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Mini-Review

Detection of the PrP^{Sc} in blood is still challenging?

Hanin Abdel-Haq*

National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161-Rome, Italy.

ABSTRACT

Transmissible spongiform encephalopathies (TSEs) or prion diseases can be efficiently transmitted by blood. Significant progress has been made, in recent years, in the field of the TSEs' pathognomonic marker (PrP^{TSE}) detection in blood; however, a routine blood test for the diagnosis of prion diseases is yet unavailable. This delay in developing a PrP^{Sc}-based blood test that can be introduced into routine clinical practice indicates that the challenges and limitations that may affect the reliability and feasibility of detecting PrP^{Sc} using blood still remain. This perspective summarizes the potential challenges and highlights those that still need to be overcome to enable the development of a routine blood test not only for prion diseases but also for prion-like neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, which involve related mechanisms. Furthermore, this mini-review highlights that in order to be able to develop a successful routine blood test for prion diseases, it may be essential to overcome all of the potential limitations not only because they hinder the identification of PrP^{Sc} in different ways, but also because these limitations are present heterogeneously in infected subjects.

KEYWORDS: limitations and challenges, PrP^{Sc} detection in blood, prion protein, prion diseases, transmissible spongiform encephalopathies, TSEs, routine test, neurodegenerative diseases.

*Corresponding author: hanin.abdelhaq@iss.it

INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) or prion diseases are fatal neurodegenerative disorders that affect both animals and humans and can be efficiently transmitted through blood [1].

The key event in the pathogenesis of prion diseases is the conversion of the cellular prion protein (PrP^{C}) to the partially protease-resistant form (PrP^{Sc} or PrP^{TSE}) [2]. PrP^{Sc} is the main marker of prion diseases and has recently been successfully detected in blood of animals and humans by several advanced methods, but especially the protein misfolding cyclic amplification (PMCA) [3, 4]. However, a routine blood test for the diagnosis of prion diseases is yet unavailable. One possible explanation for this issue, is that detecting PrP^{Sc} using blood is still unreliable, perhaps because of the presence of particular challenges that are either not taken into account at all or not considered adequately.

Some challenges that may affect the reliability and feasibility of PrP^{Sc} detection using blood still need to be overcome

Detection of the PrP^{Sc} in the blood is particularly difficult and hampered by various factors, including, high concentration of PrP^{C} and low concentration of PrP^{Sc} in the blood, sensitivity of PrP^{Sc} to proteinase-K (PK), individual variability, high-abundance proteins, fibrin clots, direct application of methods developed for PrP^{Sc} detection in the brain for the detection of PrP^{Sc} in the blood, methodological limitations, and instability of PrP^{Sc} [1]. Table 1 summarizes these factors and shows that some of

Table 1. Summary of the challenges that affect the detection of PrP^{Sc} in the blood.

Category	Challenge	Consequence	Status[a]
Related to the PrP^{Sc}	• PK-sensitivity • Aggregation • Biochemical-physical instability	• Digestion of PrP^{Sc} upon treatment with PK • Incapacity to detect hidden epitopes in PrP-aggregates by some antibodies • Increasing susceptibility or propensity of PrP^{Sc} to degradation and/or aggregation	• Overcome • Not overcome • Not overcome
Related to the blood	◦ Presence of low levels of PrP^{Sc} ◦ Ratio of PrP^{C} to PrP^{Sc} is highest in blood compared to other tissues ◦ Inhibitory interaction by blood high-abundance proteins ◦ Plasma proteins binding to fibrin clots	◦ Incapacity to detect PrP^{Sc} by conventional methods ◦ Incapacity to detect PrP^{Sc} specifically ◦ Interference with the detection of PrP^{Sc} ◦ Possible removal of PrP^{Sc} by entrapment into fibrin clots	◦ Overcome ◦ Overcome ◦ Partially overcome ◦ Not overcome
Related to the host	♦ Individual variability	♦ Incapacity to detect PrP^{Sc} in all clinically ill subjects	♦ Not overcome
Related to the detection process	▪ Using methods with low sensitivity for the detection of PrP^{Sc} ▪ Treatment with PK ▪ Direct application of methods developed for the detection of PrP^{Sc} in brain, to the blood ▪ Methodological limitations	▪ Incapacity to detect tiny amounts of PrP^{Sc} ▪ Digestion of PK-sensitive PrP^{Sc} ▪ Theoretical estimation of PrP^{Sc} in blood ▪ False negative/positive results	▪ Overcome ▪ Overcome ▪ Not overcome ▪ Partially overcome

[a]This classification was inferred from the analysis of the literature related to these limitations.

them have successfully been overcome while others continue to represent a challenge for the development of a routine blood test.

These factors may be due to intrinsic properties of the prions that control their preferential accumulation in certain tissues and/or the properties of the blood, as after peripheral exposure, many TSE agents accumulate on follicular dendritic cells (FDCs) in lymphoid tissues prior to neuroinvasion since FDCs express high levels of PrP^{C} on their cell membrane [5]. On the other hand, the biochemical-physical properties of blood PrP^{Sc} seem to be responsible for the hypothesized-for-a-long-time and recently demonstrated PK-sensitivity of blood PrP^{Sc} [6].

The individual variability in detecting PrP^{Sc} in the blood but not the brains of some tested subjects could be attributed to the efficiency of clearance by each subject, to the fact that the PrP^{Sc} present in blood is both associated with cellular components and in a cell-free state, to the variable ratio between aggregated and non-aggregated PrP^{Sc}, and to the coexistence of multiple forms of PrP^{Sc} in the blood [1, 4]. This heterogeneity poses challenges for the test specificity, precision, accuracy and reliability.

High-abundance proteins, such as immunoglobulins and albumin, interfere with prion detection in the blood either by masking the presence of PrP^{Sc} or by interacting directly with the detection antibodies [7, 8] or reagents. All of the attempted approaches to enable the detection of low abundance proteins in the blood have demonstrated high efficiency in removing large abundant proteins; however, some limitations still remain.

Either PrP^{Sc} or the prion agent could be trapped in fibrin clots, which are often found in "*in vitro*" plasma samples [9], with the consequent removal of some, if not all, PrP^{Sc} and infectivity. This likelihood is also increased by the fact that blood from terminally 263K-infected hamsters is more viscous and clots more quickly forming fibrin clots than blood from noninfected hamsters [10]. In addition, entrapment of α2-macroglobulin and α1-antitrypsin, two important plasma protease inhibitors, in a fibrin clot [9] may result in the PK-sensitive PrP^{Sc} being spontaneously digested by the non-inhibited plasma proteases. Recently, the measurements of PrP^{Sc} in macaque-adapted vCJD (the new variant form of Creutzfeldt-Jakob disease) buffy coat replicates by enhanced-PMCA have been influenced by the quality of blood samples collected longitudinally throughout the incubation period from macaques peripherally infected with vCJD [4]. In that study, macaque-adapted vCJD buffy coat replicates, which were positive for PrP^{Sc} at previous preclinical time points, tested negative for PrP^{Sc} at later time points due to sample quality rather than a particularly low concentration of prions at that time point [4].

The direct application of methods developed for the detection of PrP^{Sc} in animal brain to human blood, may significantly affect estimates of PrP levels in blood. For example, in comparison to brain samples, estimates performed using a pool of blood samples instead of individual samples would negatively affect PrP^{Sc} detection. This is because the concentration of PrP^{Sc} is further reduced in pooled samples being subjected to dilution in comparison to individual samples, and this reduction is proportional to the size of the pool.

Since the levels of PrP^{C} in the blood are crucial in determining the levels, distribution, and site of replication of PrP^{Sc} in the blood itself, the PrP^{C}-to-PrP^{Sc} ratio can vary considerably among species. Therefore, the levels of PrP^{Sc} that are estimated for one species may not reflect the expected levels in other species. Based on these considerations, the differences between species in the expression of detectable quantities of PrP^{C} by blood cells and the type of involved cells should be also taken into account when extrapolating the results from one animal species to another animal species or from animals to humans.

Drawbacks related to the two amplification techniques most used for the detection of PrP^{Sc}, the PMCA [11] and real-time quaking-induced conversion (RT-QuIC) [12], have recently been reported. The PMCA failed to detect PrP^{Sc} in blood from patients with sporadic Creutzfeldt-Jakob disease (sCJD), which is more frequent than vCJD, suggesting that it could not be suitable for all the human prion strains [3], and therefore, it needs further optimization. Whereas, the RT-QuIC appears to be less efficient for blood samples and also for cerebrospinal fluid (CSF) samples when they are contaminated with blood [13]. Indeed, the seeding activity of PrP^{Sc}, which resulted in a false-negative RT-QuIC response in CSF from sCJD patients, was inhibited immediately after incubation of CSF samples with >1250 cells/µl of sonicated erythrocytes and after 3 days upon incubation of CSF samples with 5000 cells/µl of non-sonicated erythrocytes [13]. In both cases, the inhibition was caused by haemolysis and release of inhibitory proteins [13]. A similar inhibitory effect, by increasing the concentration of red blood cells, on the RT-QuIC reaction in CSF from patients with sCJD and E200K-mutated genetic CJD, has recently been reported by other laboratories [14].

Other challenges

The high susceptibility (instability) of PrP^{Sc} to digestion by endogenous enzymes and other factors present in blood is an additional factor that hinders the detection of PrP^{Sc} using plasma and serum [15]. This issue is controversial and still debated because some authors have stated that PrP in the blood is instable [16] while others disagreed completely [17, 18].

PrP^{C} in the blood of mice has been claimed to be enzymatically stable "*in vivo*" [17]. It has also been stated that PrP present in human plasma is stable both at –18°C (storage temperature) and at room temperature (up to 96 h) [18]. The first statement is based on the results obtained using a radioactive method to quantify PrP^{C}. This study did not verify whether the PrP^{C} glycosylation pattern or profile was altered during the experimental run. This is important because both the quantity (concentration) and quality of a substance reflect its stability. The second statement is also based on the quantitative estimate of PrP recovery from

10 randomly selected plasma samples that remained stable during the entire storage period at room temperature. This study also did not verify the qualitative status of PrP. Furthermore, it is very unusual for serum and plasma proteins to maintain their integrity during a storage period of 96 h at room temperature because protein stability and enzymatic activity are strictly reliant on temperature. Plasma and serum are rich in enzymes whose activity increases significantly *in vitro*, causing substantial changes in protein profiles as demonstrated in proteomic studies [19]. It has been observed that protein degradation occurs and is evident after only 8 h or less of storage at room temperature [10]. Consistent with this, it has been reported that marginal changes in the sample are observed within 6 h or less of storage at room temperature while severe changes are observed after 8 h [20]. Moreover, it was observed that the stability of serum and plasma proteomes is also affected by storage at 4°C for 24 h and by repeated freeze/thaw cycles [19, 20].

On the other hand, there are reports stating that PrP^{Sc} and PrP^{C} are spontaneously cleaved into the octapeptide repeat region *in vivo*, without PK digestion [16]. This auto-cleavage may be due to increased PrP instability, which is caused by oxidative stress [21] induced by some prolonged stress conditions. PrP^{C} binds copper, which is an essential element of reduction-oxidation (redox) transition which under conditions of oxidative stress can destabilize PrP. The redox reaction of the copper ion can locally generate oxygen species that can react at specific sites in the PrP itself, impairing the activity or resulting in cleavage [22]. In general, in the presence of oxidative stress, the cellular system is extremely unstable, which results in changes in the physicochemical properties of some proteins. For example, normal protein folding is one of the affected properties [21]. Improperly folded proteins are prone to aggregate into amyloid fibrils, a process known as amyloidosis. Therefore, the coexistence of multiple forms of PrP^{Sc} and the variable-ratio between aggregated and non-aggregated PrP^{Sc} in the blood, which are involved in individual variability, could be the result of an alteration in PrP^{Sc} stability. Recent findings have revealed that the differences between the profile and magnitude of the glycosylation of the hamster water-soluble PrP and classical PrP are responsible for the different level of stability observed between the two forms of PrP, and that the glycosylation-related instability of the hamster PrP could be potentially involved in the prion transmissibility within and between species [10].

CONCLUSIONS

These factors may act synergistically to cause delays in the development of a routinely applicable blood test for prion diseases. However, because the concentration of detectable PrP^{Sc} in the blood is variable, this delay may be caused primarily by individual variability. Due to recent technological advances, we can now detect PrP^{Sc} in many types of animal and human samples [1]. However, significant variability is observed between individuals. It might be essential to overcome all of the involved factors to detect PrP^{Sc} homogeneously in all infected samples because these factors hamper the detection of PrP^{Sc} differently and are present heterogeneously in infected subjects. Therefore, without overcoming all of these factors, it is difficult to obtain an accurate disease diagnosis and/or evaluate effective risk.

Similar to PrP^{Sc}, amyloid-related hallmark proteins, such as, amyloid beta (Aβ), alpha-synuclein and transactive response DNA-binding protein 43 (TDP-43), which possess properties similar to those of PrP^{Sc}, may also be subject to increased degradation by plasma proteases, to entrapment by fibrin clots and subsequent removal, to the masking effect of high-abundance proteins, and to other challenges [23], with the consequent underestimation of their actual concentrations in blood samples.

Finally, the presence of atypical agents and silent carriers associated with prions in blood, as in brain, constitutes a significant risk because they would not be diagnosed in the absence of detectable PrP^{Sc}. This would broaden the range of prion diseases that is already no longer limited to PrP^{Sc}-positive diseases that target the brain [24].

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CONFLICT OF INTEREST STATEMENT

The author declares that she has no conflict of interest.

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