Population Screening for Variant Creutzfeldt-Jakob Disease Using a Novel Blood Test Diagnostic Accuracy and Feasibility Study

Graham S. Jackson, PhD; Jesse Burk-Rafel, MSc; Julie Ann Edgeworth, PhD; Anita Sicilia, MSc; Sabah Abdilahi, BSc; Justine Korteweg, BSc; Jonathan Mackey, BSc; Claire Thomas, BSc; Guosu Wang, BSc; Jonathan M. Schott, MD; Catherine Mummery, MB, BS; Patrick F. Chinnery, MD; Simon Mead, BM, BCH; John Collinge, FRS

IMPORTANCE Our study indicates a prototype blood-based variant Creutzfeldt-Jakob disease (vCJD) assay has sufficient sensitivity and specificity to justify a large study comparing vCJD prevalence in the United Kingdom with a bovine spongiform encephalopathy–unexposed population. In a clinical diagnostic capacity, the assay’s likelihood ratios dramatically change an individual’s pretest disease odds to posttest probabilities and can confirm vCJD infection.

OBJECTIVES To determine the diagnostic accuracy of a prototype blood test for vCJD and hence its suitability for clinical use and for screening prion-exposed populations.

DESIGN, SETTING, AND PARTICIPANTS Retrospective, cross-sectional diagnostic study of blood samples from national blood collection and prion disease centers in the United States and United Kingdom. Anonymized samples were representative of the US blood donor population (n = 5000), healthy UK donors (n = 200), patients with nonprion neurodegenerative diseases (n = 352), patients in whom a prion disease diagnosis was likely (n = 105), and patients with confirmed vCJD (n = 10).

MAIN OUTCOME AND MEASURE Presence of vCJD infection determined by a prototype test (now in clinical diagnostic use) that captures, enriches, and detects disease-associated prion protein from whole blood using stainless steel powder.

RESULTS The assay’s specificity among the presumed negative American donor samples was 100% (95% CI, 99.93%-100%) and was confirmed in a healthy UK cohort (100% specificity; 95% CI, 98.2%-100%). Of potentially cross-reactive blood samples from patients with nonprion neurodegenerative diseases, no samples tested positive (100% specificity; 95% CI, 98.9%-100%). Among National Prion Clinic referrals in whom a prion disease diagnosis was likely, 2 patients with sporadic CJD tested positive (98.1% specificity; 95% CI, 93.3%-99.8%). Finally, we reconfirmed but could not refine our previous sensitivity estimate in a small blind panel of samples from unaffected individuals and patients with vCJD (70% sensitivity; 95% CI, 34.8%-93.3%).

CONCLUSIONS AND RELEVANCE In conjunction with the assay’s established high sensitivity (71.4%; 95% CI, 47.8%-88.7%), the extremely high specificity supports using the assay to screen for vCJD infection in prion-exposed populations. Additionally, the lack of cross-reactivity and false positives in a range of nonprion neurodegenerative diseases supports the use of the assay in patient diagnosis.

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Prion diseases are fatal transmissible neurodegenerative disorders, including CJD in humans, bovine spongiform encephalopathy (BSE) in cattle, and scrapie in sheep. The disease is thought to be caused and transmitted by a misfolded isoform of a host glycoprotein termed prion protein (PrP). Although classic CJD is rare, public health concerns were raised with the discovery of “variant” CJD (vCJD) acquired through exposure to BSE. Up to 3 million UK cattle may have been infected with BSE, of which around 750,000 were slaughtered for human consumption, leading to widespread exposure of the UK population via dietary and other routes. In addition to direct exposure to BSE prions, concerns of secondary, human-to-human transmission have affected endoscopy, ophthalmology, surgery, dentistry, organ transplantation, blood products/transfusion, and emerging stem cell therapies.

Establishing accurate prevalence estimates for vCJD infection in the United Kingdom has been a long-standing priority for guiding public health risk assessments. Since vCJD is associated with marked PrP colonization of lymphoreticular tissues, analysis of surgical samples has been used to estimate prevalence. A 2004 study of 12,674 appendix and tonsil specimens found 3 positives and estimated a prevalence of infection in the UK population of 237 per million (95% CI, 49-692 per million), leading the UK government’s Spongiform Encephalopathy Advisory Committee to recommend a prevalence estimate of infection per 4000 healthy individuals. In response, the Health Protection Agency in England initiated a larger appendix study. Released in August 2012, the study found 16 definitive positive appendices of 32,441 samples examined, equating to a prevalence estimate of 493 per million (95% CI, 282-801 per million). This value is consistent with previous studies but is more precise and has a higher central estimate of around 1 infection per 2000 UK citizens.

The estimated UK vCJD prevalence of 1 in 2000 contrasts with the approximately 220 recognized clinical cases of vCJD. The discrepancy may be explained in part by the extremely prolonged incubation periods seen with human prion infections and the existence of subclinical carrier states. Incubation periods in humans can span several decades. A common PrP gene polymorphism substantially influences prion disease susceptibility and incubation periods, and other modifier genes have an effect. In addition, the “species barrier” believed to limit prion disease transmission between mammalian species may be less effective than previously thought when assessed by infection rather than occurrence of neurological disease. While such subclinical carriers may live a normal life span without overt neurological disease, secondary transmission to others may result in the return of lethality, as same-species subpassage of infectivity is associated with 100% lethality in animal models. Finally, the barrier to infection may be more substantial in brain tissue than lymphoreticular tissue, raising the possibility of large numbers of vCJD prion infections uncoupled from neuroinvasion and clinical symptoms.

Although the risks of iatrogenic prion transmission through surgery and solid organ transplant should not be underestimated, the greatest threat to public safety is thought to be posed by the transfusion of prion-infected blood and blood products. Variant CJD is known to be transmissible via blood transfusion and studies of a small cohort who received blood transfusions from donors known to have subsequently developed vCJD suggest the risk is very high to such recipients. Emerging evidence from sheep scrapie unexpectedly indicates blood transfusion may be a more efficient infection route than direct brain inoculation, with transfusion of microliter quantities of infected blood 100% efficient in transmitting disease in sheep.

The risks and disruption associated with an infected blood supply have directed a critical strategic need to develop diagnostic tests suitable for screening where high specificity is crucial. A blood-based assay for vCJD has been elusive because abnormal PrP levels are extremely low in blood, and the ratio of normal PrP to chemically identical abnormal PrP is highest in blood compared with other tissues. Thus, to identify infection in blood successfully, an assay must be capable of detecting abnormal PrP at sensitivities several orders of magnitude below conventional tissue-based assays and must be highly specific to abnormal PrP. We have capitalized on the observed affinity of PrP for metal surfaces to develop quantitative infectivity assays approaching the sensitivity needed to detect abnormal PrP levels in blood. We subsequently adapted these approaches to capture and enrich disease-associated PrP using stainless steel powder, avoiding proteolytic processing and thus maximizing available disease-associated PrP in samples. When coupled with direct immunodetection, our prototype blood assay has a detection limit for vCJD infection orders of magnitude superior to any reported, to our knowledge, and a sensitivity of 71.4% for vCJD-infected patient blood samples. The assay specificity was nominally determined as 100% but with only 169 normal donor samples, the 95% confidence lower limit was 97.8%.

NHS Blood and Transplant (NHSBT), in consultation with other European partners, has formalized a common technical specification requiring screening tests achieve a minimum diagnostic specificity of 99.5% determined from a cohort of 5000 samples. We designed this study to evaluate this criterion, obtaining 5000 blood samples from the American Red Cross as representative of a population with negligible exposure to BSE in which no true positives would be expected in such a sample size. We also tested several large cohorts of potentially cross-reactive patient populations and reconfirmed the assay sensitivity for vCJD infection in blood.

Methods

Source of Blood Samples
Anonymous, unlinked blood samples from healthy consecutive donors were obtained from the American Red Cross (USA Normals) and NHSBT of England and Wales (UK Normals). Patient blood samples were obtained with written informed consent from patients enrolled in the MRC PRION-1 trial or National Prion Disease Monitoring Cohort study and/or referred to the National Prion Clinic or Dementia Research Centre at the National Hospital for Neurology and Neurosurgery or the Institute of Genetic Medicine, Newcastle, England. These studies were approved by the local research ethics committees of...
the UCL Institute of Neurology and the National Hospital for Neurology and Neurosurgery. USA Normals can be safely assumed not to contain subclinical cases of vCJD because of low population exposure to the BSE agent. Similarly, our cohort of 200 UK Normals is unlikely to contain any such samples, with an estimated prevalence of 1 in 2000. Diagnoses of CJD were made according to established criteria. Positive control samples consisting of 0.1% weight to volume ratio vCJD-infected brain homogenate in normal human blood (vCJD Spikes) were used to preserve scarce stocks of samples from patients with vCJD. All samples were whole-blood samples collected in EDTA tubes and stored frozen at −70°C in multiple aliquots of 500 μL and thawed before use.

Sample Masking and Honesty Control
Twenty-five positive control exogenous vCJD spiked samples were randomly included in the USA Normals cohort and the entire panel was masked. Spiked samples normally allow for a modification to the standard testing protocol, which would preclude use in a blinded manner. However, using a high concentration of infected brain spiked into blood (10⁻² dilution) allows handling such spikes in an identical manner to endogenous patient blood samples.

Testing Procedure
Samples were tested as previously described. Briefly, 8 μL of each sample was diluted 1:100 into buffer (200mM TRIS, 4% weight to volume ratio bovine serum albumin, 4% weight to volume ratio CHAPS, 2 tablets complete protease inhibitors [Roche], 80 units of Benzonase [grade II; Merck]) containing 23 mg of Capture Matrix (CM) (sub-45 μm stainless steel particles; Goodfellow) and incubated overnight. Capture Matrix was isolated using a magnetic rack, supernatant discarded, and washed repeatedly with 1 mL of phosphate-buffered saline plus 0.05% volume to volume ratio Tween-20 (PBST). After the final wash, all liquid was removed and the CM was heated at 110°C. To each tube, 50 μL of biotinylated primary antibody (ICSM18; D-Gen, Ltd) prepared at 1 μg/mL in phosphate-buffered saline plus 1% volume to volume ratio Tween-20 (PBST*) was added and incubated at 37°C for 1 hour. Samples were washed repeatedly with 1 mL of PBST, isolating CM each time. Each sample was then incubated with High Sensitivity NeutrAvidin–HRP (Pierce) prepared at 1 μg/mL in phosphate-buffered saline plus 1% volume to volume ratio Tween-20 (PBST*) and using log-transformed data, but these selected approach (mean +3σ) was superior.

Positivity Criteria and Data Analysis
Samples were scored reactive if the mean chemiluminescence from 3 replicate wells exceeded an on-plate cutoff threshold. Various cutoffs were validated using a receiver operating characteristic curve (Figure 1). The cutoff was ultimately set at the mean plus 3 SDs of 6 negative control normal blood samples on each plate, which maximized specificity at minimal cost to sensitivity. Thus, samples with a “ratio relative to cutoff” greater than 1 were scored reactive. Samples reactive on initial testing were retested. Samples that were repeat-reactive were considered positive for vCJD; nonreactive and single-reactive samples were considered negative for vCJD.

The testing data were visualized in MATLAB R2007a (MathWorks). Log-normal fits were overlaid on the histograms using the lognfit and lognpdf functions. All confidence intervals for proportions used the “exact” method, which guarantees strict conservatism (ie, ensures at least 95% confidence). Likelihood ratios (LRs) were calculated using the Haldane correction, which adds 0.5 to all counts in contingency tables containing zero counts. Likelihood ratio confidence intervals were calculated using a derived approximation for risk ratios.
Results

Specificity

We tested 5000 anonymous blood samples from the American Red Cross where exposure to BSE is minimal and no true positives would be expected (USA Normals). No samples tested positive, resulting in a specificity estimate of 100% (95% CI, 99.93%-100%) (Table 1). The high specificity was confirmed in a healthy UK cohort (UK Normals). In both normal cohorts, a small fraction of samples (about 1.8%) were reactive following the first test, suggesting a theoretical false-positive rate (repeat-reactive) due purely to chance of about 1 in 3000 normal samples (99.97% specificity). Although there were no false positives in the USA and UK Normals, the expected specificity based on chance repeat-reactivity is within our specificity confidence interval.

Despite excellent specificity with normal controls, it is crucial the assay does not produce false-positive reactions in other neurodegenerative disease types, particularly those where abnormal PrP accumulation can occur. To explore the potential role of cross-reacting species on assay specificity, we tested blood samples from patients with a range of nonprion neurodegenerative diseases (Other Neurol) collected from different centers and from National Prion Clinic referrals where a prion disease diagnosis was likely (NPC Referrals). No nonprion neurodegenerative disease samples tested positive (Table 1). Among NPC Referrals, 2 patient samples tested positive of 105 in total. Around 72% of referrals received a diagnosis of sporadic CJD (sCJD) and both patients with a positive blood test had an sCJD diagnosis, indicating the test has some sensitivity for sCJD infection under testing conditions optimized for vCJD.

Sensitivity

All 25 blinded vCJD Spikes included in the USA Normals cohort tested positive, demonstrating that the assay’s high sensitivity does not appear to be an artifact at the expense of sensitive detection of vCJD-affected blood. To confirm the assay was capable of detecting vCJD-affected patient samples, an additional small blind panel comprising 5 USA Normals samples and 10 endogenous Patients With vCJD samples was tested. No normal controls tested positive (100% specificity; 95% CI, 47.8%-100%), while 7 of 10 Patients With vCJD samples tested positive (70% sensitivity; 95% CI, 34.8%-93.3%).

Overall Performance

We pooled ratio relative to cutoff data in each cohort and analyzed the relative frequency of test values. The underlying distribution in each cohort was approximately lognormal (Figure 2), as illustrated by overlaid lognormal probability density curve fits and the normality achieved on log-transformation of the data (Figure 3). These fits were sensitive to binning and sample size and thus were not used to extract underlying parameters but rather to show the general test value distribution by cohort. The separation of test value distributions highlights the assay’s ability to discriminate normal blood samples from vCJD Spikes (Figure 3A) and Patients With vCJD (Figure 3B).

Table 2 summarizes assay performance. The observed sensitivity indicates that 71.4% of Patients With vCJD correctly tested positive. Conversely, the specificity indicates that 100% of healthy persons correctly tested negative. Sensitivity and specificity are uninformative when screening persons with unknown disease status; in contrast, LRs describe how a test result changes an individual’s probability of having disease.23 Because the true prevalence of vCJD infection is not known and was not measured in this study, positive and negative predictive values were not appropriate. The assay’s positive LR indicates a positive test result (repeat-reactive) is more than 7000 times as likely to occur in a person with vCJD (true positive) than in a healthy person (false positive). Although the positive LR confidence interval is wide, the lower limit still indicates a positive test result is extremely suggestive of vCJD. The negative LR indicates a negative test result (non- or single-reactive) is more than 3 times as likely to occur in a healthy person (true negative) than in a person with vCJD (false negative). These LRs can convert pretest disease odds to posttest probabilities.43 Using the recent UK Health Protection Agency vCJD prevalence estimate of 1 in 2000, the pretest probability of disease in a randomly screened individual may be about 0.05%. A positive test would increase the probability of dis-
ease in that individual to about 78%, while a negative test would decrease the probability of disease to about 0.01%. Despite the modest diagnostic sensitivity, the assay has excellent discriminatory value.

Discussion

We validated a prototype blood-based assay for vCJD based on the immunodetection of abnormal PrP captured on metal particles. In a cohort of 5000 normal US blood samples, there were no false positives, yielding assay specificity greater than 99.93%, which substantially refines earlier estimates and exceeds the NHSBT recommended minimum of 99.5% for screening tests. Additionally, testing more than 300 blood samples from patients with other neurodegenerative conditions, including Alzheimer disease and frontotemporal dementia, did not elicit any false positives. A small fraction of patients with sCJD tested positive, indicating the assay has some sensitivity for sCJD. There is no historical evidence for the presence of infectivity in the blood of patients with sCJD, and to our knowledge, no cases have ever been ascribed to receipt of prion-infected blood or blood
products. However, although peripheral pathogenesis in general is limited in sCJD, abnormal PrP and infectivity can be detected in peripheral tissues, albeit at significantly lower levels than in cases of vCJD. It is therefore not surprising that the assay could detect abnormal PrP in a small proportion of sCJD cases, although when low with the incidence of sCJD (about 1-2 cases per million/annum), this would be an extremely rare event in samples from a general population. Although the primary thrust of this work was to refine our specificity estimate, we also reconfirmed the assay’s sensitivity to detect vCJD-infected blood. Using masked samples, the assay correctly detected 100% of exogenous spikes and 70% of samples from patients with vCJD, confirming but not refining previous sensitivity estimates.

This study rigorously validated the specificity of a prototype vCJD test using large samples from healthy and potentially cross-reactive patient populations. We believe this work unambiguously establishes the extremely high specificity of the assay—a critical performance characteristic when screening for rare conditions. Most importantly, this specificity did not come at the expense of our previously established sensitivity. The modestly high sensitivity combined with the exquisite specificity result in LR that dramatically change an individual’s pretest disease odds to posttest probabilities, effectively ruling out or confirming vCJD infection. The only other large-scale study to establish specificity of a potential blood test achieved respectable results of 99.9% (95% CI, 99.85-99.94) with 20,000 plasma samples. Unfortunately, the assay used had no demonstrable sensitivity with authentic samples from patients with vCJD and has since been abandoned by the developer.

Our study has some limitations. It remains unclear whether the assay has sufficient sensitivity to detect asymptomatic individuals with vCJD who are subclinical carriers or are early in their disease progression. Others have suggested that asymptomatic individuals would have lower concentrations of abnormal PrP compared with individuals with clinical vCJD. However, animal models indicate preclinical blood involvement and efficient transmission of scrapie infection via blood in preclinical sheep. The National Prion Monitoring Cohort is collecting serial samples from individuals deemed to be at risk of developing vCJD as a result of prion exposure and who may in the future become symptomatic. Alternative samples could be considered from large cohorts of at-risk individuals, particularly recipients of contaminated plasma products who have been identified and notified of their at-risk status. Such groups could be used to assess our test performance in preclinical stages of infection but this could only be considered following the analysis of large numbers of UK control samples to establish pre-existing background levels of prionemia in the general population. There is currently no way to determine if the sensitivity we have observed reflects missed cases of vCJD with significant prionemia or, alternatively, that not all vCJD cases have prionemia that is detectable in the analyte volume used (8 μL). However, the detection limit of the assay for spiked vCJD brain homogenate at a 10^-10 dilution suggests that the test is detecting at or below the level of a single infectious particle and therefore that in about 30% of cases there is no infectivity to detect in the analyte volume used. Unfortunately, sensitivity has been determined using all available vCJD blood samples and refining our sensitivity estimate may not be possible.

A highly sensitive and specific blood test for vCJD infection is urgently needed. Current measures to prevent secondary infections, including leucodepletion of donor blood and the non-UK sourcing of plasma, have led to significant disruption and costs to the National Health Service (cumulative costs to NHSBT estimated at £540 million [US$895 million], with a recurrent annual cost of £40 million [US$60 million]). If the incidence of vCJD remains low over time, many of these expensive protective measures may no longer be justified. However, the rapidity of policy changes depends on formal risk assessments, for which the prevalence of prionemia is a critical parameter. Additionally, screening blood donors, high-risk groups, and surgical patients would enable more directed risk management and early diagnosis.

Despite some limitations and uncertainties, our findings indicate the prototype vCJD assay is suitable for both clinical diagnostics and screening. The assay is already used diagnostically and could see wider use by clinicians without fear of false positives. Most importantly, the prototype vCJD assay has sufficient performance to justify now screening a large UK population sample and at-risk groups to produce an initial estimate of the level of prionemia in the UK blood donor population. Using our assay to compare vCJD prevalence in the BSE-exposed UK population with the BSE-unexposed US population would require 20,000 samples from each population (80% power, 5% two-sided significance level). A blood prevalence study would provide essential information for policy makers for deciding if routine vCJD screening is needed for blood, tissue, and organ donations and patients prior to high-risk surgical procedures.

Table 2. Sensitivity, Specificity, and LRs of Prototype vCJD Assay

<table>
<thead>
<tr>
<th>Statistic</th>
<th>% (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>71.4 (47.8-88.7)</td>
</tr>
<tr>
<td>Specificity</td>
<td>100 (99.9-100)</td>
</tr>
<tr>
<td>Positive LR</td>
<td>7047 (435-114 146)</td>
</tr>
<tr>
<td>Negative LR</td>
<td>0.30 (0.16-0.56)</td>
</tr>
</tbody>
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Abbreviations: LR, likelihood ratio; vCJD, variant Creutzfeldt-Jakob disease.

* Calculated from the analysis of 5000 presumed negative blood samples obtained from the American Red Cross (zero samples tested positive) and 21 samples from patients with confirmed vCJD (15 samples tested positive).

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Blood Test for Variant Creutzfeldt-Jakob Disease

Critical revision of the manuscript for important intellectual content: Jackson, Burk-Rafel, Edgeworth, Abdollahi, Korteweg, Mackley, Thomas, Wang, Schott, Mummery, Chinnery, Mead, Collinge.

Statistical analysis: Burk-Rafel.

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REFERENCES


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