Reference range review for the RSR acetylcholine receptor antibody assay

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Abstract

Myasthenia Gravis (MG) is the most common autoimmune condition to affect the neuromuscular junction and is characterised by progressive skeletal muscle weakness and fatiguability. Detection of anti-acetylcholine receptor (ACHR) antibodies is frequently used as a first line investigation for MG. Our laboratory currently uses the RSR ELISA kit for this test, with a quoted manufacturer's cut-off value of 0.45 nmol/L.

Initial verification of the assay with healthy blood donor sera indicated that the manufacturer's cut-off value may be too low, with further observations from routine testing showing a high incidence of low-positive results. These findings plus a recent NEQAS non-conformity has prompted a review and revision of the established cut-off.

Anti-ACHR values were obtained from a cohort of 29 Neurology and 11 Ophthalmology Patients with established MG. Here we describe the use of Receiver Operator Curve (ROC) analysis to establish a new optimal cut-off value for the assay. Preliminary analysis implies that the specificity is much improved, the new cut-off is more clinically relevant, in line with published data, and should not lead to further EQA misclassifications.

Introduction

The Greater Manchester Immunology Service currently uses the RSR ELISA kit for the testing of anti-ACHR antibodies. During verification of the assay, 48 healthy blood donor samples were used to verify the reference range (0 - 0.44 nmol/L) supplied by the manufacturer. Three of these samples had results above the manufacturer's cut-off suggesting that the manufacturer's range may not be appropriate for use. In practice, we have observed a relatively high incidence of very-low positive results on our runs which supports this indication.

Additionally in 2020, the laboratory received a misclassification for UK NEQAS acetylcholine receptor antibody distribution 203, where we reported sample 203-2 as positive when the consensus result was negative. The sample used for the distribution was a single donor normal human serum. The RSR method group target for this sample was 0.4 nmol/L which was close to the assay cut-off of 0.45 nmol/L and the distribution of responses from other labs using the RSR method was up to 1.0 nmol/L, well above the manufacturer's cut-off.

In a recent audit conducted at the Manchester Royal Eye Hospital, 124 anti-ACHR antibody results from the neuro-ophthalmology department were analysed and the sensitivity was calculated as 73% and the specificity as 83%. RSR quote their ELISA to have a sensitivity of 92% and specificity of 99.8% while the literature accepts a sensitivity of 40-70% and specificity of 91% [1-3]. Based on the audit data, the assay's specificity is lower than expected. When discussing this with the neuro-ophthalmologists at the Manchester Royal Eye Hospital, they feel that this has become an unreliable test, which has resulted in multiple patients being falsely diagnosed with MG and unnecessarily started on pyridostigmine trials.

Following the above findings the recommendation was to revise the reference range for this assay.

Methods

A list was obtained from Neurology of 52 patients with clinically diagnosed Myasthenia Gravis (MG) that were diagnosed between 2018 and 2022. Another cohort of 12 patients with ocular MG diagnosed between 2020 and 2022, was identified with help from the Ophthalmology team. A data set was compiled which included patient identifiers, date of MG diagnosis, ACHR antibody levels at the time of diagnosis, and salient clinical information. Patients were excluded from analysis due to lack of relevant ACHR antibody result or unavailable clinical records on the EPR systems.

In total, ACHR antibody results from a total of 40 clinically diagnosed MG patients (29 Neurology and 11 Ophthalmology), with an ACHR antibody result within the same year of diagnosis, were included in the ROC analysis.

ACHR antibody results from 48 healthy control samples sourced from NHSBT were used as a "disease negative" population.

Results

The assay specificity, sensitivity, False Positive Rate (FPR) and True Positive Rate (TPR) were calculated using the patient and healthy control cohorts across a range of cut-off values between 0.3 to 1.2 nmol/L. A ROC curve was plotted (see Figure 1) using the FPR and TPR values at the various cut-off values (see Figure 2).

From this ROC curve a new cut-off of 0.6 nmol/L was chosen. Our data shows that between the original cut-off of 0.45 nmol/L and the new cut-off of 0.6 nmol/L, there is no change in TPR while the FPR reduces from 0.0625 to zero.
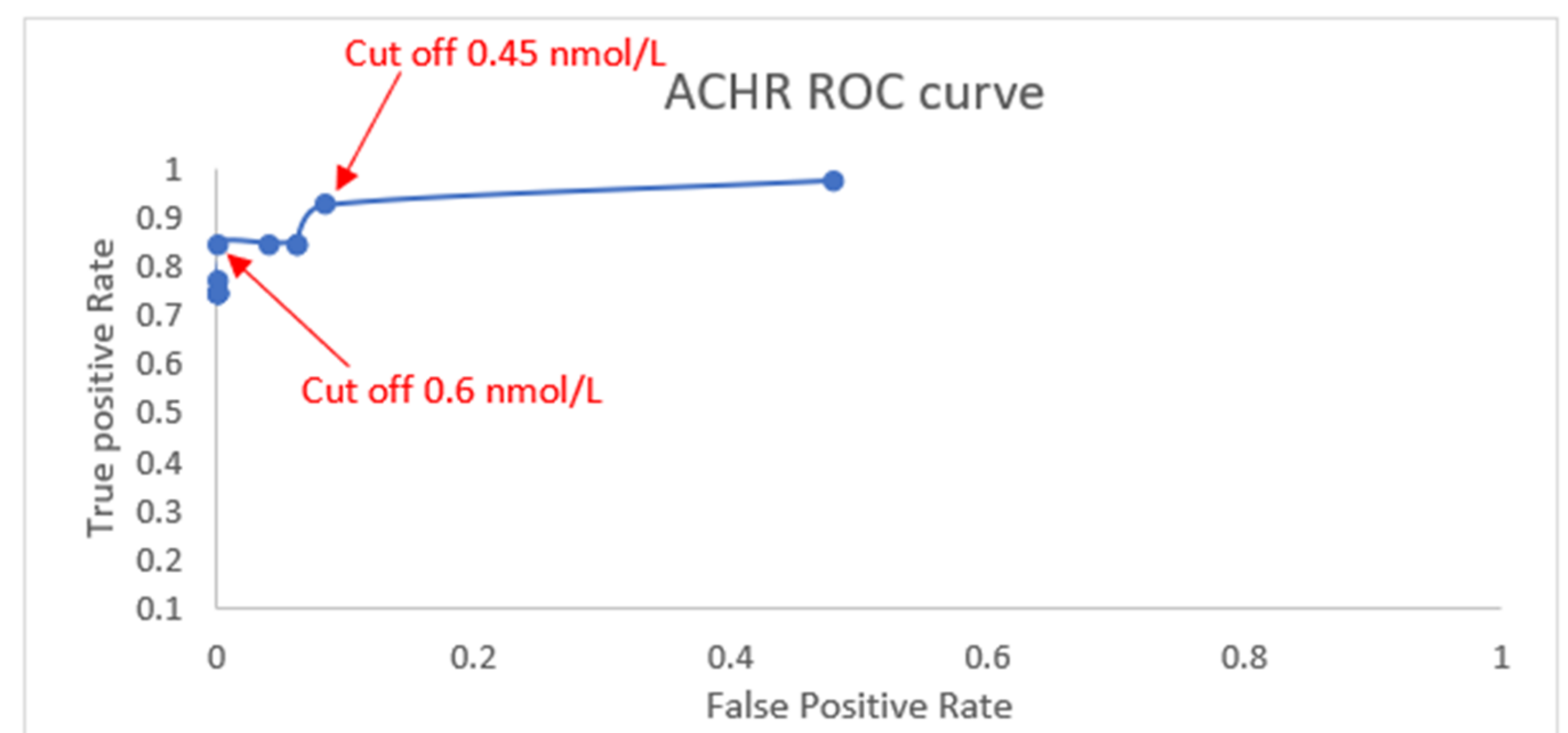
Results

Figure 1: Acetylcholine receptor antibody assay ROC curve

Cut-off (nmol/L)	FPR	TPR	Sensitivity	Specificity
0.3	0.479	0.974	97.40%	52.10%
0.4	0.0833	0.923	92.30%	91.67%
0.45	0.0625	0.846	84.60%	93.75%
0.5	0.0625	0.846	84.60%	93.75%
0.55	0.0416	0.846	84.60%	95.84%
0.6	0	0.846	84.60%	100.00%
0.7	0	0.769	76.90%	100.00%
0.8	0	0.743	74.30%	100.00%
0.9	0	0.743	74.30%	100.00%
1	0	0.743	74.30%	100.00%
1.1	0	0.743	74.30%	100.00%
1.2	0	0.743	74.30%	100.00%

Figure 2: False Positive Rates (FPR), True Positive Rates (TPR), sensitivities, and specificities calculated at different cut-off points ranging from 0.3 to 1.2 nmol/L.

Conclusion

Myasthenia gravis is a treatable disease that has better outcomes with early intervention[4]. It is important that the ACHR-ab test provides the maximum sensitivity to single out patients with true MG and treat them, but it must also be specific enough to avoid over-diagnosing MG and subjecting patients to unnecessary trials of pyridostigmine and steroids, as this can delay finding the true diagnosis, and could result in unwanted side effects from the medications.

Using the ROC curve, the proposed new cut-off of 0.6 nmol/L would increase assay specificity to 100% based on the 48 healthy controls in our original verification and would reduce the incidence of very-low positive results we have been observing on our runs.

The sensitivity (84.6%) at the higher cut-off value of 0.6 nmol/L was deemed to be acceptable, given that 15% of patients with generalized MG do not show anti-ACHR antibodies[5].

The new cut-off of 0.6 nmol/L would bring our previously reported results for NEQAS sample 203-2 into consensus with the method group and with previous classifications when the same sample had been distributed before (as 202-3 and 194-1). Historical results for all NEQAS samples tested between January 2020 and January 2023 were re-assessed using the proposed new cut-off and our results remain in consensus with the targets (data not shown). This suggests that the new cut-off is suitable for implementation.

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