

Common Sense Pathology

A REGULAR CASE-BASED SERIES ON PRACTICAL PATHOLOGY FOR GPs

Contents:

- Acute phase proteins and inflammation
- Measurement and interpretation of CRP
- Diagnostic value of ESR
- Case studies

Making sense of **INFLAMMATORY** markers

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**Australian
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CRP and ESR are a simple, cost-effective and valuable diagnostic tool to distinguish inflammatory from non-inflammatory conditions.

Introduction

The 'acute phase response' refers to a large number of pathophysiological changes that occur in the setting of inflammation. Despite the name, many of the features of the acute phase response also occur in response to more chronic conditions, including malignancy and infection.

Acute-phase reactants are proteins whose concentrations in blood increase or decrease by 25% or more during inflammation. The acute-phase reactants most commonly used for diagnostic purposes are C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR). Although these two markers lack the specificity to distinguish among the many possible causes of inflammation, they are a simple, cost-effective and valuable diagnostic tool to distinguish inflammatory from non-inflammatory conditions and to monitor the response to treatment for a number of disorders.

Acute-phase proteins

Inflammation can result from a variety of tissue insults including infections, trauma, autoimmunity and malignancy. These stimuli lead to the production of cytokines (small molecules that communicate between cells), which trigger the up- or down-regulation of acute-phase proteins.¹ Acute-phase proteins are primarily produced in the liver by hepatocytes. These proteins can be separated into groups with different functional properties, as outlined in table 1 (see next page).

There is marked variation in the timing and magnitude of response among different acute-phase proteins. As an example, ceruloplasmin and complement proteins increase by around 50% while CRP and serum amyloid A can be induced by 1000-fold.² CRP begins to rise within 4-6 hours after an inflammatory stimulus and reaches its peak level in blood within 48-72 hours.³

Fibrinogen, the major determinant of the ESR, can increase by threefold and peaks over a week after initial stimulus.

Regulation of the acute-phase response

Acute-phase proteins are regulated by a complex system of cytokine signalling. The main cytokines involved in up-regulating acute-phase proteins (as well as modulating other components of the acute inflammatory response such as fever) are the cytokines interleukin-6 (IL-6), interleukin-1 (IL-1), tumour necrosis factor alpha (TNF-alpha) and interferon gamma (IFN-gamma).⁴ These cytokines are generally released at the site of injury by cells of the innate immune system such as tissue macrophages and monocytes. IFN-gamma is predominantly produced by T-cells and natural killer cells. The inflammatory response is not always beneficial, and may cause tissue damage in the host. Where the inflammatory response is harmful, a potential therapeutic approach is to block the action of these cytokines with monoclonal antibodies resulting in a diminished response. Examples of these antibodies include anakinra (anti-IL-1), tocilizumab (anti-IL-6), adalimumab and infliximab (anti-TNF-alpha).

Function of CRP

CRP was first described in 1930 as a component in the sera of patients with lobar pneumonia that could precipitate the polysaccharide from "fraction C" of pneumococcus.⁵ It is a member of the pentraxin family of proteins — a class of pattern recognition receptors that is highly conserved in evolutionary terms.³ Structurally, CRP is a pentameric molecule made up of five identical subunits that bind and interact with a number of important targets or ligands. CRP binds a number

Table 1: Acute-phase protein changes observed with inflammation ^{1,2}		
Functional group	Increased	Decreased
Complement proteins	C3, C4, C9, Factor B, C1 inhibitor, C4b-binding protein, Mannose-binding lectin	Properdin
Coagulation system proteins	Fibrinogen, Plasminogen, Tissue plasminogen activator, Factor VIII, Urokinase, Protein S, Vitronectin, Plasminogen-activator inhibitor 1	Factor XII
Proteinase inhibitors	Alpha-antitrypsin, Alpha-antichymotrypsin, Inter-alpha-trypsin inhibitors	
Transport proteins	Ceruloplasmin, Haptoglobin, Haemopexin, Ferritin	Transferrin, Transthyretin, Thyroxin-binding globulin
Others	CRP, Serum amyloid A, Secreted phospholipase A, Lipopolysaccharide-binding protein, Interleukin-1-binding protein, Interleukin-1-receptor antagonist, Granulocyte colony stimulating factor, Alpha-acid glycoprotein, Fibronectin, Angiotensinogen	Albumin, Alpha-fetoprotein, Insulin-like growth factor

of bacterial and human cell components including phosphocholine, C1q and Fc receptors for antibodies.⁶ Phosphocholine, a well-described ligand of CRP, is a component of most cell membranes — including bacterial, fungal and human cells. After binding phosphocholine, CRP can interact with antibody receptors on phagocytic cells to facilitate phagocytosis. More importantly, complexed CRP is able to interact with the protein C1q — an early component of the classical complement pathway.¹ This pathway facilitates three main functions: enhanced opsonisation of cell targets by phagocytic cells, recruitment of inflammatory cells and formation of the membrane attack complex by the complement cascade. CRP, therefore, has an important role in clearing not only pathogens but also damaged, necrotic or apoptotic human cells.

Measurement and interpretation of CRP levels

Measuring CRP is perhaps the most practical way to detect and monitor the presence and progress of a systemic inflammatory response, due to its large dynamic range, rapidity of response, short half-life and relative simplicity of measurement. CRP is stable in serum or plasma and can be measured by relatively simple and inexpensive analytical methods such as enzyme-linked immunosorbent assay (ELISA), turbidimetry and nephelometry. The plasma half-life of CRP is 19 hours and its clearance is constant, meaning that it is the rate of synthesis that determines its concentration.¹

Given the wide range of conditions associated with an elevated CRP level, measurement of CRP lacks the required specificity to be used in isolation as a diagnostic test for any condition. The magnitude of the rise in CRP can, on occasion, point towards a possible cause of inflammation. As a general rule, mild inflammatory stimuli, such as viral infections, are associated with CRP levels of 10-40mg/L. More serious conditions, such as bacterial infections or active connective tissue diseases, can be associated with levels of 50-200mg/L. Levels of

greater than 200-300mg/L are typically seen in the setting of severe conditions or injury such as sepsis or burns.⁷

A normal or only slightly elevated CRP does not exclude serious or significant inflammation. There are a number of well-described inflammatory conditions that are associated with a discordant CRP and other markers of inflammation such as ESR (which is discussed in more detail below). Two well-known examples are SLE and ulcerative colitis.

Despite extensive inflammation and demonstrated elevation of IL-6 with raised ESR levels during disease flares in patients with lupus, CRP levels are typically muted. This can be used to advantage as CRP levels may help differentiate a flare of autoimmune disease from infection in patients with lupus. It has been suggested that this phenomenon is due to the cytokine IFN-alpha, which is up-regulated in SLE and acts as an inhibitor of CRP.⁸ An exception to this is lupus serositis, which is often associated with marked elevation in CRP.

In inflammatory bowel disease, active Crohn's disease is typically accompanied by a strong CRP response; whereas ulcerative colitis, in the absence of other inflammatory stimulus, is often associated with an absent or only modest CRP elevation.⁷ Other disorders with a muted CRP response include scleroderma and dermatomyositis.

Conventional methods of CRP measurement detect levels in the order of 3-10mg/L. More recently, high-sensitivity CRP assays with the ability to detect levels of less than 3mg/L have become available. As these results fall within the normal CRP reference range, measurements of high-sensitivity CRP do not provide additional information in the assessment of patients with clinical evidence of systemic inflammation. They have a role in conditions not previously thought to be associated with significant inflammation.⁹ There is a body of evidence exploring the role of high-sensitivity CRP as an independent risk measure in cardiovascular disease.

Conditions in which the measurement and/or monitoring of CRP may be clinically useful are listed in



CRP lacks the required specificity to be used in isolation as a diagnostic test for any condition.

table 2. In the setting of rheumatoid arthritis, it is worth noting that CRP correlates well with disease activity in terms of symptoms and radiologic progression of joint changes.¹⁰

Erythrocyte sedimentation rate (ESR)

The ESR is defined as the distance that erythrocytes settle in anticoagulated whole blood, under gravity, in one hour. It is measured as the length, in mm, of clear plasma at the top of a vertical tube. Thus it actually measures the amount of sedimentation in an hour as opposed to the rate per hour. The ESR is a simple and inexpensive test that has been used for almost a century. It can, and has in the past, been performed at the bedside to rapidly provide an indication of illness severity. In the laboratory, there are two main methods for measuring ESR: the Westergren and Wintrobe methods. These two methods use different standardised tubes and anticoagulants, and the results are not interchangeable.¹¹

The ESR differs from CRP in that it is not a measurement of a single acute-phase protein. Rather, it measures not only several acute-phase proteins, but also a host of other factors. Erythrocyte sedimentation is dependent on the ability of red blood cells to aggregate and form rouleaux. This, in turn, is determined by factors such as red blood cell number, size and shape, electrostatic charges and plasma viscosity.¹¹ It is estimated that the acute-phase protein fibrinogen accounts for approximately 60-70% of the increase in ESR during an inflammatory state due to its ability to neutralise sialic acid residues on red blood cells that normally prevent rouleaux formation.¹² As with CRP, fibrinogen is produced in the liver and is regulated primarily by IL-6, IL-1 and TNF-alpha.

Fibrinogen levels do not always correlate with ESR; other proteins in blood also affect red blood cell sedimentation. These proteins include, in order of decreasing influence: beta-globulins, alpha-globulins,

Table 2: Conditions where CRP is routinely measured¹¹

Assessment of disease activity of autoimmune/ autoinflammatory conditions
Rheumatoid arthritis
Juvenile idiopathic arthritis
Seronegative arthritis
Ankylosing spondylitis
Reactive arthritis
Psoriatic arthritis
Crohn's disease
Rheumatic fever
Vasculitis
Behcet's syndrome
ANCA-associated vasculitis
Polyarteritis nodosa
Pancreatitis
Periodic fever syndromes
Assistance with diagnosis and monitoring of infection
Bacterial endocarditis
Abscess
Postoperative infection
Response to antibiotic therapy
Differentiation between inflammatory conditions
Semic lupus erythematosus vs rheumatoid arthritis
Crohn's disease vs ulcerative colitis

gamma-globulins and albumin. Globulins have roughly half the aggregating power of fibrinogen; albumin makes the least contribution.¹⁰ Other factors not necessarily related to inflammation that can influence ESR include anaemia (increased sedimentation), polycythaemia (low sedimentation), and advancing age in women (increased sedimentation). These factors are taken into account when



determining normal reference ranges. Medications can also affect ESR; for example, both the oral contraceptive pill and heparin increase sedimentation.

The value of ESR in addition to CRP

There are a small number of conditions for which an elevated ESR is included in the diagnostic criteria. These include giant cell arteritis (GCA) and polymyalgia rheumatic (PMR). There are no specific serological tests for these conditions. A provisional diagnosis is generally made on the basis of suggestive signs and symptoms in a patient aged more than 50-65 with an ESR of greater than 40-50mm/hour (though it is typically higher than this). Although there is some evidence to suggest that CRP may be more sensitive than ESR, it is the ESR that is included in the majority of published diagnostic criteria.¹³

As many of the factors that determine the ESR have long half-lives, it is less valuable in assessing acute changes than CRP. An elevated ESR may be due to events that have occurred weeks to months previously and may have resolved at the time of measurement.

In the investigation of a patient with an unclear source of inflammation, a mild to moderate elevation in ESR by itself lacks the specificity to be of great diagnostic value. Marked elevation of ESR (ie, greater than 100mm/hr), on the other hand, is almost always significant. In patients with an ESR greater than 100mm/hr, a significant diagnosis will be made in over 90% of cases.¹⁴

As a general rule, an ESR of over 100mm/hr should prompt consideration and investigation for the conditions listed in table 3.

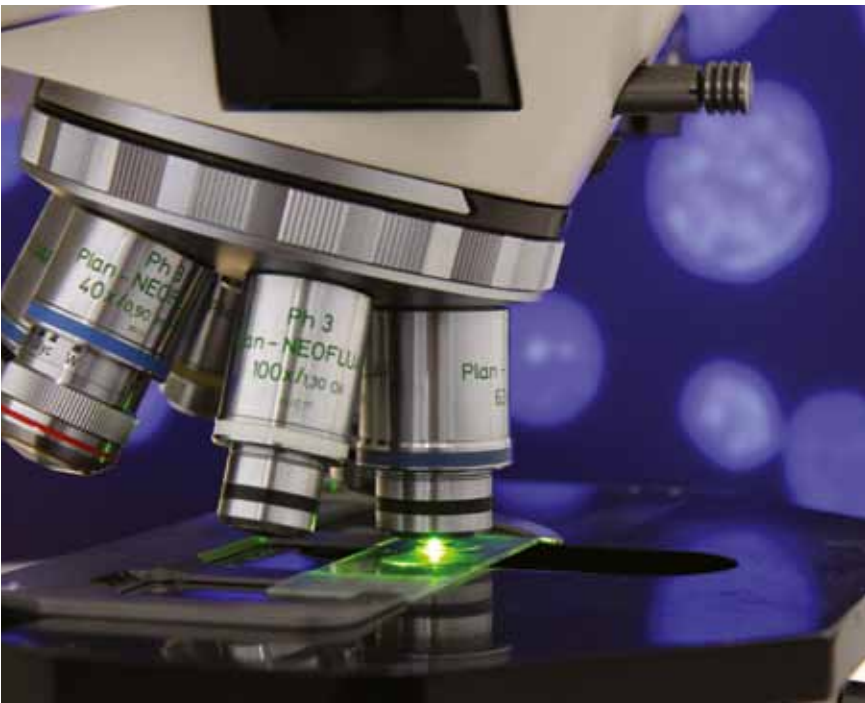
Some clinicians use the term “vasculitic screen” to describe a set of tests ordered when vasculitis is suspected

Table 3: Potential causes of markedly elevated ESR (>100mm/hr)

Infection	Tuberculosis
	Endocarditis
	Abscess
Malignancy	Dysproteinaemia (ie, multiple myeloma)
	Metastatic cancer
Rheumatological	Vasculitis
	Inflammatory arthritis
	Rheumatoid arthritis
	Crystal arthropathies

or needs to be excluded. The composition of this panel of tests may vary between requestors, but generally includes a mix of autoimmune serology including antinuclear antibodies (ANA), antibodies to extractable nuclear antigens (ENA) and ANCA. It will often also include serology for SLE (double-stranded DNA), rheumatoid arthritis (rheumatoid factor and cyclic-citrullinated peptide antibodies), as well as ESR and CRP.

The term used to describe this panel is a misnomer. A requirement of a good screening test is a high sensitivity. These tests may detect a proportion of patients with either ANCA-associated or lupus-related vasculitis, but — with the exception of ESR — will likely miss the many forms of vasculitis such as GCA, polyarteritis nodosa (PAN), Behcet’s syndrome and others, which do not have specific serological tests. The ESR is perhaps the closest test to an actual vasculitis screen, as a normal ESR would make active vasculitis very unlikely, with the exception of exceedingly rare forms such as primary vasculitis of the central nervous system.



An elevated ESR may be due to events that have occurred weeks to months previously.

CASE 1

An 82-year-old woman presents to her GP with several weeks of progressive fatigue. She has been feeling hot intermittently but her husband has not detected a fever with a thermometer at home. Her visit was prompted by concern regarding her 5kg weight loss in four weeks. On further questioning she reveals that, in the past few days, she has developed a new frontal headache that she attributes to tiredness. She has no localising symptoms or signs of infection. Her past medical history is significant only for mild hypertension, which has responded well to treatment. Her examination reveals no significant abnormalities. The results of initial blood tests are:

Investigation	Result	Reference Range
Haemoglobin	105g/L	120-150
White cell count	5.0×10 ⁹ /L	120-150
Platelets	180×10 ⁹ /L	120-150
Albumin	28g/L	120-150
ESR	80mm/hour	120-150
CRP	74mg/L	<5

In the absence of clinical history and examination, these results are non-specific. In the setting, however, of a new onset headache and significantly raised inflammatory markers, giant cell arteritis (also called temporal arteritis) is a strong possibility. Other diagnoses to be considered, in light of her constitutional symptoms and anaemia, would include chronic infection, multiple myeloma or other malignancy. In this case, the patient requires prompt referral to a specialist (such as a rheumatologist or clinical immunologist) for definitive diagnosis and treatment. This will generally involve

arranging a temporal artery biopsy followed by immunosuppression.

Giant cell arteritis is a vasculitis of large- and medium-size blood vessels. It most commonly affects the extracranial branches of the carotid arteries. In the appropriate age group (ie, over the age of 50), it is the most common form of systemic vasculitis. Rapid diagnosis and commencement of treatment is essential due to the possibility of vascular complications such as irreversible visual loss. ESR and CRP typically correlate in this condition and tend to be moderately to highly elevated.

ESR is traditionally the test that is used to confirm clinical suspicion of GCA and is a component of the American College of Rheumatology diagnostic criteria. It is possible that ESR has a prognostic significance as an ESR of greater than 70mm/hr may be associated with an increased risk of severe eye/visual symptoms.¹³ It is important to realise that a low to moderate ESR does not exclude GCA. Indeed, over 10% of patients who are found to have biopsy-proved GCA have an ESR of less than 30 mm/hr.¹³

CASE 2

A 34-year-old woman presents with over 12 months of moderate to severe lethargy and fatigue. On further questioning, she describes dryness of her eyes and mouth — though not severe enough to have required specific treatment. She also reports intermittent arthralgia to her hands, wrists and back that does not sound particularly inflammatory in nature. Her past medical history is significant for menorrhagia. Careful examination reveals no concerning features such as masses or lymphadenopathy. Her doctor feels that an underlying autoimmune disease is unlikely and orders

systemic inflammatory markers in anticipation of a normal result to reassure the patient prior to pursuing treatment for her menorrhagia. Her results are as follows:

Investigation	Result	Reference Range
Haemoglobin	88g/L	120-150
MCV	75fL	80-100
White cell count	6.0×10 ⁹ /L	4-10
Platelets	240×10 ⁹ /L	150-400
Albumin	38g/L	38-48
ESR	50mm/hr	0-12
CRP	8mg/L	<5

In this case, the ESR and CRP are mildly — but non-specifically — elevated. The elevation in ESR may be due to the presence of anaemia leading to a faster sedimentation rate. Microcytic anaemia, in the clinical setting of menorrhagia, points towards iron deficiency anaemia as a cause for her fatigue. Given her sicca symptoms, and the fact that the ESR and CRP appear to be discordant with each other, autoimmune serology, including ANA, ENA and testing for lupus, should be considered to explore the possibility of conditions such as Sjögren's syndrome.

CASE 3

A previously well 62-year-old man presents seeking analgesia after developing sudden onset lower back pain two days earlier. Of concern, he describes a three-month history of worsening fatigue and generalised weakness associated with several kilograms of weight loss. There has been no recent trauma or fever. On examination, he is tender in the midline of the lumbar spine but has no focal neurological deficit.

Given his localised tenderness and the presence of a number of “red flags”, his doctor arranges a number of further investigations to look for a sinister cause.

Investigation	Result	Reference Range
Haemoglobin	95g/L	130-170
MCV	90fL	80-100
White cell count	4.0×10 ⁹ /L	4-10
Platelets	160×10 ⁹ /L	150-400
Creatinine	140µmol/L	50-90
Albumin	34g/L	38-48
ESR	104mm/hr	0-30

An X-ray of his spine reveals a crush fracture of the L4 vertebral body in addition to lytic lesions in several of his vertebrae. His doctor subsequently arranges for further blood tests, including serum electrophoresis, which confirms the presence of an IgG kappa paraprotein, 30g/L. A provisional diagnosis of multiple myeloma is made and he is referred to a haematologist for further investigation and treatment.

Acute back pain, in the setting of constitutional symptoms and a markedly elevated ESR, prompts investigation for infection, such as discitis or a paravertebral abscess, as well as malignancy, such as myeloma or metastatic disease. Myeloma is one of the archetypal diseases associated with an elevated ESR. Although there may not necessarily be an acute phase reaction, the paraprotein is a gamma globulin that acts to promote rouleaux formation.

Key points

1. The acute-phase response is characterised by changes in the concentrations of a large number of proteins in response to tissue damage.
2. In many cases, measuring parts of the acute-phase response is simple, inexpensive and useful in confirming and monitoring the presence of systemic inflammation.
3. The most commonly measured components of the acute-phase response are CRP and, indirectly, the ESR. With some important exceptions, these markers of inflammation are elevated in the presence of infections, autoimmunity, trauma and malignancy.
4. While low-grade elevation of these markers is non-specific, marked elevation of CRP and ESR is generally associated with significant illness. For example, a CRP of greater than 200mg/L is typically seen in the setting of bacterial infection/sepsis, while an ESR of greater than 100mm/hour should prompt investigation for infection, malignancy and autoimmunity depending on the clinical circumstances.

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