Estimation of variant Creutzfeldt-Jakob disease infectivity titers in human blood

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BACKGROUND: Blood of individuals with variant Creutzfeldt-Jakob disease (vCJD) is infectious but the titer is unknown. Current estimates of possible vCJD infectivity titers in blood have largely relied on an assumption that the titers of vCJD agent in human blood are likely to be similar to those in blood of rodents infected with model transmissible spongiform encephalopathy agents, assayed by intracerebral inoculations of rodents of the same species.

STUDY DESIGN AND METHODS: We analyzed published descriptions of experimental transfusion-transmitted (TT) bovine spongiform encephalopathy and scrapie in sheep and reports of TTvCJD in humans, applying statistical approaches to estimate the probable number of intravenous infectious doses (ID_{iv}) per unit of transfused blood (ID_{iv}/unit). For humans, ID_{iv}/unit of nonleukoreduced red blood cells (NLR-RBCs) were estimated by two statistical models.

RESULTS: Sheep blood collected at or near onset of clinical illness contained a mean of 0.80 ID_{iv}/unit. Estimates of infectivity in NLR-RBCs from donors incubating vCJD indicated a probable mean infectivity of 0.29 ID_{iv}/unit (Model 1) and 0.75 ID_{iv}/unit (Model 2). The analysis predicted a mean of 21 vCJD-infected recipients expected in a cohort transfused with vCJD-implicated NLR-RBCs in the United Kingdom.

CONCLUSION: Our analysis suggested that, while less than one ID_{iv} is likely to be present in a given unit of NLR-RBCs collected from a donor incubating vCJD, there is a high probability of TT infection among recipients of vCJD-implicated blood components. The analysis supports continuing measures currently recommended to reduce the risk of TTvCJD.

Transmissible spongiform encephalopathies (TSEs) are rare fatal neurodegenerative diseases with long preclinical asymptomatic incubation periods (IPs). In the United Kingdom and other countries, a new TSE of cattle, bovine spongiform encephalopathy (BSE), has infected more than 220 humans, most of them exposed to the BSE agent through consumption of contaminated beef products; the new human disease was named variant Creutzfeldt-Jakob disease (vCJD). Although food-borne vCJD cases are in decline, secondary transmissions of vCJD through blood transfusions, first reported in 2003, continue to pose a risk to public health. A lookback study identified three blood donors among 25 individuals that developed vCJD in France. The implicated blood donations were traced back

ABBREVIATIONS: BC = buffy coat; BSE(s) = bovine spongiform encephalopathy(-ies); ID_{ic} = intracerebral infectious dose; ID_{iv} = intravenous infectious doses; IP(s) = incubation period(s); NLR-RBC(s) = nonleukoreduced red blood cell(s); TMER = Transfusion Medicine Epidemiology Review; TSE(s) = transmissible spongiform encephalopathy(-ies); TT = transfusion transmitted; vCJD = variant Creutzfeldt-Jakob disease; WB = whole blood.

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to recipients; until now, there is no evidence of secondary transfusion transmissions.[1] The UK Transfusion Medicine Epidemiology Review (TMER) has identified 66 recipients transfused with blood from donors who subsequently died of vCJD.[2] Three of these recipients died with clinical vCJD;[3-6] one individual died of vCJD-unrelated causes, but had abnormal prion protein (PrP^{TSE}) in lymphoid tissues.[7] Forty-three individuals died with no vCJD symptoms but were not tested for PrP^{TSE}, and 19 other individuals in the TMER cohort remain alive to date with no clinical signs of disease. All four transfusion-transmitted vCJD (TTvCJD) infections were in recipients of nonleukoreduced red blood cells (NLR-RBCs). More recently a probable case of TTvCJD was reported in a patient treated with coagulation Factor VIII[8,9] manufactured from plasma of UK donors. TTvCJD was recently identified as one of three emerging infectious diseases posing the greatest immediate threat to the safety of the blood supply.[10]

Large animals such as nonhuman primates and sheep are especially relevant experimental models to study TSE infectivity in blood because transfusions can be conducted using large volumes of blood components under conditions that closely mimic transfusions in humans. These models have consistently shown that infectivity is present in blood, even during the preclinical phase of the disease, and it is transmissible by transfusion.[11-14] Early studies with TSEs in small animal models frequently detected low concentrations of infectivity circulating in blood.[15-17] TSE rodent models have been particularly important, providing reproducible quantitative measurements of infectivity titers in blood during IP and overt illness; infectivity titers were measured by bioassay: intracerebral inoculations of rodent blood into a large number of susceptible animals of the same species.[15-17] Some blood-inoculated animals failed to develop TSE, suggesting that not every inoculum contained an intracerebral infectious dose of agent (ID_{ic}).

One ID_{ic} has been operationally defined as the minimum dose of agent that, when inoculated intracerebrally, transmitted an infection with 100% probability. Infectivity titers were calculated based on the probability, described by a Poisson distribution, that an inoculum contained one or more infectious doses of agent. Using this method, the blood of clinically ill hamsters infected with the 263K strain of scrapie agent was found to contain, on average, 10 ID_{ic}/mL.[15,18] Similar values were reported with TSE mouse models.[16,17,19] Extrapolated to humans, these experimental models suggested that a unit of TSE agent–infected human blood (approx. 450 to 500 mL) might contain a few thousand ID_{ic}. Such values have been used repeatedly as assumptions to estimate the probable number of infectious doses in units of human blood for vCJD risk assessments and to develop public health policies.[20-22] For several practical reasons, actual titers of vCJD infectivity in blood of infected individuals cannot be measured directly. In this article, we present two quantitative statistical models based on available transfusion data to estimate the probable number of intravenous infectious doses (ID_{iv}) in the blood of TSE-infected sheep and humans.

MATERIALS AND METHODS

Calculation of infectious doses per transfused unit of blood

We used a binomial distribution, binomial (S, p), to describe the possible number of transmissions randomly occurring among a certain number of transfusions. The binomial probability p corresponds to ID_{iv} per transfused unit of blood. When n infections are observed after S transfusions, the binomial probability p can be calculated using a beta distribution,

$$ID_{iv}/unit = Beta(n+1, S-n+1) \quad (1)$$

When n is equal to S, p is mostly likely 1 and, thus, transfusion of one ID_{iv}/unit corresponds to a 100% probability of transmission.

Statistical model for quantifying infectivity in the blood of TSE-infected sheep

We analyzed data from the transfusion study of Houston and colleagues[13] in which donor sheep, either naturally exposed to scrapie—a TSE of sheep—or dosed orally with BSE agent, were bled at different times during the IP. Typically 2 units of blood (400 mL each) were removed from the same animal to transfuse into two recipient sheep. The units were not leukoreduced. One unit was transfused as whole blood (WB) and the second unit as separated buffy coat (BC) containing nucleated cells. We combined the results for sheep infected with BSE and scrapie agents as well as data for WB and BC based on statistical analyses showing that neither the rates associated with transfusions of WB and BC (Prob > F = 0.9653) nor the transmission rates associated with BSE and scrapie transfusions (Prob > F = 0.5219) were significantly different. Because the infectivity titers in blood of rodents with TSEs increase progressively during IPs, we grouped the sheep data based on the percentage of the IP that had elapsed at the time a blood unit was collected: 0% to 25%, more than 25% to 50%, more than 50% to 75%, and more than 75% to 100%. The ID_{iv}/unit for a specific IP group was calculated using Equation (1), where n is the number of TT infections and S is the total number of transfusions associated with that specific IP group. We considered a transfusion to have transmitted infection if the recipient animal developed typical clinical signs of TSE or had an accumulation of abnormal TSE-associated prion protein (PrP^{TSE}) detected in peripheral tissues. We made three assumptions: 1) each

unit of blood represented a random and independent sampling of the infectivity circulating in the blood of the sheep at the time it was drawn; 2) all recipient sheep had PrP-encoding genotypes conveying similar susceptibility to TSE infectivity; and 3) all donor sheep were infected with TSE. A caveat regarding the last assumption is that, for technical reasons, not all donor sheep could be confirmed to have had TSE. However, it is likely that all donor sheep were infected, because they had been selected to have PrP genotypes conveying very high susceptibility to the TSE infections studied.[13]

Statistical models for quantifying infectivity in the blood of vCJD-infected humans

Model 1

Retrospective data from the TMER study were used to estimate the infectious doses in the blood of infected blood donors ("vCJD-implicated" donors later diagnosed with vCJD). Because, unlike the experimental sheep study, we do not know when a person with food-borne vCJD was infected with the agent, it is not possible to group vCJD-implicated blood units by elapsed fraction of IP at the time of donation. (However, because published intervals between donation and clinical onset of vCJD in donors ranged from 1.4 to 3.5 years and the mean IP of food-borne vCJD has been estimated at approximately 12 years, it seems likely that all four implicated donations were drawn during the last third of the IP.) As above, we assumed 1) that each donation represented a random and independent sampling of the infectivity circulating in the donor's blood and 2) that humans of all PrP-encoding (*PRNP*) genotypes (at Codon 129) are susceptible to vCJD. We restricted analysis to RBCs, representing 82% of all transfused components (54 of 66) and we specifically analyzed NLR-RBCs (27 of 54), the only labile blood cellular component implicated so far in vCJD transmissions.[1] Of 27 NLR-RBC recipients, four have died with evidence of vCJD infection while 11 recipients survived for 5 years or longer after transfusion without signs of vCJD.[23] Twelve individuals dying without evidence of vCJD were excluded from analysis in Model 1 because they underwent no postmortem examination of lymphoid tissues for PrP^{TSE} accumulation to rule out preclinical vCJD infection. Infectivity was calculated as ID_{iv}/unit using Equation (1) where n is the number of known vCJD infections transmitted by transfusion of NLR-RBCs (4) and S is the total number of NLR-RBCs recipients (15).

Model 2

We analyzed the three clinical TTvCJD cases with IPs of 6.5, 7.8, and 8.3 years after transfusion and applied a uniform distribution with 6 and 9 years as the lower and upper bounds to describe the IP for TTvCJD. As of November 15, 2010, of 66 recipients of vCJD-implicated blood components, 47% (31 of 66) had survived longer than 6 years after transfusion and 29% (19 of 66) survived for longer than 9 years after transfusion.[2] The same percentages were applied to a subgroup of NLR-RBC recipients composed of infected individuals with the *PRNP* Codon 129 MM genotype (MM). If we assume a 6-year IP, then 47% of this MM subgroup is predicted to have exceeded the IP and progressed to clinical cases while the remaining 53% presumably remains in the preclinical phase of infection. Assuming a 9-year IP, 29% of the MM subgroup is predicted to have exceeded the IP and have become clinical cases. Dividing the three known clinical TTvCJD cases ($TTvCJD_{clin\text{-}MM}$ in Equation (2)) by the percentage of recipients that survived for longer than the assumed minimum IP ($Percent_{clin}$, 29% to 47%), we back-calculated the total number of NLR-RBC–infected cases (clinical and preclinical) with the MM genotype. We called this value $TTvCJD_{inf\text{-}MM}$, represented by the ratio in parentheses in Equation (2). We further assumed that transfusion recipients having the *PRNP* non-MM genotype are equally susceptible to infection as those with the MM genotype (albeit with longer IPs). Dividing $TTvCJD_{inf\text{-}MM}$ by the percentage of recipients with MM genotype ($Percent_{MM}$) we calculated the total number of infected recipients of all genotypes ($TTvCJD_{inf\text{-}tot}$). The calculations are summarized by the equation

$$TTvCJD_{inf\text{-}tot} = (TTvCJD_{clin\text{-}MM} / Percent_{clin}) / Percent_{MM} \quad (2)$$

Although approximately 40% of the general UK population has the MM genotype, in a small group of 27 individuals this percentage might be slightly different by chance. To develop a distribution of the possible $Percent_{MM}$ in such a small group, we used a bootstrap resampling approach. This method randomly draws samples of 27 individuals from a general population (of 40% MM and 60% non-MM genotypes) and then calculates the percentage of MM genotype each time for each draw.[24] The results of 10,000 repeat draws were aggregated into a distribution that allowed us to estimate with 90% confidence the percentage of persons who might be of MM genotype in a group of 27 individuals. The ID_{iv}/unit was calculated using Equation (1), where n is equal to $TTvCJD_{inf\text{-}tot}$ and S is the total number of NLR-RBCs recipients (27 individuals).

RESULTS

Estimated infectivity in TSE-infected sheep blood

The combined data for sheep with scrapie and BSE were stratified into four groups based on the elapsed fraction of the IP of the donor sheep at time of blood donation. Table 1 shows zero cases of transmission resulting from eight transfusions in the 0% to 25% IP group, 3 of 12 in the 25% to 50% IP group, 7 of 15 in the 50% to 75% IP group,

TABLE 1. Model estimates of infectivity doses per unit of blood collected from sheep infected with BSE or scrapie agents[13]

IP_i (%)*	Mid-percent IP	Total number of transfused sheep	Number of infected recipient sheep	ID_{iv}/unit		
				Mean	5th	95th
0 to 25	0.13	8	0	0.10	0.005	0.28
>25 to 50	0.38	12	3	0.29	0.11	0.49
>50 to 75	0.63	15	7	0.47	0.28	0.67
>75 to 100	0.88	8	7	0.80	0.57	0.96

* Percentage of IP elapsed at time of donation.

and seven transmissions of eight transfusions in the 75% to 100% IP group. The numbers of transmitted cases increased as the donor sheep approached the onset of clinical illness, but in no IP group did all transfused sheep develop TSE. These observations suggest that not every blood unit drawn from an infected sheep transmitted infectivity when transfused. We infer that not all blood units contained one ID_{iv}, the quantity of agent sufficient to transmit an infection by the intravenous route. The mean ID_{iv}/unit of sheep blood using Equation (1) with 90% confidence intervals (CIs) estimated for each IP group are shown in Table 1.

Estimated infectivity in vCJD-infected human NLR-RBCs

Model 1

Eleven NLR-RBC recipients remain vCJD symptom free, all surviving longer than 5 years after transfusion. A large subset of these cases has survived for more than 10 years and several recipients more than 15 years after transfusion.[2,4,23] We interpret these observations as suggesting that not all recipients of NLR-RBCs from donors with vCJD became infected. This conclusion is supported by a report that one recipient of NLR-RBCs drawn just a few months before the donor's onset of clinical illness has survived for more than 11 years without developing vCJD; the TMER study revealed that the same donor had previously donated 2 units of blood, NLR-RBCs from each of which transmitted vCJD to a different transfused patient.[23] Thus, even though the donor had twice demonstrated infectivity circulating in blood, not every blood unit from that donor contained one ID_{iv} of agent. We applied Equation (1) to estimate the infectivity doses for NLR-RBCs based on four reported vCJD transmissions of 15 NLR-RBC transfusions. The estimated mean value was 0.29 ID_{iv}/unit of NLR-RBCs with 90% CI ranging from 0.13 to 0.48 (Table 2).

Model 2

This model assumed that the three clinical TTvCJD cases reported in the TMER study represent NLR-RBC recipients who met three criteria: 1) they were infected, 2) they were of the MM genotype, and 3) they had survived long enough to exceed the minimum IP of vCJD. The single asymptomatic-infected recipient was excluded from this analysis because no IP could be assigned.[7] Using statistical Model 2, we applied the percentage of the population transfused with any component to have survived longer than the IP of vCJD to the subgroup of NLR-RBC recipients with the MM genotype and back-calculated the total number of infected cases (clinical and preclinical) among this subgroup ($TTvCJD_{inf\text{-}MM}$). The mean total number of infections estimated from the model for this subgroup is eight cases (90% CI, 7-10 cases; Table 3). To estimate the total number of infections among recipients of all genotypes ($TTvCJD_{inf\text{-}tot}$), we first used the bootstrap resampling approach and estimated that among 27 NLR-RBC recipients 26% to 56% (90% CI) had the MM genotype, with a mean of 40%. We then applied this distribution to estimate the mean number of infections expected among recipients of all genotypes, which was 21 with a 90% CI of 18 to 27 (Table 3). Finally, we calculated ID_{iv}/unit using the beta distribution based on 21 infections out of 27 total NLR-RBC transfusions. The estimated mean value was 0.75 ID_{iv}/unit with a 90% CI ranging from 0.56 to 0.96 (Table 2).

TABLE 2. Model estimates of infectivity doses per unit of human NLR-RBCs collected from vCJD-infected donors

Model estimate	ID_{iv}/unit		
	Mean	5th	95th
Assumes 4 TTvCJD cases per 15 recipients (Model 1)	0.29	0.13	0.48
Assumes 21 TTvCJD cases per 27 recipients (Model 2)	0.75	0.56	0.96

TABLE 3. Model estimates of the number of infections among 27 vCJD-implicated NLR-RBCs recipients using Model 2

Step	Results		
	Mean	5th	95th
$TTvCJD_{clin\text{-}MM}$	3	NA	NA
$TTvCJD_{inf\text{-}MM}$	8	7	10
$TTvCJD_{inf\text{-}tot}$	21	18	27

DISCUSSION

We analyzed published data and applied statistical models to estimate the probable doses of infectivity present in units of blood from sheep and humans infected with TSEs. Our models assumed that persons of all Codon 129 *PRNP* genotypes are susceptible to TSE infection, probably with different IPs.[7,25,26] However, we cannot exclude the possibility that polymorphisms at other *PRNP* codons or in different genes might also influence susceptibility to transmission of vCJD.[27] Furthermore, our estimates could be based only on those vCJD-infected blood donors that developed overt disease and we did not consider the blood donations from asymptomatic vCJD-infected donors that never became clinically ill. Currently, there is no way to identify these individuals. This situation may change in the future if an antemortem blood test for TSE becomes available.

Models for humans and sheep yielded roughly similar estimates of infectivity, suggesting that the studies of TT TSE in sheep are relevant to humans. Endogenous blood infectivity is localized in both plasma and white blood cells (WBCs).[15,17,19] Thus, it was somewhat surprising that the infectious dose estimates for human NLR-RBCs and sheep WB (or BC) did not reflect differences in plasma volume and in WBC concentration. We have no explanation at this time but emphasize that the estimates here must be based on a very small number of cases. It is also possible that infectivity in plasma from sheep is less than that from humans. In sheep, ID_{iv}/mL of blood increased progressively during the IP, eventually reaching a mean titer close to 0.5 ID_{iv}/unit and probability of transmission as high as 1 of 2 transfused blood units with blood of sheep collected during the last half of the IP. These data confirm previously reported conclusions that both scrapie and BSE are efficiently transmitted by homologous transfusions of sheep.[11-13]

To estimate the infectious doses in vCJD-infected human blood, we specifically focused on NLR-RBCs—to date the only component clearly demonstrated to have transmitted vCJD to blood recipients. We developed two statistical models based on different assumptions. The estimates derived from Model 1 might increase in the future should reliable antemortem tests for vCJD become available to allow diagnosis of vCJD during the asymptomatic IP or more vCJD-implicated blood recipients become ill with vCJD. Thus, Model 1 represents a snapshot of the current situation and does not predict future TTvCJD cases. As of now, the amount of infectivity likely to be present in blood during the last 3 years of the IP of vCJD estimated by Model 1 is 0.29 ID_{iv}/unit. The second model estimated a mean of 0.75 ID_{iv}/unit by back-calculating the probable number of infected NLR-RBC recipients from three known TTvCJD cases. This model also predicted that a mean of 21 NLR-RBCs recipients in the TMER cohort were infected with 95% probability that at least 18 recipients were infected and a 5% probability that all of 27 recipients at risk were infected. There is an approximate 2.6-fold difference in NLR-RBC infectivity estimated by the two models. This difference in estimates may narrow in the future when the disease status of more recipients of vCJD-implicated NLR-RBCs becomes known. However, estimates from the two models can never fully converge, because Model 2 considered all 27 NLR-RBC transfusion recipients as potentially infected cases while Model 1 was restricted to those 15 NLR-RBC transfusion recipients for whom information is either available now or likely to become available in the future.

We are aware that, for many years, precautionary measures have been implemented by blood establishments to reduce the risk of TT classic forms of CJD and related TSEs. The analyses presented here cannot be applied to human TSEs other than vCJD, because no TT infections have ever been recognized.[28] However, based on available information, it can be concluded that even less infectivity must be present in blood of persons incubating sporadic CJD than those with vCJD infections.

A unit of NLR-RBCs contains approximately 20 mL of plasma while a unit of WB has approximately 10 times more plasma. Thus, the number of infectious doses in a unit of WB is likely to be 10 times higher than in NLR-RBCs. The contribution of WBCs to the final infectious doses is more difficult to establish. In studies with blood components of rodents experimentally infected with TSEs (most often scrapie), it appeared that approximately 20% of the infectivity in WB remained in the RBC component (L. Gregori and R.G. Rohwer, unpublished data). If this same ratio holds true for humans, then the total number of ID_{iv} in a 500-mL unit of vCJD-infected human WB should be on the order of only a few infectious doses. This value is substantially lower than that calculated based on actual assays of ID_{ic} of scrapie agent per milliliter of blood from infected rodents, even after the titer is corrected for the somewhat lower efficiency of transmission by the IV route;[17,19] this difference is due to the different sizes of the inocula. While vCJD infectivity in human blood was estimated as ID_{iv} per inoculum—an inoculum being an entire transfused unit of NLR-RBCs—rodent infectivity titers were calculated as ID_{ic} or ID_{iv} per milliliter of inoculum.[17-19] However, it remains possible that blood of rodents with experimental TSEs may contain more endogenous infectivity than that in blood of persons incubating vCJD. Nevertheless endogenous vCJD infectivity in human blood cannot be assayed experimentally, and experimentally infected rodents models appear to offer practical, accessible, and probably relevant models to investigate the general properties of TSE infectivity in blood.

Various models of the dose-response relationship for samples with low infectivity doses can be considered

including a linear dose-response model and "one-hit" Poisson model.[21] The linear dose-response model is based on an assumption that the probability of infection is linearly dependent on the quantity of infectious agent present with no minimum dose. The one-hit Poisson model assumes that (as for other infectious agents) a minimum infectious dose is required to transmit infection with no probability of infection below one ID_{iv}. The latter model can be expressed with a mathematical equation (Poisson distribution), and it has been used to estimate ID_{ic}/mL of TSE-infected rodent blood.[15,18,19] In this analysis, we have assumed that the recognition of one transmission of vCJD by a transfusion indicates that at least one ID_{iv} must have been present in the unit of blood transfused; we applied a binomial beta distribution instead of a Poisson distribution because the number of cases analyzed was so small. The estimated ID_{iv}/unit represents the mean ID_{iv} per transfused unit over all transfusions, and 0.5 ID_{iv} can be interpreted as meaning that 50% of recipients received one ID_{iv} and 50% received no ID_{iv}. Other models of dose-response relationship might apply to human cases, reflecting the possibility that one ID_{iv} might differ from individual to individual as a function of genetic makeup (TSE susceptibility genes), general health of the recipient, and other unknown factors. Our findings support a continued concern that even a single dose of vCJD agent, when present in a unit of transfused blood, is efficiently transmitted to a transfusion recipient. We offer our analysis to assist in the future development of risk assessments and public health policies to maintain a safe blood supply.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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