Dialysis Related Amyloidosis
Agenda

Introduction
β₂-Microglobulin (B2M): Roles, metabolism
B2M Amyloid formation
Risk Factors: B2M Amyloidosis
Clinical Manifestations of DRA
Diagnosis
Management- Treatment and Prevention
Agenda

**Introduction**

- $\beta_2$-Microglobulin (B2M): Roles, metabolism
- B2M Amyloid formation
- Risk Factors: B2M Amyloidosis
- Clinical Manifestations of DRA
- Diagnosis
- Management - Treatment and Prevention
A WF in her 50s presented with h/o subcutaneous masses on her back that she had noticed 8yrs previously & were producing postural discomfort

PMH-Hepatitis C infection for 23 years, b/l nephrectomy requiring on HD for 31 yrs, b/l carpal tunnel syndrome (CTS), flexion contractures on her fingers

P.Ex: 3 massive elongated nodules on her back

Largest -31.0 × 5.5 × 4.5cm located over spine

Others - each measuring about 27.0 × 2.5 × 1.0 cm - located symmetrically on either side of spine

Firm, covered by normal skin

Macroglossia and some yellowish nodules (about 2-4 mm each) on the sides of her tongue
Clinical Appearance of the Back of the Patient: Three massive, elongated, parallel, skin-colored subcutaneous masses.
Clinical Case (Pg 2 of 2)

- CT: Infiltration of subcutaneous fat, -ve calcification
- Bx: Subcutaneous deposition of amorphous eosinophilic material +ve with Congo red, apple-green birefringence under polarization
- Immunostaining: +ve for β₂-microglobulin amyloid (B2M Amyloid)
Figure 2. Histologic Features Related to $\beta_2$-Microglobulin Amyloidosis

A, Eosinophilic amorphous material deposited in the subcutaneous tissue (hematoxylin-eosin, original magnification $\times$20).

B, Immunohistochemical staining for $\beta_2$-microglobulin ($\beta_2$-microglobulin antibody; Dako, Denmark; 1/20,000 concentration) (original magnification $\times$100).
• In 1968, Berggard and Beam isolated $\beta_2$-Microglobulin (B2M)
• In 1980 Assenat et al. mentioned the presence of amyloid deposits in the tissue removed during CTS surgery in pts on long-term dialysis
• In 1985 Gejyo group showed that the constituent of amyloid deposits of pts on chronic hemodialysis was B2M
• B2M is a 100-amino acid single polypeptide chain
• Globular structure
• Maintained by a disulfide bond linking two cysteins in positions 25 & 80
• MW: 11,815 daltons
Dialysis-related amyloidosis (DRA)

A disabling disease characterized by accumulation & tissue deposition of amyloid fibrils consisting of B2M in the bone, peri-articular structures & viscera of patients with chronic kidney disease
Agenda

Introduction

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B2M Amyloid formation

Risk Factors: B2M Amyloidosis

Clinical Manifestations of DRA

Diagnosis

Management- Treatment and Prevention
Roles of B2M

- B2M is found on the surface of human cells expressing MHC class I, stabilize str.
- Intragranular protein within neutrophil granulocytes, released during degranulation
- Studies in hepatocytes showed B2M is a secretory protein and its synthesis & release - modulated by alpha- and gamma-interferon
- Associated with HFE protein, regulation of Hepcidin expression in liver
- Targets Fe transporter Ferroportin on membranes of enterocytes & macrophages for degradation \(\rightarrow\) decreased Fe uptake
Human cells expressing MHC class I
Metabolism

- B2M is filtered by the glom., degenerated in the Proximal Tubules
- In CKD pts: circulating B2MG levels are elevated
- In dialysis pts: B2M accumulates in the circulation far above its levels in normal subjects
  > 60x in pts receiving regular HD who have no residual renal function
Agenda

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Diagnosis

Management - Treatment and Prevention
Pathogenesis

• Although amyloid fibril-like structures can be created in vitro from intact B2M, several observations argue against simple precipitation as the sole mechanism of amyloidogenesis in DRA

• Additional pathogenic mechanisms of a more general nature exist

• S.B2M levels don’t correlate with the presence or absence of disease

• Fragmented & polymerized B2M next to monomeric intact B2M found to be a major constituent of the amyloid (modified B2M)

• Increased prevalence of DRA with age & observation that occasional pts. are spared from DRA even though they have had dialysis treatment for >15 yrs
Current knowledge in amyloidogenesis

- Mostly from in-vitro studies
- Concentration-Increased in dialysis pts
- Seeds of natural fibrils
- Low pH for protein unfolding
- Presence of Advanced Glycation End products (AGE), GAGs, Collagen, antiproteinases
Understanding Pathogenesis

• Generation and retention of amyloidogenic precursor protein in terminal RF is an absolute prerequisite – A misfolding event
• Misfolded variants self-aggregate in a highly ordered manner, generating protofilaments that interact to form fibrils
Dialysis is pro-inflammatory

- Complement activation occurs on some dialysis membranes → + Cellular monokine mRNA production → preconditions cells to inc. monokine release upon subsequent stimulation
- Blood cells can release several mediators including IL-1, TNF, ROS & PGs
- Inflammatory response → increased production of B2M
- Acetate (dialysate buffer) and especially endotoxin in the dialysate induce cellular monokine release (in the past)
AGE Modified B2M

- Incubating proteins with glucose leads through early products such as Schiff’s base and Amadori products, to Advanced Glycation End products (AGE)
- Eg-Pentosidine, Pyrrole aldehyde etc
- AGE proteins (MW < 10kD) levels much higher in dialysis pts
- Endowed with chemotactic properties capable of attracting monocytes and stimulating macrophages → proinflammatory cytokines
- Released cytokines stimulate the synthesis of collagenase → bone loss, cysts
- +ve osteoclast-induced bone resorption, net calcium efflux
- Interacts with matrix proteins like Collagen
Role of collagen

- High affinity of the precursor B2M for collagen, predilection of B2M amyloid deposits for OA tissues
- Offers wide hydrophobic surfaces
- Flow of a physiologic fluid, at the interface between polar & hydrophobic surfaces, can generate sufficient forces to partially or totally unfold a globular protein
Schematic picture of the hypothetical events occurring in the interstitial space where globular soluble proteins undergo fibrillar conversion.

Monica Stoppini, and Vittorio Bellotti J. Biol. Chem. 2015;290:9951-9958
Proteases

• Incomplete proteolysis of B2M is the prerequisite for amyloid fibril formation
• Complete proteolysis is prevented by "amyloid-enhancing factors" eg such as the anti-protease α₂-Macroglobulin (A2MG)
• A2MG also inhibits the protease production of macrophages following specific receptor binding
• Campistol *et al.* showed that several anti-proteases are co-deposited in B2M natural fibrils & presence of A2MG is particularly abundant
Glycosaminoglycans

- Fibrillogenesis is promoted by glycosaminoglycans (GAGs)
- Amyloid deposits are stabilized and protected from proteolysis by GAGs and serum amyloid P (SAP), both universal components of amyloid deposits.
- Tissue amyloid burden is determined by the relative rates of amyloid formation and degradation - imbalance in dialysis pts
Overview: Pathogenesis
B2M Amyloid Development

Stage I

• Asymptomatic
• Diagnosed by pathology
• Precedes by several years the onset of clinical & radiologic signs
• Neither macrophages nor bone destruction is detectable in the vicinity of amyloid deposits
• Amyloid deposition in 21% pts receiving hemodialysis for <2 yrs, 50% at 4 - 7 years, 90% at 7 - 13 years, & 100% >13 years (low flux studies)
Stage II

- Symptomatic
- +ve Inflammatory reaction
- Macrophages surround B2M deposits, and cysts develop within bones
- Mechanism transforming silent early deposits into clinically manifest bone and joint destruction remains to be elucidated
- Clinical prevalence of the disease as 0% -5 years, to 50% - 12 yrs ,almost 100% -20 years
Agenda

Introduction

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Clinical Manifestations of DRA

Diagnosis

Management - Treatment and Prevention
Risk Factors

• Age of the patient
• Duration of dialysis - Inflammatory state
• Residual Renal Functions: B2M levels twice as high in HD patients with a GFR < 1 ml/min Vs 4-5 ml/min
• High Flux vs Low Flux HD
• Dialysis Modality - PD, HD
Increased frequency of dialysis-related amyloidosis in older adults

Relative risk of radiologic signs of bone amyloidosis according to age and membrane type. The risk is increased in older patients and those treated with bioincompatible cellulosic membranes. Patients who are over age 60 at the start of dialysis have a fivefold increase in likelihood of developing bone amyloidosis if they are dialyzed with cellulosic membranes compared with high-flux biocompatible AN 69 membranes.
• High flux eg. AN69 vs low flux membranes eg. Cuprophane (+ve complements)
• HF membranes more biocompatible-less inflammatory response
• Hydrophobic membranes- absorb circulating putative uremia associated toxins
• Both fail to meet daily B2MG production whether normal (3 mg/kg per d) or enhanced (4.35 mg/kg per d)
• Assuming steady B2M generation & a 70 kg anuric individual: Net yearly B2M retention is 111gm in Low flux conventional HD, 97gm in HF, 77gm short daily HD, 53gm nocturnal HD, and 51 grams short daily hemofiltration, respectively.
PD vs HD

• PD: Small, daily removal of B2M due to the overall slow rate of convective transport, dialysate flow rate, despite the peritoneal membrane being highly permeable to small proteins.

• In one study, clearance was significantly higher with HF HD Vs PD (29 versus 6 l/week per 1.73 m).

• However, residual renal function is generally higher among patients undergoing peritoneal dialysis compared with hemodialysis; this may increase overall clearance of B2M.

Evenepoel P et al KI 2006
Agenda

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Diagnosis
Management - Treatment and Prevention
Carpal Tunnel Syndrome (CTS)

• Usually the first manifestation
• 3-5 yrs after starting dialysis
• Prevalence nearly 100% >20 yrs of hemodialysis
• Symptoms similar to those in non-dialysis pts-pain, dysesthesia in median nerve distribution
• Usually on side of longest functioning access-worsens 2/2 steal
Amyloid Arthropathy

- Large & medium-sized joints
- From synovitis, effusions to periarthritis, erosions & destruction
- Involves carpal bones, but may also occur in the femoral neck, phalanges of the hands, humeral head, acetabulum, tibial plateau & distal radius
- Pain worsens in supine position- esp on dialysis
- ~8 to 10 yrs after the start of dialysis
- Often coincides with CTS
Bone Fractures

• Development of typical bone cysts may eventually culminate in pathologic fractures, especially of the femoral neck
• More than 90% > 7 yrs of HD
• Both in peripheral joints, vertebrae
• Sternoclavicular joints & the cervical vertebrae are preferentially involved compared with shoulder/thoracic vertebrae
Figure 1. Ultrasonographic (A) and postmortem (B) findings in a patient dialyzed for 17 yr. Femoral neck capsule is markedly thickened (arrows), measuring 13.6 mm both on ultrasonographic and postmortem. Histologic examination of femoral neck capsule disclosed extensive β2 microglobulin (β2m) amyloidosis. Reprinted with permission. From reference 142.
## B2M amyloid cysts vs Brown tumors

<table>
<thead>
<tr>
<th>B2M amyloid cysts</th>
<th>Brown tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Subperiosteal bone erosion are missing</td>
<td>• Decrease in size with PTH-ectomy</td>
</tr>
<tr>
<td>• Bone cysts increase in size despite PTH-ectomy</td>
<td>• Rarely are located in carpal bones</td>
</tr>
<tr>
<td>• Subchondral bone cysts appear to be very specific</td>
<td>• Metaphysis and epiphysis of tubular bones, jaws, and ribs</td>
</tr>
<tr>
<td>• Rapid enlargement</td>
<td></td>
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<tr>
<td>• Restricted to the vicinity of synovial joints</td>
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Destructive spondyloarthropathy

- Marked narrowing of the intervertebral discs combined with erosions & cysts of the adjacent vertebral plates without significant osteophyte formation
- First appeared in the cervical discs, lumbar & upper thoracic discs, middle & lower thoracic discs
- Can involve epidura
- Neurological symptoms such as pain and paralysis due to vertebral destruction often affect the quality of life (QOL)
- Multifactorial origin: Age, mechanical stress and/or severe hyperparathyroidism
DSA in PD vs HD

- Retrospective study, DSA in 67 pts on Long-Term Peritoneal Dialysis (n=23) or Hemodialysis (n=44)
- Pts on dialysis for 10-19yrs
- Overall frequency of DSA similar in PD vs HD (39% vs 34% p=0.83)
- Mean age of DSA higher than non DSA pts

Hayami et al Therapeutic Apheresis and Dialysis 2015
• S.B2M level was significantly higher in PD patients than in HD patients (38.4 mg/L vs. 27.4 mg/L, P = 0.0025)
• No difference of cervical DSA between HD & PD pts
• Lumbar DSA was more frequent in patients on PD compared with pts on HD
• Mechanical stress such as elevation of the intra-abdominal pressure due to infusion of PD fluid (1500 mL to 2000 mL) for over 10 years might contribute to lumbar DSA in patients on long-term PD
**FIG 1.** Plain radiograph: narrowing or loss of the intervertebral disc spaces at L3-4 and L4-5 (large arrows). Small arrows show intravascular coils introduced to treat renal bleeding at the age of 57 years.
**FIG. 2.** On MRI, both T1- and T2-weighted images show a relatively low signal intensity at the L3-4 and L4-5 disc spaces and adjacent vertebral bodies (arrows).
DRAQ score: a self-administered 19-item questionnaire focused on symptoms and disability associated with DRA, was determined to be reliable, internally consistent, and valid.
DRAQ score

Do you have tingling sensations in your hand (circle one)?
1. No tingling
2. Mild tingling
3. Moderate tingling
4. Severe tingling
5. Very severe tingling

How often did hand or wrist pain wake you up during a typical night in the past two weeks (circle one)?
1. Never
2. Once
3. Two or three times
4. Four or five times
5. More than five times

How severe is the hand or wrist pain that you have at night (circle one)?
1. No pain
2. Mild pain
3. Moderate pain
4. Severe pain
5. Very severe pain

How often do you have hand or wrist pain during the daytime (circle one)?
1. Never
2. Once or twice a day
3. Three to five times a day
4. Four or five times a day
5. The pain is constant

Do you have numbness (loss of sensation) in your hand (circle one)?
1. No numbness
2. Mild numbness
3. Moderate numbness
4. Severe numbness
5. Very severe numbness

Do you have weakness in your hand or wrist (circle one)?
1. No weakness
2. Mild weakness
3. Moderate weakness
4. Severe weakness
5. Very severe weakness

How severe is your shoulder pain (circle one)?
1. No pain
2. Mild pain
3. Moderate pain
4. Severe pain
5. Very severe pain

How severe is your hip pain (circle one)?
1. No pain
2. Mild pain
3. Moderate pain
4. Severe pain
5. Very severe pain
Systemic (Nonosteoarticular) Manifestations

- Mostly >12 yrs of HD
- Can cause GI involvement (M/C) bleed, bowel perforation, infarction or pseudo-obstruction, chronic diarrhea, macroglossia, or lingual nodules
- Heart failure with pulmonary hypertension
Cutaneous manifestations

- Rare
- 3 types - Lichenoid plaque, Hyperpigmentation & subcutaneous masses
- Chronic pressure or trauma favors deposit
- Appear at a late stage of the disease
- Presence reveal progression from osteoarticular → systemic
Prognostic Implications

• Prognostic Implications: S.B2M levels for the survival of hemodialysis patients was examined in HD pts in Japan, 2 set of pts – low and high B2M levels
• S.B2M level is a significant predictor of mortality
• Particularly non-cardiovascular mortality, after adjustment for age, gender, hemodialysis duration, presence of diabetes, serum albumin and serum CRP
• Lower S.B2M concentrations were significantly preferable for better survival of hd patients

Senji et al NDT 2009
Fig. 3. Kaplan–Meier analysis of all-cause mortality of 490 haemodialysis patients, classified according to lower (<32.2 mg/L, n = 245) and higher (≥32.2 mg/L, n = 245) β2-microglobulin (β2-M) concentrations. Patients with higher β2-M concentrations (thick line) exhibited a significantly higher death rate compared to those with lower β2-M concentrations (thin line) (log-rank test, P < 0.001).
• B2M serve as a surrogate marker of uremic toxins of other middle molecules that have similar systemic or extracorporeal kinetics in dialysis patients
• S.albumin levels were significantly lower and serum CRP were significantly higher in patients with higher serum B2M, compared to those with lower serum B2M correlation with nutrition & inflammation
Agenda

Introduction

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Diagnosis

Management- Treatment and Prevention
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- Elevated levels do not correlate with disease activity

Histology:
- +ve Congo red, not routinely tested, also limited as easily accessible sites may not be involved/limited deposit size. Apple green birefringence under polarized light
- +ve serum amyloid P (SAP) component, therefore also stain with anti-SAP antibodies
- EM: In contrast to the straight, longer, & thinner fibrils seen in other types of amyloid, shorter & thicker curvilinear fibrils aligned in parallel & aggregated in bundles
Congo Red Staining of Amyloid
Imaging studies

- Plain radiography
- MRI recently contributed to more precise detection of intraosseous, periarticular, and soft-tissue lesions
- SAP scintigraphy - entails radiation exposure
- USG-esp for joint swelling – precedes cyst formation
Agenda

Introduction

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B2M Amyloid formation

Risk Factors: B2M Amyloidosis

Clinical Manifestations of DRA

Diagnosis

Management - Treatment and Prevention
Rx & Prevention (1 of 4)

• Chronic pain- NSAIDs, tylenol
• Surgical Management
• Aim to preserve Residual Renal Functions
• High Flux dialyzers, Longer duration of HD, aim B2M < 15-20mg/L
• Role of On-line HDF – decreased levels of B2M with daily sessions in small studies
• Use of adsorbent columns
Renal Transplant

• Striking, almost immediate improvement of B2M amyloid joint symptoms and signs
• Although this beneficial short-term effect has been ascribed to the high doses of steroids, the effect lasts despite their reduction and, sometimes, the interruption of steroid treatment
• S.B2M levels WNL
• Progression of B2M deposits is halted but number of bone cysts remains constant
Rx & Prevention (3 of 4)

_in vitro_ antibiotic doxycycline

- Inhibit the amyloidogenesis of WT B2M,
- Concentration of doxycycline in the tissue targets proved to be much higher than in plasma and most likely sufficient to inhibit aggregation of oligomers and B2M toxicity
- Preliminary data obtained in the first three patients affected by DRA and treated with doxycycline, suggest that a therapeutic response can be achieved even with a plasmatic concentration that is apparently insufficient to inhibit fibrillogenesis _in vitro_
• The pathogenic effects of glycosylated B2M might be prevented by the administration of aminoguanidine, an agent that inhibits advanced glycation
• However, aminoguanidine has not been approved for human use by regulatory agencies
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