Immunology for the Orthopaedic Surgeon

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Portsmouth
No clinical data and multiple requests - more thought needed:

~£150 worth of tests requested here
Tests delayed if there’s no clinical data
Functions of IgG

Constitutes 75% of total serum immunoglobulins

Present in vascular and extra-vascular spaces

4 subclasses (IgG1 – 4)

Crosses placenta (IgG1,3 & 4 >>2)

Fixes complement (IgG3>1>2>4)

Binds to macrophages, thus “arming” them so they can act as cytotoxic cells
Functions of IgA

Constitutes 15% of total serum immunoglobulins

Predominant antibody found in body secretions

Provides the primary defence mechanism against some local infections because of its abundance in saliva, tears, bronchial and nasal secretions, etc.

Probably prevents access to the general immune system rather than destruction of antigen

1/400 people have no IgA
Functions of IgM

Constitutes 10-15% of total serum immunoglobulins

From an evolutionary point of view, this is the oldest antibody.

Predominantly found within the intra-vascular space because of its large size.

Fixes complement very efficiently indeed with a single molecule bound to antigen sufficient to initiate the complement cascade, thus along with complement it has lysing properties.

Involved in the primary immune response against blood borne bacteria.

Reaches adult levels within a few weeks of birth.
Childhood Development of Immunoglobulin Concentrations

- IgM
- IgG
- IgA

Total Ig's post birth

IgE subclasses post birth
Fig. 1 - News of the World montage about potato allergy. (Photograph courtesy of The Independent.)
Autoimmunity

- As users, you request tests but you cannot order them.
  - You could try ordering GZ to do a neck dissection!
- We perform tests according to clinical data and to investigate specific autoimmune conditions.
  - No point in asking for things like an “autoimmune profile” or “exclude autoimmune disease”
  - No clinical data generally means no tests.
    - ?? Reasonable course of action
### Spectrum of autoimmune diseases

<table>
<thead>
<tr>
<th>Organ-specific</th>
<th>Non-organ-specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hashimoto's thyroiditis</td>
<td></td>
</tr>
<tr>
<td>primary myxoedema</td>
<td></td>
</tr>
<tr>
<td>thyrotoxicosis</td>
<td></td>
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<tr>
<td>pernicious anaemia</td>
<td></td>
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<tr>
<td>autoimmune atrophic gastritis</td>
<td></td>
</tr>
<tr>
<td>Addison's disease</td>
<td></td>
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<tr>
<td>premature menopause (few cases)</td>
<td></td>
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<tr>
<td>insulin-dependent diabetes mellitus</td>
<td></td>
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<tr>
<td>Goodpasture's syndrome</td>
<td></td>
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<tr>
<td>myasthenia gravis</td>
<td></td>
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<tr>
<td>male infertility (few cases)</td>
<td></td>
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<tr>
<td>pemphigus vulgaris</td>
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<tr>
<td>pemphigoid</td>
<td></td>
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<tr>
<td>sympathetic ophthalmia</td>
<td></td>
</tr>
<tr>
<td>phacogenic uveitis</td>
<td></td>
</tr>
<tr>
<td>multiple sclerosis (?)</td>
<td></td>
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<tr>
<td>autoimmune haemolytic anaemia</td>
<td></td>
</tr>
<tr>
<td>idiopathic thrombocytopenic purpura</td>
<td></td>
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<tr>
<td>idiopathic leucopenia</td>
<td></td>
</tr>
<tr>
<td>primary biliary cirrhosis</td>
<td></td>
</tr>
<tr>
<td>active chronic hepatitis (HBs Ag negative)</td>
<td></td>
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<tr>
<td>cryptogenic cirrhosis (some cases)</td>
<td></td>
</tr>
<tr>
<td>ulcerative colitis</td>
<td></td>
</tr>
<tr>
<td>Sjogren's syndrome</td>
<td></td>
</tr>
<tr>
<td>rheumatoid arthritis</td>
<td></td>
</tr>
<tr>
<td>dermatomyositis</td>
<td></td>
</tr>
<tr>
<td>scleroderma</td>
<td></td>
</tr>
<tr>
<td>mixed connective tissue disease</td>
<td></td>
</tr>
<tr>
<td>discoid lupus erythematosus</td>
<td></td>
</tr>
<tr>
<td>systemic lupus erythematosus (SLE)</td>
<td></td>
</tr>
</tbody>
</table>

### Types of autoimmunity

#### Organ-specific diseases

- Thyroid
  - Hashimoto's thyroiditis
  - primary myxoedema
  - thyrotoxicosis

- Stomach
  - pernicious anaemia

- Adrenal
  - Addison's disease

- Pancreas
  - insulin dependent diabetes mellitus

#### Non-organ-specific diseases

- Muscle
dermatomyositis

- Kidney
SLE

- Skin
scleroderma

- Joints
rheumatoid arthritis
Conditions

- Rheumatoid or other arthritis
- Wegener’s or other systemic vasculitides
- Multiple Myeloma
- Coeliac Disease
Rheumatoid or other arthritis

- Rheumatoid factor: very non-specific and not a diagnostic tool
- Present in any condition that elicits a polyclonal immune response.
- Absence will sometimes delay an appropriate clinical referral.
- Presence often means an inappropriate referral to the Rheumatologist.
Is There a Need for a Diagnostic Test for RA?

- Patients present with common symptoms
- A simple blood test with high sensitivity and specificity can lead to more appropriate referral patterns
- Earlier diagnosis and treatment
Clinical diagnosis difficult

- RA has a heterogeneous presentation and variable clinical course
- Many conditions can resemble RA
  
  Osteoarthritis
  Psoriatic arthropathy
  Pyrophosphate arthritis
  Gout, Reiter’s,
  Sjögren's, SLE etc.
Clinical diagnosis difficult

Mariana by Milais

“Gout”: James Alexander Gilray
Rheumatoid Factor

- Present in RA but also in any process that elicits a polyclonal immune response.
  - Sensitive test but hopelessly non-specific
  - Patients stratified wrongly
  - Inappropriate referrals
  - Used as a prognostic marker rather than a diagnostic one
Anti-CCP et al

- Anti-Cyclic Citrullinated Peptides
  - Also CCP2, CCP3, citrullinated vimentin antibodies
- Much greater specificity (>90%) with slightly better sensitivity (80%)
  - Offers more in patient stratification
Anti-nuclear antibodies

- Homogenous
  - Most common and least disease specific
  - Found in most SLE cases and 50% liver disease and a multitude of other conditions

- Speckled
  - Found in SLE, liver disease, Sjogren’s etc.
Centromere: CREST variant of scleroderma
Fine speckled ANA: JO-1, SSA, SSB (RO / LA)
Lady standing has RA?
Jakob Jordaens-family portrait
Rafael’s “School of Athens”
Apostolic Palace - Vatican

Ruebens’ self portrait
Albrecht Dürer 1497 The Fürleger sisters. Both have arthritis? RA or Jaccoud’s arthritis (Note that one has a goitre!)

![Painting of the Fürleger sisters by Albrecht Dürer]
ANCA

- c-ANCA
- p-ANCA
Wegener’s or other systemic vasculitis

- ANCA - two types: cytoplasmic and perinuclear
  - cANCA disease association:
    - Wegener's granulomatosis
    - Microscopic polyangiitis
    - Churg-Strauss syndrome
    - Polyarteritis
Perinuclear p-ANCA

- **pANCA disease association:**
  - Primary vasculitis
  - Microscopic polyangiitis (MPO)
  - Churg-Strauss syndrome (MPO)
  - Polyarteritis nodosa (MPO)
  - Collagenosis
  - Felty's syndrome (lactoferrin)
  - Systemic lupus erythematosus (lactoferrin)
  - Rheumatoid arthritis (MPO/lactoferrin)
  - Sjögren's syndrome (MPO/lactoferrin)
  - Chronic inflammatory bowel disease
  - Ulcerative colitis (cathepsin G/elastase)
  - Crohn's disease (elastase)
  - Chronic liver disease
  - Primary sclerosing cholangitis (cathepsin G)
ANCA associated vasculitis

- Small vessel disease (pulmonary capillaritis and glomerulonephritis). +/- granulomata

- Conventionally classified: Wegener’s granulomatosis; microscopic polyangiitis or Churg Strauss Syndrome

- Extent and rate of recognition of disease undoubtedly increased by availability of rapid, reliable serological testing

- Animal models clearly demonstrate the capacity of anti-MPO antibodies to incite a pauci-immune GN/vasculitis (Xiao H et al JCI 2002; Little MA et al Blood 2005)
When is ANCA testing most helpful?

- Active urine sediment +/- renal impairment +/- compatible systemic Sx
- Pulmonary-Renal Syndromes
  - 54% ANCA+ve
  - 7% anti-GBM+ve
  - 8% ANCA+ve & GBM+ve
  - 31% unrelated kidney + lung diseases

Niles et al 1996

- Monitoring?
ANCA – International Guidelines

- Acute or chronic renal failure,
- Polyarteritis nodosa,
- Wegener's granulomatosis (established or tentative diagnosis),
- Proteinuria/haematuria requests from Renal Unit,
- Churg-Strauss syndrome,
- Nephrotic syndrome,
- Chest X-ray or lung biopsy abnormality,
- Haemoptysis,
- Nasal septum perforation,
- Henoch-Schonlein purpura
- Peripheral Neuropathy
- Vasculitis with systemic features.
The first tumour marker?

“Saturday, Nov. 1st 1845:

Dear Dr Jones,

The tube contains urine of very high specific gravity. When boiled it becomes slightly opaque. On the addition of nitric acid, it effervesces, assumes a reddish hue, and becomes quite clear; but as it cools, assumes the consistence and appearance which you see. Heat reliquifies it. What is it?”

Letter from Dr Thomas Watson, a London GP to Dr Henry Bence Jones about a grocer named Thomas Alexander McBean “of temperate habits and exemplary conduct”.
Immunoglobulin
2 identical heavy chains (Gene chromosome 14)
2 identical light chains (Gene chromosome 2)
Either Kappa (Gene chromosome 2)
Or Lambda (Gene chromosome 22)

Normal plasma cell secretion of whole immunoglobulin and free light chains

Kappa Plasma cells

Lambda Plasma cells
Paraproteins in myeloma

- Original and best!!
- Serum monoclonal immunoglobulins
- Urine Bence Jones protein
- β2-microglobulin
- Serum free light chains
Paraproteins in myeloma

  - Paraprotein concentration/ presence of immune paresis.
- Bence Jones protein: monoclonal κ* or λ light chain fragments excreted into urine.
  - * Named after Korngold and Lapiri in 1956
- β2-microglobulin: useful prognostic marker
- Serum free light chain ratio: very useful for monitoring after chemotherapy.
Free light chains in MGUS?

- MGUS will progress in a proportion of cases: IgG < IgA and IgM
  - Paraprotein <30g/L
  - Bone marrow plasma cells <10%
  - No evidence of other B cell neoplasm
  - No related organ or tissue impairment
- Urine Bence Jones protein is an important prognostic marker
- Could serum free light chains be better?
hypogamma →

AAT deficient

ALCOHOLIC CIRRHOSIS
BISALBUMINAEMIA.

NEPHROTIC SYNDROME.

POLYCLONAL INCREASE WITH MINOR MONOCLONAL BAND

ACUTE PHASE RESPONSE
PK 22/7/35: role of lab staff!

- Presented with # neck of femur 8/4/5
- Acute renal failure (urea 40.1 creat 489) 15/4/5
- Left leg DVT; abdo mass; ESR >140; HB 8.6 16/5
  - Tumour markers: AFP, PSA, CEA, CA19-9 all normal
- Urine retention; oedema; renal failure 19/5
  - IgG 6.4; IgA 1.22; IgM 0.21g/L; CRP 307
  - Slightly low gamma region; faint band visible: Please ensure we exclude Bence Jones proteinuria.
- 24/5 urine sample arrives
Urine protein: 1.32g/L with significant lambda band visible

Urine Bence Jones lambda: 1.08g/L
- Significant Bence Jones proteinuria; refer to Haematologists.

8/6/5
- IgG 8.3; IgA 1.36 IgM 0.21; free lambda band visible between the two beta bands and not easily quantifiable; suggest free light chains monitoring.
PK 22/7/35

- 17/8/5
  - Free kappa = 14.30 mg/L (ref 3.3-19.4)
  - Free lambda = 732 mg/L (ref 5.71-26.3)
  - Ratio = 0.02 (ref 0.26-1.65)

- 6/9/5
  - Free kappa = 12.47 mg/L (ref 3.3-19.4)
  - Free lambda = 200.37 mg/L (ref 5.71-26.3)
  - Ratio = 0.06 (ref 0.26-1.65)
Electrophoresis and immunofixation studies

- A tiny paraprotein band (patient number 26) - Immunofix against IgG, A, M, D, E, kappa and lambda light chains

Figure 1 - electrophoresis

Figure 2 - immunofixation: free lambda
Coeliac Disease

- adults and children with coeliac disease run a significant risk of increased hip fractures and fractures of any type.
- These increased risks continue for at least 20 years following diagnosis.
Endomysium antibodies

- Endomysium is the connective tissue covering individual smooth muscle fibres.
- IgA antibodies usually detected using primate oesophagus or human umbilical cord tissue.
Tissue transglutaminase antibodies

- Replacing endomysium antibodies as the method of choice for coeliac screening.
- TTG is an intracellular enzyme and is the major auto-antigen involved in the EMA response.
- ELISA: Similar sensitivity and specificity to EMA
- Small bowel biopsy still the gold standard but does this removes the need for more invasive tests?
When should these be tests be performed?

- Family history of coeliac disease
- Type I diabetes
- Irritable bowel
- Fe deficiency anaemia
- Chronic diarrhoea, chronic fatigue
- Weight loss, short stature, FTT
- Unexplained transaminase enzyme increases
- Osteoporosis
I'm so sorry this stinks!
Your's must be the worst
job in the world!

Love Fiona (the patient)
Won’t get far on Masterchef with this combination!
<table>
<thead>
<tr>
<th>Test</th>
<th>Result 1</th>
<th>Result 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>135.0</td>
<td>7.6</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.0</td>
<td>13.0</td>
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<tr>
<td>Total Bilirubin</td>
<td>0.2</td>
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<tr>
<td>Alkaline Phos.</td>
<td>87.1</td>
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<tr>
<td>AST</td>
<td>12.0</td>
<td>40.0</td>
</tr>
<tr>
<td>ALT</td>
<td>3.0</td>
<td>12.0</td>
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<tr>
<td>Alpha F. Prote.</td>
<td>33.4</td>
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<tr>
<td>Prog. Cell</td>
<td>0.2</td>
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<tr>
<td>Carc. Embry. A</td>
<td>47.4</td>
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</tbody>
</table>

**Request Reason:** Mites in hair.

**LTG Comments:**
Raised CEA, AFP and CA19-9 levels.
BIOCHEMISTRY REQUEST FORM

**URGENT REQUESTS MUST BE TELEPHONED TO LABORATORY**

**THE FORCE IS STRONG WITH THIS ONE? JEDI!**

<table>
<thead>
<tr>
<th>ELECTROLYTE</th>
<th>Urea, Cr, K, No.</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUCOSE</td>
<td>Glucose</td>
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</tr>
<tr>
<td>LIVER</td>
<td>T.Bil, T. Protein, Alb, Alb. Phos, AST</td>
<td>Maldi-Chlorian Count</td>
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<tr>
<td>BONE</td>
<td>Alb, Ca, P, Alb. Phos.</td>
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<tr>
<td>CARDiac 1</td>
<td>CPK</td>
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<tr>
<td>CARDiac 2</td>
<td>AST</td>
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</tr>
<tr>
<td>LIPID 14 hour Freq</td>
<td>Cholesterol, Triglycerides</td>
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</tr>
</tbody>
</table>

**CLINICAL DETAILS AND DRUG THERAPY**

|_for drug analysis time of last dose:| |

<table>
<thead>
<tr>
<th>DOCTOR'S SIGNATURE</th>
<th>BLEEP No.</th>
<th>2A</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Ward, Address or Dept.</th>
<th>SMPH</th>
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<tbody>
<tr>
<td>Sex: M/F</td>
<td>Prev. Tests Yes/No</td>
<td>Consultant/G.P.</td>
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<tr>
<td>TICK STATUS OF PATIENT</td>
<td>Private Input Day</td>
<td>NHS</td>
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<tr>
<td>Urea</td>
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<td>TBN</td>
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<td>Ca</td>
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<td>Crea</td>
</tr>
<tr>
<td></td>
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<td>CRP</td>
</tr>
<tr>
<td>DATE RECEIVED</td>
<td>FOR LABORATORY USE ONLY</td>
<td></td>
</tr>
</tbody>
</table>

Date: Signature:

**PRED**
P3
CRP

**BB 692472 B F**
Tick serum test panel required:

| 1. ELECTROLYTE | Urea, Creat, K, Na. |
| 2. RENAL | CO₂, P, Alk, Phos, Ca, Alb. |
| 3. GLUCOSE | Glucose |
| 4. LIVER | T.Bil, T. Protein, Alb, Alk. Phos, AST |
| 5. BONE | Alb, Ca, P, Alk. Phos. |
| 6. CARDIAC 1 | CK |
| 7. CARDIAC 2 | AST |
| 8. LIPID 14 hour Fast | Cholesterol, Triglycerides |

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**URGENT REQUESTS MUST BE TELEPHONED TO LABORATORY**

**CLINICAL DETAILS AND DRUG THERAPY**

Collected in church while singing. Jesus calls me for a sunbeam. Now better. Clearly services as a sunbeam not currently required.

**DOCTOR'S SIGNATURE**

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PORTSMOUTH HEALTH DISTRICT PATHOLOGY SERVICE

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**HOSPITAL**

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**WARD, ADDRESS OR DEPT.**

---

**SEX: M/F**

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**PREV. TESTS YES/NO**

---

**CONSULTANT/G.P.**

---

**TICK STATUS OF PATIENT**

---

**DATE RECEIVED**

---

**FOR LABORATORY USE ONLY**

---

**BLEEP No.**

---

**DATE COLLECTED**

---

**SURNAME:**

---

**HOSPITALS USE RENADDRESS BLOCK LETTERS**

---

**FIRST NAMES:**

---

**DATE OF BIRTH:**

---

**CASE NO.:**

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