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# Development of an automated capillary nano-immunoassay—Simple Western assay—to quantify total TDP43 protein in human platelet samples

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## Abstract

Frontotemporal lobar degeneration syndrome is the second cause of young-onset dementia. Unfortunately, reliable biomarkers are currently lacking for the diagnosis of this disease. As TDP43 protein is one of the proteins pathologically involved in frontotemporal lobar degeneration, many studies have been performed to assess TDP43 protein diagnostic performances. Mixed results were obtained using cerebrospinal fluid and plasma samples so far. The aim of the study was to develop an automated capillary nano-immunoassay—Simple Western assay—to detect and quantify TDP43 protein simultaneously in human blood-based samples. Simple Western assay was developed with two different cell lysates used as positive controls and was compared to Western blot. TDP43 protein profiles in plasma samples were disappointing, as they were discordant to our positive controls. On the contrary, similar TDP43 patterns were obtained between platelet samples and cell lysates using both assays. Simple Western assay provided good quantitative performances in platelet samples: a linearity of signals could be observed ( $r^2 = 0.994$ ), associated to a within-run variability at 5.7%. Preliminary results based on a cohort of patients suffering from frontotemporal lobar degeneration showed large inter-individual variations superior to Simple Western's analytical variability. Simple Western assay seems to be suitable for detecting and quantifying TDP43 protein in platelet samples, providing a potential candidate biomarker in this disease. Further confirmation studies should now be performed on larger cohorts of patients to assess diagnostic performances of TDP43 protein in platelet samples.

**Keywords** Dementia · TDP43 proteinopathies · Capillary electrophoresis/electrophoresis · High-throughput screening assays · Biomarkers

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## Introduction

Frontotemporal lobar degeneration (FTLD) is a clinically and pathologically heterogeneous syndrome, characterized by progressive atrophy of frontal and/or anterior temporal lobes [1]. FTLD spectrum is the second cause of young-onset dementia [2]. Different clinical syndromes exist, defined on the basis of leading features at presentation. They include behavioral frontotemporal dementia (bvFTD) and other cognitive syndromes impairing language capabilities primarily [3, 4]. In addition, both syndromes can overlap with motor neuron diseases [5]. The diagnosis of FTLD with definite pathology relies on the association of a clinical diagnosis with different core features depending on the syndrome and either presence of histopathologic evidence of specific protein aggregates or a known pathogenic mutation [6, 7].

In 2006, Neumann et al. discovered TDP43 protein as the major component of ubiquitin-positive, tau- and alpha-

synuclein negative inclusions in FTLD [8]. TDP43 cerebral inclusions contain an accumulation of native protein, but also hyperphosphorylated and truncated forms of TDP43 protein [9]. Numerous studies have been performed so far to elucidate TDP43 protein's functions, metabolism and misprocessing [10]. Otherwise, a lot of research has been done to detect TDP43 protein in patients' biofluids, potentially providing a diagnostic, prognostic and/or therapeutic evaluation biomarker [11]. Cerebrospinal fluid (CSF) markers are commonly evaluated in neurodegenerative diseases [12]. Plasma samples are less invasive, but neurodegenerative markers may exhibit lower concentrations than in CSF. Developing blood-based biomarkers could then become challenging in the absence of sensitive enough technologies [13]. Currently, no CSF or plasma biomarker can positively identify living FTLD patients [14]. Some studies showed differences in TDP43 protein between FTLD patients and other neurodegenerative diseases or controls in both matrices [15, 16]. However, quantification of TDP43 protein in CSF and plasma gave contradictory results so far. Potential reasons to these discrepancies could be the very low concentration of this protein or the low-binding affinity of the antibodies in the presence of abundant immunoglobulins and albumin, underscoring the necessity to identify disease-specific patterns [17]. Interestingly, different high-throughput Western blot platforms exhibit qualitative patterns associated to quantitative results and offer simplified workflows compared to traditional Western Blot [18–20]. Moreover, some studies highlighted an important role of platelets in neurodegenerative diseases, thus providing a new potential matrix for biomarkers' discovery [21].

The main objective of this study was to evaluate Simple Western technology developed on Peggy Sue analyzer (Protein Simple®) to detect TDP43 protein in platelet samples, in the context of lacking validated markers for FTLD. This automated technology coupled both electrophoresis and a chemiluminescent detection, providing qualitative and semi-quantitative measurements for tested samples. After initial development using cell culture lysates, Simple Western assays were performed to detect TDP43 protein in plasma and platelets samples. This first step was followed by an evaluation of automated quantification of TDP43 protein. Finally, the suitability and analytical performances of Simple Western assay were assessed on platelet samples from FTLD patients.

## Material and methods

### Samples

#### Endogenous TDP43 protein in cell culture lysates

The human embryonic kidney cell line HEK293T was obtained from the Généthon (Evry, France). Cells were cultured

in DMEM-Glutamax medium supplemented with 10% Fetal Bovine Serum (FBS) and penicillin/streptomycin (Invitrogen). The human neuroblastoma cell line SH SY5Y was obtained from Sigma-Aldrich® (Saint-Louis, USA) and was cultured in DMEM-F12 (1/1) Glutamax supplemented with 10% FBS, non-essential amino acids and penicillin/streptomycin (Invitrogen®, Carlsbad, USA). SH SY5Y and HEK293T cells were recovered in 1× PBS and pelleted by centrifugation at 4 °C. Cell pellets were suspended and lysed in Laemmli lysis sample buffer (6% Tris HCl pH 6.8, 10% β-mercaptoethanol, 4% SDS, glycerol and bromophenol blue) and sonicated at 4 °C. Cell lysates were frozen at –20 °C until further analyses.

#### Study population and *C9ORF72* genetic testing

The cohort included 2 male and 7 female patients aged between 59 and 76 years old. All the patients met the diagnosis of probable behavioral variant of frontotemporal dementia (bvFTD) according to Rascovsky criteria [6] and exhibited a family history of neurodegenerative diseases including FTD and/or ALS [22]. Following genetic counseling in the framework of their medical follow-up, the patients included in this study or their legal next of kin gave two informed written consents: the first one for FTLD genetic testing and the second one authorizing a change of biological purpose for remaining parts of samples. Blood sampling (EDTA tubes) was collected. After DNA extraction, the screening for GGGGCC repeat expansions in *C9ORF72* (the genetic variant with the highest prevalence in FTLD) was performed using a repeat-primed PCR (RP-PCR), adapted from the international study from Akimoto et al. [23]. RP-PCR products were separated by capillary electrophoresis using an ABI 3130xL (Life Technologies®, Carlsbad, USA) with Genescan™ 500 ROX size standard (Life Technologies®). Data were analyzed using GeneMapper® software 5 (Applied Biosystems®, Foster City, USA). RP-PCR gave negative results for *C9ORF72* expansion screening in all patients.

#### Blood fractionation of samples

In parallel to classical genetic testing, a part of whole-blood samples was centrifuged at 200g during 15 min (without acceleration and deceleration) to obtain platelet-rich plasmas (PRP). PRP were then centrifuged at 2000g during 15 min. Platelet-poor plasmas (PPP) were then saved, aliquoted, and stored at –80 °C. Pellets were suspended in 4 mL of washing buffer (Tris 50 mM, NaCl 150 mM, EDTA 2.7 mM in water, pH 7.35). A second centrifugation at 2000g during 15 min was performed, supernatant was discarded and pellets were suspended again in 4 mL of washing buffer. Platelet counts were performed on Sysmex® (Kobe, Japan) XN-2000 analyzer. Another centrifugation was performed at 2000g during

15 min and supernatant was discarded. Pellets were suspended in a variable volume of buffer (Tris 50 mM, NaCl 150 mM in water, pH 7.35/Triton X-100 20%, ratio 40:1) to obtain a similar final platelet concentration in all samples (200 G/L). Platelet samples were heated at 37 °C during 15 min, aliquoted and stored at -80 °C until further analysis. Diluted solutions of albumin (Vialebex®, LFB Biomedicaments®, France, 40 g/L) and immunoglobulins (Privigen®, CSL Behring®, USA, 100 g/L) were analyzed in parallel to plasma samples (final dilution for both solutions: 0.5 g/L).

### Recombinant TDP43 protein

Recombinant human TDP43 protein (Ag1231) was purchased from Proteintech® (Rosemont, USA). This truncated recombinant protein includes TDP43 1-260 amino acids, with N-terminal GST tag and its approximated molecular weight is 56 kDa. Serial dilutions from a stock solution (0.5 mg/mL) were performed to assess semi-quantitative performances of Simple Western assay.

### Antibodies

Rabbit polyclonal 10782-2-AP antibody (ProteinTech®, Rosemont, USA) was chosen to detect total TDP43 protein in the different tested matrices. This antibody is one of the most popular anti-TDP43 antibodies and detects TDP43 aminoacids 203–209 and aminoacids near N-terminus [24]. Rabbit polyclonal 12892-1-AP antibody (ProteinTech®, Rosemont, USA) and mouse monoclonal 2G10 antibody (AJ Roboscreen GmbH®, Leipzig, Germany) were used to confirm the specificity of TDP43 protein profiles. These antibodies are directed against TDP43 aminoacids 288–414 and aminoacids 201–220 respectively.

### Simple Western assays

Simple Western 12–230 kDa size-assays were carried out using Peggy Sue® analyzer (Protein Simple, San Jose, CA, USA). First, we prepared a 384-microwell plate containing samples mixed with fluorescent standards and denatured at 95 °C during 5 min, a ladder including 6 size markers, primary antibodies (dilution 1/50 in our study), secondary antibodies, the detection solution (equivalent volumes of luminol and peroxide), stacking and separation matrices, according to the manufacturer's preparation template [25]. After centrifugation at 1000g during 5 min, the 384-microwell plate was loaded into Peggy Sue® platform. Then, the complete automated workflow, ranging from samples' uptake on the plate to the chemiluminescent detection and quantification of proteins, was launched. The first step consisted in samples' loading into 12 individual single-use capillaries per cycle (1 cycle comprising 11 samples and 1 ladder), followed by a size-based separation (45 min in our

study). A detection of fluorescence of standards, mixed with every sample, ensured a control of electrophoresis. Sample proteins were captured onto capillary beds after illumination with UV light. Incubations of primary and secondary antibodies lasted 1 h and 30 min respectively in our protocol. Finally, a chemiluminescent detection of target proteins was automatically performed with different exposure times, ranging from 4 to 512 s [20]. Target proteins' sizes were determined for each sample in comparison to the migration of the size markers of the ladder. Heights and areas of all detected peaks were systematically calculated on Compass for Simple Western® software, version 3.1.7, using the 4-s exposure time.

### Western blot assays

According to their nature, samples could be preliminary diluted in sodium chloride. Then Laemmli lysis sample buffer (6% Tris-HCl pH 6.8, 10% β-mercaptoethanol, 4% SDS, glycerol and bromophenol blue) was added to samples (ratio 1:1). Samples were denatured at 95 °C for 5 min and 10 µL of samples were separated on NuPAGE 4–12% Bis-Tris protein gels, 1.0 mm (Thermo Scientific®, Waltham, MA, USA) with MOPS SDS running buffer (Thermo Scientific®, Waltham, MA, USA) according to manufacturer's instructions. Proteins were then transferred to polyvinylidene fluoride (PVDF) membrane and blocked during 2 h with 5% nonfat-dried milk in Phosphate Buffered Saline (PBS). Membranes were incubated overnight at 4 °C with rabbit polyclonal 10782-2-AP antibody (dilution 1/2000). Three washes were then performed in PBS-0.1% Tween. Membranes were incubated 30 min with secondary HRP anti-rabbit antibody (Agilent Dako®, Santa Clara, USA, dilution 1/2000), washed four more times with PBS-0.1% Tween and then three times with PBS. Finally, membranes were incubated with SuperSignal West Dura Extended Duration Substrate (Thermo Scientific®, Waltham, MA, USA) and exposed on Amersham Hyperfilm ECL (GE Healthcare®, Buckinghamshire, UK). Apparent sizes of proteins were determined using MagicMark XP Western Protein Standard (Thermo Scientific®, Waltham, MA, USA).

### Analytical performances of Simple Western assay

The determination of the limit of detection (LOD) and the lowest limit of quantification (LLOQ) was performed using 6 samples containing only Sample Buffer, the reagent used to perform dilutions with Simple Western assay. These "blank" samples were incubated in 6 different capillaries with both primary and secondary antibodies. Heights' and areas' peaks were determined for each of these 6 "blank" samples. LOD and LLOQ were then calculated as: LOD = average signal (height or area) + 3\* (standard deviation); LLOQ = average signal (height or area) + 10\* (standard deviation). In platelet

samples, capillary-to-capillary variability was evaluated using 6 determinations of the same sample in the same cycle. The correlation between calculated heights and areas of TDP43 protein signals was determined with the Pearson correlation coefficient  $r$ . The linearity observed for TDP43 protein signals was assessed performing a linear regression.

## Results

### Development of a Simple Western assay to detect TDP43 protein

Identical 1/50 dilutions of SH SY5Y and HEK293T cell lysates were compared using both Simple Western and Western Blot assays. First assays were performed using rabbit polyclonal 10782-2-AP antibody (Fig. 1a).

Identical TDP43 protein profiles were observed in SH SY5Y and HEK293T cell lysates using Western Blot assay, with a band of strong intensity between 40 and 50 kDa and faint bands between 30 and 40 kDa. The same patterns were obtained with Simple Western assay, but bands were around 10 kDa higher than those observed with the manual technique (54 kDa and 44 kDa respectively). In both assays, the intensity of signals was stronger in HEK293T cell lysate. The shift of TDP43 protein sizes was confirmed using two other anti-TDP43 antibodies presenting a different epitope (rabbit polyclonal 12892-1-AP antibody) or coming from a different species (mouse monoclonal 2G10 antibody) compared to rabbit polyclonal 10782-2-AP antibody (Fig. 1b).

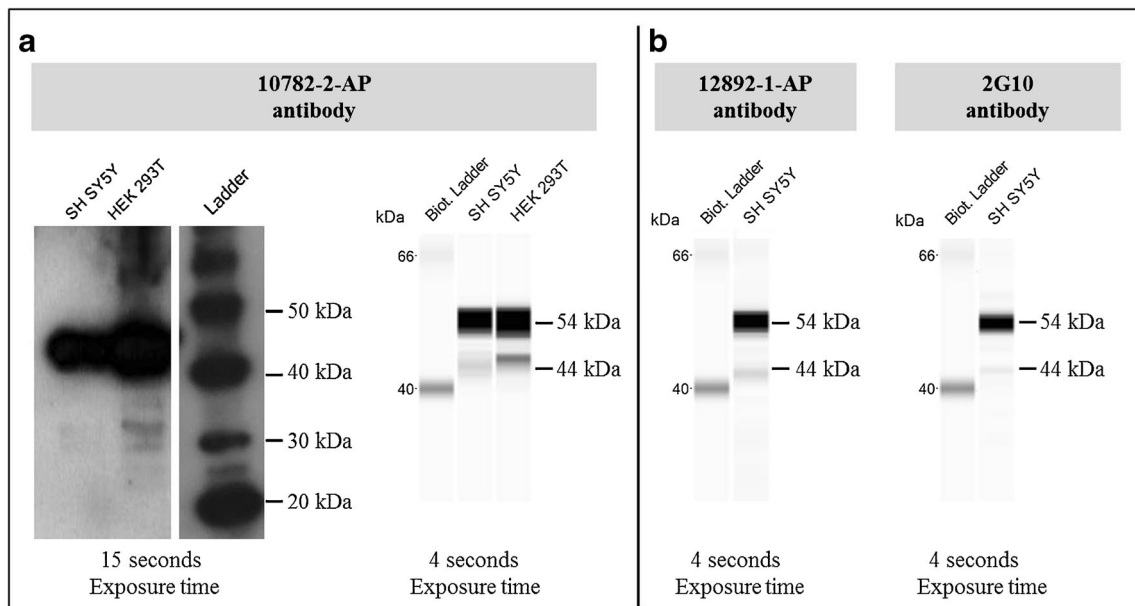
Blood-based samples obtained from one patient were then compared to SH SY5Y cell lysate using both Western Blot and Simple Western assays (Fig. 2a).

Western Blot and Simple Western TDP43 protein profiles were once again concordant, except for the shift of apparent sizes previously described (Fig. 2a). Both assays exhibited similar patterns in platelet samples and SH SY5Y cell lysate (lanes 3 and 4). On the contrary, protein profiles were different between plasma samples and SH SY5Y cell lysate: the bands that were previously described in platelet samples and SH SY5Y cell lysate were not detected in plasma samples (lanes 1, 2, and 4). However, plasma samples harbored other bands of different molecular weight. The electrophoretic profile of diluted PPP was compared to diluted solutions of immunoglobulins (IgG) and albumin (Alb.), as these two compounds are abundant in plasma and therefore could serve as migration controls (Fig. 2b). A similar pattern could be observed between diluted PPP and immunoglobulins. No reactivity was detected with diluted albumin.

### Quantitation of TDP43 protein using Simple Western assay

Quantitation performances of Simple Western assay were first performed using a stock solution of recombinant GST-tagged TDP43 protein (0.5 mg/mL according to manufacturer's instructions). Serial dilutions were performed, ranging from 0 to 20  $\mu\text{g/L}$  (Fig. 3).

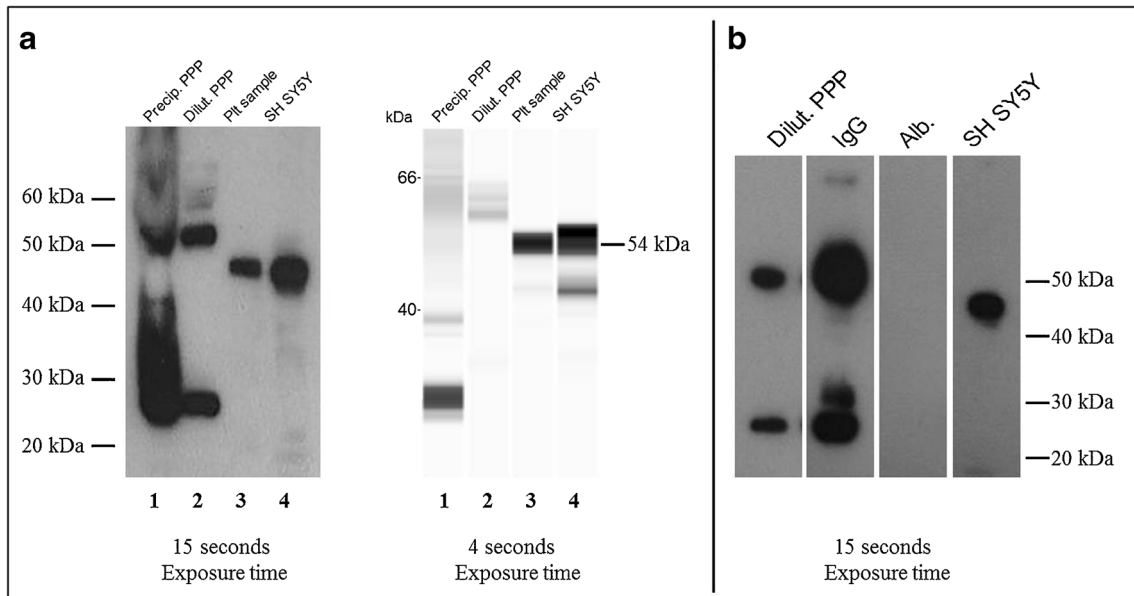
A band of 57 kDa apparent mass was observed in all the dilutions of recombinant GST-tagged TDP43 protein. No



**Fig. 1** Comparison of Western Blot and Simple Western TDP43 protein profiles in HEK293T and SH SY5Y cell lysates. **a** Comparison of Western Blot and Simple Western protein profiles of SH SY5Y and HEK293T cell lysates (dilution 1/50) using rabbit polyclonal 10782-2-AP antibody. **b**

Identical Simple Western profiles were obtained in SH SY5Y cell lysate using both rabbit polyclonal 12892-1-AP and mouse monoclonal 2G10 antibodies



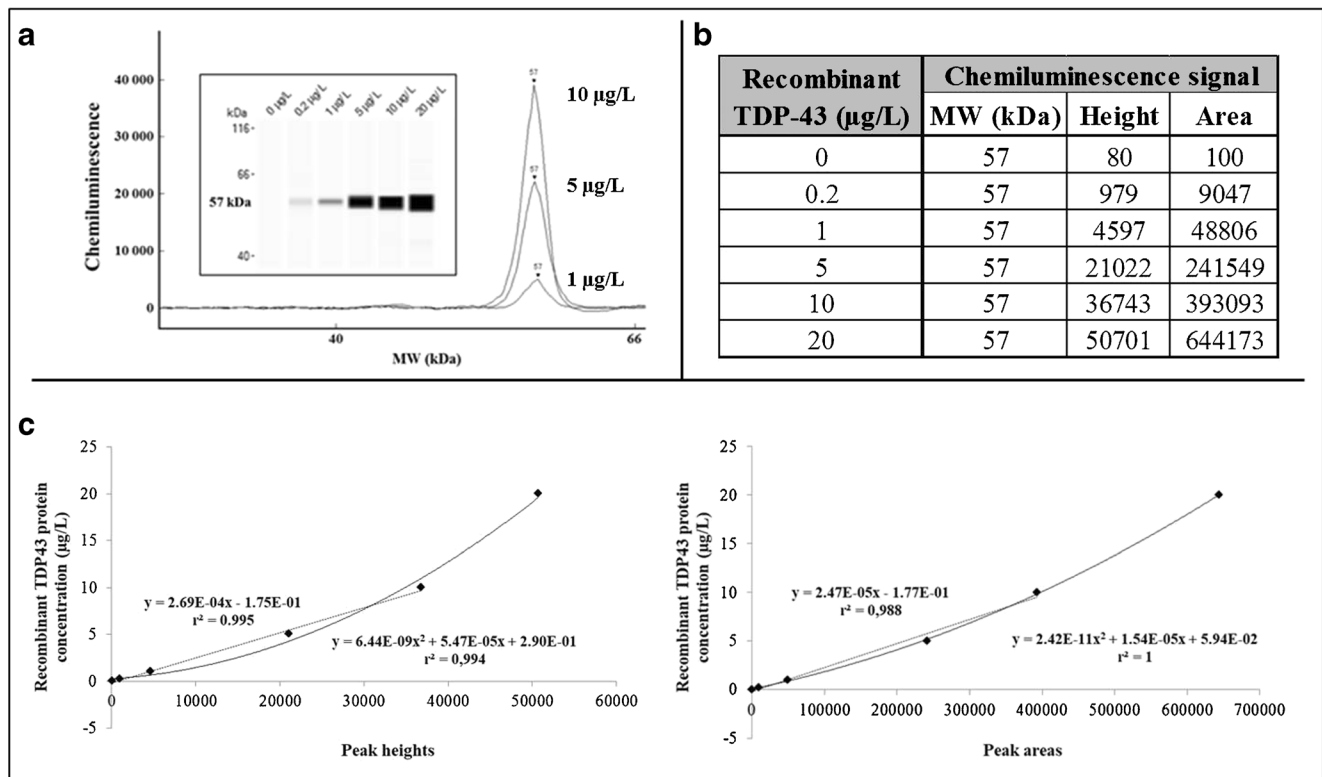


**Fig. 2** TDP43 protein profiles in blood-derived samples using rabbit polyclonal 10782-2-AP antibody. **a** Comparison of Western Blot (left) and Simple Western (right) assays representing blood fractionation of one patient: platelet-poor plasma (PPP) sample precipitated with ice-cold acetone (lane 1), PPP sample - dilution 1/100 - (lane 2) and

platelet sample (lane 3) are compared to SH SY5Y cell lysate (lane 4). **b** Western Blot assays of diluted immunoglobulins (IgG) and albumin (Alb) solutions with anti-TDP43 antibody compared to a diluted PPP sample and SH SY5Y cell lysate

signal was visible when no recombinant TDP43 protein was added and increased signals were observed for increased

recombinant protein concentrations (Fig. 3a). The intensity of signals was automatically calculated by Compass for



**Fig. 3** Serial dilutions of recombinant GST-tagged TDP43 protein using Simple Western assay. **a** Graph view and lane (Compass for Simple Western® software) of serial dilutions of recombinant TDP43 protein (4-s exposure time). **b** Molecular weights, heights, and areas for all the

dilutions of recombinant protein (from 0 to 20 µg/L) automatically calculated by Compass for Simple Western® software. **c** Representation of calibration curves obtained for heights and areas signals

Simple Western® software (heights and areas are provided in Fig. 3b). The analysis of 6 “blank” samples, containing Sample Buffer only, gave a limit of detection (LOD) of 371 for peak heights and 1595 for peak areas. Using the same raw data, the lower limit of quantification (LLOQ) was 930 and 4322 for peak heights and areas respectively. Next, calibration curves were generated: a linear calibration curve ( $r^2 = 0.995$  for heights and  $r^2 = 0.988$  for areas) was obtained for concentrations below 10  $\mu\text{g/L}$  (Fig. 3c), as a saturation of signals was observed for the highest concentration (20  $\mu\text{g/L}$ ). Interestingly, a quadratic model fitted correctly to all concentrations ( $r^2 = 0.994$  for heights and  $r^2 = 1$  for areas). Using this last fitting model, the corresponding LLOQ of recombinant TDP43 protein were then 0.35  $\mu\text{g/L}$  and 0.13  $\mu\text{g/L}$  for heights and areas respectively. The lowest standard included in the calibration curve (0.2  $\mu\text{g/L}$ ) gave peak heights and peak areas close to these calculated values (Fig. 3b).

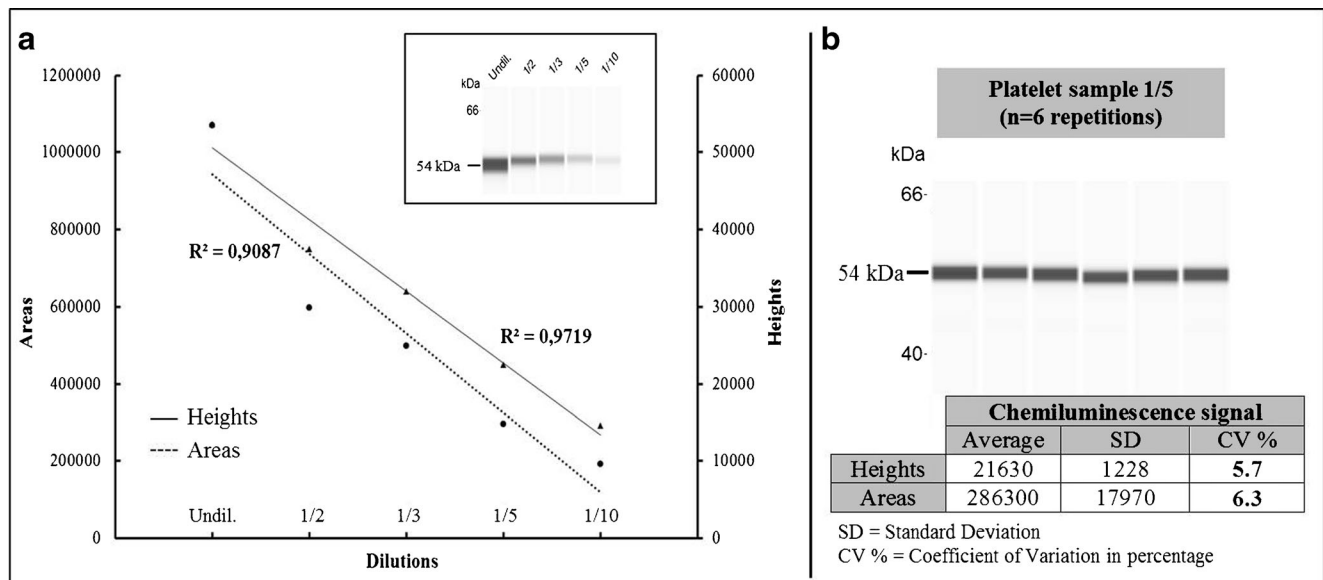
Further assays were performed to assess Simple Western assay's suitability to detect and quantify TDP43 signals in platelet samples (Fig. 4). Signals were detectable until a ten-fold dilution, which was the strongest dilution performed in this assay (Fig. 4a). A linearity of signals was observed ( $r^2 = 0.9719$ ) for peak heights. Concordant results ( $r^2 = 0.9087$ ) were obtained with peak areas. The variability of Simple Western assay was also assessed with repeated measurements of the same sample 6 times within one cycle: Simple Western assay exhibited an interesting 5.7% coefficient of variation for heights' determination (Fig. 4b). The coefficient of variation for areas' determination was 6.3%.

Finally, platelet samples were tested in duplicates in the cohort of nine patients suspected of familial FTLD (Table 1).

Calculated parameters by Compass for Simple Western® software gave concordant results for signals detection and calculation. Indeed, average heights and areas measured for a same patient were well-correlated (Pearson correlation coefficient  $r = 0.986$ ). Duplicates variation was correct for both calculations, the highest duplicate variability for average heights and areas being 13.1% and 13.6% respectively. Average heights exhibited a lower variability than average areas, with an average coefficient of variation at 4.9% versus 6.2%. In this cohort of 9 samples with normalized platelet concentration (200 G/L), a 38-fold difference was observed between the lowest and the highest TDP43 protein concentrations. Indeed, these two concentrations were 0.39  $\mu\text{g/L}$  and 14.76  $\mu\text{g/L}$  respectively using the quadratic calibration curve obtained for height signals. This variation was significantly above the analytical variation of Simple Western assay (the coefficient of variation was 5.7%), meaning that samples exhibited TDP43 protein signals in platelet samples which were quantitatively different between patients.

## Discussion

In this study, platelets appeared as a suitable sample matrix to study TDP43 protein in frontotemporal dementia patients. Indeed, platelet samples and SH SY5Y cell lysate, considered as a positive control for our target protein, gave identical TDP43 protein patterns using two different qualitative immunodetection assays: Western Blot and Simple Western assays. Western Blot assay is a commonly used tool to identify a specific protein in a complex mixture, and was therefore



**Fig. 4** Suitability of Simple Western assay to quantify TDP43 signals in platelet samples. **a** Serial dilution of one platelet sample gives linear signals using Simple Western assay (4-s exposure time). **b** Capillary-to-

capillary precision ( $n = 6$ ) of TDP43 protein chemiluminescent signals (heights and areas) using Simple Western assay

**Table 1** Calculated TDP43 signals in normalized platelet samples (200 G/L). Calculated parameters include average heights and areas of samples duplicates, their relative standard deviation (SD) and their coefficient of variation in percentage (% CV)

Sample	Calculated parameters (Compass for Simple Western® software)					
	Average height	SD	% CV	Average area	SD	% CV
Patient 1	19,750	25	0.1%	363,476	2580	0.7%
Patient 2	43,363	79	0.2%	974,709	15,054	1.5%
Patient 3	19,675	2569	13.1%	379,628	50,736	13.4%
Patient 4	28,583	2742	9.6%	601,856	37,526	6.2%
Patient 5	28,717	3176	11.1%	523,724	71,382	13.6%
Patient 6	41,743	1015	2.4%	827,149	12,269	1.5%
Patient 7	37,042	1829	4.9%	693,959	36,997	5.3%
Patient 8	25,686	158	0.6%	479,711	18,352	3.8%
Patient 9	1496	28	1.9%	28,569	2883	10.1%

considered as the reference method in our study [26]. Simple Western assay, a new automated capillary nano-immunoassay, provided qualitative protein patterns like Western Blot. First steps of Simple Western assays' development included the assessment of different dilutions of SH SY5Y and HEK293T cell lysates, ranging from 1/200 to 1/20 using rabbit polyclonal 10782-2-AP anti-TDP43 antibody. A band of strong intensity at 54 kDa and a fainter band at 44 kDa were visible. The comparison of Simple Western and Western Blot assays gave similar TDP43 protein patterns, at the exception of apparent molecular weight (Fig. 1a). Many factors can affect proteins' motility including the type of gel or matrix used for electrophoresis, running buffers and even the intrinsic characteristics of target protein [27]. TDP43 protein profiles obtained with Simple Western technology were confirmed using two other anti-TDP43 antibodies presenting a different epitope—rabbit polyclonal 12892-1-AP antibody—or coming from a different species—mouse monoclonal 2G10 antibody—(Fig. 1b). Our results were concordant with those of an industrial company providing another mouse monoclonal anti-TDP43 antibody (clone 671834), which was tested on A431 human epithelial carcinoma and NR8383 rat alveolar macrophage cell lines ([https://www.novusbio.com/products/tdp-43-tardbp-antibody-671834\\_mab7778](https://www.novusbio.com/products/tdp-43-tardbp-antibody-671834_mab7778)) [28]. Despite the shift of protein sizes observed between both assays, Simple Western was successful in providing qualitative TDP43 protein patterns. Differences were observed between plasma samples and SH SY5Y cell lysate. Indeed, the 54 kDa band observed in our positive control was not detected in plasma samples. On the other hand, additional bands were detectable in plasma samples, corresponding to immunoglobulin light and heavy chains (Fig. 2b). This cross-reaction with immunoglobulins was already described by Steinacker et al. assessing TDP43 protein in CSF samples [29]. Interestingly, contrary to plasma samples, platelet samples and SH SY5Y cell lysate exhibited similar TDP43 protein profiles.

In addition to protein profiles assessment, Simple Western assay allowed an automated semi-quantification of all the

peaks obtained in each capillary. An evaluation of the analytical performances of the protocol previously developed was then performed. When performing serial dilutions from a TDP43 recombinant protein solution, linear signals were observed for concentrations below 10 µg/L (Fig. 3c). With increased concentrations, a loss of linearity was observed, leading to a potential underestimation of signals [30]. Moreover, a burn-out phenomenon was visible, leading to the presence of split peaks or to a complete disappearance of signals for the most concentrated samples (data not shown). This phenomenon was already described for Simple Western assays [25] and is known as ghost or hollow bands in traditional Western Blot assays, occurring when too much target protein is applied, leading to a total depletion of substrate [26].

The linearity of chemiluminescent signals was observed for one platelet sample (Fig. 4a). In parallel, Simple Western assay provided a low capillary-to-capillary variability below 6.3% for both TDP43 heights and areas (Fig. 4b). These performances were similar to other variability assays performed using Simple Western assay or enzyme-linked immunosorbent assays (ELISA) for CSF markers [31, 32]. Thus, Simple Western protocol seems suitable to detect and quantify total TDP43 protein in platelet samples.

In our cohort of nine platelet samples obtained from FTD patients, the lowest and the highest height values were 1496 and 43,363 respectively, despite a normalized platelets' concentration of 200 G/L per sample. The associated TDP43 protein concentrations were then 0.39 µg/L and 14.76 µg/L respectively. These values were above the LOD and LLOQ of this assay, validating the suitability of Simple Western assay to quantify TDP43 protein in platelet samples. Furthermore, this preliminary exploratory study indicates that TDP43 protein presents an important inter-individual variability in platelet samples superior to its analytical variability. This observation strengthens the fact that TDP43 protein's detection and quantification in platelet samples could become a potential candidate biomarker for diagnosis' purposes, reflecting pathological mechanisms in FTLD and ALS patients [33]. However,



this variability could also be due to physiological variations such as age and/or sex. To address these questions, a prospective study based on our newly validated Simple Western assay, combining coupled qualitative and quantitative measurements, should be performed to assess TDP43 protein diagnostic performances in larger cohorts of patients with neurodegenerative definite pathology.

## Compliance with ethical standards

**Conflict of interest** Ingolf Lachmann provided 2G10 antibody and reports that he is employee of AJ Roboscreen GmbH®, Leipzig, Germany. No other conflict of interest is reported.

**Ethical approval** This study was based on biological samples collected for routine diagnosis in the framework of a medical follow-up recommended for FTL D patients. The samples associated to written consents were stored in a bio bank with the authorization from the French Ministry of Health (Declaration Number DC-2008-304). Authorization for handling personal data was granted by the French data protection commission [Commission Nationale de l'Informatique et des Libertés (CNIL)].

The patients included in this study or their legal next of kin gave two informed written consents: the first one for FTL D genetic testing and the second one authorizing a change of biological purpose for remaining parts of samples.

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