

## Aplasia versus pancytopenia, including the pure red cell variant

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This is the first in our third series of clinical vignettes that are centred on everyday presentations. Each emphasises practical aspects of team-based care, having relevance for general practitioners, specialists and paramedical professionals alike. Disorders arising in blood and bone marrow result in some of the commonest symptoms and signs for which patients seek medical advice. A carefully taken history and meticulous physical examination, complemented by thoughtful and judicious use of laboratory tests, provide the clinical basis for a working diagnosis. Advanced haematology may be needed to extend evaluation to the bone marrow and plasma while supplementary imaging or radionuclide technology further links primary care practitioner or specialist to the experienced haematologist. Such a multidisciplinary and fully interactive approach offers the preferred way to problem solving and optimum management.

### Aplasia

A frequent clinical and haematological question is what pathological process has caused the full blood count result, also sometimes called the haemogram, to become abnormal? This is particularly important when one or other peripheral value is significantly raised or, conversely, reduced. In the latter instance involvement of a single lineage, giving rise to anaemia or thrombocytopenia, has vastly different implications than when all three cell types are below normal – the entity of pancytopenia. The cardinal first step is to separate global bone marrow failure or aplasia from hypercellularity with ineffective production or intramedullary destruction, also known as shunting, that occurs with vitamin B<sub>12</sub> and folate deficiency. This needs to be distinguished from the myelodysplastic (preleukaemic) syndromes. The variability in presentation can be misleading and, to rapidly arrive at the correct interpretation of the patient's problem, it is prudent to seek explanation in how normal (i.e. physiological) structure and particularly function are disturbed – causation or pathophysiology.

### Physiology

The bone marrow continuously produces, and delivers daily, to the circulating blood 2.5 billion new red cells, 100 - 500 million granulocytes and a further vast number of lymphocytes, together with 2.5 billion platelets at a rate to exactly match loss by the body through utilisation and natural ageing or death in the process, described as apoptosis. These balanced processes dynamically and rapidly respond to any changes in the internal environment where individual lineages selectively accelerate singly or in any appropriate combination, to maintain levels within narrow ranges and so protect tissue or organ functional integrity.

The spongy medullary cavity, sandwiched between layers of dense supporting cortical bone, is where the immunohaematopoietic stem cells home to receptors through particular patterns of adhesion molecules, grow and mature in specialised endosteal areas called niches (Fig. 1). In this specialised microenvironment they are nurtured by

the supporting architecture derived largely from mesenchyme and its derivatives with a wide range of locally secreted growth factors (Fig. 2).

With appropriate stimuli the first division leads to replenishment of this stem cell pool as a unique and defining characteristic. The daughter cell undergoes *commitment* to blood formation via the haemangioblast that can give rise to vascular endothelium and the full range of haematopoietic cells, collectively known as the haematon. *Differentiation* separates lineages – one will result in both B and T lymphocytes that fulfil immunological function. The second, collectively described as myeloid, generates red cells or the erythron, granulocytes and monocytes as well as megakaryocytes from which platelets will ultimately be derived (Fig. 3). Within each lineage the earliest progeny are known as *progenitors* and not morphologically identifiable, needing the use of *in vitro* laboratory culture techniques. These give rise to *precursors* that are microscopically recognisable as normoblasts comprising the red cells series that include erythroid islands (Fig. 4)

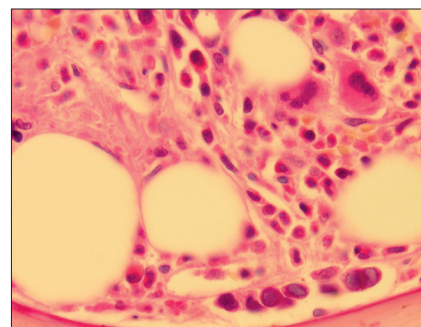


Fig. 1. The bone marrow niche.

## Practical haematology

central to regulation of iron traffic between the stores in macrophages and haemoglobin synthesis in this population.

At the same time, and occurring approximately 3 times more frequently, a range of granulocytes and monocytes develop that acquire quite different cytomorphological characteristics during their growth (Fig. 5). Megakaryocytes are

also formed during myelopoiesis and these ultimately liberate cytoplasmic contents into the circulation as platelets.

*Proliferation* is best examined by focusing on granulocytes. Consecutive phases vary, giving rise to a kinetically distinct mitotic pool in which numbers expand to include the myeloblasts, progranulocytes and myelocytes – loosely

referred to as the fixed components of the microenvironment. *Maturation* occurs in a post-mitotic compartment, and is made up of metamyelocytes, band forms and mature neutrophils, and allows the cells to reach full functional state prior to the release into the circulation: also called the labile compartment or pool. *Apoptosis*, intramedullary death that is sometimes known as shunting, provides a system

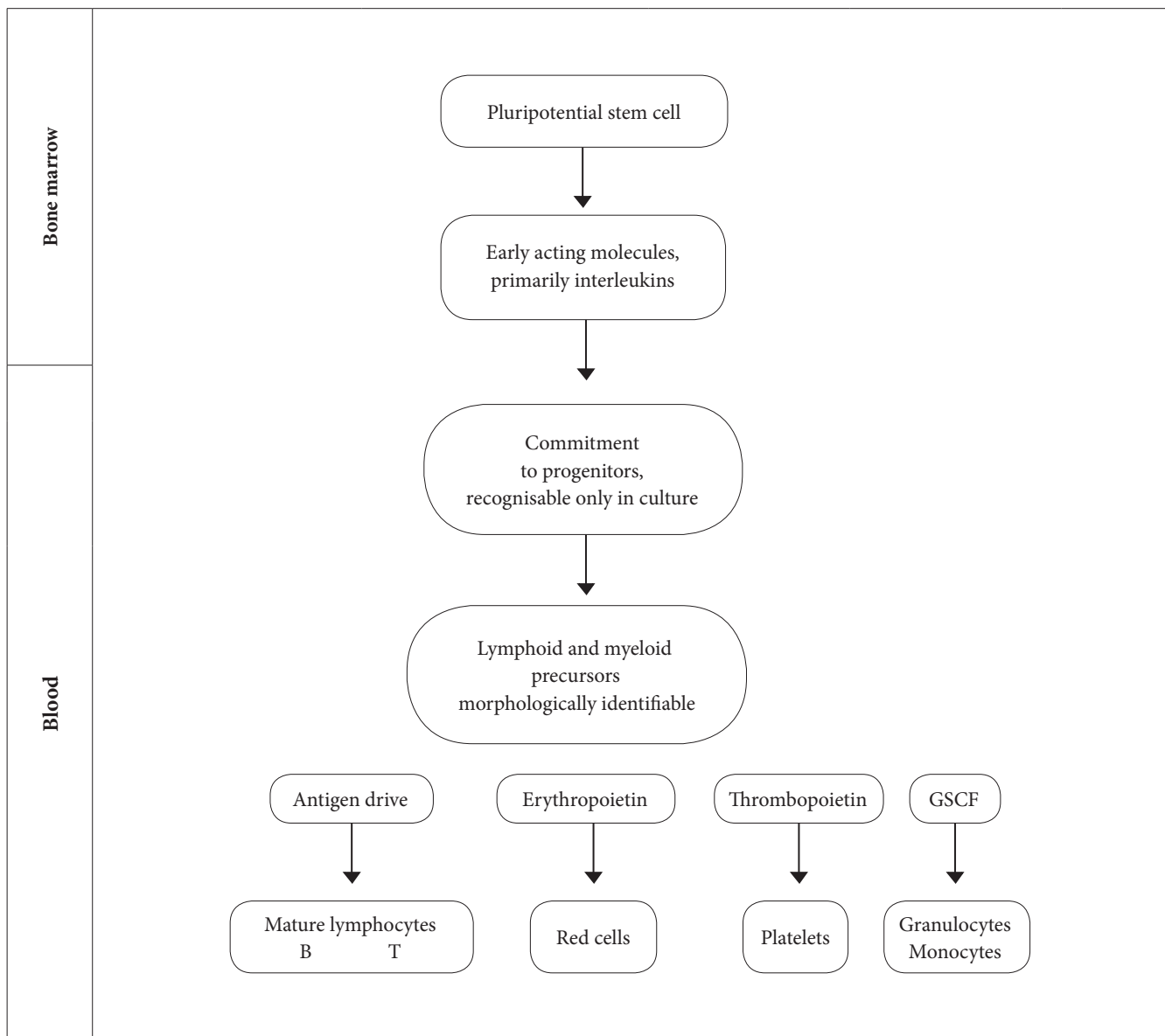


Fig. 2. Growth or stimulatory factors. The orderly development, and then delivery of mature cells to the peripheral blood is moderated by changing patterns of migration through the marrow. An array of molecules, or peptides, has a distinct pattern of expression during the various stages of differentiation, proliferation and maturation. These steps are coupled with alterations in adhesion factors that govern release from the niche and balance this exactly to intramedullary destruction or apoptosis, as dictated by physiological requirements. The focused activity of these cytokines can be recapitulated with commercially available equivalents to selectively drive each line in therapy: e.g. erythropoietic stimulating factors for erythrocytes and granulocyte or monocyte stimulating agents (G-CSF) in the case of these two lineages.

Progenitors		Pluripotential stem cell				Lymphoid	
		Myeloblast	Monoblasts	Megakaryocyte	Erythroid		
Marrow pool precursors	Mitotic	Promelocytes Myelocytes	Promonocytes	Maturing megakaryocytes	Normoblasts	Pre-B	Pro-T
	Post-mitotic	Metamyelocytes bands					
Blood and tissue function		Neutrophils, eosinophils and basophils  Innate immunologic function	Monocytes  Macrophage	Platelets  Haemostasis	Erythrocytes  Oxygen transport	B lymphocytes and plasma cells  Adaptive immunologic function	T-lymphocytes and NK cells

Fig. 3. Kinetics of blood cell generation – the concept of pools or compartments. Totipotentiality is found only when sperm fuses with ovum to create a zygote. Immediately there is restriction described as pluripotentially governing all tissue and organ development, well-exemplified in immunohaematopoiesis. Here the different lineages diverge into lymphoid and myeloid with the latter further narrowed down to give rise to red cells, the platelet-megakaryocyte system in parallel with earliest monocytes and granulocytes.

of quality control in which about 15% of the daily production is removed by the phagocytic cells of the reticuloendothelial system and never circulate – a similar pattern is seen in the other cell lines.

These stages can be disordered at a number of different levels and may affect one or more lines. Global production failure defines aplasia while a single lineage is described as cytopenia. This is a fundamental distinction upon which is based classification that will, in turn, dictate further investigation en route to individualised comprehensive management.

## Pathophysiology

### Clinical<sup>1</sup>

Many individuals with aplasia, particularly in the early phases or during disease evolution, are asymptomatic. Presentation is typically fatigue, with decreasing tolerance and palpitations that correlates roughly with the rate as well as fall in haemoglobin level.<sup>2</sup> Less commonly pale skin, headache and dizziness are the first warning signs. Recurrent bacterial infections occur due to neutropenia that may be compounded by leucocyte dysfunction.

Low platelet counts result in a primary haemostatic defect with prolonged bleeding

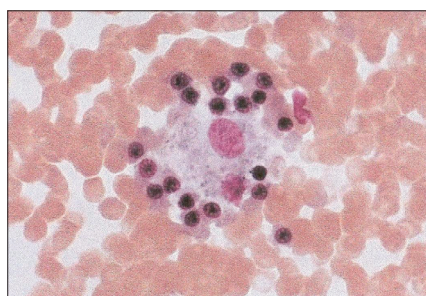
from cuts and less often accompanied by epistaxis while oozing from the gums is an early warning sign.<sup>3</sup> Once levels drop below 10 or 15 x 10<sup>9</sup>/l mucocutaneous bruising, particularly at sites of relatively minor injury, is characteristic and may be aggravated by concurrent use of antiplatelet drugs such as aspirin or non-steroidal anti-inflammatory agents.

### Haematological

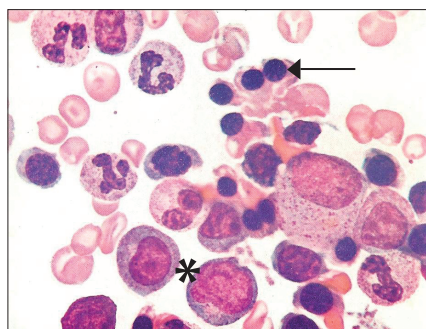
The first step is meticulous examination of peripheral blood count values to make the initial crucial distinction between single cytopenia such as anaemia, low white count or reduced platelets, in contrast to

pancytopenia in which all the levels are sub-normal or pancytopenia is a problem.

It is difficult to overemphasise the importance of supplementing instrument-generated figures in the haemogram by careful scrutiny of a well-prepared blood



**Fig. 4. Erythroblastic island.** Iron traffic to maturing red cell population, the normoblasts, broadly described as the erythron, are in part furnished by recovery from the diet via enterocytes in the small bowel. Simultaneously, recycling of this trace metal from effete red cells in the macrophage system is exemplified by those in the bone marrow. The regulation is genetically determined and modulated by expression of the iron exporter known as ferroportin. The latter is under surveillance and regulation by the master regulator – the hepatic peptide known as hepcidin.



**Fig. 5. Bone marrow precursors – aspirated sternal sample.** The ratio of myeloid to erythroid cells (shown by arrow), or the M-E ratio is physiologically 3 to 1. A range of granulocytes are recognisable from neutrophils to the earlier myelocytes (shown by \*) distinguished by azurophilic granules in the cytoplasm. Megakaryocytes are large and prominent (not present). Both erythroid and myeloid lines undergo varying degrees of maturation prior to release into the circulation.

film or smear. Cytomorphologically, the distribution of mature cells in the peripheral blood can be enumerated in a differential leucocyte count. Particularly relevant at this point is establishing total marrow activity as reflected in the reticulocyte count, corrected for the haemoglobin level, to generate the production index that provides a ready clinical assessment to quantitate overall function of the haematon reflected primarily in the red cells series.<sup>4</sup>

With this information it is logical to directly examine the bone marrow by means of aspiration coupled with trephine biopsy (Fig. 6). This remains the central investigation to secure the initial working diagnosis.

Once a production defect due to global marrow failure or aplasia is confirmed, a number of different explanations arise for loss of precursor cells in the marrow (Fig. 7). Here the experienced haematologist is needed to direct confirmatory investigations as a basis for treatment.

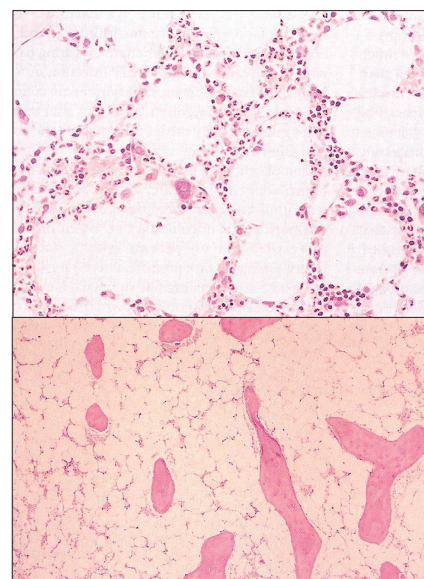
### Causation

Of the *inherited* disorders, Fanconi anaemia may present late and with minimal anatomical abnormalities which can be overlooked, making recognition of the syndrome difficult. With the advent of molecular testing telomerase mutations and other genetic defects are becoming diagnosable. *Acquired* injuries include irradiation and a wide spectrum of cytotoxic drugs and industrial toxins, among which are benzene and a number of relatively commonly used medications. A surprising range of viral infections have been incriminated, including hepatitis, parvovirus and, in sub-Saharan Africa of particular significance, HIV (Fig. 8).

Once these possibilities have been excluded immune-mediated mechanisms, supported by experimental and clinical observations, become the basis for selection of suppressive interventions. These range from antithymocyte globulin, with or without cyclosporin, determined by severity of disease. Other choices are mycophenolate mofetil and cyclophosphamide.<sup>5</sup>

### Diagnostic algorithm

It cannot be over-emphasised that this possibility requires immediate referral to an appropriate academic centre, so avoiding problems with both under- and over-diagnosis and preventing inappropriate treatment or side-effects.



**Fig. 6. The trephine biopsy.** Normally between 60% and 80% of the marrow is occupied by blood-forming tissue with the architectural arrangement best appreciated from trephine biopsy shown in the top panel. In contrast, loss of haematopoiesis depletes these precursors, leaving residual stroma comprising delicate reticulin, lymphocytes, monocytes and abundant fat spaces.

#### Ineffective haematopoiesis

- B<sub>12</sub> or folate deficiency
- Myelodysplasia
- Severe infections

#### Extensive infiltration

- Malignancy
- Fibrosis

#### Aplasia

- Congenital
- Acquired

**Fig. 7. Pancytopenia due to marrow failure.** Diagnosis may be reflected in the peripheral blood smear. The cardinal investigation is examination of the bone marrow aspiration and trephine biopsy.

<b>Cytotoxic drugs and radiation</b>
<b>Idiosyncratic drug reaction</b>
Chloramphenicol
Non-steroidal anti-inflammatory
Sulphonamides
<b>Industrial toxins</b>
Benzene
<b>Viral infections</b>
Hepatitis
HIV
Epstein-Barr virus
<b>Immune disorders</b>
Systemic lupus erythematosus
<b>Miscellaneous</b>
Paroxysmal nocturnal haemoglobinuria
Thymic tumours
Pregnancy
<b>Idiopathic or primary</b>

Fig. 8. Causes of bone marrow aplasia. In some cases the cause is apparent and predictably reversible – only careful observation is needed. In others it may be obscure and have an immunological basis. The relevance of accurate diagnosis is choice of therapy and options extend from support and monitoring to active intervention ranging from immunosuppression to allogeneic bone marrow transplantation.

While symptomatic anaemia or thrombocytopenic mucocutaneous bleeding may be the presenting symptoms, the most ominous association is with recurrent bacterial infections as a result of profound neutropenia and dysfunctional innate immune system. It is impossible to sufficiently stress the importance of a thorough history and clinical examination preceding laboratory studies.<sup>6</sup> Disease severity is characterised after confirmation on bone marrow aspiration and scrutiny of the trephine biopsy (Fig. 9). This also excludes inherited bone marrow failure syndromes, hairy cell and chronic lymphocytic leukaemia, collagen vascular diseases and particularly systemic lupus erythematosus.

Do not overlook the presence of abnormal populations or clones due to

<b>Moderate aplasia</b>
Bone marrow cellularity <30%
Absence of severe pancytopenia
Depression of at least two of three blood elements below normal
<b>Severe aplastic aplasia</b>
A bone marrow biopsy showing <25% of normal cellularity
or
A bone marrow biopsy showing <50% normal cellularity in which fewer than 30% of the cells are haematopoietic
And at least two of the following are present:
Absolute reticulocyte count <40 000/microliter
Absolute neutrophil count (ANC) <500/microliter
Platelet count <20 000/microliter
<b>Very severe aplasia</b>
The criteria for severe aplastic anaemia are met and the ANC is <200/microliter

Fig. 9. Diagnostic criteria and grading for aplasia. The correlation of survival is with absolute neutrophil count at diagnosis. Where this is under  $0.2 \times 10^9/l$  outcome is substantially and significantly inferior predicating a more aggressive approach to interventions and moving therapeutic options towards early immunohaematopoietic stem cell allogeneic transplantation. ANC = absolute neutrophil count; 500 ANC =  $0.5 \times 10^9/l$ .

paroxysmal nocturnal haemoglobinuria or myelodysplasia, the impact of HIV or infiltrative lesions causing hypersplenism on the basis of congestive splenomegaly. However, in these cases the marrow is not devoid of haematopoietic tissue.

**Therapeutic decisions and management**

Supportive care is often adequate in mild and stable to improving forms, anticipating repopulation of the marrow where recovery is variable and independence may occur from transfusion. This approach requires detailed record-keeping with antigen-reduced packed red cells sufficient for symptomatic relief given with chelation therapy to avoid iron overload.

Granulocyte stimulatory peptides have limited value since the precursor cells are generally absent. However, be aware of the local prevalence of specific bacterial pathogens in order to treat infections empirically where necessary. An infectious disease or microbiology consultant on the team is invaluable. Thrombocytopenia is also challenging and replacement should be limited to single apheresis units given only to

maintain haemostasis. The local practice of replacement without documenting response should be avoided, as such practices may accelerate isoimmunisation. The major emphasis is to restrict this period and proceed to specific curative interventions as rapidly as necessary.

Allogeneic immunohaematopoietic stem cell transplantation from a fully matched sibling is preferable in severe forms of the disease. Where this is not possible a matched unrelated volunteer donor is the next choice. Depending on conditioning programmes and other variations in these procedures, complications range from rejection through acute and possibly subsequent chronic graft-versus-host disease to even graft failure. Nevertheless, in younger individuals cure rates have continually improved over the years and currently exceed 90%.<sup>2,3,6</sup>

Immunosuppressive regimens have emerged over the years with varying responses to regimens that include antithymocyte globulin through high-dose corticosteroids and cyclosporin or novel agents. Preliminary data suggest that all three products in com-

bination may have a better outcome. Late relapse and clonal malignant disease arise in a quarter of those treated. Additionally, in comparison to transplanted individuals, while a good life is possible, cure remains infrequent despite prolonged cyclosporin and steroid maintenance.<sup>5</sup>

These realities should be fully understood by referring doctors and mandate access to experienced multidisciplinary clinics who regularly audit and report outcome.

*Alternative or innovative options* include unconfirmed observations that a single course of cyclophosphamide may prolong survival in about half the patients. Attempts to combine this with other immunosuppressive regimens, particularly antithymocyte globulin, were unsuccessful and further evaluation of this approach is awaited. Another promising approach, currently unconfirmed, is response to a monoclonal antibody that binds to the interleukin-2 receptor, thereby modulating T-cell-mediated immunosuppression.

**Current practice**

The treatment of choice, when a suitable donor is available, remains a fully matched immunohaematopoietic stem cell transplant from a sibling as an early priority. When this is not possible alternative options include timeous use of matched unrelated volunteer donors. Success depends upon performance status and absence of significant co-morbidity.

Conditioning is immunosuppressive rather than myeloablative. Cyclophosphamide with antithymocyte globulin and fludarabine produces a survival rate of approximately 70% at 15 years in a cohort under 20 years of age. In older patients, certainly up to 60, good results are possible even following previous treatment.

One major point is the need to blunt potential graft-versus-host disease. Campath monoclonal antibodies, particularly *ex vivo* or using in-the-bag technique, have shown success.<sup>7</sup> These results were from centres with experienced multidisciplinary teams.

<p><b>Primary</b></p> <p>Associated with thymoma in 10 - 15% of cases</p> <p>Idiopathic causes</p> <p><b>Secondary</b></p> <p><i>Neoplasia</i></p> <p>Chronic leukaemia, leukaemia, lymphomas; large granular lymphocytic proliferative disorders; prodrome to myelodysplastic syndromes</p> <p>Systemic lupus erythematosus or rheumatoid arthritis</p> <p>Associated with pregnancy and autoimmune diseases</p> <p><i>Drugs</i></p> <p>Phenytoin, chlorpropamide, zidovudine (AZT), trimethoprim-sulfamethoxazole, isoniazid</p> <p><i>Infections</i></p> <p>Infectious mononucleosis, viral hepatitis, parvovirus infection, HIV</p>
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Fig. 10. Causation of red cell aplasia. The majority of cases have no demonstrable associated pathology and are therefore momentarily accepted as idiopathic or primary. It is nevertheless important to search carefully for possible causes – particularly if they are reversible.

**Pure red cell aplasia**

In adults this is acquired, with profound anaemia, characteristically without reticulocytes in the blood and erythroid precursors absent from the marrow while the other elements are preserved.

**Pathophysiology**

The total loss of erythropoiesis is believed to be immune-mediated by both antibody and T-cells at a point of development prior to the pro-erythroblasts. Additionally there may be humorally directed inhibition of erythropoietin but the alternative explanation is that erythropoietin-stimulating activity leads to the loss of these precursors. In many instances there is a specific attack on the precursors by parvovirus B19 and increasing concern of an underlying early myelodysplasia.

The aetiology may be primary or secondary (Fig. 10).

Diagnosis depends upon usually severe degrees of anaemia that is, initially at least, asymptomatic but typically preceded by fall in exercise tolerance and relentlessly progressive fatigue. Reticulocytes are absent but the remainder of the peripheral blood findings are normal.

**Haematology**

Bone marrow aspiration and particularly trephine biopsy are diagnostic. Interestingly there may be giant pro-normoblasts in the marrow that many consider pathognomonic (Fig. 11).

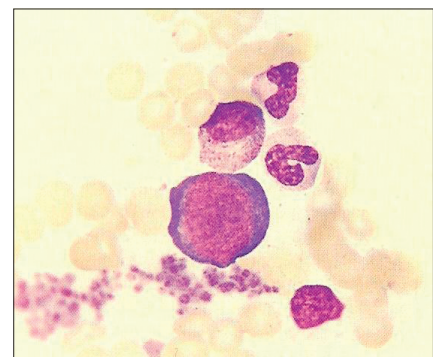


Fig. 11. Bone marrow failure due to parvovirus B19 infection. This particular and unusual morphological feature is distinctive and regarded by many as unique, signalling the underlying causation as that of this particular infection.

In evaluating associated and possibly causative mechanisms, use tomography of the chest to exclude thymoma, flow cytometry to identify large granular lymphocyte proliferation and cytogenetics together with a fluorescent *in situ* hybridisation panel to

avoid overlooking myelodysplasia as well as antibody tests for parvovirus. Ferrokinetic studies demonstrate selective absence of erythropoiesis but these investigations are still largely in a research setting.

### Treatment

Treatment requires antigen-reduced packed red cells to control symptoms, and iron overload should be diminished by concurrent use of chelation therapy. With an accurate diagnosis the course may be self-limiting, including parvovirus B19 infections, and patients can be managed expectantly, although intravenous gammaglobulin remains an option, particularly in those associated with HIV.

Pathophysiologically the explanation is an inability of the immune system to clear

the offending virus. In refractory cases interventions parallel those for aplastic anaemia, with significant response rates following immunosuppression, particularly with antithymocyte globulin and prednisone, and possibly in combination with anti-CD 20 monoclonal antibody, among other therapeutic possibilities.

### Conclusion

As for aplasia, this latter entity is also relatively uncommon but requires consideration in patients whose anaemia is otherwise not readily explained. Distinction is necessary from the more common nutritional deficiencies or the anaemia of chronic disorders where marrow findings are usually diagnostic. Once the diagnosis is established, the

importance of protocol management, preferably on clinical trial by an accredited multidisciplinary group in an academic centre of excellence, is regarded worldwide as standard of care.

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References available at [www.cmej.org.za](http://www.cmej.org.za)

## SINGLE SUTURE

### *Fingertip tingle enhances a surgeon's sense of touch*

Our fingers are precision instruments, but there are plenty of things they are not sensitive enough to detect. Now we can augment their talents – using wearable electronic fingertips that provide tingling feedback about whatever we touch.

John Rogers of the University of Illinois at Urbana-Champaign and colleagues have designed a flexible circuit that can be worn over the fingertips. It contains layers of gold electrodes just a few hundred nanometres thick, sandwiched between layers of polyimide plastic to form a 'nanomembrane'. This is mounted on a finger-shaped tube of silicone rubber, allowing one side of the circuit to be in direct contact with the fingertips. On the other side, sensors can be added to measure pressure, temperature or electrical properties such as resistance.

People wearing the device receive electrotactile stimulation – a tingling sensation caused by a small voltage applied to the skin. The size of the voltage is controlled by the sensor and varies depending on the properties of the object being touched.

Surgical gloves are one potential application. Rogers, who worked with colleagues at Northwestern University in Evanston, Illinois, and Dalian University of Technology in China, says gloves fitted with the nanomembrane could sense the thickness or composition of tissue via its electrical properties. A surgeon could also whittle away at the tissue using a high-frequency alternating current supplied by a battery attached at the wrist and delivered via the nanomembrane itself, says Rogers.

Fiorenzo Omenetto at Tufts University in Medford, Massachusetts, is impressed. 'The work sets the stage for a new generation of devices,' he says.

There are applications beyond surgery, too. MC10, the company commercialising the technology, is running animal trials of a nanomembrane 'sock' that can be wrapped around the heart. This provides a 3D map of its electrical activity, useful in treating irregular heartbeat.

MC10 is also working with medical device company Medtronic to use the membrane inside the heart, sending it in on a limp balloon, which is then inflated to push the membrane onto the heart's interior walls.

Rogers says MC10 is also collaborating with sportswear firm Reebok on a product to be launched by the end of this year. The aim is to build a 'body-worn piece of electronics' designed for contact sports, although Rogers declined to say exactly how it will be used.

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