Transmission of vCJD by blood transfusion

Sanquinavond
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Transmissible Spongiform Encephalopathies (TSEs or prion diseases)

- Transmissible fatal neurodegenerative disorders
- Brains display neuronal loss, spongiform vacuolation, astrocytosis and amyloid plaques containing misfolded prions.
- Clinical signs: Cognitive and motor dysfunction (memory and personality changes, ataxia)
TSEs are caused by an aberrant form of the cellular prion protein

- Prion = **Proteinaceous Infectivity Only**
- Prions are unconventional infectious agents composed by the misfolded prion protein (PrPSc) that replicates by converting the host prion protein (PrPC)
- Infectivity resistant to procedures that destroy nucleic acids such as heat and radiation
- Host PrP is absolutely required for prion replication. PrP-null mice are resistant to prion infection
- Prion strains with different neuropathological properties and incubation times
Prion replication

Infection → Prion replication → Disease

Incubation time:
- 60–120 days
- 3–5 years
- 7–40 years

Clinical symptoms

TRENDS in Neurosciences
Human and animal TSEs

HUMANS

• Creutzfeldt-Jakob disease (CJD)
  • sporadic CJD (sCJD): unknown cause; 85-90% of cases
  • genetic CJD (gCJD): mutations in the PRNP gene; 10% of cases
  • iatrogenic CJD (iCJD): neurosurgery
    tissue transplants (cornea, dura mater)
    cadaveric growth hormone
  • kuru: ritual cannibalism in New Guinea
  • variant CJD (vCJD): consumption of BSE-contaminated meat
    blood transfusion

• Fatal familial insomnia (FFI) mutations in the PRNP gene

• Gerstmann-Straussler-Scheinker syndrome (GSS) mutations in the PRNP gene

CATTLE: Bovine spongiform encephalopathy (BSE)
SHEEP: Scrapie
DEER: Chronic wasting disease (CWD)
Variant CJD (vCJD)

- New form of CJD first described in the UK in 1996 following a BSE epidemic starting in the 1980’s
  - Worldwide 219
  - UK 172
  - France 25
  - The Netherlands 3
- Incubation time in primary transmission: 10 years
- Median age at death is 28 with a range from 14 to 75
- All individuals with clinical symptoms are homozygous for methionine at the polymorphic codon 129
- Possible silent vCJD carriers
  - vCJD diagnosed in an MV individual in 2009
  - PrPSc in spleen of blood recipient with MV genotype
  - PrPSc in appendixes with VV genotype
vCJD secondary transmission through blood in the UK

22 donors who later developed vCJD

66 recipients of whole blood and blood components

- non-leukodepleted RBCs: 3
  - 1 (MV, death from unrelated cause; PrPSc in spleen)

- leukodepleted RBCs: 0

- plasma-derived product (Factor VIII): 1
  - (death from unrelated cause; PrPSc in spleen)
vCJD infectivity in blood

- Estimated titer from experiments in hamsters: 1-10 IU/ml (0.1-1 pg PrPSc)
- Infectivity associated with mononuclear cells (30%) and plasma (50%)
- Isolated platelets and RBCs carry little infectivity
- Infectivity is protease-sensitive
- Blood transmission is 100 times more effective than infection via oral route with BSE (no species barrier)
Efficient prion transmission through blood

Donation & Transfusion | Recipient dies | Donor dies
3.5 yrs | 6.5 yrs
0.8 yrs | 7.8 yrs
1.4 yrs | 8.3 yrs
Prevalence of vCJD in asymptomatic carriers


Screening of 12,674 samples of tonsil and appendix specimens by immunohistochemistry

- 3 positive appendixes (2 were 129VV)

- Estimated prevalence = 237 per million → 3000 infected people in UK; ~300 infected blood donors

- Existence of asymptomatic carriers
  - New wave of clinical cases?
  - Risk for secondary transmission?
Control of vCJD blood transmission

- Risk reduction measures
- Screening tests
- Prion removal filters

Medische Advies Raad (MAR) of Sanquin is informed on developments of prion test and removal
Risk reduction measures

- Leukodepletion
- Deferral of donors previously transfused after 1980
- Sourcing of plasma from countries unaffected by vCJD
Blood screening tests

• PrPSc is the most specific marker for vCJD, however its concentration is very low (1 PrPSc per $10^6$ PrPC molecules)
• Tests require high specificity and sensitivity
• Test should detect both protease-sensitive and –resistant PrPSc
• Fear for negative impact in number of donors (fatal disease with no available treatment)
• Confirmatory test for infectivity

No prion test for blood screening is currently available
Amorfix Life Sciences: EP-vCJD™

Epitope protection assay

1. Find the infectious prion that can be hidden in an excess of the normal protein.

2. Mask the normal protein (and surface of the aggregate) by chemical modifications.

3. Disaggregate the prion and release protected, unmodified proteins.

4. Magnetic beads (coupled to 6H4)

Eu chelate containing beads (coupled to 3F4)

Fluorescent beads with bound PrP are magnetically collected
Detection of PrPSc in blood of pre-clinical non-human primates with EP-vCJD
Detection of PrPSc in human plasma with EP-vCJD

- **Sensitivity**: $10^6$ dilution of brain homogenate
  - 100% in a panel of human plasma spiked with human vCJD brain homogenate
  - 3 plasma samples from 3 different vCJD patients tested negative
    new test development will achieve 10 times more sensitivity

- **Specificity**: 99.95% after screening 39,000 donations in two blood transfusions centers in France
PMCA
Protein Misfolding Cyclic Amplification

C. Soto (Univ of Texas)

Possible confirmatory test with high sensitivity (also detects PrPSc)
**PMCA**: increased sensitivity with number of cycles

**Serial PMCA**: 7 rounds of 144 cycles each

- 3000 million times more sensitive than WB

*Detection of 1 ag (10^{-18} g) of PrPSc BUT it takes 3 weeks!!!*

- Detection of PrPSc in blood of pre-symptomatic hamsters

**Development of PMCA for PrPSc detection in human plasma**
Prion protein laboratory (National CJD Surveillance Unit, UK)
Prion reduction filters

- Filtration of leukodepleted RBCs using affinity resins for PrPSc
- No issues related to notification, verification and management of false positives
Prion reduction filters

P-Capt (PRDT, MacoPharma) (CE mark in Sept 2006)
  • Removal of > 7 log10 infectivity (ID50) per ml of sample
  • Octapharma has incorporated PRDT resin in the production of OctoplasLG (Solvent/Detergent plasma)

Pall leukotrap affinity prion reduction filter (LAPR) (CE mark in May 2005)
  • Combined leukodepletion and prion removal filter
  • > 3log10 infectivity reduction
Prion reduction of red blood cells: impact on component quality

Wiltshire et al 2009 Transfusion

272 leukoreduced RCC units filtered through p-Capt

- Hemolysis increased after filtration but units remained within UK specifications
- Loss of 7-8 g of Hb and 6-8% reduction in hematocrit
- No major changes in potassium, 2,3-DPG and ATP levels
- No evidence for immunological changes on the membrane of RBC
P-Capt: Safety studies

- Volunteer Clinical Study - 48 persons transfused with P-Capt filtered RCC
- Irish Patient Clinical Study - 120 persons transfused with P-Capt filtered RCC
- UK Patient Clinical Study: Prion-filtered vs Standard Red cells in Surgical and Multi-transfused (PRISM), 540 patient multi-centre study nearing completion
- Routine use in selected Irish hospitals
Phase I/II safety study of transfusion of prion-filtered red cell concentrates in transfusion-dependent patients (IBTS Ireland)

20 haematology patients were transfused with one unit of p-Capt filtered leukodepleted RCCs (6 of them were retransfused): no adverse events were observed during transfusion and follow-up (24h; 6 weeks)

Fig. 1 Pre- and post-haemoglobin levels of 20 patients transfused with prion-filtered red cell concentrate units.
Latest advances in the policy for implementation of prion filters

27 October 2009:
SaBTO (UK Advisory Committee on the Safety of Blood, Tissues and Organs) said that there is enough evidence for a reduction of prion infectivity by p-Capt and recommends prion filtration for recipients born after 1 January 1996 (non-exposed to BSE) provided satisfactory completion of the PRISM study.

18 March 2010
Motion made at the House of Commons on the implementation of blood filtration.

The Minister Public Health replied that the Government will wait for the results of the PRISM clinical trial and are currently evaluating costs, benefits and impacts before making a decision on implementation of filtration.

Still open question: Filter for universal use or limited to children?
Koch’s postulates

(1) the causative agent of the disease must be present in every diseased animal

(2) the agent must be isolated from the diseased animal and grown in pure culture

PMCA: Protein Misfolding Cyclic Amplification
- in vitro amplification of PrPSc from diseased brain (seed) using normal brain (template)
- in vitro amplification of PrPSc using purified components

(3) the isolated infectious organism must reproduce the disease when injected into a healthy susceptible animal

Synthetic PrPSc generated from purified components is infective at a concentration a million times higher than brain isolates: requirement for cellular cofactors

(4) the same infectious agent must be re-isolated from the experimentally diseased animal

Mice infected with synthetic prions can transmit disease to healthy animals