Sickle Cell Disease

Pathophysiology and Clinical Manifestations

Severe Sickle Cell Disease

HbS/S (β6Glu>Val/ β6Glu>Val)	Sickle cell anaemia – the commonest form of SCD
HbS/β^0thalassaemia	Most prevalent in Eastern Mediterranean, India
Severe HbS/β^+thalassaemia	Most prevalent in India, Eastern Mediterranean; 1-5% HbA present
HbS/OArab (β6Glu>Val/ β121Glu>Lys)	Found in North Africa, Middle East and Balkans; fairly rare
HbS/D Punjab (β6Glu>Val/β121Glu>Gln)	Predominant in Northern India but occurs all over the world
HbS/C Harlem (β6Glu>Val/ β6Glu>Val/β, β73Asp>Asn)	Electrophoretically resembles HbSC, but clinically severe; double mutation in β globin gene; very rare
HbC/S Antilles (β6Glu>Lys/ β6Glu>Val, β23Val-Ile)	Double mutation in β globin gene resulting in severe SCD when coinherited with HbC; very rare
HbS/Quebec-CHORI (β6Glu>Val/ β87Thr>Ile)	Two cases described; resembles sickle cell trait with standard analytical techniques

Less Severe Sickle Cell Disease

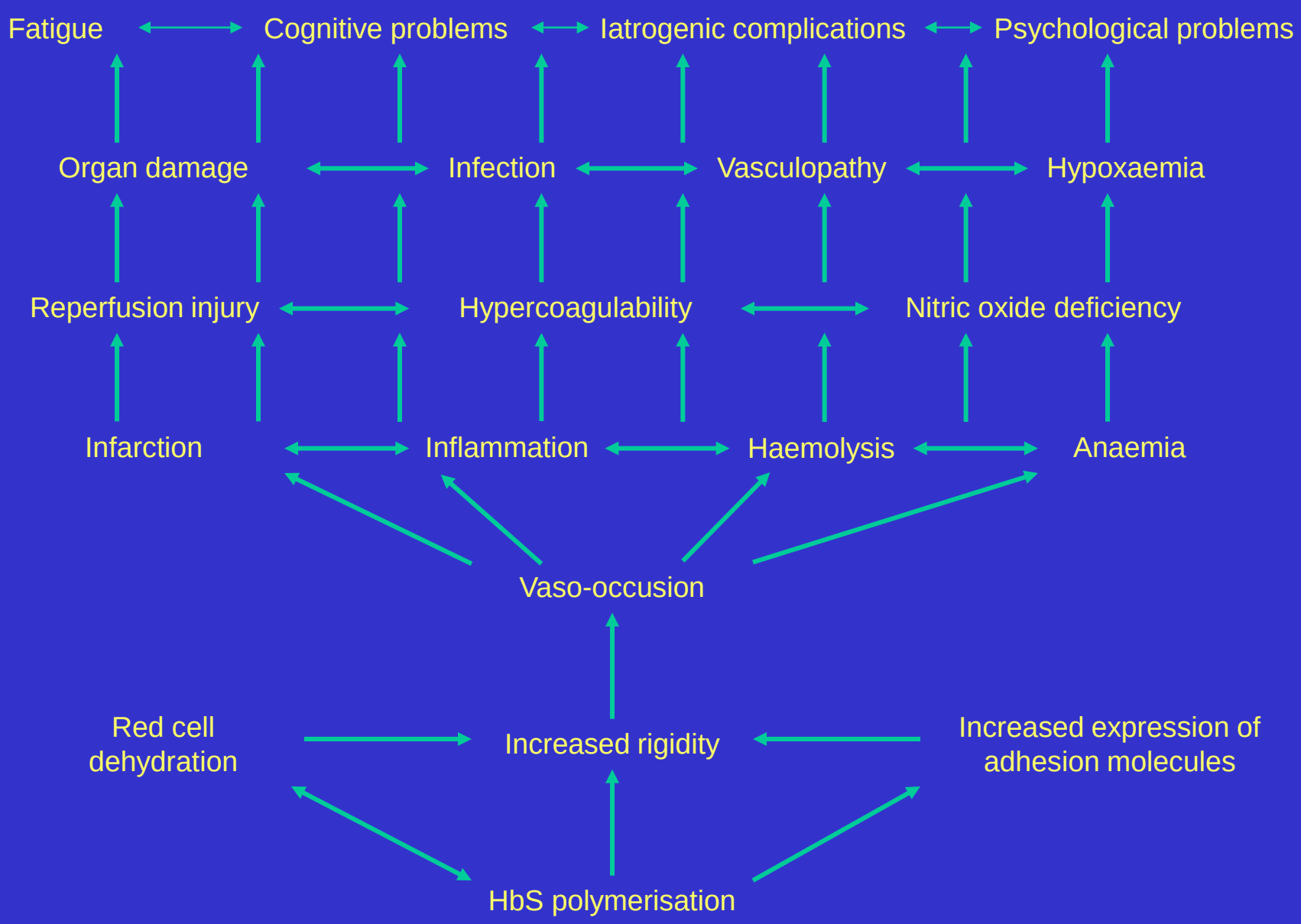
Moderate Sickle Cell Disease	
HbS/C (β6Glu>Val/β6Glu>Lys)	25-30% cases of SCD in populations of African origin[13]
Moderate HbS/β^{+}thalassaemia	Most cases in Eastern Mediterranean. 6-15% HbA present[14]
HbA/S Oman (β^{A}/ β6Glu>Val,β121Glu>Lys)	Dominant form of SCD due to double mutation in β globin gene. Very rare[18]
Mild Sickle Cell Disease	
Mild HbS/β^{++}thalassaemia	Mostly in populations of African origin. 16-30% HbA present[14]
HbS/E (β6Glu>Val/β26Glu>Lys)	HbE predominates in Southeast Asia and so HbSE uncommon, although increasing with population admixture[19]
HbA/Jamaica Plain (β^{A}/ β6Glu>Val,β68Leu/Phe)	Dominant form of SCD; double mutation results in Hb with low oxygen affinity. One case described[20]
Very Mild Sickle Cell Disease	
HbS/HPFH	Group of conditions caused by large deletions of the β globin gene comples. Typically 30% HbF[14]
HbS/other Hb variants	HbS is co-inherited with many other Hb variants, and symptoms develop only in extreme hypoxia

Pathophysiology

HbS
Codon 6 GAG-GTG
glutamic acid - valine

Pathophysiology

- Glutamic acid to valine substitution causes haemoglobin to polymerise when deoxygentated
- Polymers distort and damage red cell
  - Reduce flexibility
  - Damage membrane and cause cellular dehydration
  - Haemolyse releasing haemoglobin
- Rate and extent of HbS polymerisation determines degree of red cell damage, which in turn determines other pathological events


Fatigue
Cognitive problems
Iatrogenic complications
Psychological problems
Organ damage
Infection
Vasculopathy
Hypoxaemia
Reperfusion injury
Hypercoagulability
Nitric oxide deficiency
Infarction
Inflammation
Haemolysis
Anaemia
Vaso-occusion
Red cell dehydration
Increased rigidity
Increased expression of adhesion molecules
HbS polymerisation

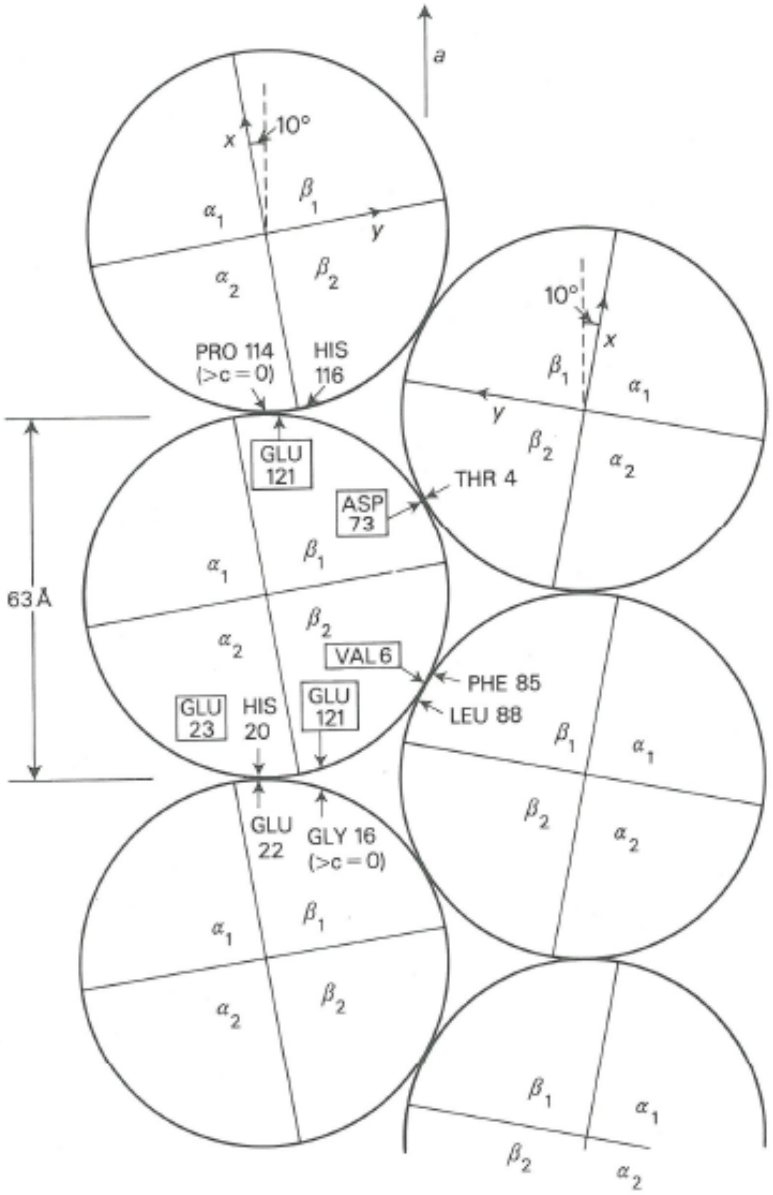
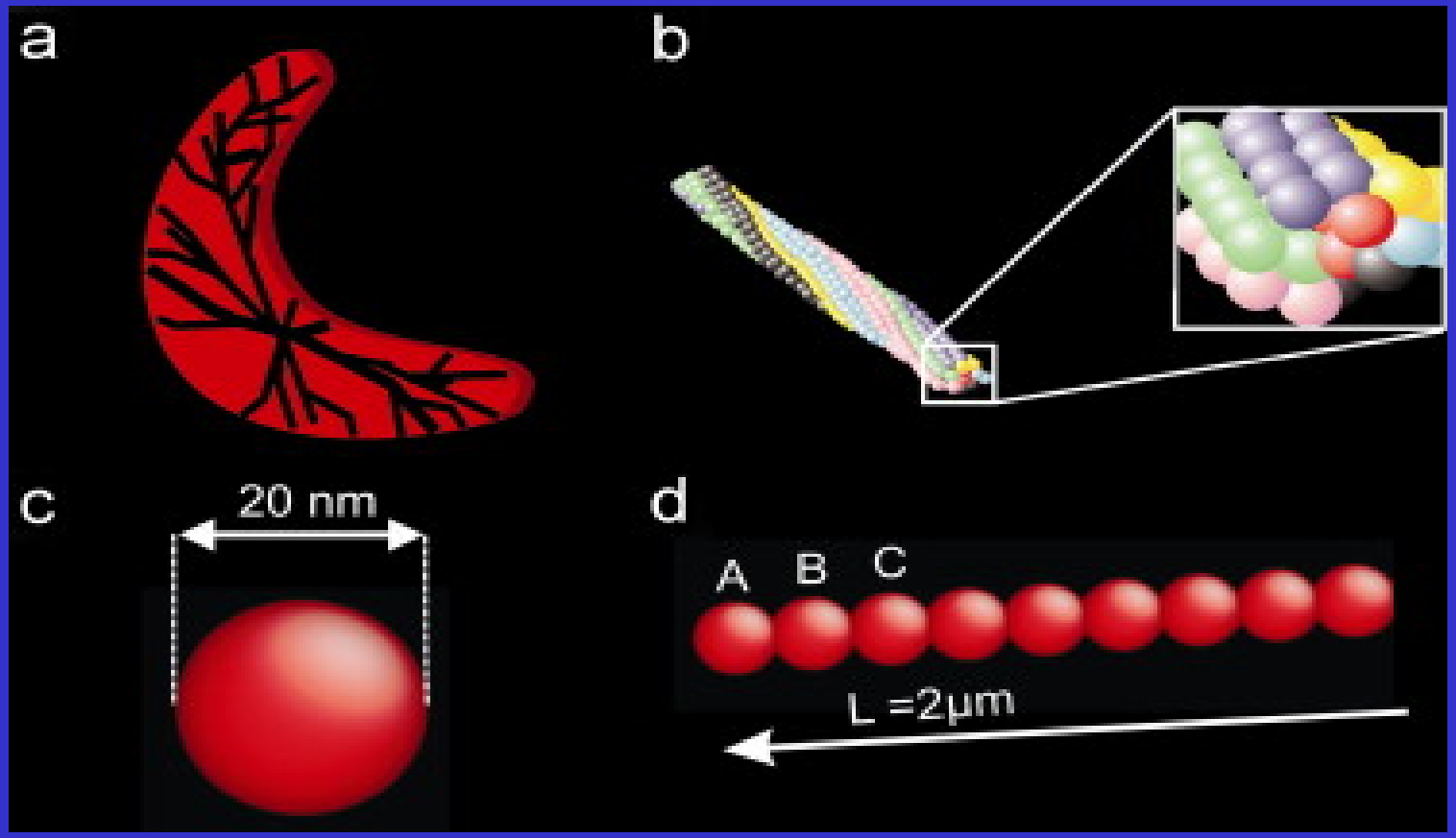


Fig. 5.4 Diagram of two strands of tetramers in deoxy HbS illustrating the intermolecular contacts. Residues deduced to be contacts from studies on mutants are enclosed in boxes. (From Wishner *et al.* 1975).

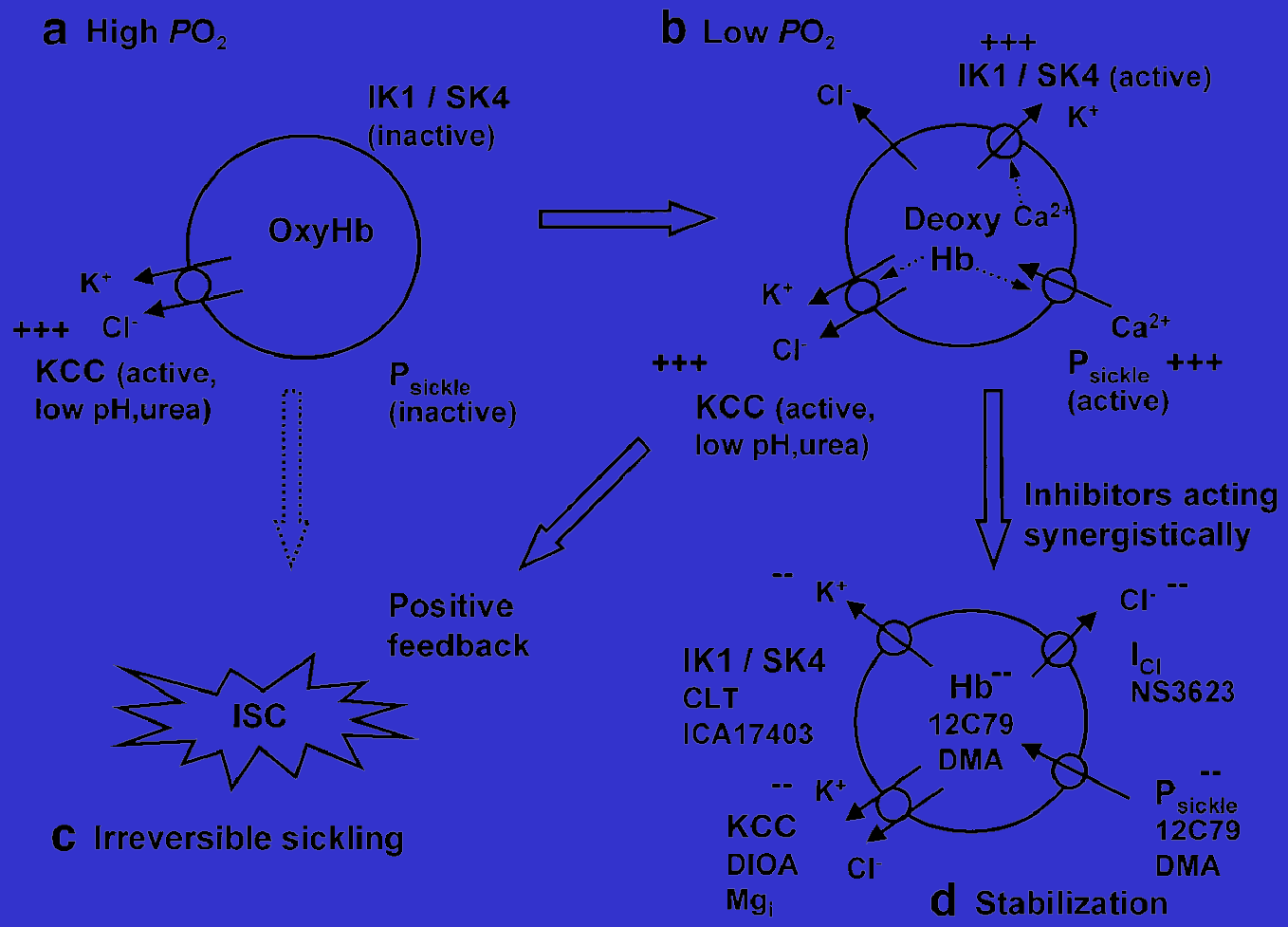
HbS Polymerisation



Determinants of the Rate and Extent of HbS Polymerisation in Red Cells

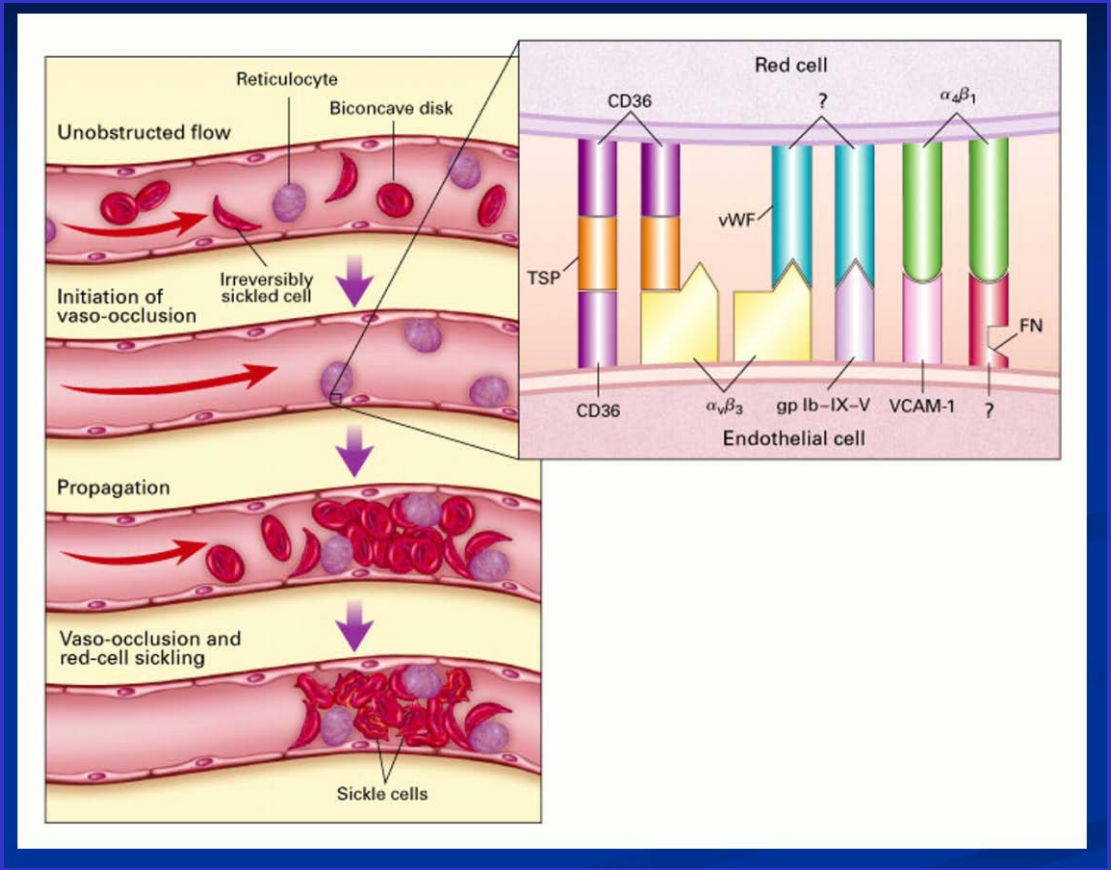
- Concentration of deoxygenated HbS molecules in the red cell
  - Time spent in hypoxic conditions
  - Degree of tissue hypoxia
  - pH
  - Mean cellular haemoglobin concentration
    - Reduced by co-existent α thalassaemia
  - Red cell dehydration
- Presence of molecules which inhibit polymerisation
  - HbF concentration
  - Possibly other molecules
- Temperature
  - Polymerisation slower at lower temperatures

Red Cell Dehydration



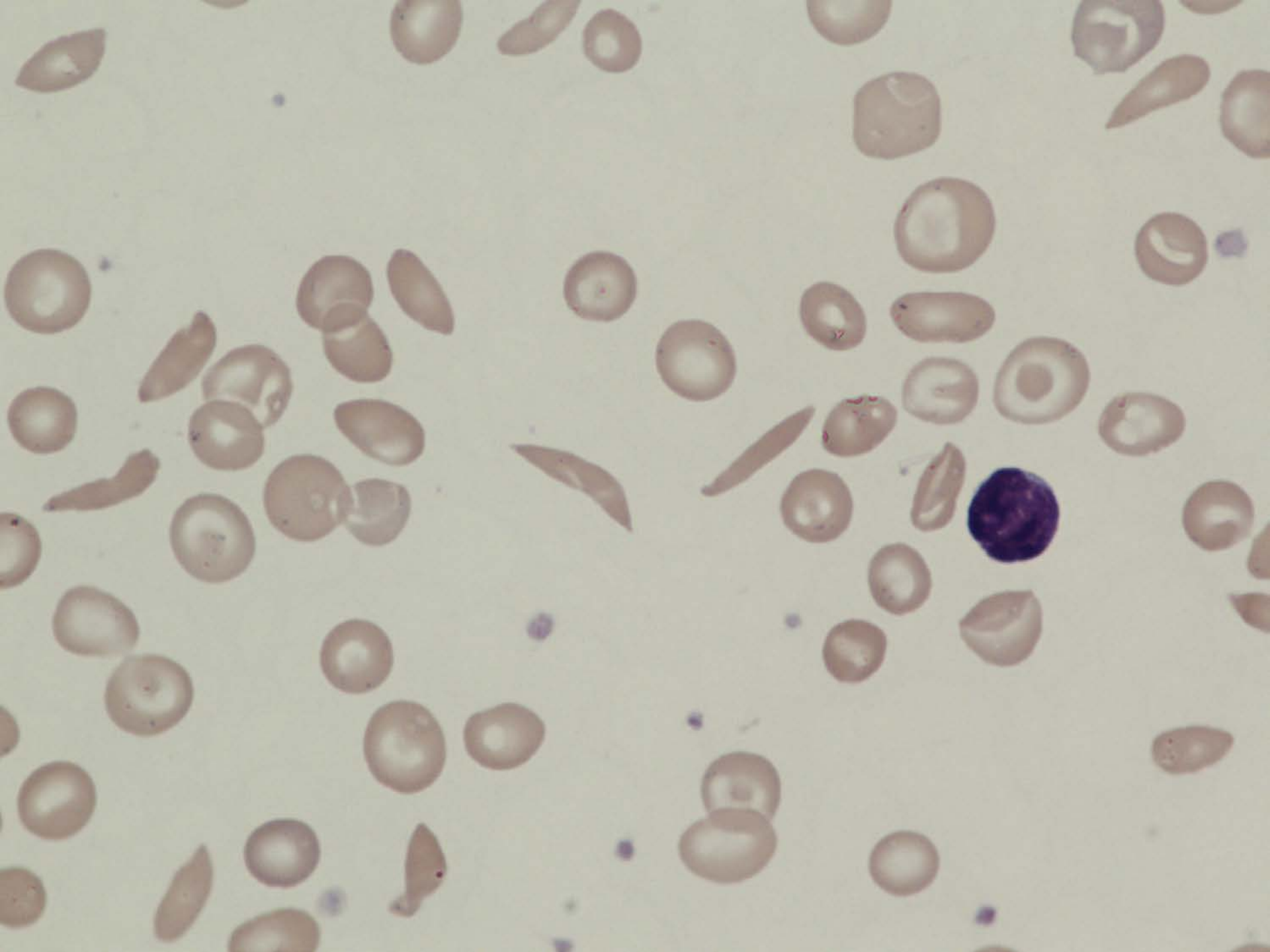
Gibson and Ellory, Blood Cells, Molecules and Diseases 2002, 28, 303-314

Altered Expression of Adhesion Molecules



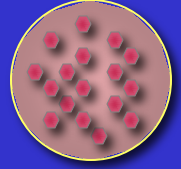
Hebbel RP. N Engl J Med 2000; 342: 1910-1912

Adhesion molecule	Expression	Ligand	Significance
sICAM-1	Vascular endothelium	White cell LFA-1 and Mac-1	High in SCD and reduced by hydroxycarbamide
sVCAM-1	Vascular endothelium	Erythrocyte VLA-4	High in SCD and reduced by hydroxycarbamide
P-selectin	Platelets, vascular endothelium	White cells	Marker of endothelial dysfunction high in SCD
E-selectin	Vascular endothelium	White cells	Marker of endothelial dysfunction, correlating with pulmonary hypertension and death
Fibronectin	Plasma	Erythrocyte $\alpha_4\beta_1$, collagen	Reduced in steady state SCD and further reduced during acute pain

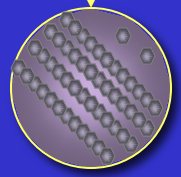


Pathophysiology of Vaso-occlusion

- Sickled cells block microvasculature due to
  - Reduced flexibility
  - Increased expression of adhesion molecules
- Vaso-occlusion causes further hypoxia
- Other factors include
  - Increased white cells and platelet counts
  - Fat embolism
  - Inflammation
- Development of vasculopathy

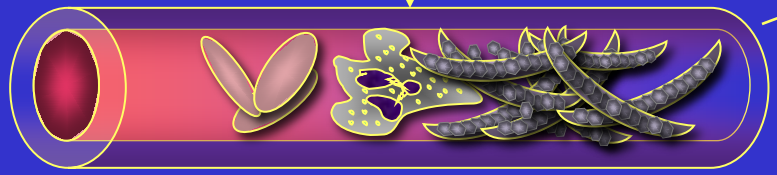


Oxygenated red cell containing HbS

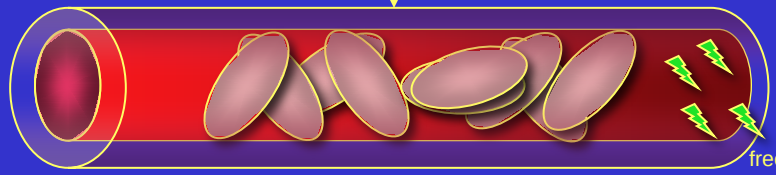


Deoxygenated red cell with HbS polymerising

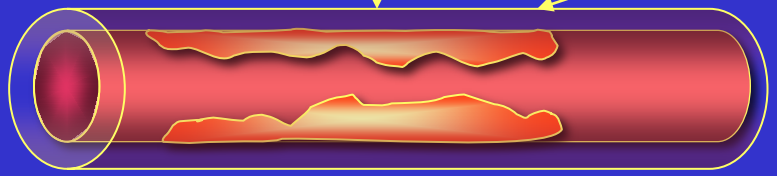
Dehydrated, sickled red cell



Occlusion of postcapillary venules



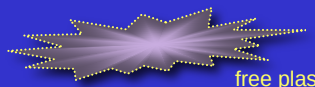
Reperfusion



Vasculopathy and endothelial dysfunction

infarction

haemolysis



free plasma haemoglobin inactivating nitric oxide

free radicals, causing tissue damage

functional nitric oxide deficiency

- acute pain
- acute chest syndrome
- hyposplenism
- osteonecrosis
- nephropathy

- Inflammation
- Increased expression of VCAM-1 and other adhesion molecules
- Hypercoagulability

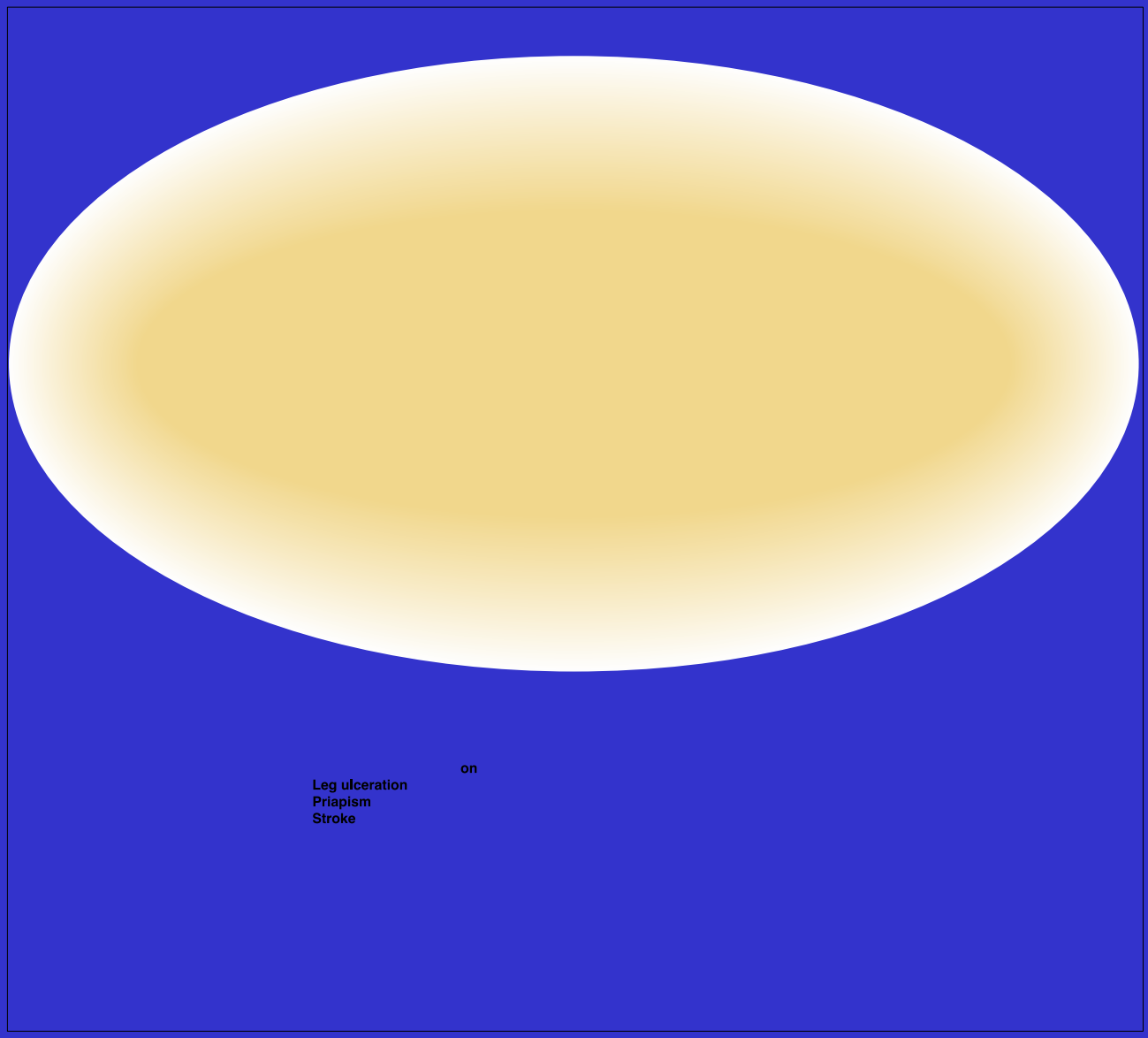
- pulmonary hypertension
- priapism
- leg ulcers
- cerebrovascular disease

Pathophysiology of Vasculopathy

- Complex, interrelated processes result in vasculopathy
  - Inflammation
  - Macrophage activation
  - Oxidant stress
  - Coagulation activation
  - Endothelial activation and dysfunction
  - Reperfusion injury
  - Vascular stasis
  - Increased levels of free plasma haemoglobin
  - Functional nitric oxide deficiency

Intravascular Haemolysis

- Controversial role of haemolysis in contributing to vasculopathy
  - Intravascular haemolysis releases free plasma haemoglobin, arginase
    - Free plasma haemoglobin binds to and inactivates nitric oxide
    - Arginase reduces substrate for nitric oxide synthetase
  - State of functional nitric oxide deficiency
  - Development of vasculopathy responsible for some complications including pulmonary hypertension, leg ulcers, priapism and possibly cerebrovascular disease


on
Leg ulceration
Priapism
Stroke

Haemolysis – Endothelial Dysfunction

- Central importance of haemolysis is debatable
  - Lack of marked vasculopathy with other haemolytic condition
  - No established link between vasculopathy and increased nitric oxide consumption
  - Evidence for link based entirely on association
  - Haemolysis one of a number of pathological events
  - Failure of nitric oxide based therapies

Inflammation

	function	Steady-state levels in SCD	Other associations
IL-1β	Inflammatory mediator	Increased	Low levels associated with increased stroke risk
IL-2	Stimulates T-cell response	Increased	
IL-3	Stimulates erythropoiesis	Increased	Positive correlation with Hb and HbF
IL-4	B- and T-cell proliferation	Normal	Increased in acute pain
IL-6	Inflammatory mediator	Increased	Predicts increased tricuspid regurgitant jet velocities. Increased in acute pain
IL-8	Neutrophil chemotaxis	Increased	Increased in acute complications
IL-10	Anti-inflammatory	Increased	Decreased in acute pain

Oxidative Stress

	Origin or Function	SCD associations
Modified serum albumin	Post-translational MDA modification of albumin	Increased with high tricuspid regurgitant jet velocities
TBARS, tocopherol	Lipid peroxidation	High in steady state, increasing when unwell
MDA, eicosanoids	Oxidative degradation of arachidonic acid	High in steady state, increasing when unwell
Red cell glutathione and glutamine	Red cell anti-oxidant	Low, associated with high tricuspid regurgitant jet velocities
Red cell glutathione peroxidase	Red cell anti-oxidant enzymes	Low activity, increased by hydroxycarbamide
Advanced glycation end products	Glycated and oxidized proteins	High in SCD, correlating with increased haemolysis
Melatonin	Antioxidant	Low levels

Hypercoagulability

Coagulation	Steady-state levels in SCD and significance
Coagulation system	
D-dimers	High – activated coagulation
Thrombin-antithrombin	High – activated coagulation
Prothrombin fragment F1+F2	High – activated coagulation
Fibrinopeptide A	High – activated coagulation
Factor V	High – prothrombotic
Factors VII and VIIa	Low – increased consumption
Fibrinogen	High – prothrombotic
Anticoagulant and fibrinolytic systems	
Protein C and S	Low – increased consumption and prothrombotic
Plasminogen activator inhibitor	High – decreased fibrinolysis
Platelets and microparticles	
Platelet numbers	High – prothrombotic
Platelet CD62, CD63, GPIIb/IIIa	Increased – platelet activation
Reticulated platelets	Increased – thrombopoiesis
Platelet aggregometry	Increased –platelet activation
Microparticles	Increased – prothrombotic
Von Willebrand factor	Increased with ultralarge multimers – prothrombotic
Other	
Homocysteine	Moderately increased - prothrombotic
Antiphospholipid antibodies	Increased frequency

Other Pathological Processes

	Relevance
Urinary hepcidin	Low in SCD suggesting anaemia predominates over inflammation in controlling iron metabolism.
Free plasma DNA	Elevated during acute pain and reduced by hydroxyurea
Soluble CD163	Haptoglobin-Hb receptor expressed on monocytes. Reduced by hydroxyurea
Plasma adenosine	Potentially harmful by causing decreased oxygen binding by haemoglobin
Serum zinc	Low zinc associated with poor growth, acute pain, leg ulcers
Red cell Duffy expression	Duffy-negative phenotype associated with chronic organ damage, lower white cell count

Pathophysiology

- Acute complications mainly related to vaso-occlusion
  - Acute pain (‘crisis’)
  - Acute lung damage
- Chronic complications
  - Damage to blood vessels – stroke, avascular necrosis, retinopathy, pulmonary hypertension
  - Organ damage – splenic infarction, renal failure, lung damage
- Secondary complications
  - Infections due to hyposplenism
  - Gall stones/liver problems

Pathophysiology

- Complications of treatment
  - Iron overload from transfusions
  - Infections from transfusions
  - Analgesia side-effects
- Psychological problems
  - Chronic ill health
  - Cognitive problems

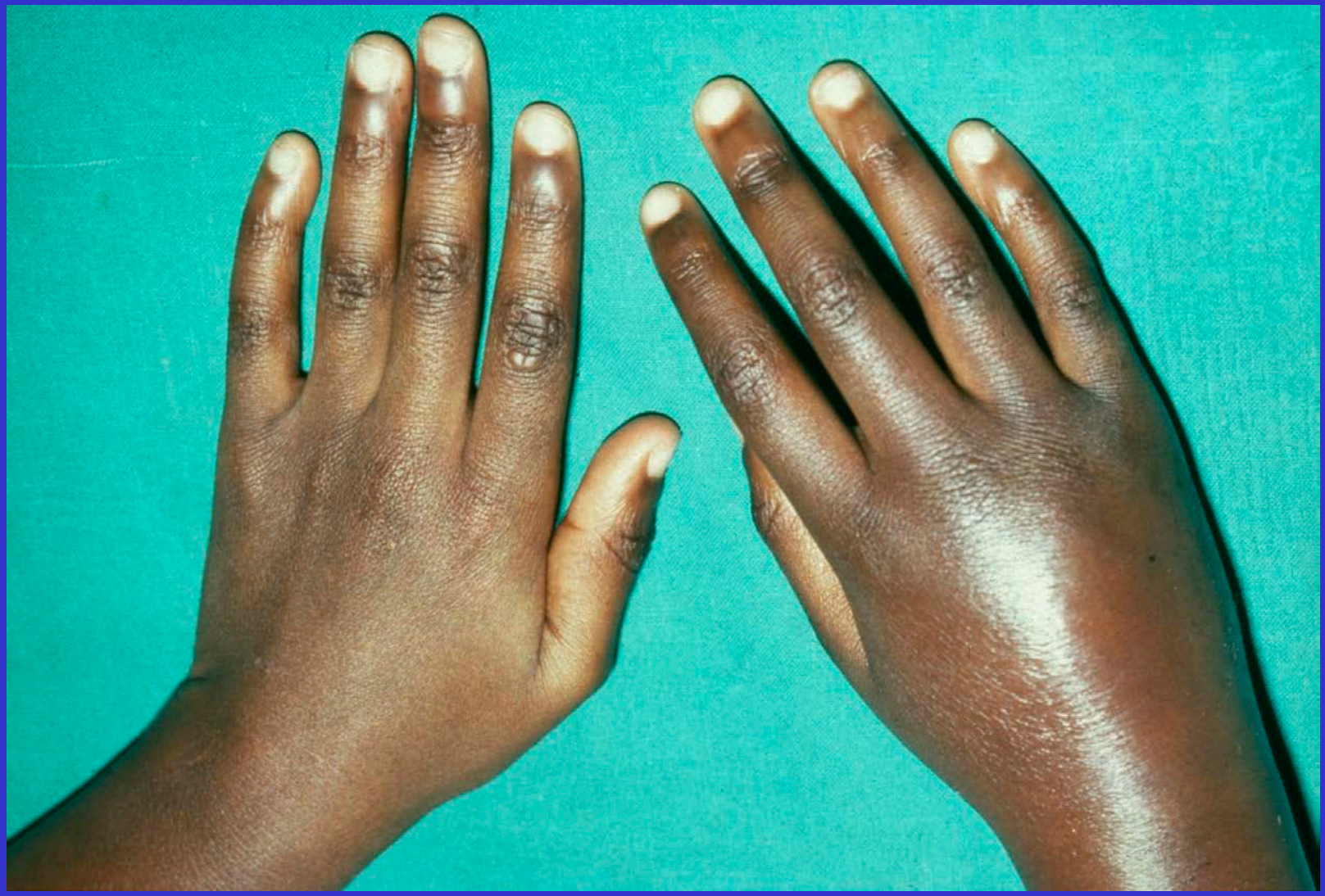
SCD in Infants

- Problems unusual before three months, due to high HbF levels
- Clinical problems include
  - Dactylitis
  - Infections
  - Splenic sequestration
  - Acute chest syndrome

Pneumococcal Infection in Sickle Cell Disease

- Children with SCD have 300-fold increased risk of pneumococcal sepsis
  - Associated with overwhelming infection, sudden death
  - Trials in USA and Jamaica have shown benefit of penicillin prophylaxis in under 5's, reducing risk of death
  - Emerging evidence that Pneumococcal infection important in Africa
  - Failure to get/take penicillin probably major cause of death in young children with SCD in UK
- Increased susceptibility to other capsulated organisms - Haemophilus, Meningococcus

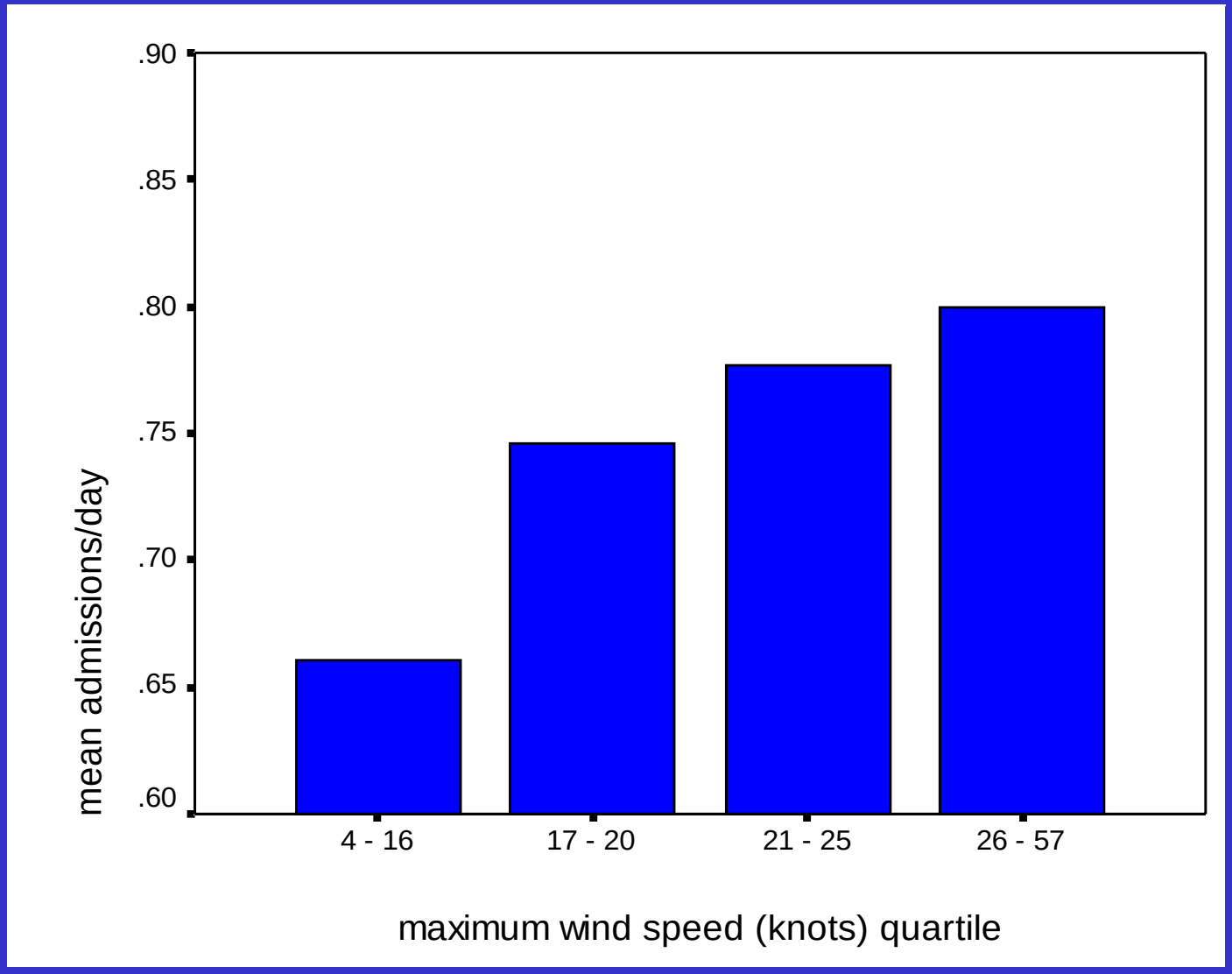
Dactylitis



Acute Pain

- Commonest complication - average of one severe episode per year but varies widely
- Primarily related to acute vaso-occlusion causing acute tissue ischaemia
- Precipitated by
  - Climatic factors
  - Exposure to cold
  - Infections
  - Stress, unidentified factors

Climatic Effects in London



Neurological Complications

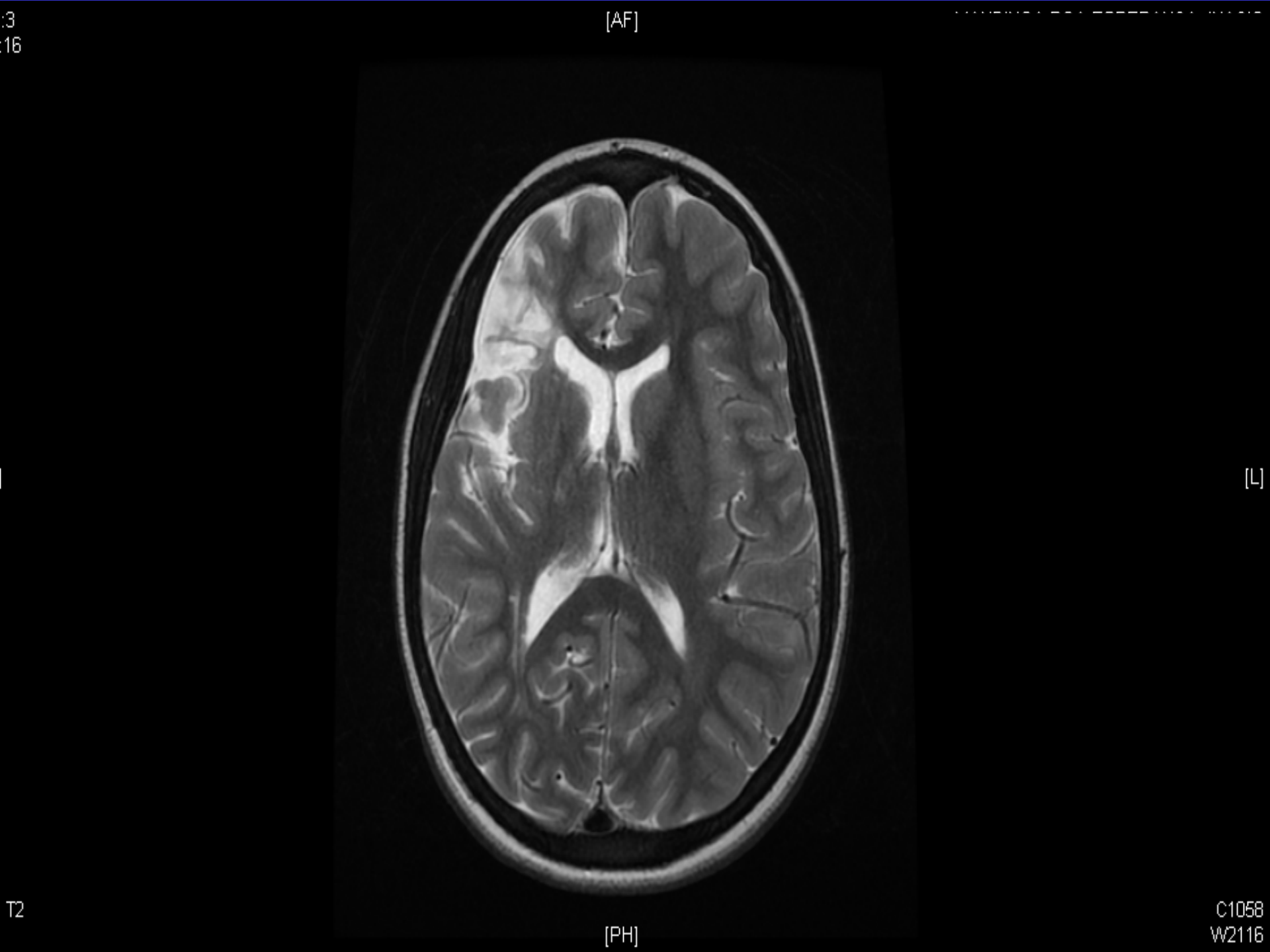
- Brain is site of major morbidity in children with SCD
- Complications include
  - Infarctive stroke
  - Haemorrhagic stroke
  - Silent infarcts
  - Transient ischaemic attacks
  - Subarachnoid haemorrhage
  - Cognitive impairment
  - Fits
  - Headaches

Epidemiology of Stroke

- Sickle cell anaemia (HbSS)
  - Overall incidence of 0.61/100 patient years
  - Peak incidence ages 2-5, at 1.02/100 patient years, and over age of 50
  - 300 times more common than in general paediatric population
  - 11% age 20 years, 15% age 30, 24% age 45
- Other types of sickle cell disease
  - HbS/β^0thalassaemia 0.24/100 patient years,
  - HbS/β^+thalassaemia 0.13/100 patient years,
  - HbSC 0.084/100 patient years

Pathology of Infarctive Stroke in SCD

- Most frequently associated with blockage of intracranial carotid and middle cerebral arteries
- 20% strokes not associated with overt intracranial vasculopathy
  - extracranial ICA stenosis
  - arterial dissection
  - patent foramen ovale
- Pathological basis not established
  - failure of control of intracerebral blood flow
  - endothelial damage
  - functional nitric oxide deficiency
  - hypoxia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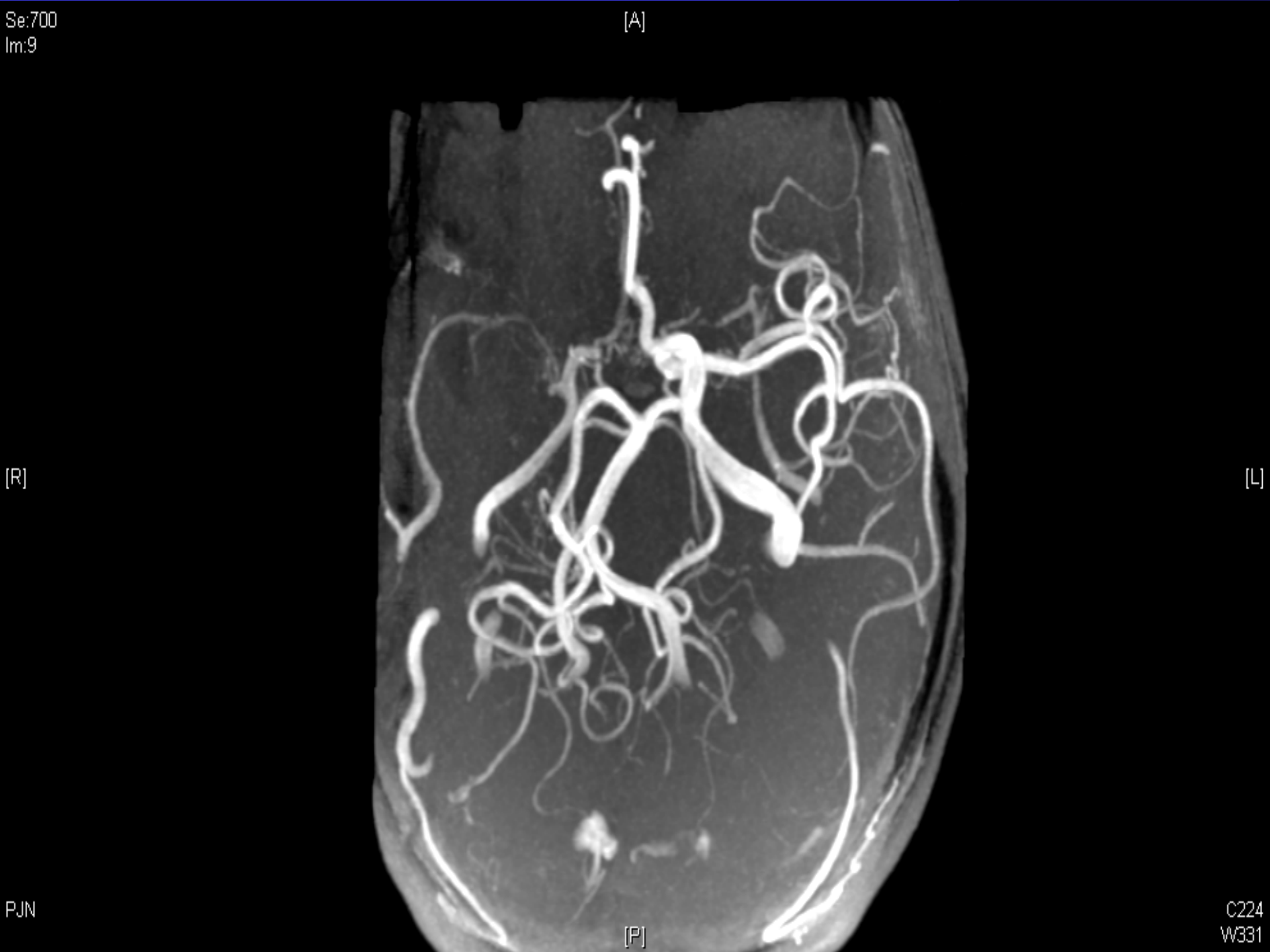
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Risk Factors for Infarctive Stroke

- Abnormal Transcranial Doppler scan
  - Detects increased blood velocity corresponding to stenosis
  - Ranges defined by STOP study
    - Abnormal: >200cm/s in MCA or dICA
    - Conditional: 170-200cm/s in MCA or dICA
    - Inadequate: MCA, dICA, bifurcation not seen
  - Abnormal TCD associated with stroke risk of 10% per year

Secondary Prevention of Infarctive Stroke

- Without intervention, recurrent stroke occurs in 50-90% cases
- Regular blood transfusion
  - Maintain HbS<30%, Hb>9g/dl
  - Reduces risk of recurrence to about 10%
  - 3-4 weekly top-up transfusions
  - less frequent exchanges
- Management of complications of blood transfusion
  - Iron overload
- Sibling allogeneic bone marrow transplantation

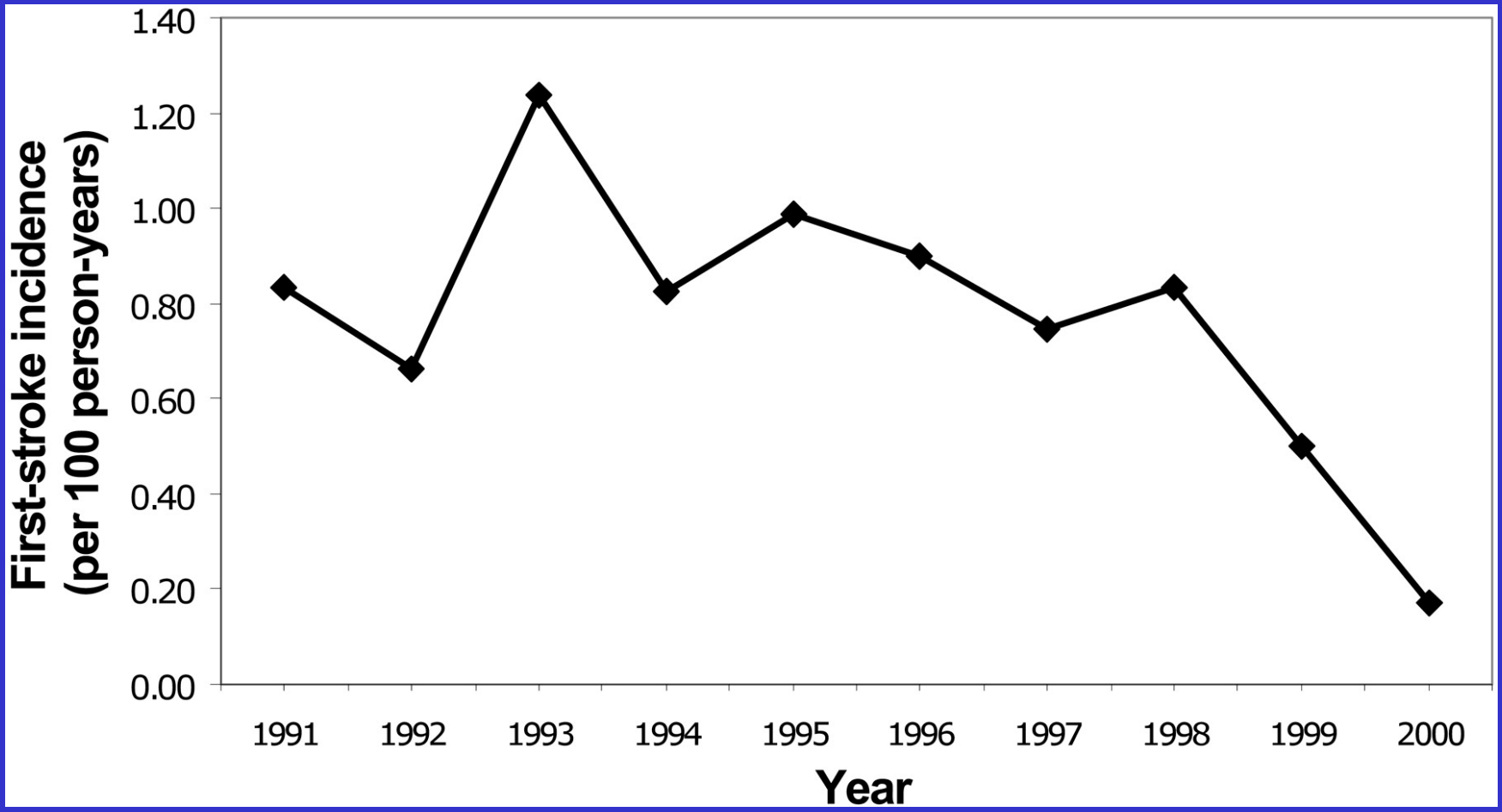
Primary Prevention of Infarctive Strokes

- Stroke prevention trial in Sickle Cell Anemia (STOP)
  - Children with HbSS, no history of stroke, TCDs>200cm/s
  - 63 assigned transfusions – 1 infarction
  - 67 standard care – 10 cerebral infarctions, 1 intracerebral haematoma

Primary Prevention of Infarctive Strokes

- Optimizing Primary Stroke Prevention in Sickle Cell Anemia (STOP 2)
  - Children with HbSS, on transfusions because of abnormal TCD's for at least 30 months, with normalised TCD's
  - 41 stopped transfusion: 14 redeveloped abnormal TCD's, and 2 strokes
  - 38 continued transfusion: no abnormal TCD's or strokes

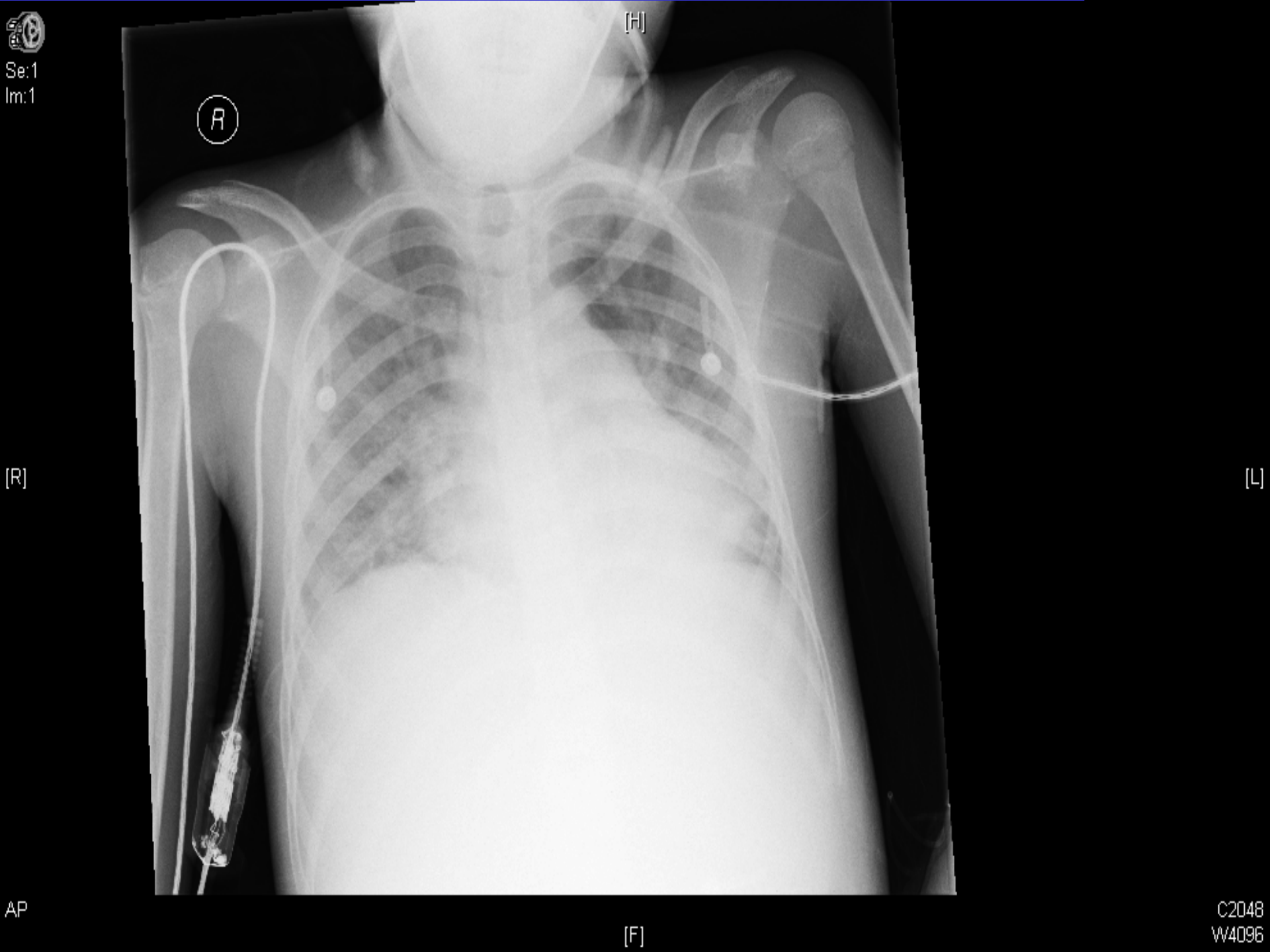
Incidence rates of first stroke in Californian children with SCD



Fullerton, H. J. et al. Blood 2004;104:336-339

Acute Chest Syndrome

- Definition varies
  - Any new shadow on chest x-ray
  - Usually associated with chest pain, generalised pain, fever, hypoxia, tachypnoea, tachycardia, chest x-ray changes
- Aetiology - complex
  - Infection (Chlamydia), fat embolism, rib infarction, thromboembolism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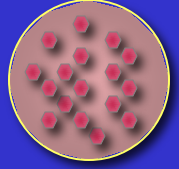
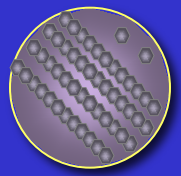
Causes

- 40-50% initially admitted to hospital with acute pain or other problem and then develop ACS
  - Pain causing reduced lung ventilation
    - Back and rib pain
    - Abdominal pain
  - Sedation from analgesia
- Infection
- Fat embolism
- Vaso-occlusion by sickled red cells
- No specific cause identified in 45% cases
- Increased risk with smoking



Oxygenated red cell containing HbS

hypoxia



Deoxygenated red cell with HbS polymerising

Dehydrated, sickled red cell

Occlusion of pulmomary blood vessels

lung infarction

- acute chest syndrome

Infection

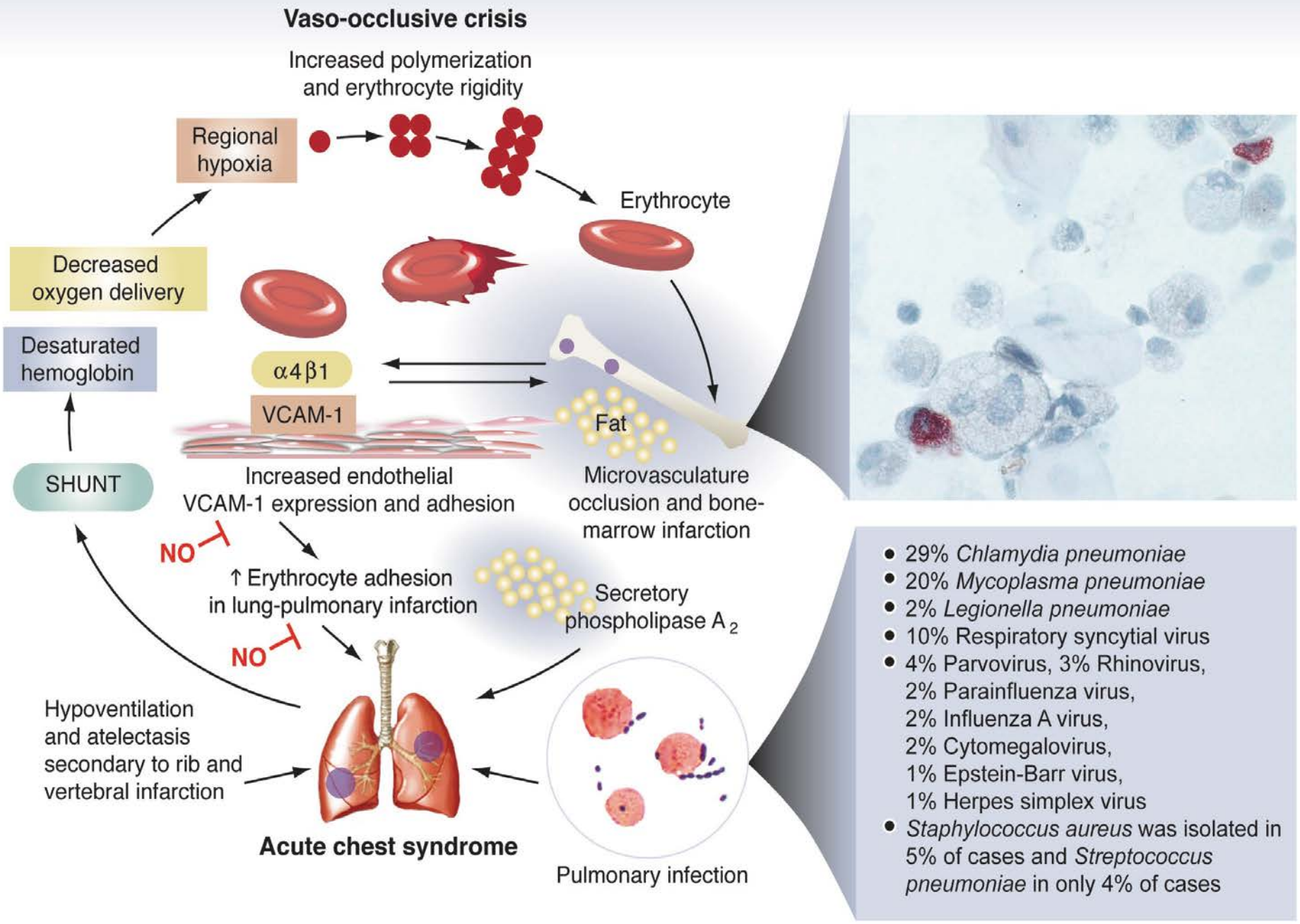
- Chlamydia pneumoniae – 7%
- Mycoplasma – 7%
- Respiratory syncytial virus – 4%
- Streptococcus pneumoniae – 2%
- Legionella – 0.6%
- Others
  - Tuberculosis, atypical mycobacteria, Salmonella
- Influenza
  - 25% cases of pandemic influenza A (H1N1) develop ACS

Vichinsky et al, 200, NEJM, 342, 1855-1865

Other Pathological Mechanisms

- Worsening anaemia
  - Reduced oxygen-carrying capacity of blood
  - Increased tissue damage
- Haemolysis
  - Release of free haemoglobin into plasma
  - Free haemoglobin inactivates nitric oxide
  - Constriction of pulmonary arteries, vascular inflammation
- Inflammation
  - Increased adhesion of red cells to blood vessels
- Reperfusion
  - Reoxygenation generates free radicals, tissue damage
- Acute heart failure
  - Over hydration
  - Pulmonary oedema and pleural effusions

Vicious cycle of vaso-occlusive crisis and acute chest syndrome



Symptoms of Acute Chest Syndrome

• Fever	80%
• Cough	62%
• Chest Pain	44%
• Shortness of breath	41%
• Pain in arms and legs	37%
• Abdominal pain	35%
• Rib or sternal pain	21%
• Wheezing	13%
• Neurological symptoms	4%
• Cyanosis	2%
• Heart failure	1%

Vichinsky et al, 200, NEJM, 342, 1855-1865

Outcome

- Outcome worse in over 20's
  - Longer hospital stay
  - More complications
  - Higher risk of death
- Mean length of hospital stay – 10.5 days
- Mechanical ventilation – 13%
- Neurological complications
  - Confusion – 5%
  - Seizures – 1%
  - Stroke – 2%
  - Posterior leukoencephalopathy syndrome
- Death – 3%

Management of Acute Chest Syndrome

- Hydration, antibiotics, oxygen/ventilatory support, analgesia, incentive spirometry
- Blood transfusion if clinical or radiological deterioration, falling oxygen saturations not corrected by oxygen
  - Top-up transfusion if Hb low
  - Exchange transfusion if severe, higher Hb
  - CPAP, ventilation, ECMO
  - Inhaled nitric oxide, corticosteroids

Pulmonary Hypertension

- Initial SCD studies used echocardiography
  - PH defined as triscupid jet velocity>2.5m/s
  - 32% adults found to have condition
  - Increased mortality rate in this population
- Subsequent studies using catheterization suggest lower prevalence
  - 6% have true pulmonary hypertension
    - 1.5% pulmonary arterial hypertension
    - 4.5% hyperdynamic state

Other Complications of Sickle Cell Disease

- Leg ulcers
- Gall stones, cholecystitis, hepatopathy
- Chronic lung syndrome
- Avascular necrosis of femoral and humoral heads
- Retinopathy
- Renal failure
- Priapism
- Chronic pain
- Sudden death

Summary

- Complex cascade of interrelated pathological processes follow on from primary events of HbS polymerisation and vaso-occlusion
- Unpredictable disease characterised by acute and chronic illness
  - Acute problems mostly caused by infection and infarction
  - Chronic problems caused by vasculopathy and organ failure
- Iatrogenic and social factors very important determinants of severity