Chemiluminescent detection of oligoclonal immunoglobulins after isoelectric focusing and affinity-mediated immunoblotting

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Background and Aims. Detection of oligoclonal IgG (o-IgG) in the cerebrospinal fluid (CSF) not found in serum is the principal laboratory test to support a diagnosis of multiple sclerosis. The aim of this study was to compare chemiluminescent and chromogenic detection of oligoclonal immunoglobulins in the cerebrospinal fluid and serum after their separation by means of isoelectric focusing followed by immunoblotting.

Methods. A set of experiments was designed to detect oligoclonal immunoglobulins by means of alkaline phosphatase BCIP/NBT substrate and chemiluminescent peroxidase substrate.

Results. Based on visual evaluation of signals, chemiluminescent detection requires about a 4 times lower amount of applied protein than very sensitive BCIP/NBT chromogenic detection. Very good correlation between methods has been shown for oligoclonal IgG. Antigen-specific oligoclonal IgG could be demonstrated by both methods although the pattern was clearer using chemiluminescence. In one patient, oligoclonal IgD bands barely visible by BCIP/NBT were convincingly demonstrated by chemiluminescence.

Conclusion. Chemiluminescent detection is a feasible option for oligoclonal immunoglobulin detection and could be used in cases when the sensitivity needs to be improved. Further studies and method optimisation are warranted.

Key words: cerebrospinal fluid, chemiluminescence, immunoglobulins, isoelectric focusing, immunoblotting

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INTRODUCTION

Detection of oligoclonal IgG (o-IgG) in the cerebrospinal fluid (CSF) not found in serum is the principal laboratory test to support a diagnosis of multiple sclerosis^{1,2}. Although the presence of o-IgG is not disease-specific, it confirms the presence of an inflammatory process within the central nervous system^{3,4}. Oligoclonal IgM (ref.^{5,6}), IgD (ref.⁷) and free light chains⁸⁻¹⁰ detection was reported to provide useful additional information. The concentration of IgG in CSF lies between 10 – 100 mg/L and IgG can be easily detected by means of chromogenic detection method. However, the concentrations of IgM, IgD and free light chains are below 1 mg/L in most CSF samples, and chromogenic detection may not be sensitive enough for detection of these proteins.

According to two international consensus reports^{1,2}, isoelectric focusing (IEF) is the preferred separation method for demonstration of o-IgG. On principal grounds, the same should hold true for the demonstration of oligoclonality of other immunoglobulins or free light chains. IEF is mostly done using commercial kits from Sebia or Helena in clinical laboratories. The former method uses immunofixation of IgG within the gel by means of horseradish peroxidase (HRP)-conjugated anti-IgG antibody, while the latter is based on a passive blotting onto a nitrocellulose membrane and labelling

with HRP-conjugated antibody. Both methods, as well as various in-house methods used in research laboratories, use chromogenic substrate for detection.

Luminescent detection, using either fluorescence or chemiluminescence, is routinely used after electrophoretic separation of proteins, mainly after sodium dodecyl sulfate (SDS) electrophoresis¹¹. Although chemiluminescence has been mentioned as one of the recommended techniques for IgG immunodetection in recently published guidelines on CSF analysis¹², we were able to find few reports on luminescent detection of proteins separated by IEF as antigen-specific oligoclonal IgG (ref.¹³⁻¹⁵) or oligoclonal IgM (ref.¹⁶).

Chemiluminescent detection was also used by Kaplan and co-workers for CSF studies employing their innovative method of SDS electrophoresis in non-reducing conditions for separation of free light chain monomers and dimers^{17,18}.

Luminescent detection provides significant advantages such as higher sensitivity¹⁹ and possibility of signal quantification²⁰. The precise quantification of protein using chemiluminescent signal reveals the necessity to perform dilution series and calibration curves to estimate range of linearity between primary antibody and protein amount²¹.

Whether luminescent detection could provide an advantage for routine o-IgG detection has not been systematically investigated. Of note, however, 1 of 17 o-IgG-

negative patients in routine workup (immunofixation with peroxidase labelled anti-IgG, commercially available Hydragel Isofocusing Sebia Kit) in the study of Stich et al.¹⁵ turned out to be positive when using chemiluminescent detection.

Unfortunately, many CSF studies do not report their detection method in detail. Peroxidase labelled antibody is often the last step described, without referring whether chromogenic or luminescent detection followed. All reports of chemiluminescent detection of oligoclonal immunoglobulins separated by IEF refer to detection on an X-ray film. We were not able to find one single report using CCD camera for this purpose.

Using chemiluminescence instead of chromogenic detection may be required for two main reasons: 1. when one wants to improve detection sensitivity, 2. for the purpose of signal quantification. The first issue has been the matter of this study.

In the present study, we performed a set of experiments aimed at comparing chemiluminescent detection to very sensitive chromogenic detection using biotinylated antibody, alkaline phosphatase labelled streptavidin and 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium (BCIP/NBT) substrate.

MATERIAL AND METHODS

Reagents

Hydragel 9 CSF isofocusing kits (Cat. No. 4355) and CSF control (Cat. No. 4794) were from Sebia (Evry Cedex, France). Agarose IEF (Cat. No. 17-0468-01), Pharmalyte™ 3-10 (Cat. No. 17045601), 4-6.5 (Cat. No. 17045201), 5-8 (Cat. No. 17045301) and 8-10.5 (Cat. No. 17045501), electrophoresis wick (Munktel filter paper, Cat. No. 80-1129-52), electrofocusing strips (Cat. No. 18-1004-40), Amersham™ Protran™ 0.45 µm (Cat. No. 10600002) or Amersham™ Protran™ Premium 0.45 µm (Cat. No. 10600003) nitrocellulose blotting membrane were from GE Healthcare Bio-Sciences AB, Uppsala, Sweden, distributed through AP Czech, Prague, Czech Republic. Polyclonal rabbit anti-human kappa free light chains (Cat. No. A0100), lambda free light chains (Cat. No. A0101), IgD (Cat. No. A0093) and IgE (Cat. No. A0094) were from DAKO (Medesa, Czech Republic). Native human kappa light chain (Cat. No. PHP280) and lambda light chain (Cat. No. PHP281), purified goat anti-human IgG (Cat. No. 5211-8004) and native *Borrelia burgdorferi* purified protein (Cat. No. 1440-0006) was from Bio-Rad (formerly AbD Serotec). AffiniPure Goat Anti-Human IgM (Cat. No. 109-005-043), Biotin-SP-conjugated AffiniPure F(ab')₂ fragment Goat Anti-Human IgM (Cat. No. 109-066-129) and IgG Fc (Cat. No. 109-066-008) were from Jackson ImmunoResearch (West Grove, PA, USA). Horseradish peroxidase streptavidin ELISA grade (Cat. No. SA-5014), alkaline phosphatase streptavidin (Cat. No. SA-5100) and BCIP/NBT alkaline phosphatase substrate kit (Cat. No. SK-5400) were from Vector Laboratories (Burlingame, CA, USA).

Bovine serum albumin (Cat. No. 11924) and Tween 20 (Cat. No. 37470-01) were from Serva Electrophoresis (Heidelberg, Germany). D-Sorbitol (Cat. No. 85529) and Tris(hydroxymethyl)aminomethane (Cat. No. 252859) were from Sigma-Aldrich. Recombinant HIV-1 p17-p24, gp41-gp120 antigen (Cat. No. Hiv-110) was from Prospe (Ziona, Israel). Finally, Super Signal Western Blot Pico PLUS chemiluminescent substrate (Cat. No. 3480) and Super Signal West Femto maximum sensitivity substrate (Cat. No. 34095) were from Thermo Fisher Scientific (Waltham, MA, USA).

Samples

Only surplus CSF and serum samples remaining after aliquoting for routine analyses were used for the purpose of this methodical study.

Samples were diluted with 0.75% (w/v) saline. For IgG, dilution to 0.5 and 1.0 mg/L was used for chromogenic and 0.25 and 0.5 mg/L for chemiluminescent detection, respectively. For IgM, samples were diluted to 1.0 mg/L for chromogenic and 0.5 mg/L for chemiluminescence method. CSF samples with immunoglobulins below these concentrations were used undiluted and sera were diluted exactly to the estimated CSF IgM concentration; only if amount of CSF IgM was lower than 0.2 mg/L, sera were diluted to 0.2 mg/L to prevent undetectable signal in the serum since CSF IgM measurements in this range are known to be unreliable. Before IgM separation by IEF, samples were pretreated subsequently with dithiothreitol and acrylamide as described previously²². For fLC analysis, CSF samples were used neat and serum was diluted 1/80. For IgD, neat CSF and 1/250 serum dilution was used in most experiments.

Isoelectric focusing

IEF was performed on Multiphor II apparatus (GE Healthcare) at 10 °C. Interelectrode distance was 8.5 cm. Agarose gels were prepared in-house (1.0% gels with Pharmalytes 4-6.5 and 5-8 for IgM and IgD; 1.2% gels with Pharmalytes 5-8 and 8-10.5 for IgG and Pharmalyte 3-10 for fLC).

0.5M (for IgG and fLC) or 0.25M (for IgM and IgD) sodium hydroxide served as a catholyte whereas 0.04M DL-Glutamate (for IgG), 0.04M H₂SO₄ (for fLC) or 0.25M acetic acid (for IgM and IgD) served as anolytes, respectively.

Single step method was used for fLC analysis (1200 Vh with limiting values 1700 V, 50 mA, 10 W). 7 µL of samples were applied 2 cm from the anode. For other proteins investigated, gradient methods were used:

For IgG separation, 6 µL of samples were applied 2 cm from the anode. The separation program used was as follows: 1. 1 Vh, 500 V, 25 mA, 5 W; 2. 150 Vh, 500 V, 25 mA, 5 W; 3. 500 Vh, 1275 V (gradient), 25 mA, 8 W; 4. 550 Vh, 1275 V (constant), 25 mA, 8 W.

For IgM and IgD analysis, a prefocusing step was used (250 Vh, 600 V, 25 mA, 4W); then, the apparatus was set off and 7 µL of samples were pipetted 2 cm from the cathode. Then, the following gradient method was applied:

1. 1 Vh, 300 V, 25 mA, 3W; 2. 125 Vh, 300 V, 25 mA, 3 W; 3. 500 Vh, 1275 V (gradient), 25 mA, 8 W; 4. 575 Vh, 1275 V (constant), 25 mA, 8 W.

Affinity-mediated immunoblotting

Membranes coated with the respective antibodies at a concentration of 10 mg/L (20 mg/L for o-fLC) were incubated at least 4 h or overnight at room temperature on a rocking platform, washed in tris-buffered saline (TBS) and blocked in 3% BSA for 75–90 min. After washing twice in TBS, membranes were applied on the gel, covered by 1 sheet of moistened filter paper, 5–7 dry filter papers, a glass plate and a weight of approximately 1 kg. After 45–50 min, the membranes were washed in PBS and proteins were fixed by 0.25% (v/v) glutaraldehyde in PBS for 15–20 min at 4 °C. After washing with water and three changes of TBS, membranes were re-blocked in 0.3% BSA for 15–20 min and washed in TBS. Next, the biotinylated antibody was applied. Incubation times were 90 min for IgG, 105–115 min for fLC, and 120–130 min for IgM and IgD. After washing, streptavidin-enzyme conjugate was added and membranes were left incubated for 45–50 min, followed by final washing cycles and appropriate detection.

Western Blot Pico Plus Chemiluminescent Substrate was used in most experiments while Western Blot Femto Maximum Sensitivity Substrate was used when higher sensitivity was required.

Image acquisition and analysis were performed using ChemiDoc Imager (Bio-Rad).

Ethics Approval

The study was approved by the Ethics Committee of FN Ostrava, Reference Number 1010/2016, on December 12, 2016. All patients gave an informed consent before lumbar puncture.

RESULTS AND DISCUSSION

A set of experiments was designed to determine the feasibility and reproducibility of the method. Suitable concentration of HRP-labelled streptavidin was found to be 0.05 – 0.2 mg/L for Western Blot Pico Plus substrate and 0.01 – 0.02 mg/L for Western Blot Femto maximum sensitivity substrate. Membrane washing with TBS for 3 – 5 min followed by two washes in TBS with 0.05% Tween 20 (5–7 min each) and two washes in TBS (3 – 5 min each) was sufficient for removing most of the unspecific background signal. The excessive washing (>30 min) was unfavourable and lead to substantial loss of sensitivity. Detection time varied between 10 seconds and 10 min; longer detection times were not useful due to increased background signal (data not shown).

Next, we determined o-IgG in 34 paired CSF and serum samples sent for routine o-IgG analysis. Routine test was performed using Hydrasys CSF IEF kit on Hydrasys II apparatus (Sebia, Evry Cedex, France). This method uses IEF in agarose gels followed by immunofixation by HRP-conjugated anti-IgG antibody and subsequent chromogenic detection. Most samples were also analysed by the IEF/AIB method with BCIP/NBT detection. In one set of samples, this method failed for unknown reasons even when repeated on the next day. Third attempt has not been performed since we aimed at analysing the samples by all methods within an interval of 3–5 days to avoid any possible influence of preanalytic factors. The results of o-IgG analysis by IEF/IF as well as by IEF/AIB using BCIP/NBT or luminescent detection were evaluated by a blinded observer to avoid any possible bias. To compare the relative sensitivity of the tests to demonstrate oligoclonal IgG bands diluted into a polyclonal background, serial dilutions of CSF control into prediluted intravenous IgG preparation (Flebogamma) was performed so that IgG of the CSF control represented 41%, 7.1% and 1.7% of total IgG in the sample applied. The bands were clearly

Table 1. Agreement between methods used for o-IgG detection evaluated by a blinded observer (PK).

Methods compared (amount of IgG in ng applied per lane)	n (negative/ positive)	Kappa statistics (95% CI)		Number of discrepant results (%)	
		positivity	Types 1-5	positivity	Types 1-5
IEF/AIB-CL (1.5) vs. IEF/IF (300)	33 (25/8)	1.000	0.476 (0.220 – 0.732)	0	11 (33 %)
IEF/AIB-CL(3.0) vs. IEF/IF (300)	34 (25/9; 26/8)	0.922 (0.771 – 1.000)	0.562 (0.330 – 0.794)	1 (3 %)	10 (29 %)
IEF/AIB-CL(1.5) vs. IEF/AIB-BCIP/NBT (3.0)	24 (19/5)	1.000	0.730 (0.483 – 0.977)	0	4 (17 %)
IEF/AIB-CL(3.0) vs. IEF/AIB-BCIP/NBT (3.0)	25 (19/6)	1.000	0.649 (0.414 – 0.883)	0	6 (24 %)
IEF/AIB-CL(1.5) vs. IEF/AIB-BCIP/NBT (6.0)	24 (19/5)	1.000	0.730 (0.483 – 0.977)	0	4 (17 %)
IEF/AIB-CL(3.0) vs. IEF/AIB-BCIP/NBT (6.0)	25 (19/6)	1.000	0.649 (0.414 – 0.883)	0	6 (24 %)

IEF/AIB, isoelectric focusing followed by affinity-mediated immunoblotting; CL, chemiluminescence; BCIP/NBT, chromogenic detection using 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium. For IEF/AIB, two IgG concentrations considered suitable for evaluation were tested for both chromogenic and luminescence detection.

Fig. 1. Total IgG, anti-HIV IgG and anti-Borrelia IgG.

Panel A (left): chromogenic detection. 6 ng of IgG were applied for analysis of total o-IgG and 30 ng of IgG for the analysis of antigen-specific o-IgG.

Panel B (right): chemiluminescent detection. 1.5 ng of IgG were applied for analysis of total IgG and 15 ng of IgG for the analysis of antigen-specific o-IgG. Exposition times were 7 s for total IgG and 63 s for antigen-specific IgG.

o-IgG reactivity against HIV, but not against *Borrelia* purified protein, can be observed in C1a (unfrozen CSF stored at +4°C for 45 days) and C1b (CSF stored frozen at -30°C). No reactivity of o-IgG against HIV in o-IgG positive samples of the other two patients (C2, S2 and C3, S3) can be seen.

Co+, positive CSF control for o-IgG test (Sebia); Co-, negative control (purified intravenous IgG preparation); C, cerebrospinal fluid; S, serum.

visible in samples with CSF control IgG 41% and 7.1% while only 2-3 faint bands were visible in the sample with CSF control IgG 1.7% concentration. For real paired CSF and serum samples, the agreement was perfect if results were evaluated as negative/positive only. Only one discrepant result (negative by IEF/IF and marginally positive by both IEF/AIB-BCIP/NBT and IEF/AIB-CL with 2-3 CSF-restricted bands) has been observed. Interestingly, the diagnosis of multiple sclerosis (MS) could be made in this case after the first clinical presentation when multiple MS-typical hyperintense lesions as well as active lesions were demonstrated by brain magnetic resonance imaging (MRI). The agreement was moderate only if samples were evaluated as type 1-5 according to an international consensus (1, 2) (Table 1). However, absence of perfect agreement concerning IEF type classification has been published several times²³⁻²⁵ and the usefulness of types 1-5 has even been questioned³.

We were able to demonstrate anti-*Borrelia* o-IgG in two patients with neuroborreliosis (not shown) as well as anti-HIV o-IgG in an HIV positive patient with unspecific neurological complaints and a few small MRI white matter hyperintensities. In the latter case, CSF examination was performed because of suspicion of coincidental MS, demonstrating that a result of this laborious analysis may be of significant value in routine clinical practice in diagnostically difficult cases. The amount of IgG applied for the analysis of antigen-specific OCB was 5 to 10 fold higher than for total IgG because even in CNS infections, only a fraction of intrathecally synthesised IgG is directed against the causative organism²⁶. As for total IgG, CL required approximately 4 times lower amount of IgG applied for comparable results, and half the concentration used for chromogenic method lead to an obviously clearer pattern if CL was used (Fig. 1).

For o-IgM, results were also comparable although the agreement was not as perfect as for IgG. Results were classified as positive (≥ 2 CSF-restricted bands) and negative only. Discrepant results were found in 8/34 paired CSF and serum samples (24%) and although the number of CSF-restricted bands was ≤ 4 in all these cases (2 cases positive by CL and negative by BCIP/NBT and 6 cases negative by CL but positive by BCIP/NBT), it is clear that the method of o-IgM detection requires further optimisation. Representative o-IgM detection by BCIP/NBT and CL is shown in Fig. 2.

The search for o-IgD, driven by our previous experience of beautiful IgD serum paraprotein patterns and an accidental finding of o-IgD bands in the CSF of one patient with neuroborreliosis (unpublished results), was unsuccessful. We were able to find o-IgD in 1 out of 23 examined paired CSF and serum samples. The diagnosis in this only positive case was relapsing meningoencephalitis of unknown aetiology. The bands were barely visible by a BCIP/NBT method but obvious using CL. Interestingly, these bands were present in a CSF sample frozen on the day of receipt at -70°C as well as in a sample stored for 5 days at $+4^{\circ}\text{C}$ but precipitate at the application site was visible in the same CSF sample with protease inhibitor added on receipt before storing at -70°C . Western Blot

Fig. 2. Oligoclonal IgM. A. BCIP/NBT staining (55 min). B-D. CL detection; exposition times were 33 s (B), 50 s (C) and 90 s (D).

Femto maximum sensitivity improved the results so that the IgD pattern could be seen in several other samples; however, we did not observe a distinct oligoclonal pattern. Notably, in one case of neuroborreliosis, some rather polyclonal IgD was found on the *Borrelia burgdorferi* purified protein coated membrane, suggesting possible occurrence of antigen-specific IgD (Fig. 3).

Free light chains (fLC) were detectable down to 0.008 mg/L (0.056 ng) for free kappa and 0.04 mg/L

Fig. 3. A. Oligoclonal IgD in the CSF of a patient with relapsing meningoencephalitis of unknown cause. Left: chromogenic detection; right: CL detection (exposition time 569 s). C1*, S1*, cerebrospinal fluid and serum sample with protease inhibitor. B. Monoclonal IgD in serum of a patient with IgD kappa myeloma (P). The amounts of IgD applied in (P) were 1.75; 0.70; 0.35; 0.07 and 0.035 ng. Exposition time was 186 s. C. Total IgD (left; exposition time 4 s) and anti-*Borrelia* IgD (right; exposition time 20 s): a signal is observed in the CSF on the *Borrelia burgdorferi* purified protein coated membrane but the pattern seems to be polyclonal rather than oligoclonal. Western Blot Pico Plus substrate was used in A. while Western Blot Femto maximum sensitivity substrate was used in B. and C.

(0.21 ng) for free lambda in the monoclonal fLC standard used, which was 5 times better than chromogenic detection (lowest detected concentrations were 0.04 mg/L for free kappa and 0.2 mg/L for free lambda light chains). However, the lowest detectable concentration was not reproducible in other experiments, and we have noticed some problems with unspecific background signal, probably due to lower dilution of serum samples than for other proteins investigated. We were able to compare oligoclonal fLC patterns in 19 paired CSF and serum samples. When evaluated as positive or negative for CSF-restricted o-fLC, 3 discrepant results (15.8%) were noted for free kappa (positive by IEF/AIB-CL and negative by IEF/AIB-BCIP/NBT in 2 cases, and vice versa in 1 case)

and 1 (5.3%) for free lambda (2 faint bands by IEF/AIB-BCIP/NBT and no clear-cut bands by IEF/AIB-CL). The number of CSF-restricted bands in these cases were 2 or 3 only. Example of o-fLC detection is provided in Fig. 4.

Of course, no detection method is able to solve separation problems. However, if these are overcome using serum samples with monoclonal component for preliminary testing, chemiluminescent detection can increase detection sensitivity, although not to an extent we had expected. Notably, in their pioneering study, Schneppenheim and co-workers¹⁹ compared luminescence to HRP chromogenic substrate (4-chloro-1-naphthol). However, ALP substrate (BCIP/NBT) has been found substantially more sensitive compared to another HRP substrate (3-amino-

Fig. 4. Chromogenic (A) and chemiluminescent (B) detection of free kappa and free lambda light chains by means of IEF/AIB. C1 was diluted 1/2 and 1/4 for free kappa and 1/4 and 1/8 for free lambda, respectively. FLC concentration were measured only after performing the qualitative IEF/AIB method and were 0.91 mg/L for CSF FLC kappa and 57.65 mg/L for CSF FLC lambda, i.e. dilutions result in a concentration of 14.41 mg/L and 7.21 mg/L for FLC lambda, leading to overloading problems. STD, standard (1.0, 0.2 and 0.05 mg/L for free kappa, 1.0, 0.2 and 0.1 mg/L for free lambda); IgG, intravenous immunoglobulin (Kiovig, 200 mg/L IgG); C1 and S1, CSF and serum sample of a patient shown in A and B; C2 and S2, cerebrospinal fluid and serum sample of a patient with multiple sclerosis. Exposition time was 180 s for free kappa and 5 s for free lambda C. Immunofixation electrophoresis in serum (normal) and unconcentrated CSF; two closely spaced lambda light chain bands can be seen in the CSF (lane L) without counterpart in lanes immunofixed with anti-gamma (G), anti-alpha (A) and anti-mi (M) antisera, respectively.

9-ethyl carbazole) in a later study²⁷. Maximum sensitivity substrate was needed if the amount of applied sample was less than 1 ng. Other factors influencing detection sensitivity should be taken into account. Polyacrylamide gels with smaller pores might offer better resolution and thus increased sensitivity. However, high-molecular weight proteins cannot be separated by polyacrylamide. It has been

reported that polyvinylidene fluoride (PVDF) membranes are 3 to 30 times more sensitive than nitrocellulose when using passive immunoblotting, but no difference in sensitivity when affinity-mediated immunoblotting has been used²⁸. We use nitrocellulose membranes due to easier handling and lower consumption of immunodetection reagents compared to PVDF.

Although we are aware that our experiments resembles a series of case studies rather than large, well-defined patient group, we are able to answer if chemiluminescent detection could be feasible on a larger scale and provide additional information compared to chromogenic method. Both questions were answered positively. CL would probably not replace routine semi-automated o-IgG detection due to its higher manual and time requirement; however, it can be considered in cases where suspicion of MS is high and routine o-IgG test is negative. It has been recently demonstrated that routine o-IgG analysis might fail to detect low-grade intrathecal humoral immune response in a proportion of MS patients²⁹. Chemiluminescence detection using a CCD camera might be of advantage over detection on X-ray film²⁰ since multiple images could be taken at different times and the most suitable image for the evaluation of a particular sample could be used. This is not easily possible with CL detection on X-ray films; using chromogenic detection, only close visual follow-up of the reaction can be used to prevent overdevelopment. Using CL, exposition times vary between a few seconds and 10 minutes compared to 20–60 minutes needed for BCIP/NBT staining.

Another interesting perspective for the future lies in the attempt to quantify the intrathecal oligoclonal immune response. Most studies have failed to find significant clinical correlations with the number of oligoclonal bands, but it should be stressed that the amount of the intrathecal immune response affects the number of bands as well as their intensities. Quantification of the amount of immunoglobulins within individual bands, compared to the known amount of the immunoglobulin applied per lane, could reveal more clear-cut correlations. Unfortunately, software developed for chemiluminescent detectors was for native or SDS electrophoresis and cannot be directly used for isoelectric focusing where many bands represent the same protein.

CONCLUSIONS

Chemiluminescent detection using the appropriate camera is a feasible option for o-IgG and o-IgM analysis although we failed to find it clearly superior. Chemiluminescent detection might be the best option for studies of antigen-specific oligoclonal immunoglobulins, since these might represent only a minor portion of total immunoglobulin in the sample, as well as for the research of less abundant free light chains, IgD and IgE. Using maximum sensitivity substrate, we were able to detect 0.035–0.070 ng of monoclonal IgD which corresponds to a concentration of 0.005 – 0.01 mg/L in 7 µL of the applied sample. We have also shown that detection times longer than 10 minutes did not improve the sensitivity due to increase in background signal. Further efforts are needed to achieve better sensitivity.

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Author contributions: OD, PK, MC and DZ conceived the study. OD performed all CL detection experiments and prepared the documentation from the experiments for the manuscript. PK analysed and evaluated all o-IgG results. IK contributed to the design of CL experiments and brought ideas into the final version of the manuscript. MC participated in the design, execution and evaluation of IgD experiments. DZ performed the experiments with chromogenic detection, evaluated the results of o-IgM and o-fLC experiments, participated in the evaluation of IgD experiments, and wrote the first draft of the manuscript. OD, PK, IK, MC and DZ edited the manuscript and approved the final version of the manuscript.

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