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Clinical short communication

Comparison of fixed cell-based assay to radioimmunoprecipitation assay for acetylcholine receptor antibody detection in myasthenia gravis

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ABSTRACT

Objective: To compare specificity and sensitivity of a commercially available fixed cell-based assay (F-CBA) to radioimmunoprecipitation assay (RIPA) for acetylcholine receptor antibody (anti-AChR) detection in myasthenia gravis (MG).

Methods: In this retrospective diagnostic cohort study we reviewed the clinical information of suspected MG patients evaluated at the London Health Sciences Centre MG clinic who had anti-AChR RIPA and then F-CBA performed, in order to classify them as MG or non-MG. Classification of each patient as anti-AChR F-CBA-negative/positive, RIPA-negative/positive, and MG/non-MG permitted specificity and sensitivity calculations for each assay.

Results: Six-hundred-eighteen patients were included in study analysis. The median patient age at time of sample collection was 45.8 years (range: 7.5–87.5 years) and 312/618 (50.5%) were female. Of 618 patients, 395 (63.9%) were classified as MG. Specificity of both F-CBA and RIPA was excellent (99.6% vs. 100%, $P > 0.99$). One F-CBA-positive patient was classified as non-MG, although in retrospect ocular MG with functional overlay was challenging to exclude. Sensitivity of F-CBA was significantly higher than RIPA (76.7% vs. 72.7%, $P = 0.002$). Overall, 20/97 (21%) otherwise seronegative MG (SNMG) patients after RIPA evaluation had anti-AChR detected by F-CBA.

Conclusions: In our study anti-AChR F-CBA and RIPA both had excellent specificity, while F-CBA had 4% higher sensitivity for MG and detected anti-AChR in 21% of SNMG patients. Our findings indicate that F-CBA is a viable alternative to RIPA for anti-AChR detection. Prospective studies comparing F-CBA, RIPA and L-CBA are needed to determine optimal anti-AChR testing algorithms in MG.

1. Introduction

Myasthenia gravis (MG) is an antibody-mediated neuromuscular junction disorder characterized by abnormal muscle fatigability that may selectively affect ocular muscles or be more generalized. The most common antigenic target in MG is the muscle-type nicotinic acetylcholine receptor (AChR), which consists of α, β, δ and ε subunits (adult-type AChR-ε) or α, β, δ and γ subunits (fetal-type AChR-γ). Antibodies against all five AChR subunits are reported in MG, and test sensitivity is improved by using assays that incorporate both adult-type AChR-ε and fetal-type AChR-γ [1–4]. Approximately 50% of ocular and 85% of generalized MG patients are anti-AChR-positive by radioimmunoprecipitation assay (RIPA), the current gold standard test despite its main disadvantage of requiring radioactive reagents [5]. An additional 5–10% who are RIPA-negative harbour antibodies against other AChR-associated proteins including muscle-specific tyrosine (MuSK) and low-density lipoprotein receptor-related protein 4 (LRP4) [6,7]. Even with these advances in antibody discovery, a proportion of MG patients remain seronegative. This may relate to imperfect sensitivity of RIPA, which can miss antibodies with low affinity to AChR. This has spurred the development of live cell-based assays (L-CBA) expressing AChR subunits with the clustering protein, rapsyn, to mimic the high receptor density at the neuromuscular junction and facilitate detection of low-affinity anti-AChR [8]. L-CBA has higher sensitivity than RIPA, detecting anti-AChR in up to 66% of patients previously classified as seronegative MG (SNMG) [8–10]. Unfortunately, the costly and time-

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consuming nature of L-CBA restricts its use to specialized centers [5,10]. Recently, a commercial biochip has become available that is a composite of four fixed CBA (F-CBA): two F-CBA express AChR along with rapsyn (one F-CBA for adult-type AChR-ε, one F-CBA for fetal-type AChR-γ) to detect anti-AChR, one F-CBA expresses MuSK to detect anti-MuSK, and one F-CBA is a negative control. This F-CBA can more easily be implemented than RIPA or L-CBA in many clinical laboratories but studies of its diagnostic utility are lacking [5], so we evaluated the specificity and sensitivity of anti-AChR F-CBA compared to RIPA for MG.

2. Methods

Aliquots of serum samples stored at −70 °C from suspected MG patients evaluated at our MG clinic that were sent-out for anti-AChR RIPA between August 2002 and November 2015 were retrieved and tested by composite F-CBA (EUROIMMUN) following manufacturer's instructions. For each patient, AChR-ε, AChR-γ and MuSK F-CBA were each reported as negative, weak-positive or positive by two independent readers blinded to RIPA result and clinical information (P.E. by automated microscopy, A.B. by manual microscopy), with discussion to achieve consensus in discrepant cases. Weak-positive referred to staining that was faint but of sufficient intensity above the background to suggest a positive result (Fig. 1), as described previously [11]. Samples with excessive non-specific F-CBA staining precluding exclusion of AChR-specific staining (Fig. 1) were deemed indeterminable and excluded. A weak-positive or positive result reported for one or both of AChR-ε and AChR-γ F-CBA was grouped as 'F-CBA-positive' for analysis. RIPA was reported as absent/negative (<0.50 nmol/L), equivocal/borderline (0.50–0.99 nmol/L), low-positive/weak-positive (1.00–2.00 nmol/L), moderate-positive/positive (2.01–9.99 nmol/L), or high-positive/strong-positive (>10.00 nmol/L) by UBC Neuroimmunology Laboratory (August 2002 – January 2009) and London Health Sciences Centre Laboratory (February 2009 – November 2015) on a clinical service-basis. A low-positive/weak-positive, moderate-positive/positive or high-positive/strong-positive result reported for RIPA was grouped as 'RIPA-positive' for analysis. Samples reported as equivocal/borderline were excluded because of result ambiguity. Each patient was classified as MG (ocular, generalized) or non-MG based on chart review by A.M. of the clinical impression of the MG clinic director (M.W·N), independent of the F-CBA result. Clinical classification beyond non-MG (i.e. determination of specific alternative diagnoses) was not pursued because patients assessed at the MG clinic were not necessarily followed longitudinally after a neuromuscular junction disorder was excluded, and the binary classification of MG or non-MG was all that was required for this study of diagnostic test performance. Classification of each patient as anti-AChR F-CBA-negative/positive, RIPA-negative/positive, and MG/non-MG permitted specificity and sensitivity calculations. Anti-MuSK by F-CBA was also reported for each patient, and MG patients who were seronegative for both anti-AChR by RIPA and anti-MuSK by F-CBA were considered SNMG. The proportion of SNMG patients who were seropositive for anti-AChR by F-CBA was then calculated to determine the additional anti-AChR detection rate using F-CBA in SNMG.

Specificities, sensitivities and their 95% confidence intervals were determined and the McNemar test was used to compare significance of differences [12]. Fisher exact test was used to compare proportions of ocular and generalized MG. $P < 0.05$ was considered significant. Analyses were performed using SAS Studio.

This study was approved by the Western University Health Science Research Ethics Board (No. 119381) and is therefore in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

3. Results

Stored aliquots of serum samples from 641 suspected MG patients that were sent-out for anti-AChR RIPA between August 2002 and November 2015 were retrieved and tested by F-CBA. Identification of patients for inclusion in specificity/sensitivity analyses and their classifications are depicted via flow diagram (Fig. 2). Nineteen patients with equivocal/borderline RIPA and four patients with indeterminable F-CBA were excluded. In total, 618 patients were included in study analysis. F-CBA was performed a median duration of 12.1 years (range: 5.6–18.9 years) after sample collection for RIPA evaluation. The median patient age at time of sample collection was 45.8 years (range: 7.5–87.5 years) and 312/618 (50.5%) were female. Of 618 patients, 395 (63.9%) were classified as MG (144 ocular, 251 generalized). Three-hundred-four of 618 (49.2%) were positive for anti-AChR by F-CBA, while 287/618 (46.4%) were positive by RIPA.

Specificity of both anti-AChR F-CBA and RIPA was excellent (99.6% vs. 100%, $P > 0.99$) (Table 1). One F-CBA-positive patient who reported intermittent left eyelid droopiness and double vision when fatigued, but also numerous other symptoms including intermittent limb stiffness, dysesthesias and imbalance, was classified as non-MG. Her neurological examination found only equivocal, non-fatigable left ptosis confounded by blepharospasm. Electrophysiological studies as part of her MG clinic evaluation including SFEMG of the right orbicularis oculi were normal, and no follow-up was conducted at our centre. Given her numerous symptoms inconsistent with a neuromuscular junction disorder, she was classified as non-MG.

Sensitivity of F-CBA was significantly higher than RIPA for MG

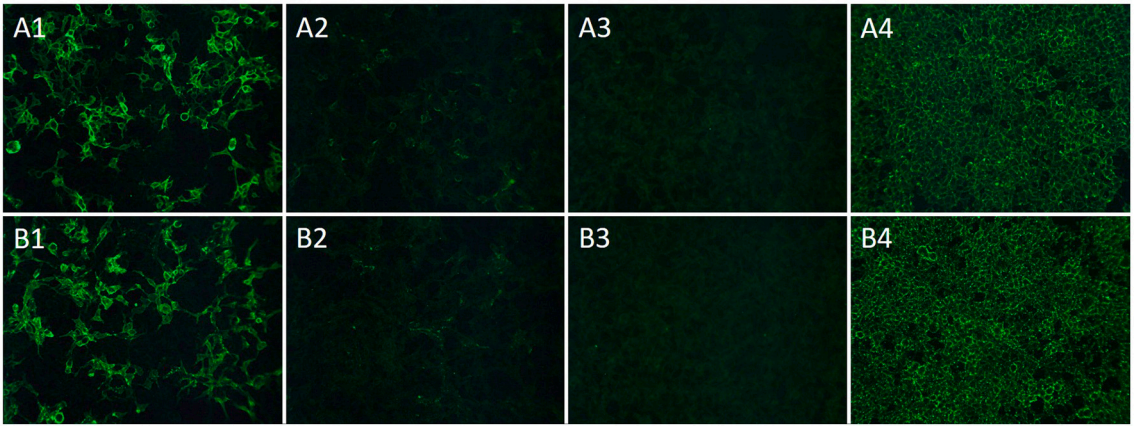
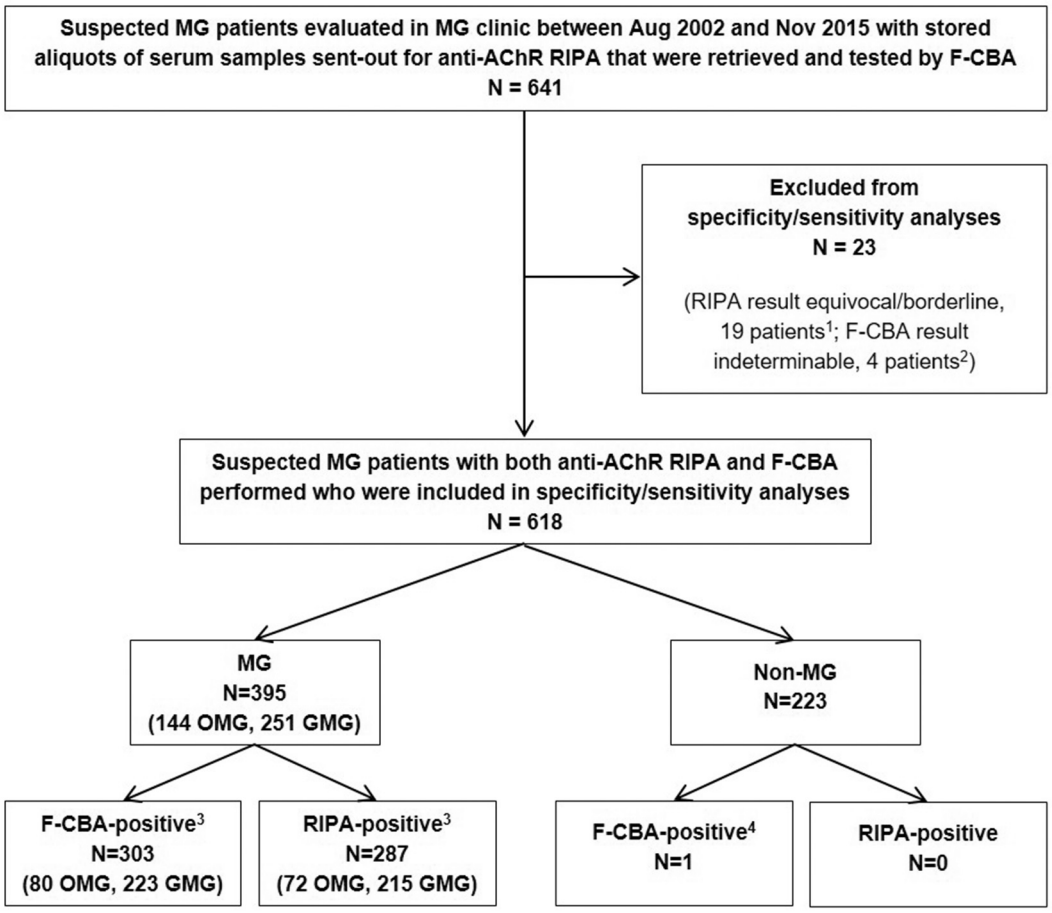


Fig. 1. Examples of positive, weak-positive, negative and excessive non-specific staining of anti-AChR F-CBA.
Examples of positive, weak-positive and negative staining of AChR-ε (A1, A2, A3) and AChR-γ (B1, B2, B3) F-CBA, as well as of excessive non-specific staining of all F-CBA including the negative control (A4, B4) precluding exclusion of AChR-specific staining.



AChR = acetylcholine receptor; F-CBA = fixed cell-based assay; GMG = generalized myasthenia gravis; MG = myasthenia gravis; OMG = ocular myasthenia gravis; RIPA = radioimmunoprecipitation assay

[1]Of 19 RIPA equivocal/borderline patients, 15 were F-CBA-positive (11 weak-positive/positive for both anti-AChR-ε and anti-AChR-γ, 4 weak-positive/positive for anti-AChR-ε only), all of whom were classified as MG (6 OMG, 9 GMG).

[2]Of 4 F-CBA indeterminable patients, 2 were RIPA-positive (1 moderate-positive/positive (2.01-9.99 nmol/L), 1 low-positive/weak-positive (1.00-2.00 nmol/L)), both of whom were classified as MG (1 OMG, 1 GMG).

[3]Of 303 F-CBA-positive patients classified as MG, 272 were weak-positive/positive for both anti-AChR-ε and anti-AChR-γ, 24 were weak-positive/positive for anti-AChR-ε only, and 7 were weak-positive/positive for anti-AChR-γ only. Of 287 RIPA-positive patients classified as MG, 110 were high-positive/strong-positive (>10.00 nmol/L), 127 were moderate-positive/positive (2.01-9.99 nmol/L), and 50 were low-positive/weak-positive (1.00-2.00 nmol/L). Twenty MG patients (10 OMG, 10 GMG) were F-CBA-positive but RIPA-negative (8 weak-positive/positive for both anti-AChR-ε and anti-AChR-γ, 12 weak-positive/positive for anti-AChR-ε only) while 4 MG patients (2 OMG, 2 GMG) were RIPA-positive but F-CBA negative (1 moderate-positive/positive (2.01-9.99 nmol/L), 3 low-positive/weak-positive (1.00-2.00 nmol/L)).

[4]One F-CBA-positive patient classified as non-MG (weak-positive for both anti-AChR-ε and anti-AChR-γ) is discussed in the text.

Fig. 2. Identification of patients for inclusion in specificity/sensitivity analyses and their classifications.

Table 1
Specificities and sensitivities of anti-AChR F-CBA and RIPA for MG.

Diagnosis (No. Patients)	F-CBA	RIPA	*P*-Value
Specificity, % (95% CI)			
All MG (395)	99.6 (97.5–100.0)	100 (98.4–100.0)	>0.99
Sensitivity, % (95% CI)			
All MG (395)	76.7 (72.5–80.9)	72.7 (68.3–77.1)	0.002
Ocular MG (144)	55.6 (47.4–63.7)	50.0 (41.8–58.2)	0.04
Generalized MG (251)	88.8 (85.0–92.7)	85.7 (81.3–90.0)	0.04

AChR, acetylcholine receptor; F-CBA, fixed cell-based assay; MG, myasthenia gravis; RIPA, radioimmunoprecipitation assay.

(76.7% vs. 72.7%, $P = 0.002$) (Table 1). Twenty of 395 MG patients (5.1%) were F-CBA-positive/RIPA-negative, while only 4/395 MG patients (1.0%) were RIPA-positive/F-CBA-negative. Eleven of 395 MG patients (2.8%) were positive for anti-MuSK by F-CBA. Of the 97 MG patients who were negative for anti-AChR by RIPA as well as anti-MuSK by F-CBA and thus considered SNMG, 20 (21%) were re-classified as seropositive MG due to anti-AChR detection by F-CBA. The proportion of patients with generalized SNMG who were re-classified as seropositive (10/25, 40%) was significantly higher than ocular SNMG (10/72, 14%) ($P = 0.009$).

4. Discussion

In our study, anti-AChR F-CBA had similarly excellent specificity and 4% higher sensitivity for MG compared to RIPA, detecting anti-AChR in 21% of patients otherwise considered to be SNMG. However, this additional anti-AChR detection rate in SNMG using F-CBA is lower than some studies reporting rates of up to 66% using L-CBA [8,10]. It is possible that some alteration of antigen conformation during the fixation procedure impacts sensitivity, like has been suggested for F-CBA used to detect other antibodies [5]. Alternatively, the long duration of

sample storage prior to F-CBA testing could have hindered anti-AChR detection due to sample degradation [13]. This may also account for the 1% of MG patients that were RIPA-positive but F-CBA-negative, despite overall higher sensitivity of F-CBA.

Interestingly, one F-CBA-positive patient who reported eyelid droopiness and double vision when fatigued, but also numerous other symptoms inconsistent with a neuromuscular junction disorder, was classified as non-MG. In retrospect, ocular MG with functional overlay was challenging to exclude. Nonetheless, this possible false-positive result emphasizes the importance of clinical-serological correlation when interpreting antibody testing, because rare false-positives may occur by virtually any test methodology including F-CBA, L-CBA, RIPA and enzyme-linked immunosorbent assay [11,14–16].

Our findings indicate that F-CBA, which is more easily implemented in many clinical laboratories, is a viable alternative to RIPA for anti-AChR detection. Limitations of our study include its retrospective nature, limited clinical characterization of patients with non-MG beyond not having the disease of interest, and lack of L-CBA for comparison. Prospective studies comparing diagnostic performance, cost and turn-around time of F-CBA, RIPA and L-CBA are needed to determine optimal anti-AChR testing algorithms in MG.

Author contributions

A.M. and A.B. designed/conceptualized the study, acquired/analyzed the data, drafted the manuscript and composed the tables/figures. M.W.N and P.E. acquired/analyzed the data, and revised the manuscript for intellectual content.

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Declaration of Competing Interest

None.

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