Survey (1988-1994). Arch Intern Med 2002; 162: 1401-1408.
5. El Achkar TM, Ohmit SE, McCullough PA, et al. Higher prevalence of anaemia with diabetes mellitus in moderate kidney insufficiency: The Kidney Early Evaluation Program. Kidney Int 2005; 67: 1483-1488.
6. Bennett CL, Luminari S, Nissenson AR, et al. Pure red cell aplasia and epoetin therapy. N Engl J Med 2004; 351: 1403-1408.
7. Lee GR. The anaemias associated with renal disease, liver disease, endocrine disease, and pregnancy. In: Lee GR, Foerster J, Lukens J, et al, eds. Wintrobe's clinical haematology. Baltimore: Williams and Wilkins; 1999: 1497-1517.
8. Jaffe ES, Harris NL, Stein IL, Vardman JW, eds. World Health Organization Classification of Tumours. Pathology and genetics of tumours of haemopoietic and lymphoid tissues. Lyon: IARC Press; 2001.
9. Guralnik JM, Eisenstaedt RS, Ferruccl L, Klein HG, Woodman RC. Prevalence of anaemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anaemia. Blood 2004; 104: 2263-2268.