Amyloidosis: Disease Caused by Amyloid

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- What is, and what is not amyloidosis?
- Amyloid deposits
- Clinical amyloidosis
- Amyloid fibrillogenesis
- Tissue damage by amyloid
- Imaging amyloid in vivo
- Treatment of amyloidosis

Amyloidosis
- Disease caused by extracellular deposition of amyloid fibrils
- Systemic amyloidosis is usually fatal
- Causes about 1 per thousand deaths
- Diagnosis and treatment are difficult
- Major recent advances and better outcomes in specialist centers
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Medicine Demands Precision!
- Amyloid and amyloidosis in medicine >150 years
- 1984 Glenner identified β-protein in Alzheimer’s disease
- "β-amyloid", "amyloid-β", "the amyloid precursor protein", etc.
- Amyloidosis is fatal and requires precise diagnosis and often life threatening therapy

What Is, and What Is Not Amyloidosis

Amyloidosis
- Disease caused by extracellular amyloid deposits
- No disease without amyloid deposits
- Anatomical distribution: local or systemic
- Aetiology: acquired or hereditary
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Other Diseases in Which Amyloid Occurs
- Alzheimer's disease
- Type 2 diabetes mellitus
- Transmissible spongiform encephalopathy

Protein Misfolding But Not Amyloid or Amyloidosis
- Huntington's disease and other polyQ diseases
- Parkinson's disease
- Serpinopathies
- Cystic fibrosis, others

Amyloid Deposits
Amyloid Deposits

- Pathological extracellular fibrillar protein deposit
- Pathognomonic red-green dichroism in polarised light after Congo red staining
- Amyloid fibrils all share pathognomonic cross-β protein fold
- 23 different types with different fibril proteins

Electron Micrograph of Spleen Amyloid

Congo Red Staining of Amyloid
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Amyloid Fibrils and Cross-β Structure

Model of Amyloid Fibril Structure

Amyloid Deposits
- Heparan and dermatan sulphate proteoglycans universal and abundant
- Serum amyloid P component (SAP) universal
- Other plasma proteins often present: apolipoprotein E complement proteins

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Clinical Amyloidosis

- Local, confined to specific organ or tissue
- Systemic, distributed in viscera, connective tissue, blood vessels (never intra-cerebral)
- Acquired, complicating preceding primary disease
- Hereditary, caused by mutation in gene encoding amyloid fibril precursor protein

Acquired Systemic Amyloidosis

- Reactive systemic AA amyloidosis
- Monoclonal immunoglobulin AL amyloidosis
- Dialysis-associated Aβ(M amyloidosis
- Senile systemic ATTR amyloidosis
### Hereditary Systemic Amyloidosis

- Familial amyloid polyneuropathy (TTR; apoA1)
- Familial amyloidosis, Finnish type (gelsolin)
- Hereditary non-neuropathic systemic amyloidosis (apoA1, lysozyme, fibrinogen A α-chain)
- Hereditary cerebral amyloid angiopathy, Icelandic (cystatin C)

### Acquired Local Amyloidosis

- Nodular or focal AL amyloidosis
- Primary localised cutaneous amyloidosis
- Ocular amyloidosis
- Orbital amyloidosis
- Sporadic cerebral amyloid angiopathy

### Hereditary Local Amyloidosis

- Oculoleptomeningeal amyloidosis
- Cerebral amyloid angiopathy (Dutch)
- Cardiac amyloidosis
- Cutaneous amyloidosis
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Acquired Local Amyloid Deposits
- Focal senile amyloid deposits
  - brain, choroid plexus, atria, joints, prostate, seminal vesicles, atheroma, etc.
- Maturity onset diabetes mellitus
- Sporadic Alzheimer's disease
- Endocrine amyloid
- Transmissible spongiform encephalopathies
- Inclusion body myositis

Hereditary Local Amyloid Deposits
- Familial spongiform encephalopathies
- Familial Alzheimer's disease

Amyloid Fibrillogenesis

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Amyloid Fibrillogenesis In-Vivo
- Sustained high concentration of normal proteins: SAA, \( \beta_2 \)M, TTR
- Acquired production of abnormal protein: AL
- Hereditary production of abnormal protein: TTR, apoAI, lysozyme, gelsolin, fibrinogen, etc.

Amyloid Fibrillogenesis In-Vitro
- Amyloidogenic variant proteins
- Wild type of amyloidogenic variant proteins
- Normal proteins unrelated to amyloidosis

Amyloid Fibrillogenesis
- Amyloidogenic variant proteins are unstable
- Spontaneous or induced unfolding
- Population of partly unfolded intermediate states
- Spontaneous aggregation and stabilisation in cross-\( \beta \) fold
- Nucleation, seeding, fibril growth

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Lysozyme Amyloid Fibrillogenesis

Unanswered Questions

- Why do the 23 diverse known proteins cause amyloidosis?
- Intrinsic or interactive?
- Tissue distribution of deposits?
- Time of appearance?
- Clinical effects?
- Seeding?

Tissue Damage by Amyloid
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Tissue Damage by Amyloid

- No disease without amyloid deposits
- Amyloid load correlates with disease
- No effect of lifetime exposure to amyloidogenic precursor proteins in absence of amyloid
- No inflammation around amyloid deposits

Tissue Damage by Amyloid

- Amyloid deposits are directly pathogenic by their physical presence alone
- Do misfolded amyloidogenic proteins have other pathological effects?
- Aggregated amyloidogenic proteins are cytotoxic in vitro
- Relevance in vivo?

In-Vivo Compared to In-Vitro Cell Culture Conditions

- Fluid phase: plasma proteins 70 g/L versus 10% foetal calf serum
- Tissue architecture: parenchymal, endothelial, epithelial cell proximity
- Vasculature, lymphatics
- Non-parenchymal cells: fibroblasts, phagocytes, mast cells
- Extracellular matrix: collagen, elastin

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Direct Physical Damage by Amyloid Deposits

- Heart
- Spleen
- Local masses
- Eye
- Coagulopathy in AL

Organ Transplantation in Hereditary apoAI Amyloidosis

- 32 year old woman: end stage heart and renal failure
- Combined heart and kidney transplant
- No intervention affecting amyloidogenic apoAI
- Alive and well 12 years later, aged 44 years
- Normal transplanted organ function
- Minor amyloid deposits in heart, kidney
- No evidence of cytotoxicity!

Cardiac Amyloidosis
Extra-Dural AL Amyloidoma

Localised Ocular AL Amyloidosis

Diversity of AL Amyloidosis
Hereditary Transthyretin Amyloidosis

- Vitreous amyloid does not damage the retina
- No organ dysfunction without amyloid
- Amyloid after domino liver transplant

Pathogenicity of Misfolded Proteins

- Pathogenic mechanisms in non-amyloid diseases associated with protein misfolding?
- Intra-cellular vs. extra-cellular protein aggregates!
- Soluble Ab aggregates cause reversible neuropathology in Alzheimer's disease.

Cautionary Tales

- TSE and amyloid
  - no amyloid deposits in BSE
  - no amyloid deposits in FFI
  - PrPSc accumulation causes no neuropathology in the absence of PrP
- HD and inclusion bodies
  - in-vitro survival of rat neurons expressing huntingtin is better when inclusion bodies are formed than when they are absent
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Imaging Amyloid In Vivo

Diagnosis of Amyloidosis

- Before 1988
  - histochemistry
  - tissue biopsy / resection / autopsy
- Since 1988
  - SAP scintigraphy:
    - safe, non-invasive, quantitative, serial,
    - whole body
- Tissue essential for chemical identification

Serum Amyloid P Component (SAP)

- Constitutive plasma protein 20-30 mg/l, $t_{1/2}$~24 h
- Normal ECM protein in GBM and on elastic fibres
- Synthesized and catabolized only by hepatocytes
Serum Amyloid P Component (SAP)

- Pentraxin protein family, with CRP
- Homopentamer, lectin fold

3D Structure of SAP: Face View
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3D Structure of SAP: Side View

SAP-MObDG Binding

Lectin Fold Proteins

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Serum Amyloid P Component (SAP)
- Calcium dependent ligand binding
- Binds to all amyloid fibrils but not to their native precursors
- Specifically concentrated in amyloid deposits
  - in plasma: albumin:SAP ~2000:1
  - in amyloid: albumin:SAP < 1:10

Diagnosis and Quantification of Amyloid In-Vivo Using SAP
- SAP binds to amyloid fibrils in all amyloid deposits
- SAP in amyloid is in equilibrium with plasma SAP
- Injected radiolabelled SAP distributes freely between the plasma and the amyloid pools of SAP
- Free, unbound SAP is about 100 mg; amyloid bound SAP may be up to 20,000 mg
- Tracer SAP localises specifically to amyloid in proportion to amyloid load
- NHS National Amyloidosis Centre, RFH

UK NHS National Amyloidosis Center
- Diagnosis/management for ~1100 patients/year
- Unparalleled experience of amyloidosis
- SAP scintigraphy is essential
- Only center doing routine SAP scintigraphy
- ~800 SAP scintigraphy scans per year
- UK Dept of Health funding ~£1.8 million/year
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SAP Scintigraphy for Amyloidosis

Explosive Late Onset Evolution of AA Amyloidosis
Whole body SAP scans one year apart in a patient with a long history of rheumatoid arthritis

Diversity of AL Amyloidosis
Minor proteinuria
No evidence of other organ involvement
Amyloid deposits only in kidneys
Modest whole body amyloid load

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Diversity of AL Amyloidosis

- Minor proteinuria
- No evidence of other organ involvement
- Large whole body load
- Associated with poor prognosis and high mortality during stem cell transplantation

Diversity of AL Amyloidosis

- Multiple fractures over 4 yrs
- X-ray and bone scan normal
- Bone biopsy - amyloid
- Monoclonal gammapathy not identified
- Bone amyloid is frequent in AL; may be the main clinical feature

Treatment of Amyloidosis
Treatment of Systemic Amyloidosis

- Maintenance and replacement of organ function
- Reduce abundance of amyloid fibril precursor protein

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Treatment of Systemic Amyloidosis

- Elimination of fibril precursor proteins
  - Control of SAA production for AA
  - Elimination of B cell clones for AL
  - Renal transplantation for Aβ2M
  - Liver transplantation - "surgical gene therapy" for ATTR, AFib

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Regression of AA Amyloidosis

Juvenile rheumatoid arthritis
Treated with chlorambucil
Clinical remission
SAA < 10 mg/L

1999 2001
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SAA and Outcome in AA Amyloidosis

Probability of survival (%)

120 months

Complete remission
SAA > 10 mg/l (n = 42) P < 0.0001

Persistent inflammation
SAA > 10 mg/l (n = 38)

Regression of AL Amyloid

Liver and bone deposits
Treated with high dose Melphalan and stem cell rescue

1998 2000

Treatment of AL Amyloidosis

- Chemotherapy to suppress underlying plasma cell dyscrasia
- Therapy takes months
- Benefits often delayed months to years
- AL clonal disease usually subtle and impossible to monitor
- No M-protein detectable in 21% of cases
- AL patients often die before benefiting from chemotherapy
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**Serum Free Light Chain Assay**

- Automated immunoassay for free immunoglobulin light chains in serum (FREELITE assay)
- Specific and sensitive < 5 mg/l
- Positive in 98% of AL amyloid patients
- Values in retrospective 10 year study correlate with course and outcome of AL amyloidosis
- Chemotherapy can be guided by early effect on FLC

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**Serum Free Light Chains in AL Amyloidosis**

- Graph showing distribution of serum free light chains in AL amyloidosis patients, highlighting the abnormal range.
- 98% of patients have abnormal levels.

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**Survival After Chemotherapy in AL Amyloidosis**

- Graph comparing survival rates of patients whose serum FLC was suppressed by 50% or more versus those suppressed by less than 50%.
- Statistically significant difference (P<0.0001).

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DNA Screening for Hereditary Amyloidosis

- 350 patients with apparent AL amyloidosis
- 34 (9.7%) had amyloidogenic mutations, confirmed to be the cause of their disease
  - 18 fibrinogen A α-chain Val526
  - 13 variants of TTR, including 4 new ones
  - 2 apolipoprotein A1 (Pro175 and Arg26)
  - 1 lysozyme His67

Fibrinogen A α-Chain Amyloidosis

Congo red bright field fibrinogen
Congo red cross polar
Immunostaining for fibrinogen

Curative Hepatorenal Transplantation for Fibrinogen Amyloidosis

56 yr British woman
Age 33, kidney failure
‘AL’ amyloidosis, no family history
2 renal transplants within 5 years
Age 51, liver failure
Hepatorenal transplantation
Completely well 6 yr later

Baseline  42 months
New Treatments for Amyloidosis?

- Inhibition of fibrillogenesis:
  - small molecules, GAG analogues, peptides
  - stabilisation of native fold
  - inhibition of refolding or aggregation
  - depletion of fibril precursors by Abs, etc.

- Enhancement of amyloid regression:
  - antibodies to fibril proteins?
  - peptides?
  - targeting SAP?

SAP and Amyloidogenesis

- SAP is universal in amyloid deposits
- SAP values correlate with amyloid deposition in mice and hamsters
- SAP in amyloid deposits is not degraded
- SAP binding stabilizes amyloid fibrils in vitro
- SAP knockout mice show retarded, reduced amyloid deposition

Inhibitors of SAP Binding

- Ro 15-3743, IC50 = 100 nM
- Ro 63-3300, IC50 = 50 nM
- Ro 63-8695, IC50 = 0.9 mM
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**123I-SAP Scintigraphy**
Before and After CPHPC Infusion

**Targeted Depletion of Plasma SAP by CPHPC**

7 patients with systemic amyloidosis

48h i.v. infusion of CPHPC 0.25-6.0 mg/kg/day

**The SAP-CPHPC Complex**

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Clinical Studies of CPHPC in Systemic Amyloidosis

- No adverse clinical effects in >30 patient years treatment
- No laboratory test or organ function abnormalities attributable to CPHPC
- Most patients remained stable during treatment
- Further studies with improved dosing in progress
- CPHPC is tolerated and active by mouth

Conclusions

- Important questions about amyloidosis remain unanswered
- Significant recent progress
  - Molecular pathogenesis: protein misfolding
  - Diagnosis and monitoring: SAP scintigraphy
  - Management: aggressive therapy/transplantation
  - Specific anti-amyloid drugs?