Amyloid β as a novel (transfusion) transmissible agent: reason to worry?

Gustaf Edgren MD PhD
Karolinska Institutet
Stockholm, Sweden

Conflicts of interest

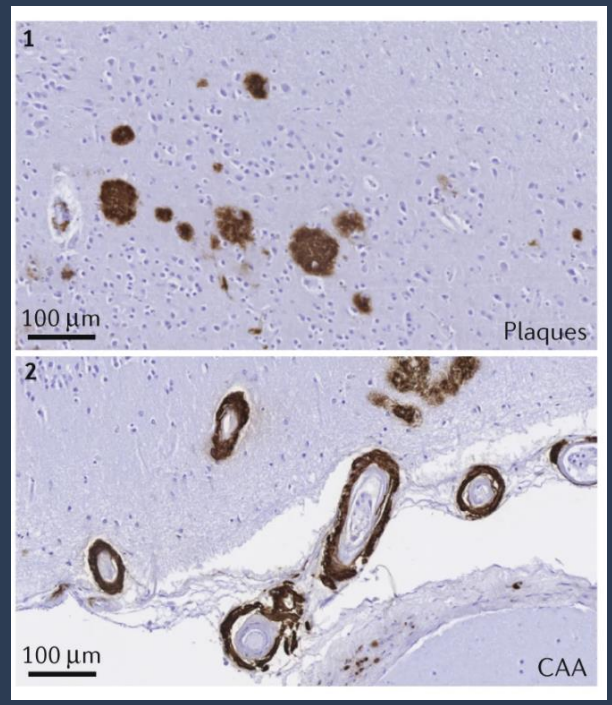
- None

4 key take home points

- Amyloid beta (Aβ) has a causative role in Alzheimer's disease and associated proteinopathies (e.g. cerebral amyloid angiopathy)
- There is ample evidence that Aβ can be transmitted experimentally in animals (both rodents and non-human primates), triggering overt clinical disease and pathological changes
- Further, evidence is accumulating that iatrogenic Aβ transmission also occurs clinically through various parental routes, e.g. dural grafts, neurosurgical procedures and cadaveric growth hormone
- Transfusion-transmission of Aβ is still speculative, but must be taken seriously given existing data and given the potentially very large public health consequences

Amyloid beta (Aβ) and cerebral amyloid angiopathy (CAA)

- In addition to its link with Alzheimer's disease, Aβ aggregation is also a key characteristic of the associated disease CAA
- CAA, in turn, is a common cause of intracerebral hemorrhage
- Typically characterized by multiple lobar intracerebral hemorrhages (ICH) and cerebrovascular amyloid-β deposits
- Estimated to co-occur in many Alzheimer's disease cases, and vice versa, but variable clinical penetrance
- Second most common cause of spontaneous ICH in older patients, after hypertension
- Lifetime risk of moderate-to-severe CAA estimated to be about 25%, high prevalence particularly >80y



Baker HF, Mol Neurobiol 1994.

Induction of β(A4)-Amyloid in Primates by Injection of Alzheimer's Disease Brain Homogenate

Comparison with Transmission of Spongiform Encephalopathy

H. F. Baker,*[*,1] R. M. Ridley,[1] L. W. Duchen,[2] T. J. Crow,[1] and C. J. Bruton[1,3]

[1]Clinical Research Centre, Harrow, Middlesex, UK; [2]Department of Neuropathology, Institute of Neurology, National Hospital, London; and [3]M. R. C. Department of Neuropathology, Runwell Hospital, Wickford, Essex

- Experimental study with intracerebral injection of brain tissue in marmosets (non-human primate)
- Brain tissue drawn from patients affected by Alzheimer's disease, elderly unaffected patients, and young unaffected patients
- A total of 22 animals injected, followed for up to 5 years



Table 1
Monkeys Injected with Brain Tissue from Patient with Alzheimer's Disease

Case	Age at death	Monkey	Time since injection	Age at death	Monkey neuropathology		
					Plaques	CAA	β-protein
1	56	Turmeric	6 yr 5 mo	8 yr 3 mo	++	++	++
1	56	Baal	6 yr 5 mo	8 yr 3 mo	++	++	++
1	56	Abel	6 yr 8 mo	8 yr 6 mo	++	++	++
1	56	Jacob	10 mo	2 yr 8 mo			
—	—	Irad	Uninjected	8 yr 2 mo	—	—	—
—	—	Apollo	Uninjected	8 yr 7 mo	—	—	—
—	—	Micah	Uninjected	8 yr 9 mo	—	—	—

Table 2
Monkeys Injected with Brain Tissue from Elderly Patients

Case	Age at death	Monkey	Time since injection	Age at death	Monkey neuropathology		
					Plaques	CAA	β-protein
2	62	Haydn	4 yr 7 mo	6 yr 0 mo	++	++	++
2	62	Mozart	5 yr 0 mo	6 yr 5 mo	—	—	—
3	79	Joachim	5 yr 10 mo	6 yr 11 mo	(+)	—	(+)
4	81	Jezebel	5 yr 10 mo	6 yr 11 mo	—	—	—
5	74	Moloch	5 yr 7 mo	7 yr 2 mo	(+)	(+)	(+)
5	74	Cain	4 yr 8 mo	6 yr 6 mo	—	—	—
5	74	Isaac	10 mo	2 yr 8 mo	—	—	NT
5	74	Beatrice	3 mo	5 yr 7 mo	—	—	NT
6	71	*See* Table 5					

Table 3
Monkeys Injected with Brain Tissue from Younger Patients

Case	Age at death	Monkey	Time since injection	Age at death	Monkey neuropathology		
					Plaques	CAA	β-protein
7	51	Adah	5 yr 8 mo	7 yr 2 mo	—	—	—
7	51	Dove	5 yr 10 mo	6 yr 10 mo	—	—	—
8	46	*See* Table 5					
9	45	Reuben	5 yr 10 mo	7 yr 3 mo	—	—	—
10	46	Hamadan	5 yr 10 mo	7 yr 2 mo	—	—	—
11	34	*See* Table 5					

Gary C, Acta Neuropath Comm 2019.

Gary et al. Acta Neuropathologica Communications (2019) 7:126
https://doi.org/10.1186/s40478-019-0771-x

Acta Neuropathologica Communications

RESEARCH Open Access

Encephalopathy induced by Alzheimer brain inoculation in a non-human primate

Charlotte Gary[1,2], Suzanne Lam[1,2†], Anne-Sophie Hérard[1,2†], James E. Koch[1,2,3], Fanny Petit[1,2], Pauline Gipchtein[1,2], Stephen J. Sawiak[4,5], Raphaëlle Caillierez[6], Sabiha Eddarkaoui[6], Morvane Colin[6], Fabienne Aujard[7], Jean-Philippe Deslys[8], French Neuropathology Network[9], Emmanuel Brouillet[1,2], Luc Buée[6], Emmanuel E. Comoy[8], Fabien Pifferi[7†], Jean-Luc Picq[1,2,10†] and Marc Dhenain[1,2*]

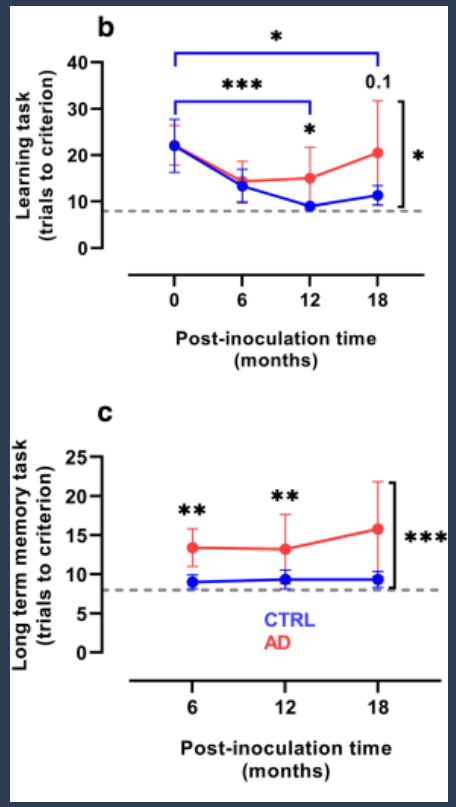


Table 1 β-amyloid and tau lesions in mouse lemurs of the Alzheimer- (AD) and control-inoculated (CTRL) groups. β-amyloid plaques or β-amyloid angiopathy (CAA) were detected only in Alzheimer-inoculated animals. Tau lesions detected in two Alzheimer-inoculated animals (AT8, MC1 and AT100 antibodies). * correspond to two animals that were euthanized at 12 months post inoculation. AD1 displayed β-amyloid angiopathy, high $A\beta_{1-40}$ and low $A\beta_{1-42}$ levels. AD2 did not display angiopathy and had low $A\beta_{1-40}$ and high $A\beta_{1-42}$ levels.

Group	Animal	β-Amyloid	Tau - AT8	Tau - MC1	Tau - AT100
AD2	265B	+ (plaques)	++	++	++
AD2	260B	+ (plaques)	++	++	++
AD1	169ABC	± (plaques)	0	0	0
AD2	190IAB	± (plaques)	0	0	0
AD1	211DBA	± (CAA)	0	0	0
CTRL	189CBD	0	0	0	0
CTRL	190IC	0	0	0	0
CTRL	169ABB	0	0	0	0
CTRL	259BB*	0	0	0	0
CTRL	213ABA*	0	0	0	0

- Similar experiment, but different primate species (mouse lemur)
- Smaller number of animals, but more sophisticated assessment of neuropathological changes
- Consideration also of phenotypes: task learning and memory

three other Alzheimer-inoculated animals Aβ deposits were also detected, but tau was not detected. Neither type of deposit was detected in the control-inoculated animals. β-amyloid deposits were found as β-amyloid plaques (Figs. 5a-b, 6) as well as bands of parenchymal β-amyloid deposits surrounding white matter tracts (Figs. 5f-g and 6) in animals inoculated with tissue homogenates from the two Alzheimer brains (AD1 and AD2). β-amyloid angiopathy was detected only in one animal inoculated with the tissue homogenate from the Alzheimer brain displaying β-amyloid angiopathy (Fig. 5c-e). Tau lesions were detected using different antibodies including AT8, MC1 and AT100 (Fig. 5h-q), and were mainly in the form of neuropil threads

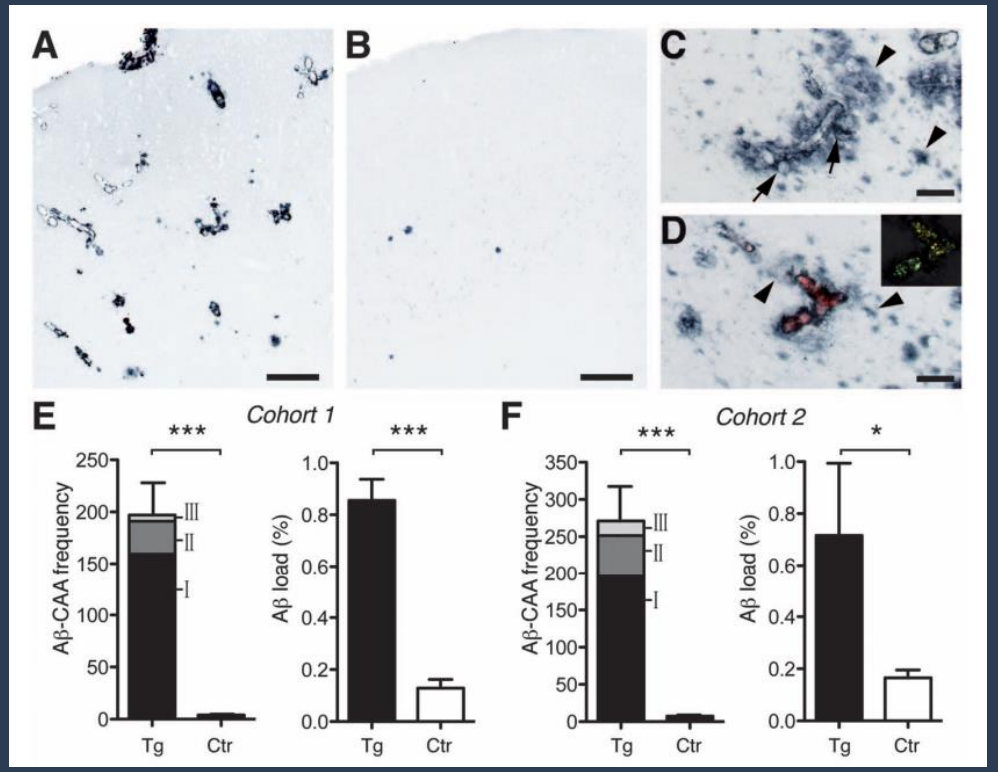
Eisele YS, Science 2010.

Peripherally Applied Aβ-Containing Inoculates Induce Cerebral β-Amyloidosis

Yvonne S. Eisele,[1,2] Ulrike Obermüller,[1,2] Götz Heilbronner,[1,2,3] Frank Baumann,[1,2] Stephan A. Kaeser,[1,2] Hartwig Wolburg,[4] Lary C. Walker,[5] Matthias Staufenbiel,[6] Mathias Heikenwalder,[7] Mathias Jucker[1,2]*

The intracerebral injection of β-amyloid–containing brain extracts can induce cerebral β-amyloidosis and associated pathologies in susceptible hosts. We found that intraperitoneal inoculation with β-amyloid–rich extracts induced β-amyloidosis in the brains of β-amyloid precursor protein transgenic mice after prolonged incubation times.

- Transgenic mouse model (APP+)
- Inoculation of Aβ-rich material from aged APP-tg-mice into the peritoneum of healthy young mice
- Mice were culled after 7 months and assessed for presence of Aβ in the CNS



Induced β-amyloidosis was strongest in the anterior and entorhinal cortices, with additional deposition in the hippocampus, resembling the regional development of endogenous β-amyloidosis in aged APP23 mice (*6*). However, whereas normal aged APP23 mice manifest mostly parenchymal deposits, the induced β-amyloid in intraperitoneally seeded mice was predominantly associated with blood vessels [cerebral β-amyloid angiopathy (Aβ-CAA)], often with massive spreading into the neighboring brain parenchyma (Fig. 1). The presence of Aβ was confirmed by immunoblotting and

Morales R, Mol Psych 2021.

Aβ transmission occurs through various routes (in mice)

Molecular Psychiatry (2021) 26:5690–5701
https://doi.org/10.1038/s41380-021-01150-w

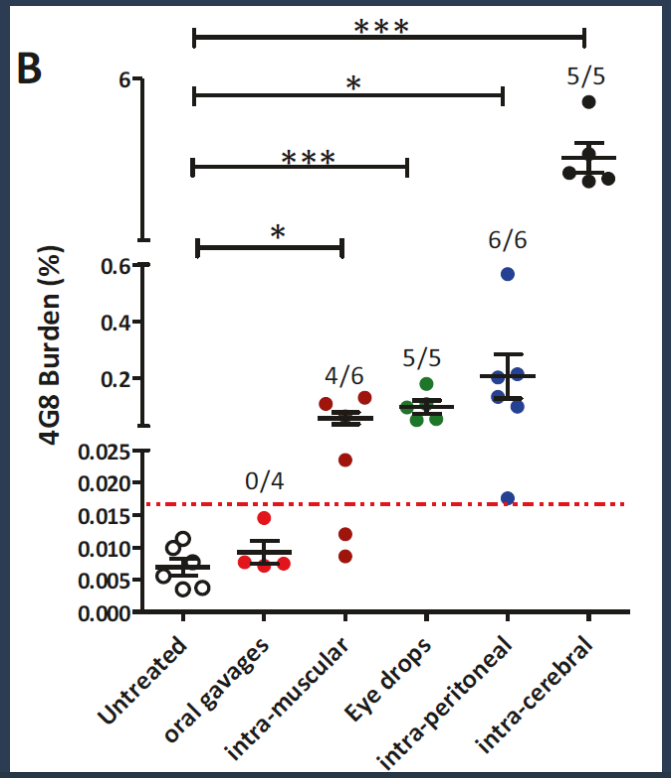
ARTICLE

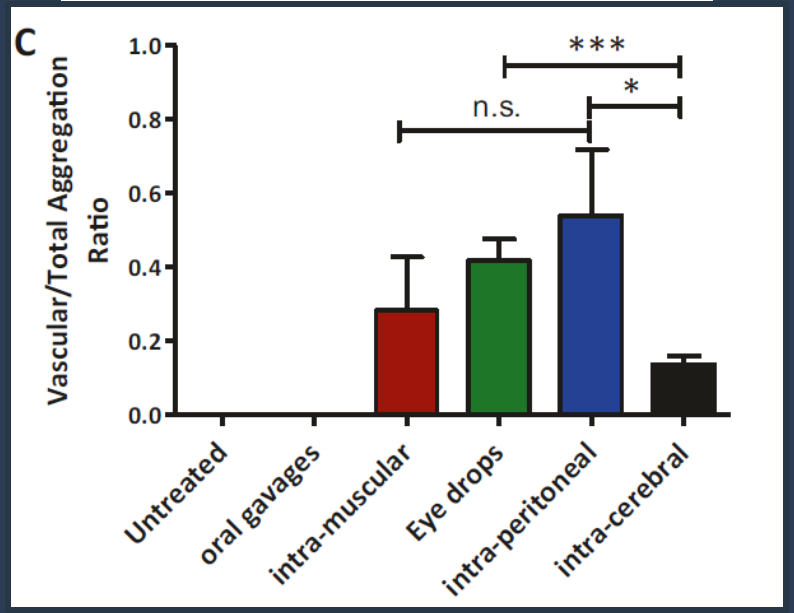
Transmission of cerebral amyloid pathology by peripheral administration of misfolded Aβ aggregates

Rodrigo Morales[1,2] · Javiera Bravo-Alegria[1,3] · Ines Moreno-Gonzalez[1,2,4] · Claudia Duran-Aniotz[1,3,5] · Nazaret Gamez[1,4] · George Edwards III[1] · Claudio Soto[1,3]

Received: 5 December 2020 / Revised: 2 April 2021 / Accepted: 26 April 2021 / Published online: 17 May 2021
© The Author(s), under exclusive licence to Springer Nature Limited 2021

- Again, transgenic mouse model (APP+)
- Inoculation using range of routes (intracerebral, intraperitoneal, eye drops, intramuscular and peroral)
- Assessment of both total Aβ and vascular deposits





So, what does this mean?

- Large numbers of experimental studies in primates and rodents show that Aβ aggregates can act as “seeds”, triggering further aggregation when injected intracerebrally or through other parenteral routes
- Interestingly, such transmission through non-intracerebral routes results in a CAA-like pathology with predominantly vascular amyloid
- In addition to the typical pathology, studies have also demonstrated early symptomatic disease in relevant animal models
- So, what about humans?

Jaunmuktane Z, Nature 2015 and Purro SA, Nature 2016.

Human-to-human transmission of Aβ demonstrated in recipients of cadaveric growth hormone

Stored vials of cadaveric growth hormone found to be contaminated with PrPSc-prion

Intramuscular injections

Autopsy study of 8 patients who died due to iCJD between 36-51y, where half had moderate to severe CAA

Shown to contain Aβ and tau proteins

Inoculated intracerebrally into mice leading to CAA

nature

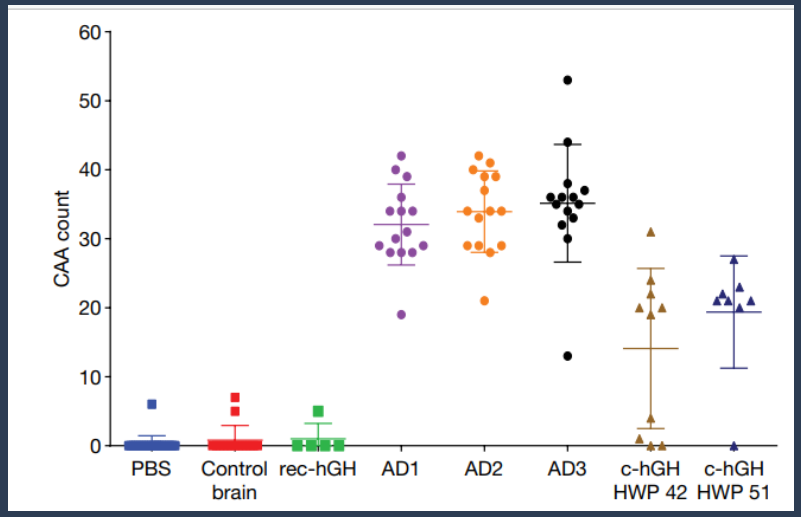
Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy

Zane Jaunmuktane[1], Simon Mead[2,3,4], Matthew Ellis[3], Jonathan D. F. Wadsworth[2,3], Andrew J. Nicoll[2,3], Joanna Kenny[2,4], Francesca Launchbury[3], Jacqueline Linehan[2], Angela Richard-Loendt[3], A. Sarah Walker[5], Peter Rudge[2,4], John Collinge[2,3,4] & Sebastian Brandner[1,2,3]

nature

Transmission of amyloid-β protein pathology from cadaveric pituitary growth hormone

Silvia A. Purro[1], Mark A. Farrow[1], Jacqueline Linehan[1], Tamsin Nazari[1], David X. Thomas[1], Zhicheng Chen[2], David Mengel[2], Takashi Saito[3], Takaomi Saido[3], Peter Rudge[1], Sebastian Brandner[1,4], Dominic M. Walsh[1,2] & John Collinge[1]*



Banerjee G, Nat Med 2024.

Aβ transmission resulting in Alzheimer's disease also seen in recipients of cadaveric growth hormone

nature medicine

Article

https://doi.org/10.1038/s41591-023-02729-2

Iatrogenic Alzheimer's disease in recipients of cadaveric pituitary-derived growth hormone

Received: 3 October 2023

Accepted: 17 November 2023

Published online: 29 January 2024

Gargi Banerjee[1,2], Simon F. Farmer[3], Harpreet Hyare[4,5], Zane Jaunmuktane[6,7], Simon Mead[1,2], Natalie S. Ryan[8,9], Jonathan M. Schott[8,9], David J. Werring[10,11], Peter Rudge[1,2] & John Collinge[1,2]

- Further follow-up of patients treated with cadaveric hGH
- 8 patients with young onset Alzheimer's disease, without other known causes
- Very long incubation periods

Table 2 | Characteristics of all c-hGH recipients

Case	Age (years) Symptom onset	Current	c-hGH treatment (range)	HWP treatment (range)	Latency from HWP, range (years)	Radiotherapy	Prior intellectual disability
1[a]	42	Deceased aged 47	4–13	4–9	33–38	Yes	No
2[a]	55	Deceased aged 57	11–17	11–17	38–44	Yes	No
3	38	54	2–8	4–8	30–34	No	No
4	46	56	6–14	6–13	33–40	No	Yes
5	49	Deceased aged 54	11–12	11–12	37–38	Yes	Yes
6	N/A	57	12–17	12–15	N/A	No	No
7	N/A	56	14–16	14–16	N/A	No	No
8	48	53	9–15	9–11	37–39	Yes	No

Zhao J, JAMA 2023

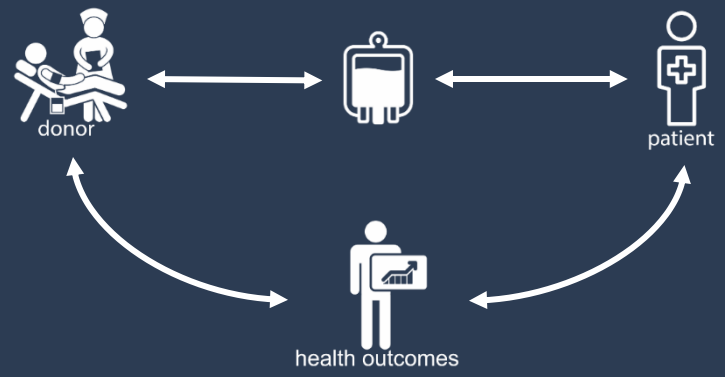
So, how could we study transfusion transmission?

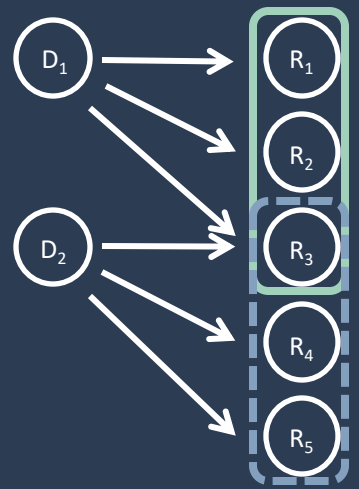
Research

JAMA | Preliminary Communication

Intracerebral Hemorrhage Among Blood Donors and Their Transfusion Recipients

Jingcheng Zhao, MD, PhD; Klaus Rostgaard, MSc; Elsa Lauwers, PhD; Torsten Dahlén, MD, PhD; Sisse Rye Ostrowski, MD, PhD, DMSc; Christian Erikstrup, MD, PhD; Ole Birger Pedersen, MD, PhD; Bart de Strooper, MD, PhD; Robin Lemmens, MD, PhD; Henrik Hjalgrim, MD, PhD, DMSc; Gustaf Edgren, MD, PhD





Are recipients' disease risk affected by donor disease?

Zhao J, JAMA 2023

Validation cohort and ‘negative control’

Negative control



Using ischemic stroke instead of spontaneous ICH

Ischemic stroke shares many of the same etiologies as spontaneous ICH (hypertension, atherosclerosis) but is not related to CAA

Main cohort



Nationwide cohort
1970-2017

Validation cohort



Nationwide cohort
1980-2017

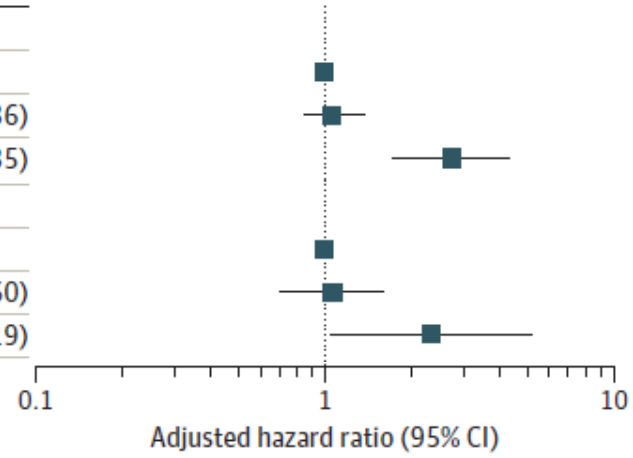
Can we replicate findings from Sweden in Denmark?

Zhao J, JAMA 2023

Increased risk of ICH in recipients who received blood from donors who eventually developed multiple but not single ICH

Cause-specific hazard ratios for ICH in recipients

Exposure	No. of events/ No. of person-years	Incidence rate per 1000 person-years	Adjusted hazard ratio (95% CI)
Sweden (main cohort)			
No sICH	5185/4611866	1.12	1 [Reference]
Single sICH	67/49583	1.35	1.06 (0.84-1.36)
Multiple sICHs	18/5704	3.16	2.73 (1.72-4.35)
Denmark (validation cohort)			
No sICH	1901/1751337	1.09	1 [Reference]
Single sICH	23/16916	1.36	1.06 (0.70-1.60)
Multiple sICHs	6/2127	2.82	2.32 (1.04-5.19)

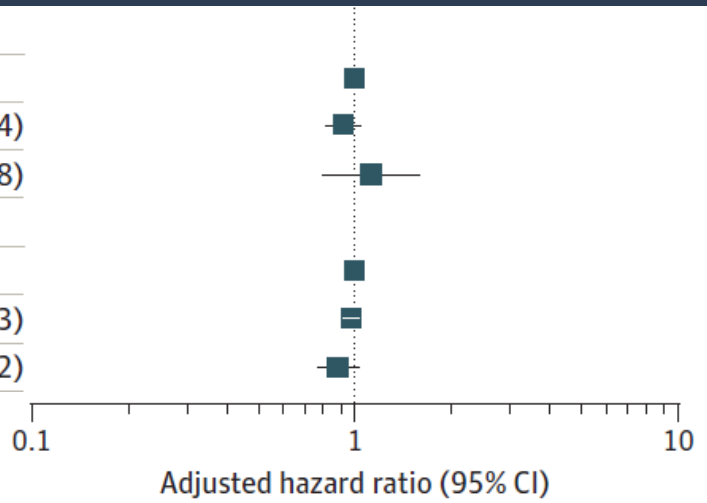


Adjusted for hospital/geographical region, calendar year, age, sex, age and sex interaction, number of transfusions, transfusion indication, and ABO and RhD blood group. All numerical covariates were treated as restricted cubic splines with 5 equally spaced knots.

Zhao J, JAMA 2023

Negative control: no effect seen for ischemic stroke

Ischemic stroke in patients as outcome[b]			
No sICH	23837/4458442	5.35	1 [Reference]
Single sICH	248/48222	5.14	0.92 (0.81-1.04)
Multiple sICHs	32/5552	5.76	1.12 (0.79-1.58)
Ischemic stroke in donors as exposure and in patients as outcome[c]			
No stroke	22591/4234502	5.33	1 [Reference]
Single stroke	1339/237163	5.65	0.97 (0.92-1.03)
Multiple strokes	187/39577	4.72	0.88 (0.76-1.02)



Adjusted for hospital/geographical region, calendar year, age, sex, age and sex interaction, number of transfusions, transfusion indication, and ABO and RhD blood group. All numerical covariates were treated as restricted cubic splines with 5 equally spaced knots.

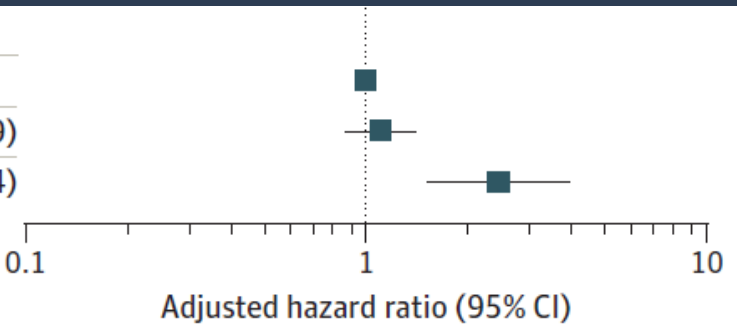
Zhao J, JAMA 2023

A donor with both ICH and dementia carried the same risk as multiple ICH: further indication that Aβ is the culprit?

In a further sensitivity analysis, we considered if donors with both dementia and single ICH—which would also be indicative of CAA—carried the same risk of ICH in transfused patients

Cause-specific hazard ratios for ICH in recipients

Adding dementia in donors as exposure[d]			
No sICH	5184/4611556	1.12	1 [Reference]
Single sICH	69/49859	1.38	1.09 (0.86-1.39)
Single sICH plus dementia	17/5738	2.96	2.44 (1.52-3.94)



Adjusted for hospital/geographical region, calendar year, age, sex, age and sex interaction, number of transfusions, transfusion indication, and ABO and RhD blood group. All numerical covariates were treated as restricted cubic splines with 5 equally spaced knots.

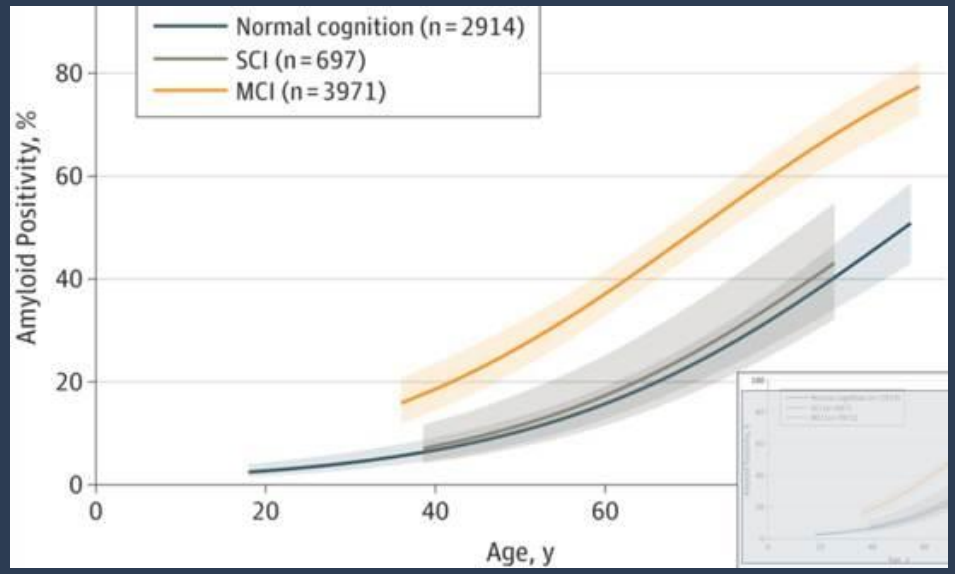
Action points?

- Although still speculative, the threat of iatrogenic transmission of Aβ must be taken seriously
- Clearly, more research is needed to replicate our findings of transfusion-transmission and possibly examine other possible mechanisms
- Meanwhile, further possible risks should be considered (Transplantation of solid organs and tissue? Other plasma-derived products?*)
- First steps?
  - Start by outlining the problem: describe the prevalence of Aβ pathology in blood and organ donors
  - Pursue animal model experiments of transfusion transmission
  - Evaluate direct transmission of Aβ using existing biobanks
  - Consider age matching of blood units (for young patients)

Ongoing follow-up studies

1. Testing for Aβ / P-Tau217 in stored samples from the Danish Blood Donor Study:
   - Effectively 4 interesting comparisons
2. RADAR repository analysis testing for transmissibility of Aβ?
3. Validation of CAA algorithm:
   - Extraction of any available CT scans or MRIs of affected donors and recipients to check if bleeds match CAA or not
   - In addition, this data will also allow assessing "transmission" of radiologically suspected CAA
4. Alzheimer's disease and spontaneous ICH in organ/corneal transplant recipients

ICH affected donor	ICH affected recipient +	ICH affected recipient -
+	a	b
-	c	d

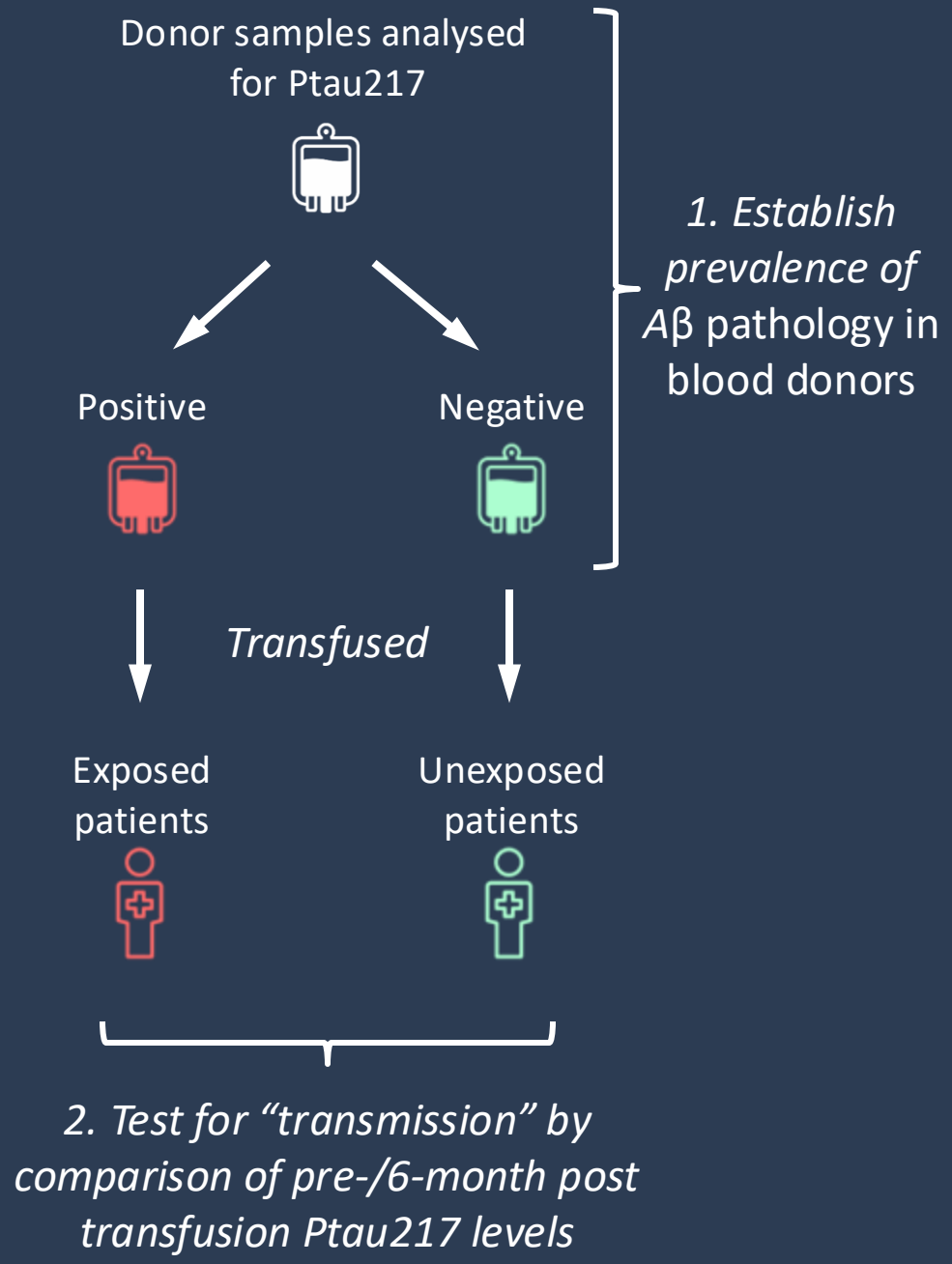


RADAR repository (1)

- From 2000 through 2003, the US NHLBI and CDC established the Retrovirus Epidemiology Donor Study Allogeneic Donor and Recipient (RADAR) repository as a tool to investigate possible transfusion-transmitted infections
- RADAR contains 13,201 donation specimens from 12,408 donors who's donated units were transfused into 3,575 recipients, who in turn completed the 6-month follow-up visit.
- The repository thus allows the coupling of donor samples to pre- and post transfusion samples for their respective recipients
- We are now using this repository to investigate possible transmission of Aβ from donors to recipients in a two-stage study

RADAR repository (2)

- Ongoing two-step study:
  1. Investigate the prevalence of pathologic Aβ/P-tau-217 among blood donors
  2. Compare exposed recipients from affected donors and age-matched controls among recipients of unaffected donors in a matched cohort design
- Power calculation prepared based on expected prevalence in unaffected donors
- Three age groups: 40-49, 50-59, and 60+ years
- A total of donor 2350 samples needed to have >80% power to detect 25% risk of transmission in all three age groups



*What about plasma derived products?

- In collaboration with professor Henrik Zetterberg at Göteborg University, we recently tested a batch of Albumin (Baxalta 200g/L) for presence of Aβ and related peptides/proteins
- Analyses were done using SIMOA, ultra-sensitive, multiplex assay

Platform	Analyte	Average conc. (pg/mL)
Simoa - 4-plex E	Ab40	N/A
Simoa - 4-plex E	Ab42	0,18
Simoa - 4-plex E	GFAP	1,00
Simoa - 4-plex E	NFL	N/A

- Super low concentrations, but mere presence is still worrying…

4 key take home points

- Amyloid beta (Aβ) has a causative role in Alzheimer's disease and associated proteinopathies (e.g. cerebral amyloid angiopathy)
- There is ample evidence that Aβ can be transmitted experimentally in animals (both rodents and non-human primates), triggering overt clinical disease and pathological changes
- Further, evidence is accumulating that iatrogenic Aβ transmission also occurs clinically through various parental routes, e.g. dural grafts, neurosurgical procedures and cadaveric growth hormone
- Transfusion-transmission of Aβ is still speculative, but must be taken seriously given existing data and given the potentially very large public health consequences