AA amyloidosis

A mouse model

Bouke Hazenberg

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Introduction

- Mouse models systemic amyloidosis
  - AA amyloidosis – long experience
  - ATTR amyloidosis – recent, resembles human clinical picture
  - AApoAII amyloidosis – senile type
  - AL amyloidosis is lacking

ATTR amyloidosis model

Amyloid deposition in a mouse model humanized at the transthyretin and retinol-binding protein 4 loci

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AA amyloidosis model

Pathogenetic mechanisms of amyloid A amyloidosis

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Background

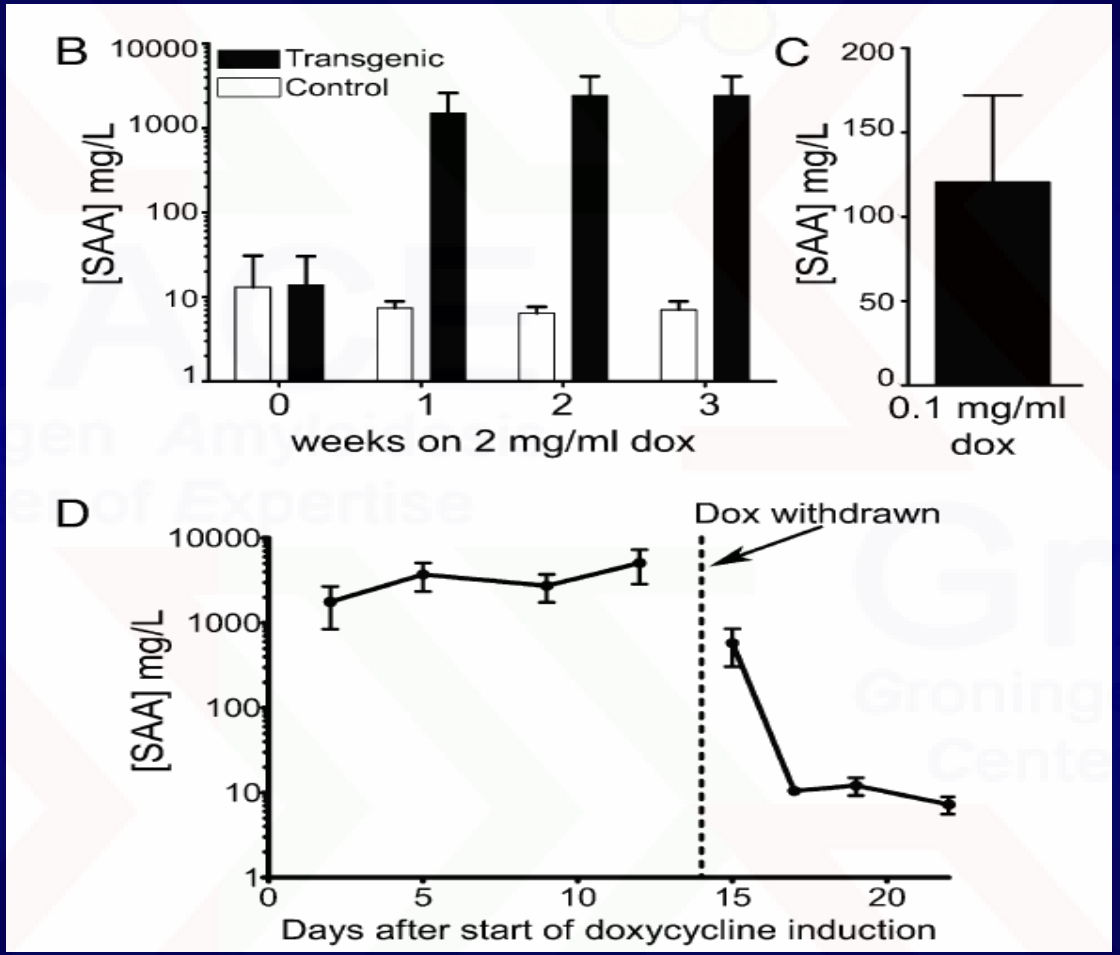
- Current mouse models use severe inflammation for a long time
- Improvement would be a model without inflammation, but with high SAA levels
- This doxycycline-inducible and tunable SAA transgene mouse model does not use inflammation

Aims of the study

- To learn more about
  - The role of inflammation and high SAA levels
  - Factors governing the onset, progression and anatomical site of amyloid deposition
  - What about the role of an amyloid enhancing factor (AEF)?

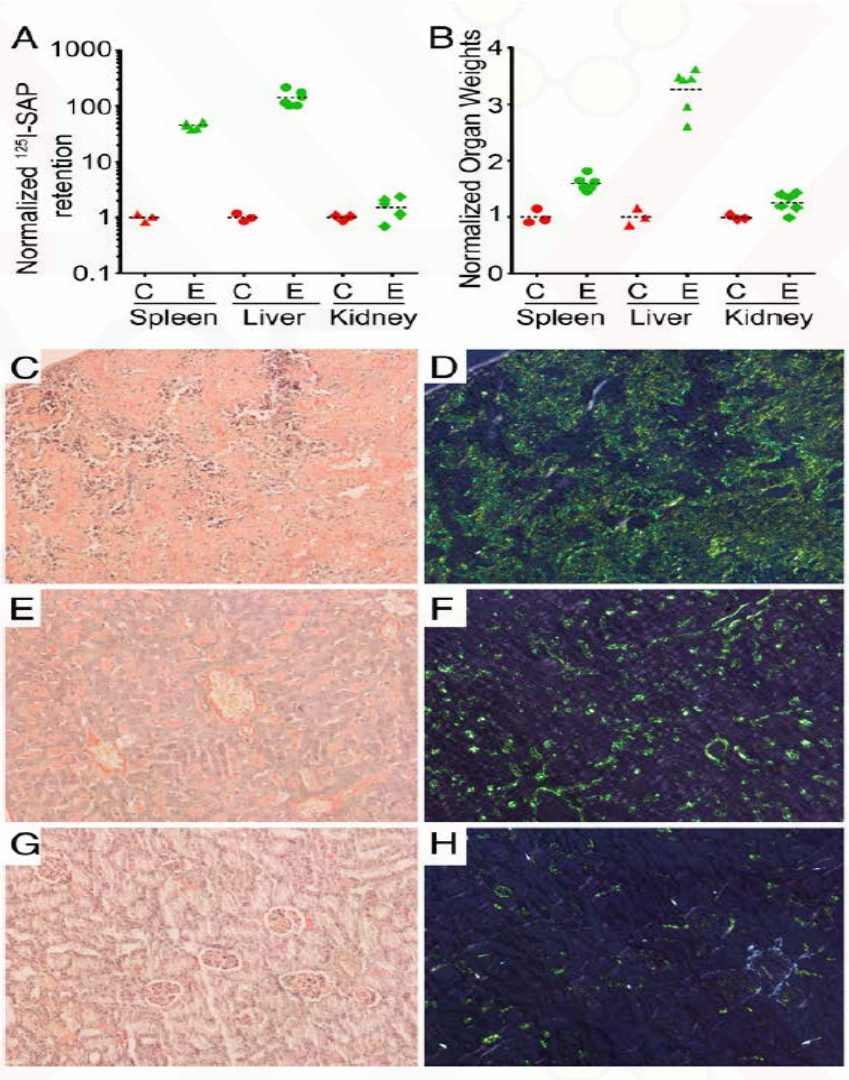
Doxycycline effect

- Dose 2 mg/ml: high SAA levels > 1000 mg/l
- Dose 0.1 mg/ml: SAA levels 100-150 mg/l
- Withdrawal of doxycycline: decrease to baseline (<10 mg/l) within 3 days.


B
10000
1000
100
10
1
[SAA] mg/L
Transgenic
Control
0
1
2
3
weeks on 2 mg/ml dox
C
200
150
100
50
0
[SAA] mg/L
0.1 mg/ml
dox
D
Dox withdrawn
10000
1000
100
10
1
[SAA] mg/L
0
5
10
15
20
Days after start of doxycycline induction

AA amyloid deposition

- No inflammation (normal SAP levels)
- 2 mg/ml doxy: AA amyloid after median 5 weeks (range 4-16) by SAP scan and autopsy confirmed
- Spleen and liver much, kidney, heart and gut modest


A
Normalized ^{125}I-SAP retention
1000
100
10
1
0.1
C E C E C E
Spleen Liver Kidney
B
Normalized Organ Weights
4
3
2
1
0
C E C E C E
Spleen Liver Kidney
C
D
E
F
G
H

Amyloid enhancing factor AEF

- Doxy 2 mg/ml + AEF 4 days later:
  - Heavy amyloid 2 days later and after 3 weeks all animals had heavy amyloid
- So, even in very high SAA levels, priming with AEF indicates the need of a priming/seeding event

Low dose doxy 0.1 mg/ml (1)

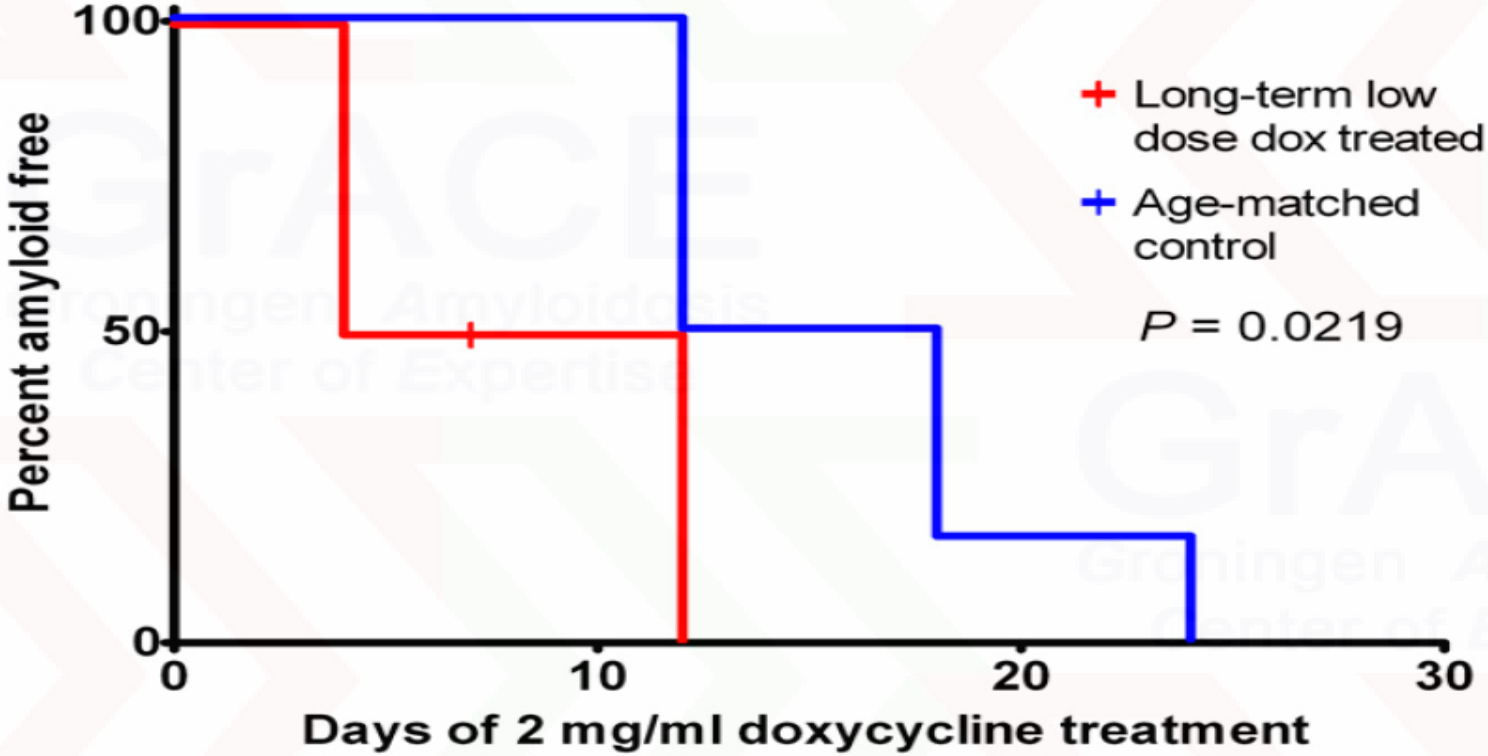
- Doxy 0.1 mg/ml: no amyloid after 1 year seen on SAP scan
- So, long latency and low incidence of clinical AA amyloidosis

Low dose doxy 0.1 mg/ml (2)

- + AEF after 4 days:
  - Little amyloid and late in 3 mice within 1 year
  - The 7 without amyloid after 1 year received doxy 2 mg/ml: all 7 had amyloid within 4 days indicating a primed state

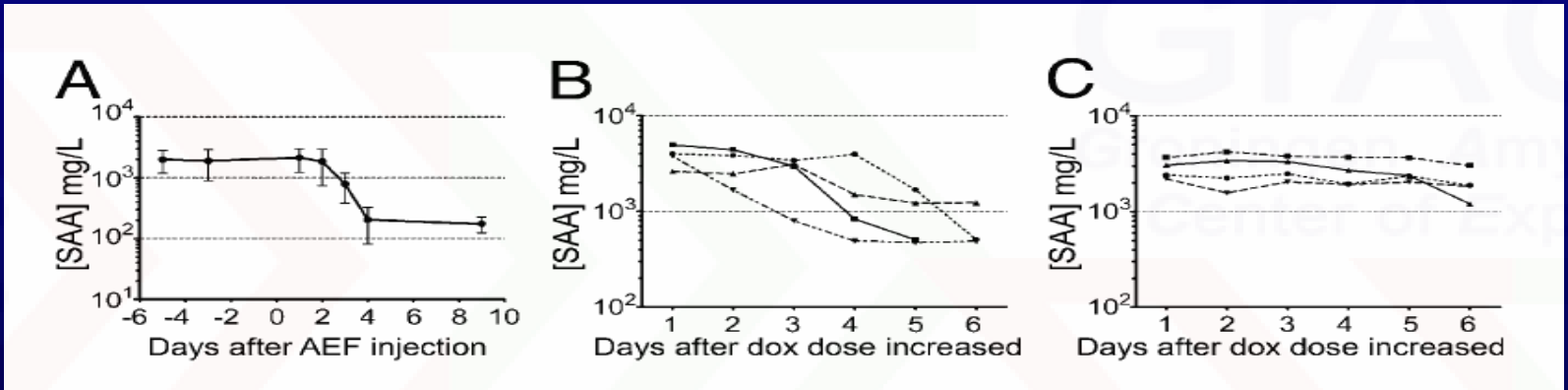
Low dose doxy 0.1 mg/ml (3)

- 8 other mice only low dose doxy for 21 months, doxy increase to 2 mg/ml:
  - 6 of 8 got amyloid within 7 days
- A control group without previous low dose doxy got amyloid later and less
- So, priming can occur with modestly elevated SAA levels insufficient for amyloid deposition


100
50
0
Percent amyloid free
0
10
20
30
Days of 2 mg/ml doxycycline treatment
Long-term low dose dox treated
Age-matched control
P = 0.0219

SAA levels during amyloid deposition

- While amyloid was deposited after AEF administration SAA levels fell down

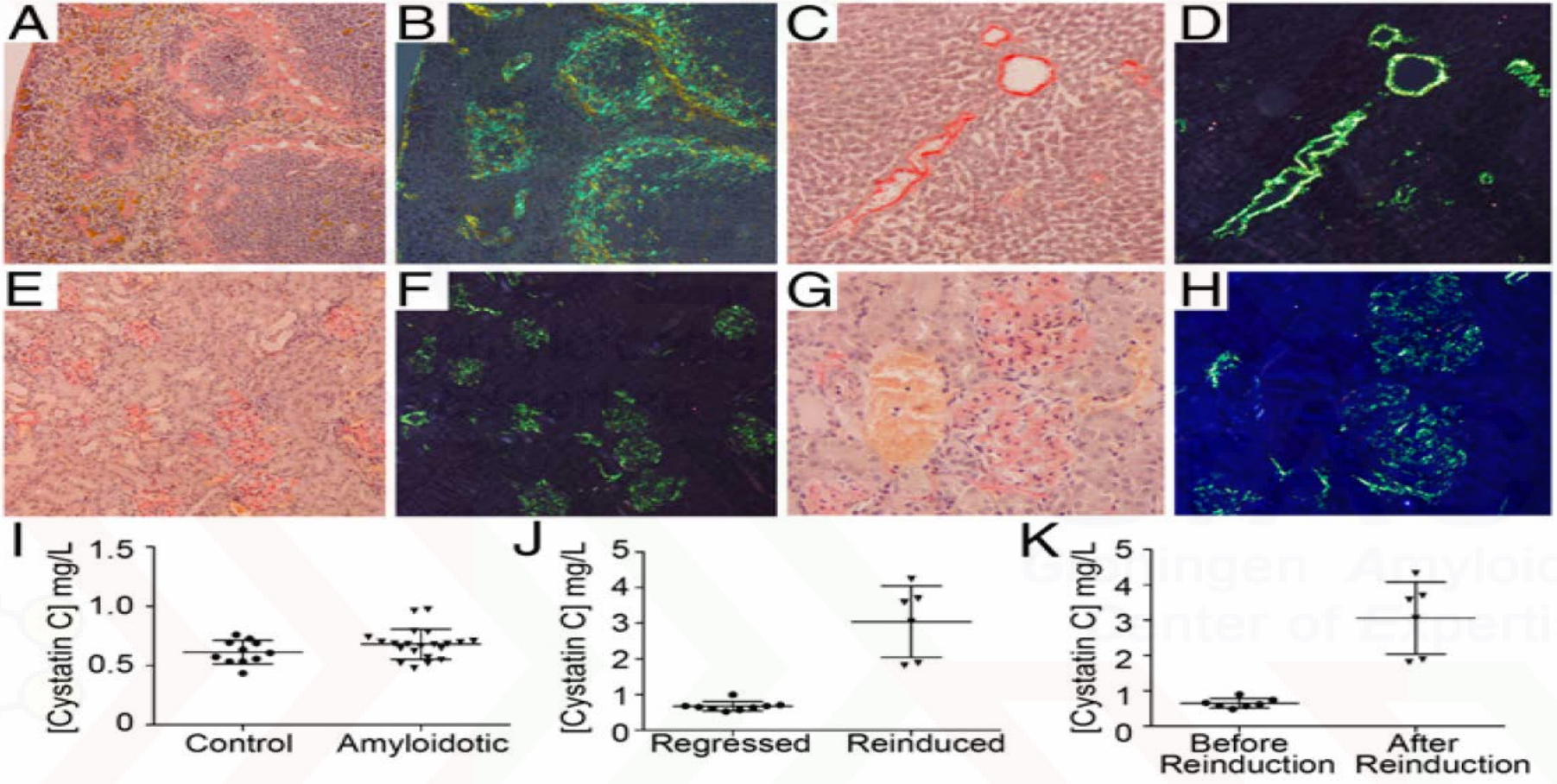


Amyloid regression

- Slow process after doxy stop
- SAP retention started to normalize after 4 months
- SAP retention abnormal in some even after 1 year
- Sometimes amyloid specks still present after 1 year

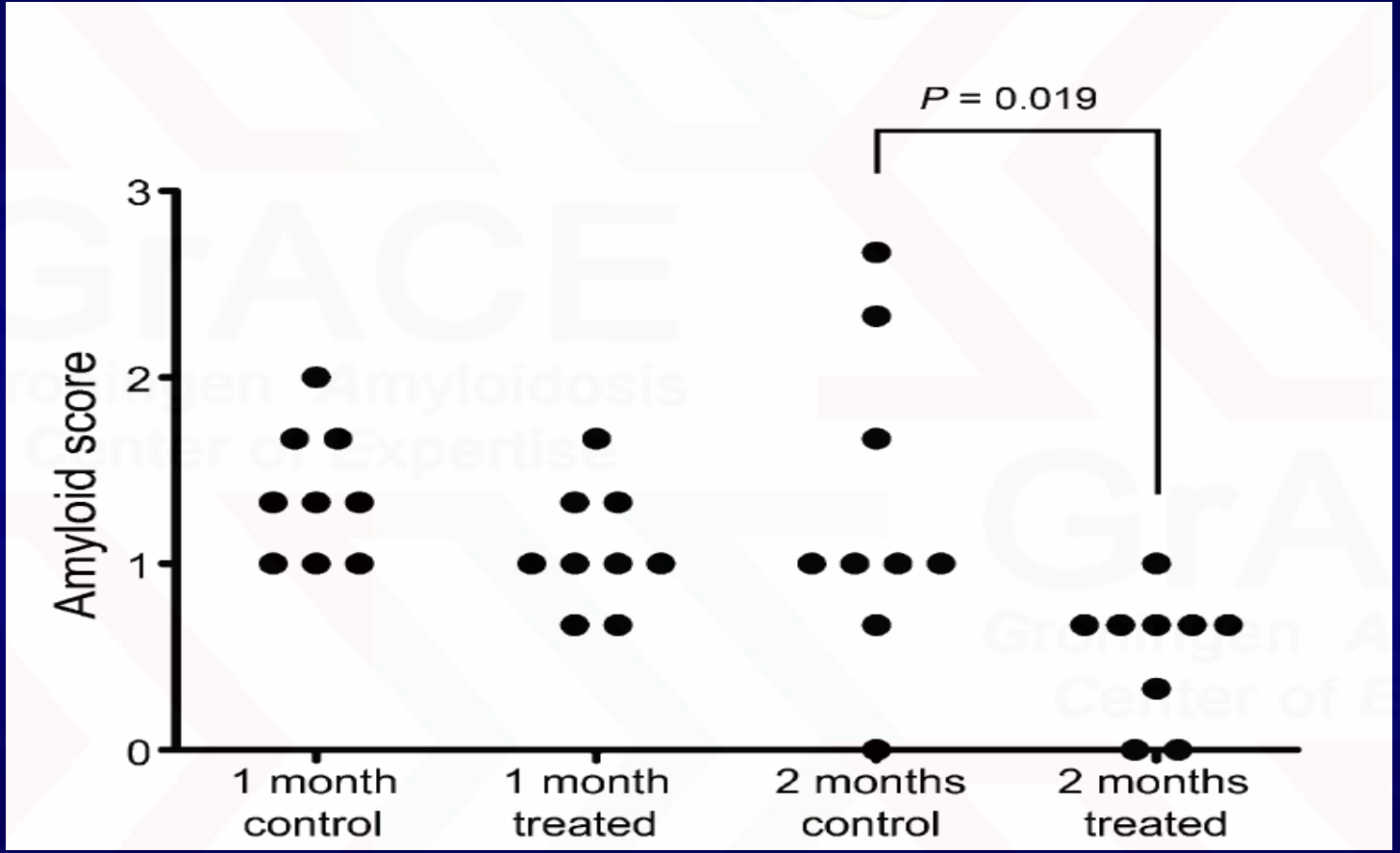
Amyloid regression (1)

- Reexposure of doxy 2 mg/ml after 6 and 17 months:
  - Extremely rapid new amyloid deposits
  - Other pattern of deposition:
  - Massive typical splenic marginal zone and interfollicular deposits
  - Hepatic deposition in and around portal tracts and central veins instead of scattered in the parynchyma
  - Major renal glomerular amyloidosis!


A
B
C
D
E
F
G
H
I
[Cystatin C] mg/L
1.5
1.0
0.5
0
Control
Amyloidotic
J
[Cystatin C] mg/L
5
4
3
2
1
0
Regressed
Reinduced
K
[Cystatin C] mg/L
5
4
3
2
1
0
Before Reinduction
After Reinduction

Anti-SAP treatment

- Hepatic and splenic amyloid regressed after anti-SAP treatment
- Cardiac amyloid only regressed after a second course of anti-SAP treatment


P = 0.019
Amyloid score
3
2
1
0
1 month control
1 month treated
2 months control
2 months treated

Conclusions (1)

- Isolated SAA overproduction is suffcient for amyloid production and inflammation is not necessary
- Amyloid is deposited after prolonged latent period of elevated SAA levels and consistently accelerated by AEF

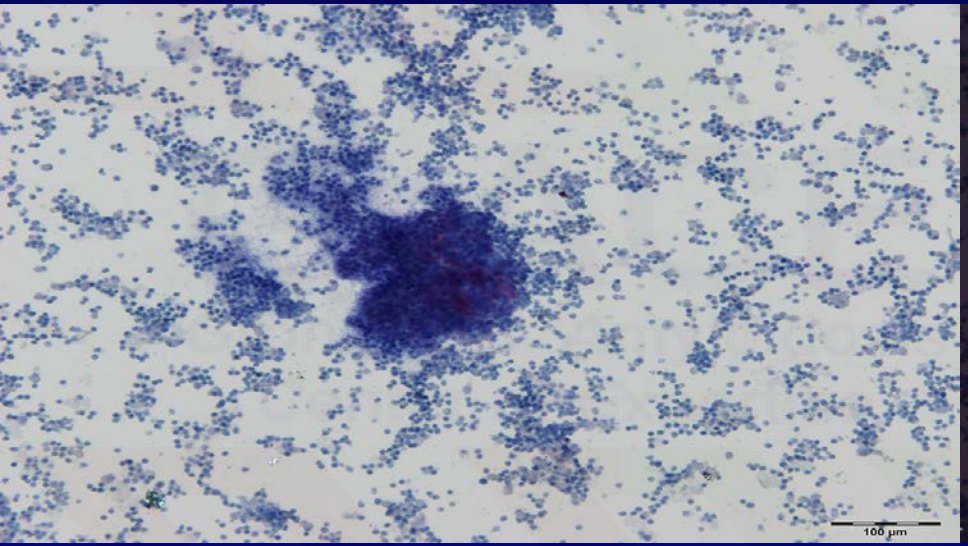
Conclusions (2)

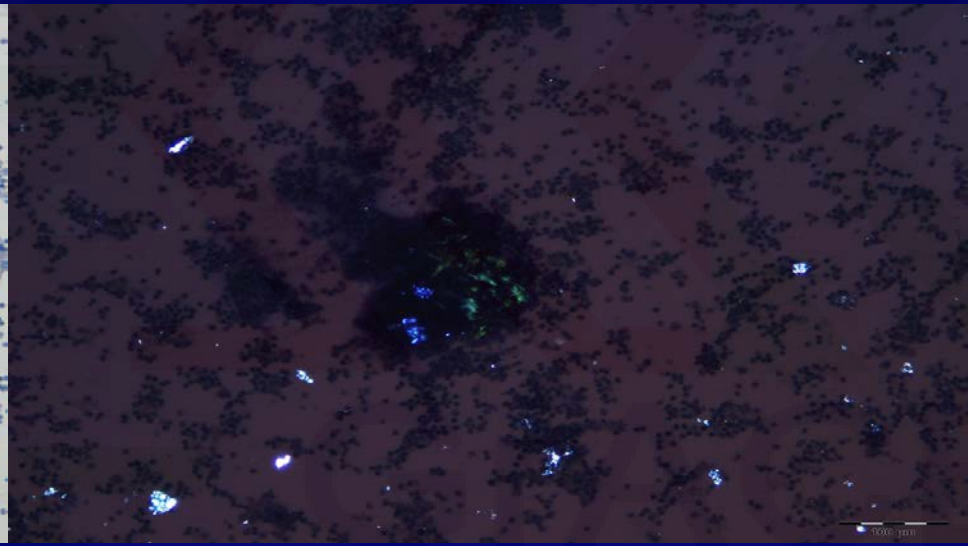
- Low dose doxy induces SAA levels resembling the human situation with infrequent amyloid
- Low dose doxy may induce a primed state
- Amyloid deposition may be precipitated by an episode of severe inflammation

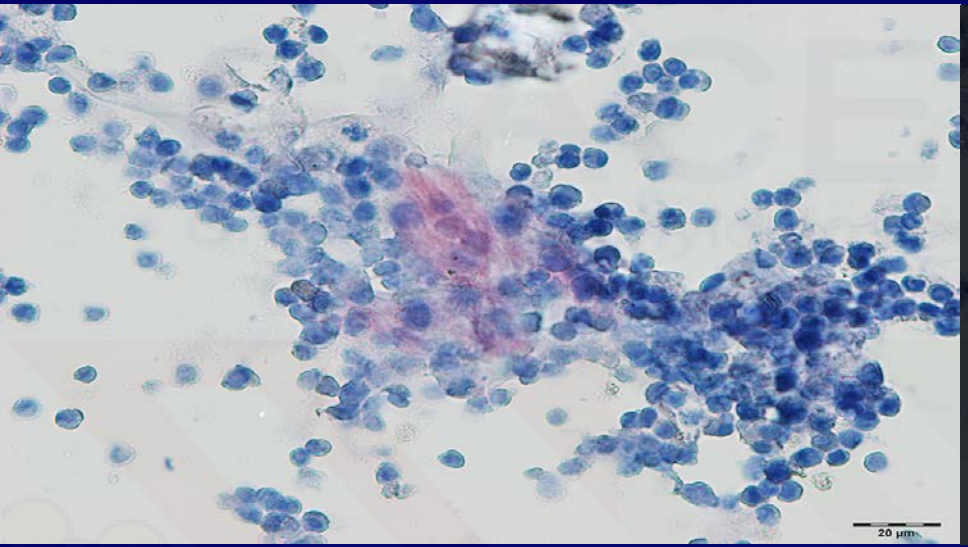
Conclusions (3)

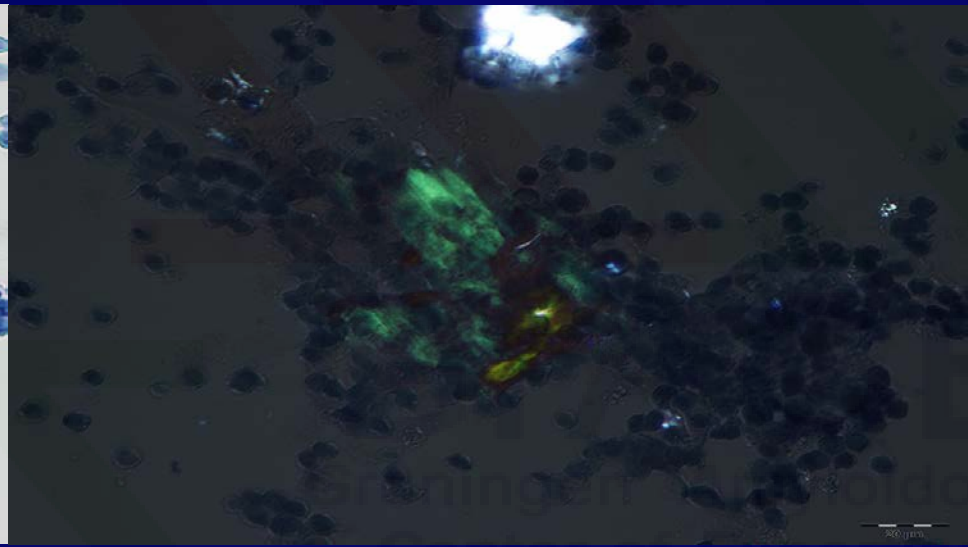
- In patients with longstanding inflammation it would be wise to treat episodes with severe inflammation aggressively and without delay, perhaps before the onset of renal failure
- SAA may fall during a period of active deposition of amyloid

Before I stop I would like to share one observation with you


100 µm




20 µm



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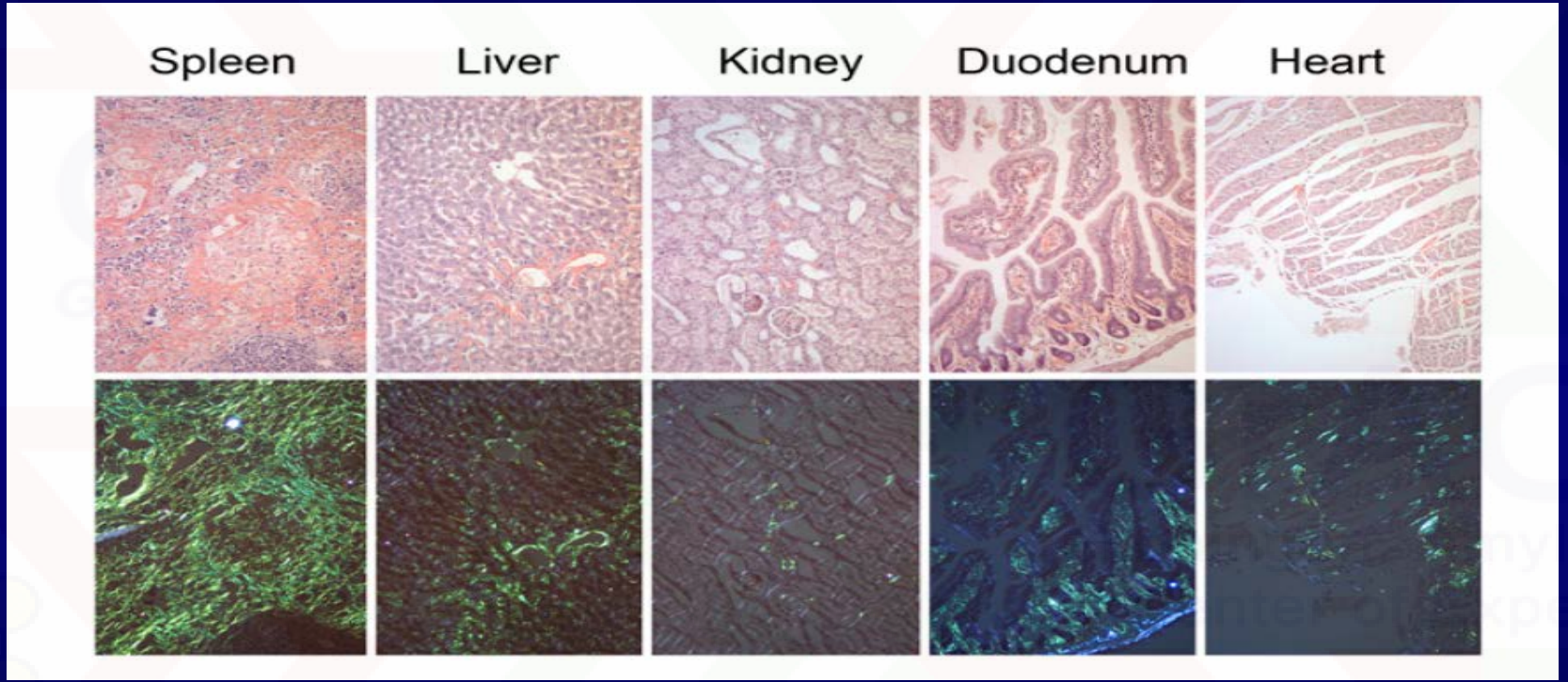
Spleen
Liver
Kidney
Duodenum
Heart