Medicines & Healthcare products Regulatory Agency

Diagnostic assays for vCJD-Challenges facing appropriate selection criteria

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“This report is independent research commissioned and funded by the Department of Health Policy Research Programme entitled CJD Research and Resource Centre, PRP ref. 007/0206. The views expressed in this presentation are those of the author(s) and not necessarily those of the Department of Health.”

Topics covered

- Intro -Transmissible spongiform encephalopathies
- Current status of vCJD cases-review
- Need for a blood test
- Assay methods
- Test performance

Characteristics of TSE disease

- Transmissible diseases caused by the change in the conformation of a normal cellular, prion protein (PrP)
- The detergent soluble alpha helical protein converts to an insoluble, protease resistant β-sheet that can form amyloid-like deposits

- Disease is typically determined by the presence of the proteinase resistant prion protein (western blotting is the gold standard method)

Variant Creutzfeldt-Jakob disease in UK (178 cases in total)

- 175 cases associated with primary transmissions (ingestion of contaminated meat)
  - 174 Methionine homozygous at codon 129
  - 1 Methionine/valine heterozygous at 129
- Secondary transmissions (blood transfusion)
  - 3 clinical cases, all Methionine homozygous
  - 1 MV heterozygous positive for markers of disease died of other cause
- Prevalence of vCJD is estimated to be as high as 1:2000

First clinical disease in MV genotype

- Clinical presentation differed from MM cases
  - Characteristics similar to classical CJD
    - CSF tested; 2 tests highly sensitive for sporadic CJD were negative

Tiny amounts of PrP^{Sc} were seen in lymphoid tissue

Blood tested negative in assay known to detect MM vCJD

Start of vCJD “second wave”?

Reducing the risk of vCJD blood transmission-measures taken

Blood:

- In 1999 leucodepletion was introduced to remove/reduce white blood cells
  - There have been no transfusion related transmissions since
- Donor deferral- recipients of transfusions excluded from blood donation

Plasma:

- Since 1999 fractionated products have been produced from non-UK sources
- Use of recombinant clotting factors for treatment of haemophiliacs under 16
- Fresh frozen plasma for treating babies and young children imported from US

vCJD transmission through blood products

Products were produced using donations from individuals who later developed vCJD. Recipients of potentially contaminated products were informed (factor VIII/Vigam). Through enhanced surveillance tissues and blood have been collected.

A haemophiliac was found positive for markers of infection

- markers of infection in lymphoid tissue-no clinical disease
- MV polymorphism

Issues to consider in developing a blood test for vCJD

- Protein only disease, no nucleic acid
- No immune response to pathogenic protein
- Novel methods needed to identify disease associated change in the conformation of a normal cellular protein
- Levels of pathogenic protein in blood are very low
- Number of samples suitable for test evaluation are small and volumes are limited

Designing protocols for test evaluation

Analytical sensitivity

- Get initial idea of sensitivity by spiking tissue homogenates into blood/blood components. Acts as a comparator between tests using well characterised materials, no inter-species reagent compatibility concerns

Diagnostic sensitivity:

1. Use blood/blood components from animals incubating TSE disease (sheep a reasonable model). Issues with reagent compatibility
2. Use primate material-less compatibility issues
3. Provide blood, blood components from clinical cases of vCJD

Specificity:

- NIBSC have collection of 1000 US donor samples
- CTS requirements of 5000 could be met by NHSBT

Current needs for vCJD blood tests

UK prevalence of disease in blood/blood donors (subject reviewed by UK government science and technology committee)

- Test must have demonstrated ability to detect a signal in a non clinical sample
  - Sheep BSE
  - Primate vCJD/BSE
- Test must be independently assessed using clinical vCJD samples
- Need assurance that test works in all genotypes of vCJD

Direct detection methods

Rely on properties unique to the altered conformation of the prion protein such as:

- Resistance to digestion by proteinase K
- Selective recognition of altered confomer by antibody
- Difference in antibody epitope pre and post denaturation of sample (surface/normal prion protein epitopes chemically altered so no longer recognised by antibody)
- Steel binding properties of the disease confomer

Performance of direct detection methods

	Sensitivity		Specificity	
	10% vCJD BH	vCJD Blood/plasma	Fresh plasma	Frozen plasma/blood
Test 1*	10^{-5}	0/3	100% (no repeat reactive)	100% (no repeat reactive)
Test 2*	10^{-5}	0/2	100% (no repeat reactive)	100% (no repeat reactive)
MRC	10^{-10}	15/21	N/A	100% (0/5000)

* Tests evaluated by NIBSC

MRC test potential screening assay ?

MRC Prion unit test-sensitivity (Clinical)

Edgeworth, J et al Lancet 2011; Jackson, G et al JAMA neurology 2014,:Mok, T et al N. Engl J Med 2017

Direct detection method

Relies on affinity of PrP^{Sc} to stainless steel

Sensitivity of assay:

Sample type	Initial reactive	Repeat reactive
Variant CJD MM MV	15/21; 7/10 0/1	Yes N/A
Other neurological disorders	5/352	0/5
Sporadic CJD*	3/105	2/3

* Test also works in a very small number of sporadic cases

14

MRC DDA pre-clinical

Sawyer *et al Scientific Reports 2015*

Model:

- RML mouse model used.
- Animals inoculated by intra-cerebral route.

Assays used to analyse samples

DDA-markers of infection were highest immediately post inoculation

Cells scrapie assay-results were opposite with infectivity starting low and increasing up to onset of clinical symptoms.

Unclear as to what DDA is measuring markers vs infectivity?

MRC test specificity

Jackson, G et al JAMA Neurology 2014.

Whole blood samples tested, specificity was 100% (no samples repeat reactive).

Sample set	Initial reactive	Repeat reactive
USA blood donors	94/5000	0/94
UK blood donors	3/200	0/3

16

Amplification methods

Rely on increasing the amount of the β-form of the prion protein to levels readily detectable

- Substrates
  - Cell extracts/brain homogenates/recombinant protein
- Methods
  - Sonication (to break down higher molecular aggregates) followed by shaking-protein misolding cyclic amplification PMCA
  - Shaking alone (Quaking induced conversion)QuIC

Often take several days-more likely application is as a confirmatory

17

Analytical sensitivity-assessed by NIBSC

Group	vCJD 10^{-6}	vCJD 10^{-8}	vCJD 10^{-10}
1	1/1	3/3	1/3
2	2/2	1/2	0/2
3	2/2	0/2	0/2

The analytical sensitivity is only a few logs greater than observed for the direct detection methods (limits of detection were 10^{-5}) and for 2 groups several logs less than for the DDA

PMCA vCJD blood samples-study 1

Lacroux et al Plos Pathogens 2014

vCJD Buffy coat/white blood cells

- 2/3 buffy coat positive
- 1 white blood cell fraction tested

Normal

- 0/6 buffy coat
- 0/135 white blood cells

vCJD samples gave a positive result even after dilution

19

PMCA results with vCJD blood-study 2

Concha-Marambio *et al Science translational medicine* (2016)

Whole blood was treated with sarkosyl and sample centrifuged, pellet washed re-suspended in PBS and re-centrifuged

samples tested:

- 14 confirmed cases of vCJD-all were positive
- 16 cases of sporadic CJD-all negative
- 137 controls-all negative

PMCA vCJD bloods study 3

Bougard *et al Science translational medicine* (2016)

Test uses an initial capture stage using plasminogen.

Plasma samples from vCJD (clinical and preclinical)

- 18 clinical cases of vCJD-all positive
- 67 cases of sporadic CJD- 1 positive
- 104 controls –all negative

PMCA preclinical sensitivity

vCJD blood

- Archived plasma from clinical cases of vCJD cases known to have donated blood were positive 31 and 16 months ahead of disease onset

Animal models:

BSE infected sheep (orally infected) white blood cells:

- Animals were positive at 6 months post exposure

vCJD infected primates:

- Animals were inoculated by transfusion and intravenously
    - White blood cells were positive 4 months ahead of onset (transfusion)
    - 40 months for IV

PMCA specificity

An issue with amplification methods is the rate of falsely scoring negative samples positive.

	Other neurological disorders	Healthy controls
Study 2	0/88	0/49
Study 3	0/134	0/104

Numbers are insufficient, NIBSC have 1000 US blood donor samples for specificity assessment

Summary of blood PMCA data

Detects markers of infection in vCJD clinical samples in

- Buffy coat
- Whole blood
- Plasma

Assay works on preclinical samples

  - Actual cases of vCJD
  - Animal models (BSE/vCJD)

Only method to have demonstrated an ability to detect vCJD preclinical blood samples positive

24

Additional considerations in test selection

Direct detection method

- Failed to work on blood from MV case
  - No infectivity in blood? Very little evidence of peripheral tissue involvement (unlike MM cases)

limited evidence of detection of pre-clinical samples except for mouse IC model

PMCA assay

- Assay is dependent on the source of normal prion protein substrate
  - Need to establish if substrates work on other genotypes to produce meaningful data from prevalence study
- Specificity requirements-full assessment needed
- Length of time to perform assay

vCJD and blood products-assessing the risk of transmission

Samples held at or collected at NIBSC:

Plasma pools and products produced (Factor VIII)

- Are disease markers detectable?

Collections of blood from “at risk” cohorts:

- Recipients of clotting factors
  - Blood and tissues collected
  - Study identified markers of infection
- Primary immuno-deficients
  - Ongoing annual blood collection from study participants