Laboratory tests in pediatric rheumatology

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10y old boy

- Since 6 weeks pain in the right knee, ‘low back pain’. Nocturnal pain.
- Swelling of right knee & intermittent limping
- No weight loss, no fever
- Clinical examination: unilateral gonarthritis, sensitive/tense paravertebral muscles

Differential diagnosis

- Reactive arthritis
- Post-streptococcal reactive arthritis
- Lyme arthritis
- Juvenile idiopathic arthritis



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Differential diagnosis ‘chronic arthritis’

- Reactive arthritis
- Post-streptococcal reactive arthritis
- Lyme arthritis
- Juvenile idiopathic arthritis

Laboratory findings

- Hb 12,7 g/dL, 383000 platelets/µL, WBC 5670/µL (50% neutrophils)
- CRP & sedimentation rate negative
- Borrelia serology IgM negative, IgG positive
- ASLO negative
- RF? ANA? HLA B27?

Which reumatic test would you ask?

- Reumafactor, anti-CCP
- ANA
- HLA B27
- All of the above
- None of the above

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Which reumatic test would you ask?

Reumafactor, anti-CCP 0%

ANA 0%

HLA B27 0%

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Lyme arthritis



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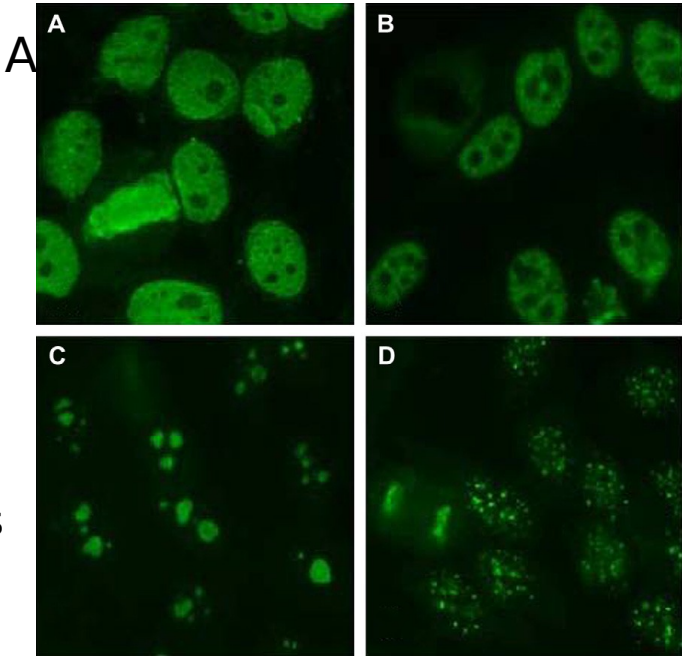
ANA

- ANA (AntiNuclear Antibodies)= autoantibodies that recognize cellular antigens found predominantly in the nucleus

- Detection
  1. Immunofluorescence=gold standard (HEp-2 cells): pattern of IF depending on the location of the target Ag
  2. ELISA: to detect specific antibodies eg. anti-histone abs, anti-SSA Abs, anti-SSB abs, anti-Sm abs, ...
  3. radio-immuno assay to detect anti-dsDNA A

Homogeneous: anti-histone and/or anti-dsDNA abs

Nucleolar: anti-scl-70 Abs



Speckled: anti-Sm Abs, anti-SSA Abs, anti-SSB Abs

Centromere

Nuclear Antigen	
Anti-ds DNA	Disease activity in **SLE**
Anti-Sm Abs	Highly specific for **SLE**, but low sensitivity (only 30% of SLE patients) Associated with less severe forms of **nephritis, CNS disease and serostis**
Anti-Ro (SSA)	**Sjögren's syndrome** and subacute **cutaneous lupus** Implicated in the pathogenesis of **neonatal lupus** 2 subtypes: Ro52-> systemic sclerosis and myositis Ro60-> sLE
Anti-La (SSB)	Often in combination with anti-Ro Abs **SLE, Sjögren's** syndrome
U1- RNP	**MCTD**, (sLE, systemic sclerosis)
Jo-1	Polymyositis typically with interstitial fibrosis, dermatomyositis, sLE, MCTD
Scl-70	Highly specific for **systemic sclerosis** (but low sensitivity) & associated with the development of pulmonary fibrosis
Anti-p155	**Dermatomyositis** with prominant skin symptoms (Gottron's papules, ulceration and edema)

ANA

ANA positivity

- Is seen in
  - Different systemic autoimmune diseases: JIA, MCTD, Sjögren’s syndrome, scleroderma, juvenile dermatomyositis
  - Organ specific autoimmune diseases: autoimmune hepatitis , autoimmune thyroiditis, …
  - Drugs: procainamide, isoniazod, minocycline, penicillamine, anticonvulsants, …
  - Infections: EBV, TBC, malaria, …
  - !Maligancies

- Healthy individuals:
  - up to 30% in low titers (1/40) and 5% in higher titers (1/160). No ANA specificity & disappear with time
  - Eldery, women, first degree relatives of patients with an autoimmune disease

ANA≠ SCREENING TOOL FOR RHEUMATIC DISEASES, BUT USEFULL IN DIAGNOSIS OF SLE

HLA B27

HLA = Human Leucocyte antigen complex, present on all cells (except on red blood cells)

Role in presentation of antigens to T cells

Important in transplantation of solid organs or in stem cell transplantation

HLA B27 ≈ MHC class I molecule

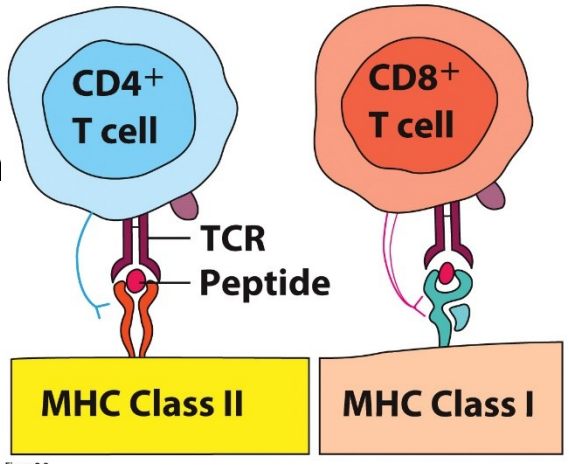
HLA B27 positivity is found in approximately 80 to 95 percent of Caucasian patients with **spondylarthritis patients** in most ethnic groups. Possible role in pathogenesis of SpA? In gut microbiome?

Also found more frequently in patients **with IBD, psoriasis, psoriatric arthritis, reactive arthritis**

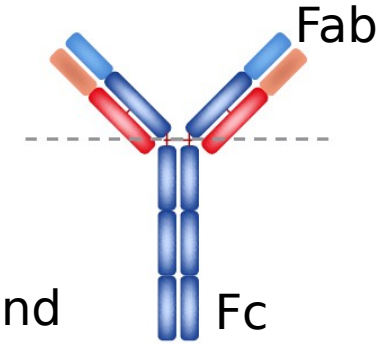
BUT

HLA B27 positivity can be found in up to 15% of healthy population

HLA B27≠DIAGNOSTIC TOOL, RISK FACTOR FOR DEVELOPING CERTAIN DISE



Reumatoid factor/anti-CCP



Reumatoid factor:

- Antibodies directed against the Fc portion of immunoglobulin (Ig) G
- Diagnosis and establishing prognosis of rheumatoid arthritis (RA)
- ! however, RF may also be present in other rheumatic diseases (Sjogren, MCTD, ….) and other conditions, including acute and chronic infections and neoplastic disease.
- RFs have been found in up to 4 percent of young, healthy individuals

RF≠SCREENING TEST, BUT USEFUL IN DIAGNOSIS/PROGNOSIS WHEN SUSPICION OF A RHEUMATIC DISEASE

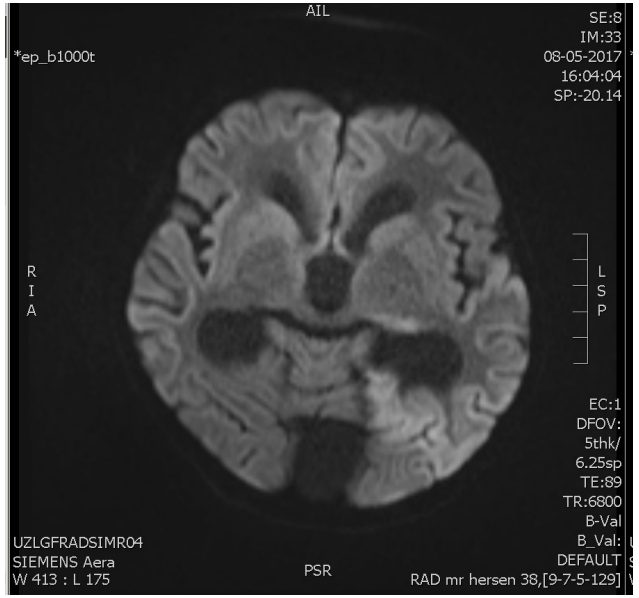
Anti-CCP Abs= Anti-citrullinated peptide antibodies

- When positive in polyarticular JIA patient: ↑ risk for joint damage/erosive disease

Systemic lupus



- 9y old boy
  - Persistent fever for 3 weeks
  - Fatigue
  - Oral ulcera, necrotizing vasculitis lesions on skin and mucosa
  - Pancreatitis
  - Partial epilepsy: fasciculations right hemiface, eye deviation. Somnolence and aphasia.
  - EEG: continuous epileptic activity (PLEDs left occipital)
  - DW-MRI: ischemic lesions left thalamus and parieto-occipital region
- Laboratory findings
  - Hb 8,4 g/dL, 115000 platelets/µL, WBC 3600/µL (1000 lymphocytes)
  - ESR 50 mm/h, CRP 0,8 mg/L
  - ANA 1/1280
  - Farr>100 IU/ml
  - Total complement<20% with low C3&C4 and increased C3d
  - Lupus anticoagulans-, anti-cardiolipin Abs-



Systemic lupus

- Heterogenous presentation, but systemic disease: fever, skin lesions (lupus rash, vasculitis), hair loss, fatigue, arthritis and athralgia, nephritis, pleuritis, pericarditis, Raynaud, neuropsychiatric manifestations, ophtalmological manifestations
- Incidence : 0.36-2.5/100000/y

- Lab:
  - Anemia, leukopenia, thrombocytopenia
  - Low CRP- high sedimentation rate
  - Positieve ANA/Farr
  - Complement use
  - HyperIgG
  - ± Risk op thrombosis (antifosfolipid syndrome)
  - Urine
- Genetics: ?, predisposition for females, african/aziatic women. Zz monogenetic forms (young children)
- Therapy: corticosteroids, Plaquenil
  - Depending on main clinical manifestation: methotrexaat, cellcept, Rituximab

Complete blood cell count:

Hb 8,4 g/dL, 115000 platelets/µL, WBC 3600/µL (1000 lymphocytes)

↳ **Microcytic anemia, haptoglobin normal, Coombs-, bilirubin nl, LDH nl**

- "**Anemia of chronic disease**"

Cytokine mediated shortened RBC survival and impaired response to EPO (TNF-α, IL-1β, IL-6 and IFNγ)

Decreased intestinal iron absorption and decreased iron release from stores ((IL-6)-hepcidin axis)

- Iron-deficiency anemia
- Autoimmune hemolytic anemia

	Anemia of chronic disease	Iron deficiency anemia
MCV	Normal (or ↓ in prolonged disease)	↓
Serum Iron	↓	↓
Transferrin	↓	↑
Ferritin	Normal or ↑	↓
Soluble transferrin receptor-ferritin index	<1,0	>2,0
Transferrin saturation %	Normal (can be ↓)	↓

Adapted from Mehta et al, 2012

Complete blood cell count

Hb 8,4 g/dL, 115000 platelets/µL, WBC 3600/µL (1000 lymphocytes)

- **“Thrombocytopenia”**

  Immune mediated: antiplatelet antibodies (+- 75% of SLE patients)

  -> Decreased survival

  (In patients with antiphopholipid syndrome and TTP)

Complete blood cell count

Hb 8,4 g/dL, 115000 platelets/µL, WBC 3600/µL (1000 lymphocytes)

- **“Thrombocytopenia”**

  Immune mediated: antiplatelet antibodies (+- 75% of SLE patients)

  -> Decreased survival

  (In patients with antiphopholipid syndrome and TTP)

- **“Leukopenia”,** particularly lymphopenia

  Increased lymphocyte apoptosis

  Antilymphocyte antibodies

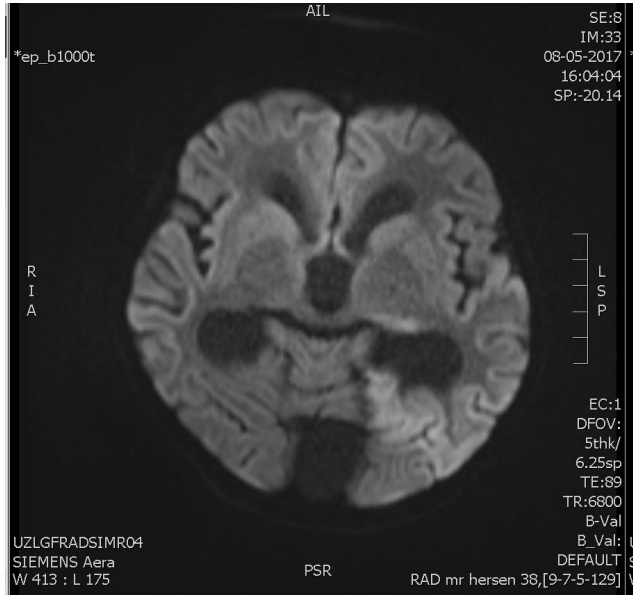
  Sequestration at sites of inflammation

Pancytopenia: autoimmune but cave malignancy (bone marrow)

Systemic lupus



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Sedimentation rate, ESR versus CRP

ESR 50 mm/h, CRP 0,8 mg/L

- **ESR** = height of the layer of red blood cells that settle in a vertical tube of anticoagulated blood in 1h (mm/h)
  - Correlates with ↑ levels of acute phase reactants (fibrinogen)
  - Aspecific (infectious and immune-mediated inflammatory conditions) , non diagnostic
  - Monitor treatment response or underlying disease activity

- **CRP** low in SLE. If increased: infection or serositis

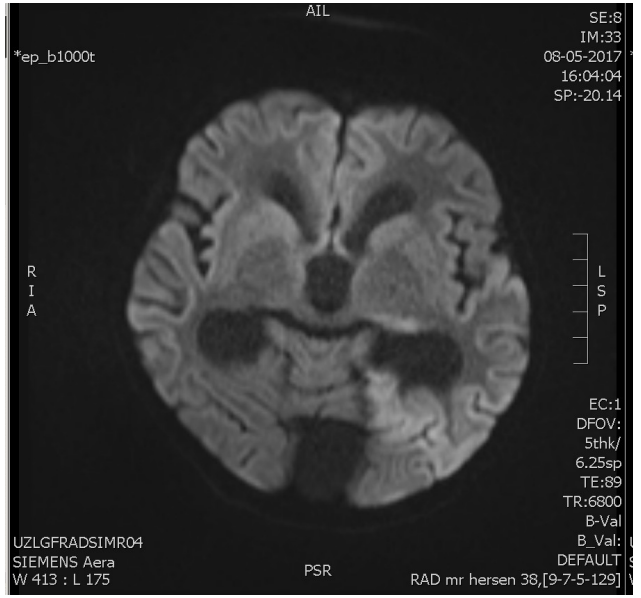
Increased ESR

Altered serum protein levels	**Elevated fibrinogen levels** (infection, inflammation, malignancy) **Hypergammaglobulinemia**	
Red cell abnormalities	Macrocytosis	
Technical issues	Dilutional problems Increased specimen temperature Inadequate mixing Tilted ESR tube	
Miscellaneous	Anemia Female sex	Increasing age Pregnancy

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ANA

ANA 1/1280 (anti-SmD 75 U/ml, anti-Ro60 >282 U/ml), Farr > 100

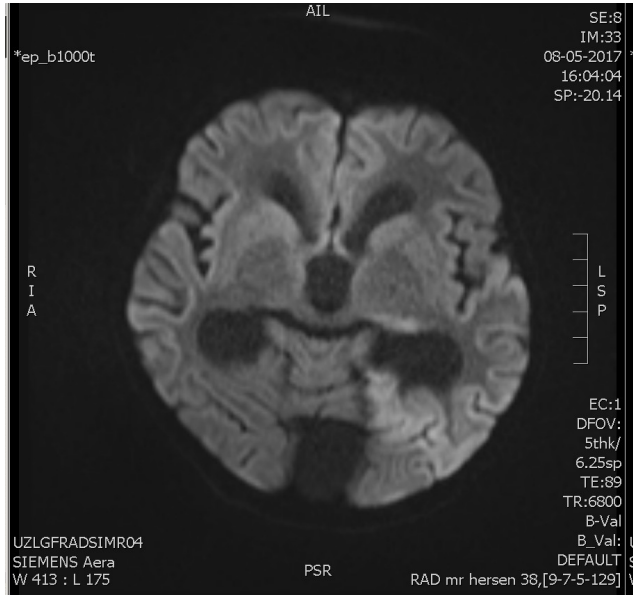
- ANA (AntiNuclear Antibodies)= autoantibodies that recognize cellular antigens found predominantly in the nucleus, usefull in diagnosis of sLE but seen in many other circumstances

Nuclear Antigen	
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Systemic lupus



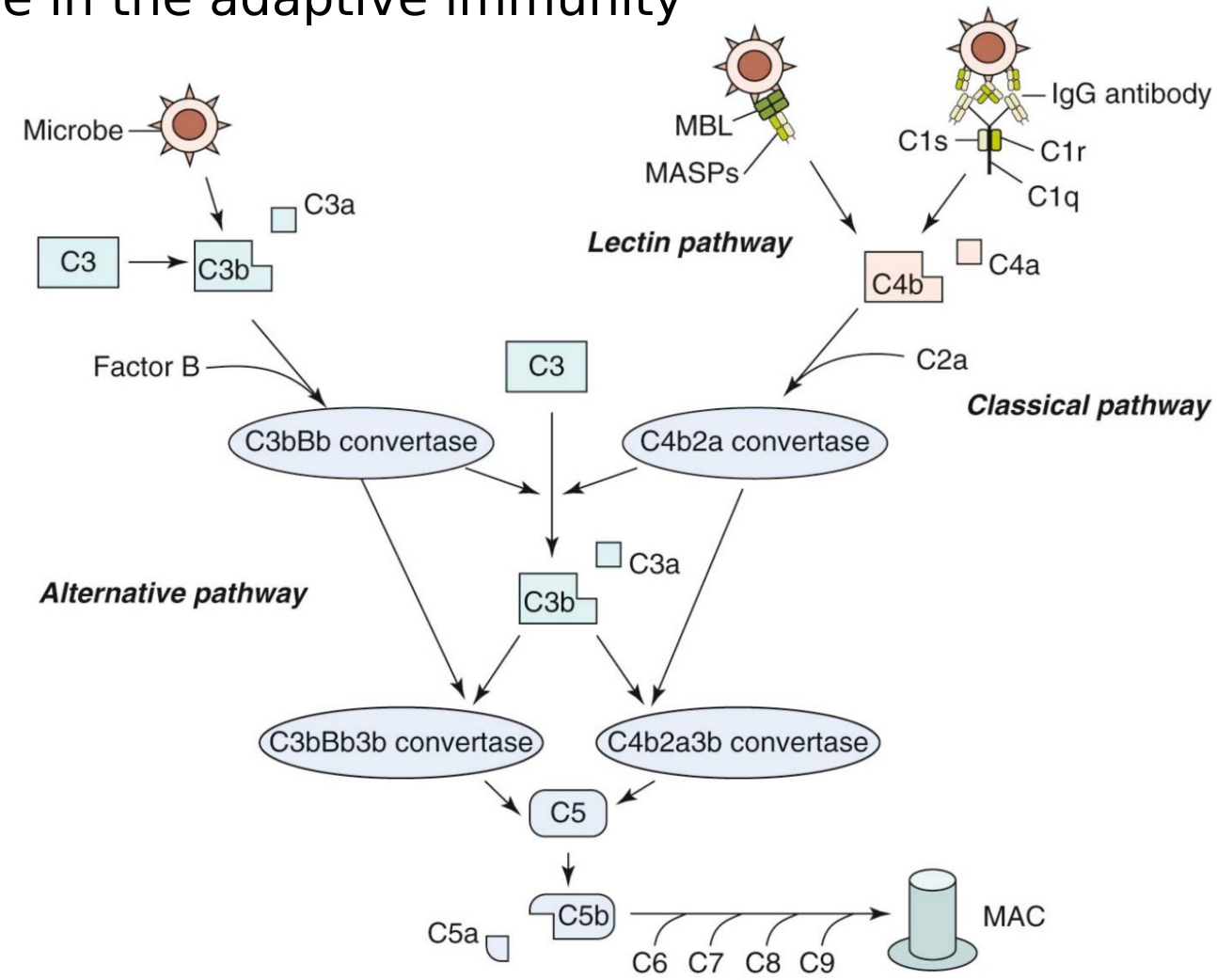
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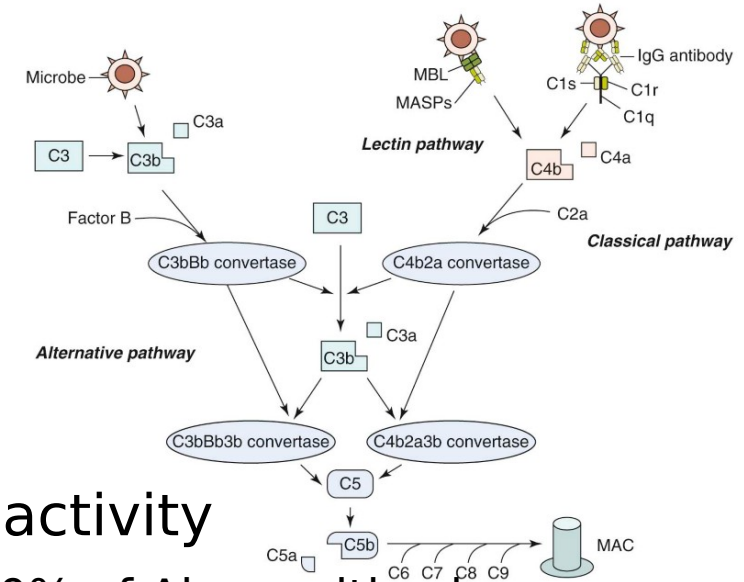
Complement system

- Innate Immune system, but also a role in the adaptive immunity
- Activated by immune complexes



Complement system

CH50<20%,
low C3&C4 and increased C3d



- CH50: assessment of the total hemolytic complement activity

= Titer at which the patients serum is able to cause hemolysis in 50% of Ab-sensitized sheep erythrocytes

↓ in sLE, acute postinfectious glomerulonephritis, membranoproliferative glomerulonephritis, liver disease

congenital deficiency of complement component

↑ inflammatory disorders (acute phase reactants)

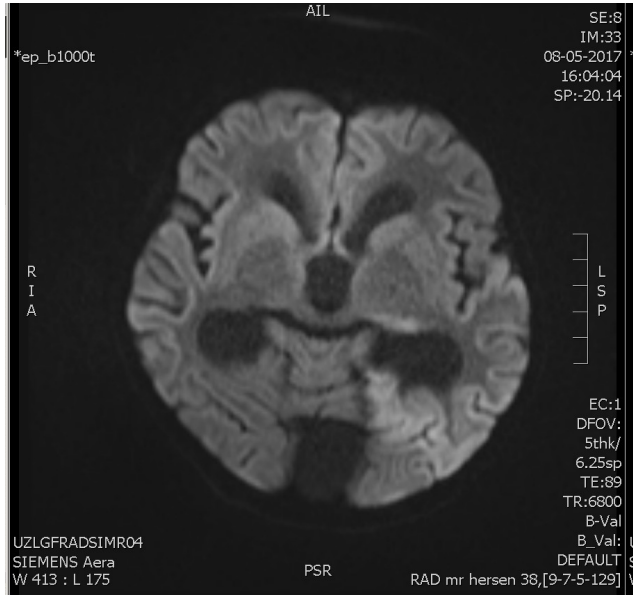
- Hereditary complement deficiencies of C1q, C4 and C2 are associated with an increased risk of development of sLE

<->deficiencies in the terminal pathway predispose to infections (Neisseria meningitidis)

Systemic lupus



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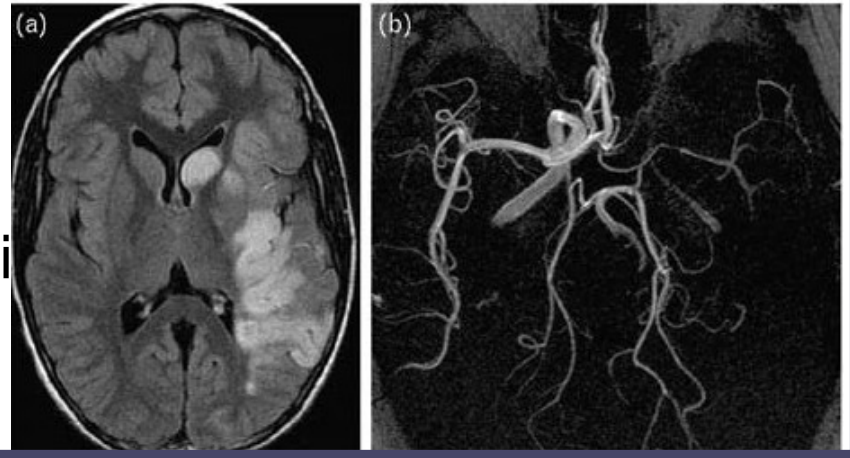
Antiphospholipid antibodies (aPL)

- Autoantibodies directed against plasma proteins that bind to cell membrane phopolipids
  1. Lupus anticoagulans (DRVVT)
  2. Anticardiolipin IgG & IgM (ELISA): most sensitive test for APS in children
  3. Anti-β2-glycoprotein-I IgG & IgM (ELISA): more specific for thrombosis

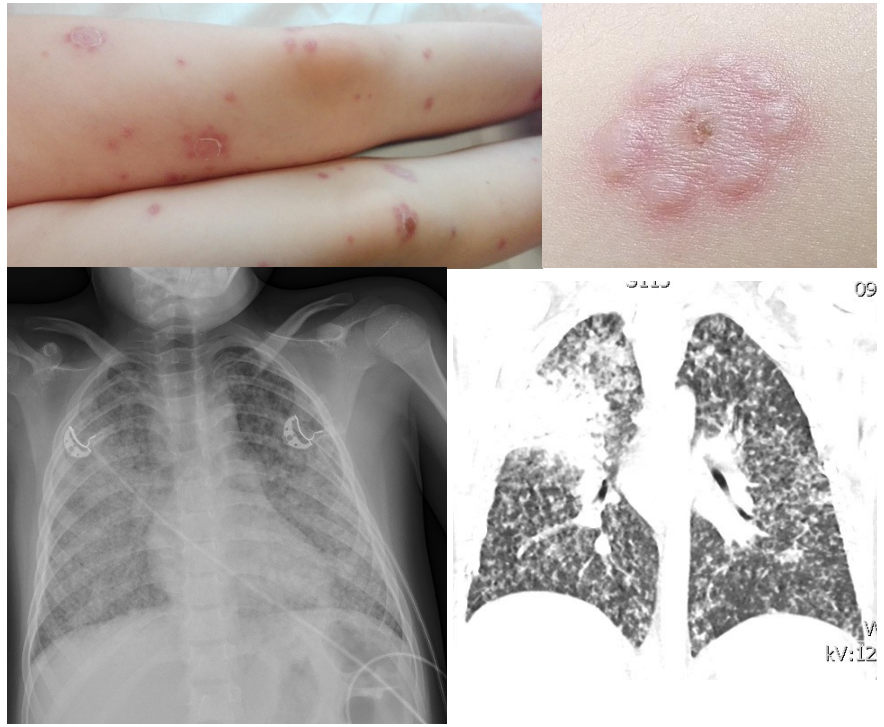
- Associated with an increased risk of arterial and venous thromboembolic events

- Primary APS versus secondary APS (sLE)

!high incidence of transient aPL following viral infecti



Systemic vasculitis



- 6y old girl
  - Fever, malaise
  - Cough with bloody sputa
  - Diffuse vasculitic skin lesions

- **Laboratory findings**
  - Hb 7 g/dL, 300000 platelets/µL, WBC 12120/µL (90,3% neutrophils)
  - ESR 78 mm/h, CRP 463,9 mg/L
  - D-dimers 15287 µg/L
  - Total complement nl with normal C3&C4
  - ANA-, ANCA+ (p-ANCA: 1/1280- MPO >134 U/ml)

ANCA

ANCA+ (p-ANCA: 1/1280- MPO >134 U/ml)

ANCA (**A**nti**N**eutrophilic **C**ytoplasmic **A**ntibodies)

- Antibodies against primary granules of neutrophils and lysosomes of monocytes. May stimulate oxidative burst and degranulation of neutrophils.
- IF, ELISA
- Group of conditions characterized by small vessel vasculitis “ANCA associated vasculitis”
- Granular cytoplasmatic fluorescence pattern: c-ANCA (PR3) -> granulomatosis with polyangiitis

Perinuclear fluorescence pattern: p-ANCA (MPO)->microscopic polyangiitis, Churg Strauss granulomatosis (eosinophilia)

Indeterminate or atypical pattern

Casus 1



What is your preferred diagnoses

- Viral infection (parvo B19, EBV, CMV)
- Leukemia
- Polyarticular JIA
- Systemic JIA
- Systemic lupus
- Systemic vasculitis

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What is your preferred diagnoses

Viral infection (parvo B19, EBV, CMV) 0%

Leukemia 0%

Polyarticular JIA 0%

Systemic JIA 0%

Systemic lupus 0%

Systemic vasculitis 0%

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What is your preferred diagnoses

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Systemic JIA 0%

Systemic lupus 0%

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Casus 1: differential diagnosis

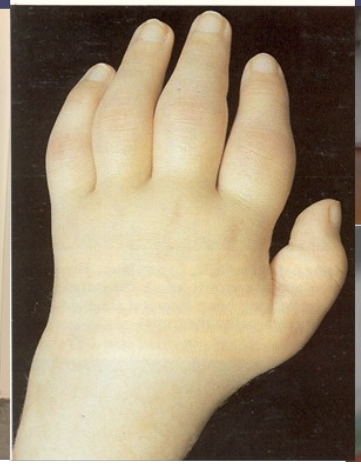
A. Viral infection (parvovirus B19, EBV, CMV) → Passed EBV en mycoplasma
Serology for CMV & parvovirus negative

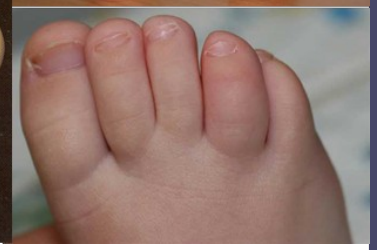
B. Leukemia → LDH slightly elevated, urinic acid nl
Blood smear nl

C. Polyarticular JIA
D. Systemic JIA
E. Systemic lupus
F. Systemic vasculitis

→ ANA + (1/1280), Farr > 100
D-dimeren2027 µg/L
IgG 16 .6g/L
Low total complement, ↓C3 en C4
+ Lupus anticoagulans/anticardiolipine Abs
Urine: milde proteinuria

Casus 2



- 6y old girl
  - Since 4 weeks pain in knees, wrists and elbows. No nocturnal pain.
  - Swelling of joints & intermittent limping
  - No weight loss
  - Clinical examination: polyarthritis (ellbows, wrists, knees, ankles, right MCP III&IV, left MCP V
- Laboratory findings
  - Hb 12,7 g/dL, 383000 platelets/µL, WBC 5670/µL (50% neutrophils)
  - Peripheral blood smear: no significant morphological abnormalities
  - AST&ALT nl, ferritin nl. LDH 424 U/L (hemolytic sample)
  - ESR 71 mm/h, CRP 23 mg/L
  - ANA 1/160

Juvenile idiopathic polyarthritis



- 6y old girl
  - Since 4 weeks pain in knees, wrists and elbows. No nocturnal pain.
  - Swelling of joints & intermittent limping
  - No weight loss
  - Clinical examination: polyarthritis (ellbows, wrists, knees, ankles, right MCP III&IV, left MCP V
- Laboratory findings
  - Hb 12,7 g/dL, 383000 platelets/µL, WBC 5670/µL (50% nuetrophils)
  - Peripheral blood smear: no significant morphological abnormalities
  - AST&ALT nl, ferritin nl. LDH 424 U/L (hemolytic sample)
  - ESR 71 mm/h, CRP 23 mg/L
  - ANA 1/160

Acute myeloid leukemia

- Treatment:
  - NSAIDs
  - Methotrexate and methylprednisolone in tapering dose
- Clinical evolution:
  - Disappearance of arthritis but intermittent pain complaints persisted.

- Laboratory findings
  - Hb 10,6 g/dL, 269000 platelets/µL, WBC 3870/µL (42% nuetrophils)
  - LDH 800 (hemolytic sample)
  - CRP 99 mg/L
  - Peripheral blood smear (after 4 months): 4% blasts

Conclusions

Laboratory test are usefull in diagnosis and follow-up

Complete blood cell count: ↑ typical for inflammatory diseases

↓ in SLE !DD malignancies

ESR/CRP are typically increased

Auto antibodies are associated with a number of autoimmune diseases

Anti-dsDNA- ENA SLE

ANCA vasculitis

Anti-phospholipid thromboembolic events

HLA B27 is not a diagnostic tool, but risk factor for developing certain rheumatic diseases

Abscence of laboratory abnormalities does not exclude a reumatic condition