DystoniaBoTXNet: Novel Neural Network Biomarker of Botulinum Toxin Efficacy in Isolated Dystonia

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Objective: Isolated dystonia is characterized by abnormal, often painful, postures and repetitive movements due to sustained or intermittent involuntary muscle contractions. Botulinum toxin (BoTX) injections into the affected muscles are the first line of therapy. However, there are no objective predictive markers or standardized tests of BoTX efficacy that can be utilized for appropriate candidate selection prior to treatment initiation.
Methods: We developed a deep learning algorithm, DystoniaBoTXNet, which uses a 3D convolutional neural network architecture and raw structural brain magnetic resonance images (MRIs) to automatically discover and test a neural network biomarker of BoTX efficacy in 284 patients with 4 different forms of focal dystonia, including laryngeal dystonia, blepharospasm, cervical dystonia, and writer's cramp.
Results: DystoniaBoTXNet identified clusters in superior parietal lobule, inferior and middle frontal gyri, middle orbital gyrus, inferior temporal gyrus, corpus callosum, inferior fronto-occipital fasciculus, and anterior thalamic radiation as components of the treatment biomarker. These regions are known to contribute to both dystonia pathophysiology across a broad clinical spectrum of disorder and the central effects of botulinum toxin treatment. Based on its biomarker, DystoniaBoTXNet achieved an overall accuracy of 96.3%, with 100% sensitivity and 86.1% specificity, in predicting BoTX efficacy in patients with isolated dystonia. The algorithmic decision was computed in 19.2 seconds per case.
Interpretation: DystoniaBoTXNet and its treatment biomarker have a high translational potential as an objective, accurate, generalizable, fast, and cost-effective algorithmic platform for enhancing clinical decision making for BoTX treatment in patients with isolated dystonia.

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Isolated dystonia is the third most common movement disorder after essential tremor and Parkinson's disease, with an estimated incidence of up to 35.1 per 100,000 in the general population.[1] Dystonia is a chronic disorder that causes abnormal, often painful, postures and repetitive movements due to sustained or intermittent involuntary muscle contractions. Chronically impaired motor control has a debilitating impact on a patient's quality of life and, in the majority of cases, leads to long-term occupational disability, continuous psychological stress, social isolation, psychiatric comorbidities, and increased suicidal risk.[2–4] Based on symptom distribution, the most common form is adult-onset focal dystonia, followed by segmental dystonia, and much rare childhood- or adolescent-onset generalized dystonia. The pathophysiology of dystonia involves complex disorganization of functional and

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structural neural networks[5,6]; however, the exact mechanism remains unclear, limiting the range of available therapies for these patients.

Currently, botulinum toxin (BoTX) injections into the affected muscles are considered the first line of therapy.[7-9] Yet, only 61% of patients are treated.[10] The highest use of BoTX is reported in laryngeal dystonia and blepharospasm, which, however, constitutes only 49% of these patients. About 40% of treated patients do not receive any benefits.[10] The poor clinical response of dystonic symptoms to BoTX injections is commonly a result of inadequate dosing, improper injecting technique, or imperfect choice of muscle location for injection (pseudo nonresponse). However, there are also primary and secondary nonresponders to BoTX treatment when patients fail to benefit from the first injection and all subsequent treatments (primary nonresponse) or receive benefit from at least one injection but lose that benefit over subsequent injection cycles (secondary nonresponse). Various reasons, including the presence of antibodies and immunogenicity, are considered to underlie this nonresponse.[11] The disorder complexity and symptom heterogeneity, as well as the experience and expertise of the treating clinician, especially in the underserved or rural areas, further contribute to the mixed outcomes or treatment underutilization. In the long-term, inadequate treatment resulting in delays in the clinical management of dystonic symptoms add to the patient's psychological and financial burden and the increasing healthcare costs due to disorder-associated disability.

One of the major factors limiting the more effective and targeted use of BoTX treatment in patients with dystonia is the absence of objective biomarkers and standardized tests for the predictive assessment of injection efficacy prior to the treatment initiation. Since its first use in patients with dystonia in the 1980s, the BoTX efficacy continues to be established following, on average, 3 to 4 treatment cycles, each lasting, on average, 3 to 4 months, during which different injecting regimens are probed to either achieve the anticipated benefit or determine that injections are not relieving the symptoms.[10,12] These cost-inefficient trial-and-error strategies often lead either to the overtreatment of patients whose symptoms are not responsive to BoTX (ie, primary or secondary nonresponders) but who are continuously injected in an attempt to achieve the desired benefit or to the undertreatment of patients who are appropriate candidates for BoTX therapy but decide to forgo or discontinue injections after the initially unsuccessful attempts (ie, pseudo nonresponders). It is, therefore, critical to accurately differentiate between true and pseudo nonresponders during treatment candidate selection in order to expand the use and improve the efficacy of BoTX therapy in patients with dystonia.

The mechanism of BoTX action includes the extracellular binding to glycoprotein structures on cholinergic nerve terminals, cleavage of the components of the soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor complex, and neuromuscular transmission via intracellular blockade of acetylcholine release, which leads to the deterrence of dystonic muscle contractions. Importantly, recent studies in different forms of dystonia determined that the central effect of BoTX therapy includes modulation of abnormal activity of sensorimotor cortical and subcortical regions at the peak of injection benefits, precuneus at the end of the injection cycle, and prefrontal and cerebellar regions over the years of treatment.[13,14] Leveraging this knowledge, we developed a deep learning algorithm, DystoniaBoTXNet, which uses brain magnetic resonance images (MRIs) of BoTX-benefiting and non-benefiting patients with laryngeal dystonia to automatically identify informative neural features of BoTX efficacy (ie, BoTX treatment biomarker). We tested and validated DystoniaBoTXNet and its treatment biomarker for predicting BoTX efficacy in patients with 4 different forms of focal dystonia, including laryngeal dystonia, blepharospasm, cervical dystonia, and writer's cramp. We hypothesized that the components of DystoniaBoTXNet biomarker will encompass brain areas contributing to both the common dystonic neural network.[15–20] and the central response to BoTX therapy[13,14] and thus provide an accurate estimate of the probability of BoTX efficacy in patients with different forms of dystonia.

Materials and Methods

Study Participants

A total of 284 patients with isolated focal dystonia participated in the study (see Tables 1 and 2 for detailed demographics). The diagnosis of laryngeal dystonia, blepharospasm, cervical dystonia, or writer's cramp and the absence of other neurological and psychiatric problems were established based on detailed case history, review of medical information, and neurological or laryngeal evaluations, as appropriate. Only patients with the confirmed diagnosis of isolated focal dystonia were included in the study. Dystonic or essential tremor was present in 34.4% of patients as a common comorbidity of dystonia phenomenology.[21] None of the patients received any centrally acting medications at the time of study participation; none had undergone surgery of the brain or dystonia-affected body region. All patients were screened for any prescribed or over-the-counter drugs and prior surgeries before the study participation; those taking medications or having a prior surgery of the brain or dystonia-affected body region were not included in the study.

TABLE 1. Patient demographics of the training set

	Training set	
N patients	106 LD BoTX-benefiting	59 LD BoTX-non-benefiting
Clinical phenotype	77 ADLD/29 ABLD	21 ADLD/38 ABLD
Putative genotype	79 sporadic/27 familial	49 sporadic/ 10 familial
Age (yr; mean ± SD)	56.2 ± 11.6	56.3 ± 11.9
Sex (F/M)	83/23	40 / 19
Dystonia duration (yr; mean ± SD)	15.6 ± 11.2	15.6 ± 10.7
Dystonia onset (yr; mean ± SD)	40.4 ± 12.5	39.2 ± 13.5
Duration of BoTX treatment (yr; mean ± SD)	7.1 ± 6.5	2.8 ± 2.9
Scanner strength	3.0 Tesla	3.0 Tesla
Scanner vendor	51 Philips/46 Siemens/9 GE	20 Philips/34 Siemens/5 GE
Head coil	60 (8)/2 (20)/44 (32)	25 (8)/2 (20)/32 (32)
MRI sequence	MPRAGE	MPRAGE
Scanning site	76 ISMMS/22 MGB/8 NIH	39 ISMMS/15 MGB/5 NIH

Abbreviations: ABLD = abductor laryngeal dystonia; ADLD = adductor laryngeal dystonia; BoTX = botulinum toxin; GE = General Electric; ISMMS = Icahn School of Medicine at Mount Sinai; LD = laryngeal dystonia; MGB = Mass General Brigham; MPRAGE = magnetization prepared rapid gradient echo; MRI = magnetic resonance imaging; NIH = National Institutes of Health.

All patients received BoTX type A injections for treatment of their dystonic symptoms but participated in the study when fully symptomatic, at least 3 months after the last injection. The efficacy of BoTX treatment was established based on the review of each patient's medical information from treating physicians, including history, physical, neurological, and laryngeal examinations, as applicable, and by questioning each patient about their treatment timelines and perceived benefits using a structured questionnaire. Taking into account both clinician-objective and patient-subjective evaluations of treatment efficacy, we stratified patients into BoTX benefiting and non-benefiting cohorts for DystoniaBoTXNet model training and testing as follows:

1. The training set included 106 patients with laryngeal dystonia (83 women and 23 men; age 56.2 ± 11.6 years; 77 adductor and 29 abductor types; 79 sporadic and 27 familial types) who received and benefited from BoTX treatment and 59 patients with laryngeal dystonia (40 women and 19 men; age 56.3 ± 11.9 years; 21 adductor and 38 abductor types; 49 sporadic and 10 familial types) who received but did not benefit from treatment (see Table 1). It is critical to train machine-learning algorithms on a well-characterized, homogeneous, large dataset in order to achieve their robust performance. This is especially important for rare diseases, such as dystonia. Our cohort of patients with laryngeal dystonia fit these characteristics best and was, therefore, chosen as a training set for DystoniaBoTX model training. Furthermore, the DystoniaBoTXNet model was trained on patients with laryngeal dystonia because of the highest use of BoTX treatment in this form of disorder.[10] Additionally, training DystoniaBoTXNet on this well-characterized patient cohort allowed us to compare its performance to the previously developed deep learning diagnostic platform, DystoniaNet, which was also trained and tested using a similar patient cohort.[22]
2. To examine the performance of DystoniaBoTXNet in patients with the same diagnosis as in the training set, the first independent test set included 29 BoTX-benefiting patients with laryngeal dystonia (25 women and 4 men; age 56.1 ± 13.2 years; 20 adductor and 9 abductor types; 23 sporadic and 6 familial types) and 15 BoTX non-benefiting patients with laryngeal dystonia (11 women and 4 men; age 61.4 ± 9.6 years; 7 adductor and 8 abductor types; 12 sporadic and 3 familial types; see Table 2).
3. To evaluate the generalizability of DystoniaBoTXNet model in predicting BoTX benefits in patients with other forms of focal dystonia, the second independent test set comprised 46 patients, including 14 patients with blepharospasm, 18 patients with cervical dystonia, and 14 patients with writer's cramp. Among these were 38 BoTX-benefiting patients (27 women and 12 men; age 57.7 ± 12.3 years) and 8 BoTX-non-benefiting patients (6 women and 2 men; age 53.3 ± 19.2 years; see Table 2).
4. To assess the translational potential of the DystoniaBoTXNet algorithmic platform, the third independent test set included 29 patients with laryngeal dystonia (21 women and 8 men; age 55.9 ± 15.1 years; 15 adductor and 14 abductor types; 25 sporadic and 4 familial types) who were BoTX-naïve (never treated) at the time of study participation

TABLE 2. Patient demographics of the independent test sets

	First independent test set	
No. of patients	**29 LD BoTX-benefiting**	**15 LD BoTX-non-benefiting**
Clinical phenotype	20 ADLD/9 ABLD	7 ADLD/8 ABLD
Putative genotype	23 sporadic/6 familial	12 sporadic/3 familial
Age (yr; mean ± SD)	56.1 ± 13.2	61.4 ± 9.6
Sex (F/M)	25/4	11/4
Dystonia duration (yr; mean ± SD)	14.4 ± 11.9	15.9 ± 14.0
Dystonia onset (yr; mean ± SD)	41.3 ± 14.3	45.8 ± 15.6
Duration of BoTX treatment (yr; mean ± SD)	9.6 ± 9.2	3.8 ± 3.4
Scanner strength	3.0 Tesla	3.0 Tesla
Scanner vendor	11 Philips/16 Siemens/2 GE	3 Philips/10 Siemens/2 GE
Head coil (channels)	13 (8)/16 (32)	5 (8) / 10 (32)
MRI sequence	MPRAGE	MPRAGE
Scanning site	22 ISMMS/7 MGB	10 ISMMS/5 MGB
	Second independent test set	
No. of patients	**38 FD BoTX-benefiting**	**8 FD BoTX-non-benefiting**
Clinical phenotype	13 BLS/16 CD/9 WC	1 BLS/2 CD/5 WC
Putative genotype	Sporadic	Sporadic
Age (yr; mean ± SD)	57.7 ± 12.3	53.3 ± 19.2
Sex (F/M)	27/12	6/2
Dystonia duration (yr; mean ± SD)	12.9 ± 9.1	10.8 ± 8.9
Dystonia onset (yr; mean ± SD)	46.7 ± 13.2	50.6 ± 12.7
Duration of BoTX treatment (yr; mean ± SD)	8.2 ± 9.1	1.0 ± 1.2
Scanner strength	3.0 Tesla	3.0 Tesla
Scanner vendor	24 Philips/13 Siemens/1 GE	4 Philips/1 Siemens/3 GE
Head coil (channels)	25 (8)/13 (32)	7 (8)/1 (32)
MRI sequence	MPRAGE	MPRAGE
Scanning site	30 ISMMS/8 MGB	5 ISMMS/3 MGB

	Third independent test set		
No. of patients			**29 LD BoTX-naïve**
Clinical phenotype	15 ADLD/14 ABLD	Scanner strength	3.0 Tesla
Putative genotype	25 sporadic/4 familial	Scanner vendor	15 Philips/14 Siemens
Age (yr; mean ± SD)	55.9 ± 15.1	Head coil (channels)	15 (8)/14 (32)
Sex (F/M)	21/8	MRI sequence	MPRAGE
Dystonia duration (yr; mean ± SD)	14.6 ± 12.4	Scanning site	20 ISMMS/9 MGB
Dystonia onset (yr, mean ± SD)	43.2 ± 15.3		

Abbreviations: ABLD = abductor laryngeal dystonia; ADLD = adductor laryngeal dystonia; BLS = blepharospasm; BoTX = botulinum toxin; CD = cervical dystonia; FD = focal dystonia; GE = General Electric; ISMMS = Icahn School of Medicine at Mount Sinai; LD = laryngeal dystonia; MGB = Mass General Brigham; MPRAGE = magnetization prepared rapid gradient echo; MRI = magnetic resonance imaging; NIH = National Institute of Health; WC = writer's cramp.

but followed up prospectively to capture their benefits to potential BoTX treatment (see Table 2).

This study was approved by the Institutional Review Board of Mass General Brigham (MGB; ID number 2020P004129), and all patients gave their written informed consent for study participation. Data from some patients were used in our previous study of the DystoniaNet diagnostic platform.[22]

Data Collection

Whole-brain T1-weighted images were acquired on a 3.0 Tesla MRI scanner using a 3D-magnetization-prepared rapid gradient echo (MPRAGE) sequence protocol between 2005 and 2021 (Table S1). The training and testing data sets were harmonized by clinically matching patient cohorts by their age, sex, disease duration, and age of onset, as well as the scanner magnetic field strength, vendor, head coil, acquisition sequence, and data collection site (all $p \geq 0.22$; see Tables 1 and 2). Clinical homogeneity was established by including only patients with a confirmed diagnosis of isolated focal dystonia and excluding those with unclear diagnosis and any other neurological (except for co-occurring tremor), psychiatric, or laryngeal disorders. To balance the potential effects of the scanner hardware and the data collection sites and to assess the generalizability of DystoniaBoTXNet, the training and testing sets included MRIs from 3 major scanner vendors (Philips, Siemens, and General Electric [GE]) and the frequently used head coils (8-, 20-, and 32-channels) that were acquired at 3 different sites (MGB, Icahn School of Medicine at Mount Sinai [ISMMS], National Institutes of Health [NIH]; see Tables 1, 2 and S2). The parameters of the 3D-MPRAGE sequence protocol across the sites and scanners were kept as stable as possible (see Table S1).

In all subjects, head movements during scanning were minimized by tightly cushioning and restricting the head inside the coil. All images were manually inspected for their quality to ensure the absence of artifacts, such as field-of-view clipping anatomy, wrapping artifacts, ringing, striping, blurring, ghosting, radio frequency noise, and signal inhomogeneity. The radiological evaluation did not reveal any gross anatomic abnormalities in any patient. Limited image preprocessing included the alignment to the anterior–posterior commissure orientation, normalization to the standard Montreal Neurological Institute (MNI) space, and N4 bias field correction. All input MRI scans used for DystoniaBoTXNet training and testing were of the same dimension at 181 × 217 × 181 voxels.

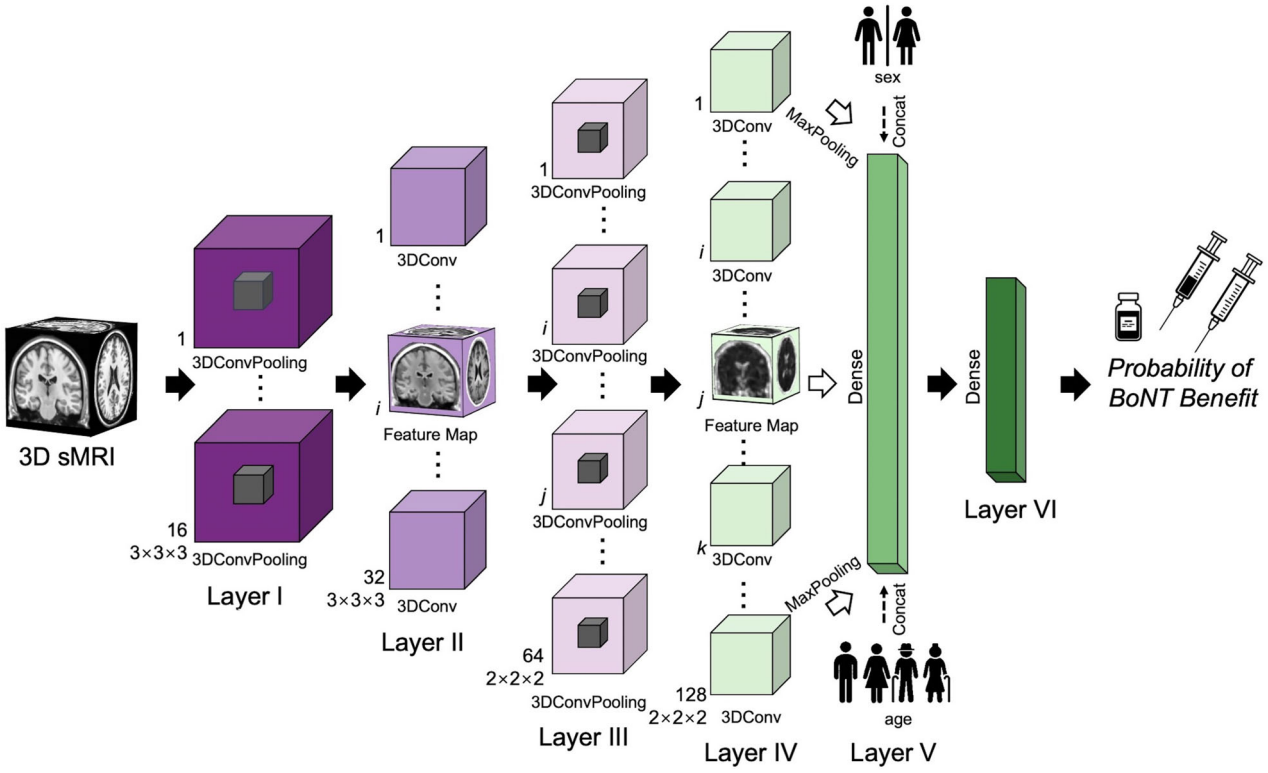


FIGURE 1: The architecture of 3D convolution neural network of the DystoniaBoTXNet model. Raw brain structural magnetic resonance imaging (sMRI) is used as input into DystoniaBoTXNet, which consists of 4 convolutional layers (3DConv) for feature extraction and representation learning (layers I–IV) and 2 fully connected dense layers (Dense) for classification (layers V–VI). A maximum pooling operation (3DConvPooling) is used to decrease the dimensions in layers I and III. The number and size of filters are set at 16 and 3 × 3 × 3 for layer I, 32 and 3 × 3 × 3 for layer II, 64 and 2 × 2 × 2 for layer III, and 128 and 2 × 2 × 2 for layer IV, respectively. The biological variables (sex and age) are concatenated with the image feature map flattened by the adaptive 3D global maximum pooling (MaxPooling) operation. The output of the DystoniaBoTXNet model is the individual probability of botulinum toxin (BoTX) benefit.

DystoniaBoTXNet Model Development and Training

DystoniaBoTXNet was developed based on the 3D-Convolutional Neural Network (CNN) architecture in the linearly aligned image space of T1-weighted brain images of 165 patients with laryngeal dystonia (training set). The DystoniaBoTXNet model included feature extraction and classifier components (Fig 1). For feature extraction and representation learning, we implemented four 3D convolutional layers (I–IV) with unit stride, 3D batch normalization, and rectified linear unit (ReLU) activation, without padding strategy. The number and size of filters were 16 and $3 \times 3 \times 3$ for layer I, 32 and $3 \times 3 \times 3$ for layer II, 64 and $2 \times 2 \times 2$ for layer III, 128 and $2 \times 2 \times 2$ for layer IV, respectively. The 3D convolution operations were followed by a maximum pooling operation (3DConvPooling) to decrease the dimensions in layers I and III. The biological variables (sex and age) were included in the model and concatenated with the image feature map flattened by the adaptive 3D global maximum pooling (MaxPooling) operation. The representations containing image and biological information were mined by the first dense layer (layer V), consisting of 1D batch normalization, ReLU activation, and a dropout with 0.5 probability. The second dense layer (layer VI) with normalized exponential function (SoftMax activation) was used to classify the probability of BoTX treatment efficacy. The adaptive moment estimation (Adam) optimizer with the number of epochs and batch size set to 150 and 8, respectively, was used for the model training. Early stopping was implemented to continuously monitor the training loss and speed up the training of the model in the last 20 epochs. Finally, to optimize the performance and minimize the predictive errors of the DystoniaBoTXNet model, a dynamic range was incorporated using 2 optimal thresholds, t_b and t_{nb}, which converted the output into 3 predictive outcomes of BoTX treatment: (1) benefit, (2) no benefit, and (3) referral. For a patient to be classified as benefiting from treatment, the output of DystoniaBoTXNet had to exceed $t_b = 0.52$; for a patient to be classified as BoTX non-benefitting, the output of DystoniaBoTXNet had to be lower than $t_{nb} = 0.42$. The referral rate was set to ≤10% of the predicted probability of treatment efficacy to balance the cost of mistreatment and the cost of additional treatment while avoiding the deflation of true negatives. As such, the integration of the dynamic range into the DystoniaBoTXNet model allowed the referral of uncertain cases instead of providing a potentially wrongful treatment outcome.

The DystoniaBoTXNet model was trained and tested on an NVIDIA Tesla A100 GPU (40 GB GDDR5 memory).

DystoniaBoTXNet Treatment Biomarker Visualization

To visualize brain areas that were automatically discovered and used by DystoniaBoTXNet as components of treatment biomarker, we reconstructed the end-to-end algorithmic model by computing four 3D average feature maps corresponding to each convolutional layer. These feature maps were then zoomed in using third-order spline interpolation to match the size of the standard MNI space. Each 3D average feature map was threshold at the top 10% of voxelwise absolute maximum weight. The identified brain areas used by DystoniaBoTXNet to discriminate BoTX-benefiting versus BoTX-non-benefiting patients were overlaid on the standard MNI brain template to visualize the DystoniaBoTXNet treatment biomarker.

DystoniaBoTXNet Treatment Biomarker Performance Evaluation

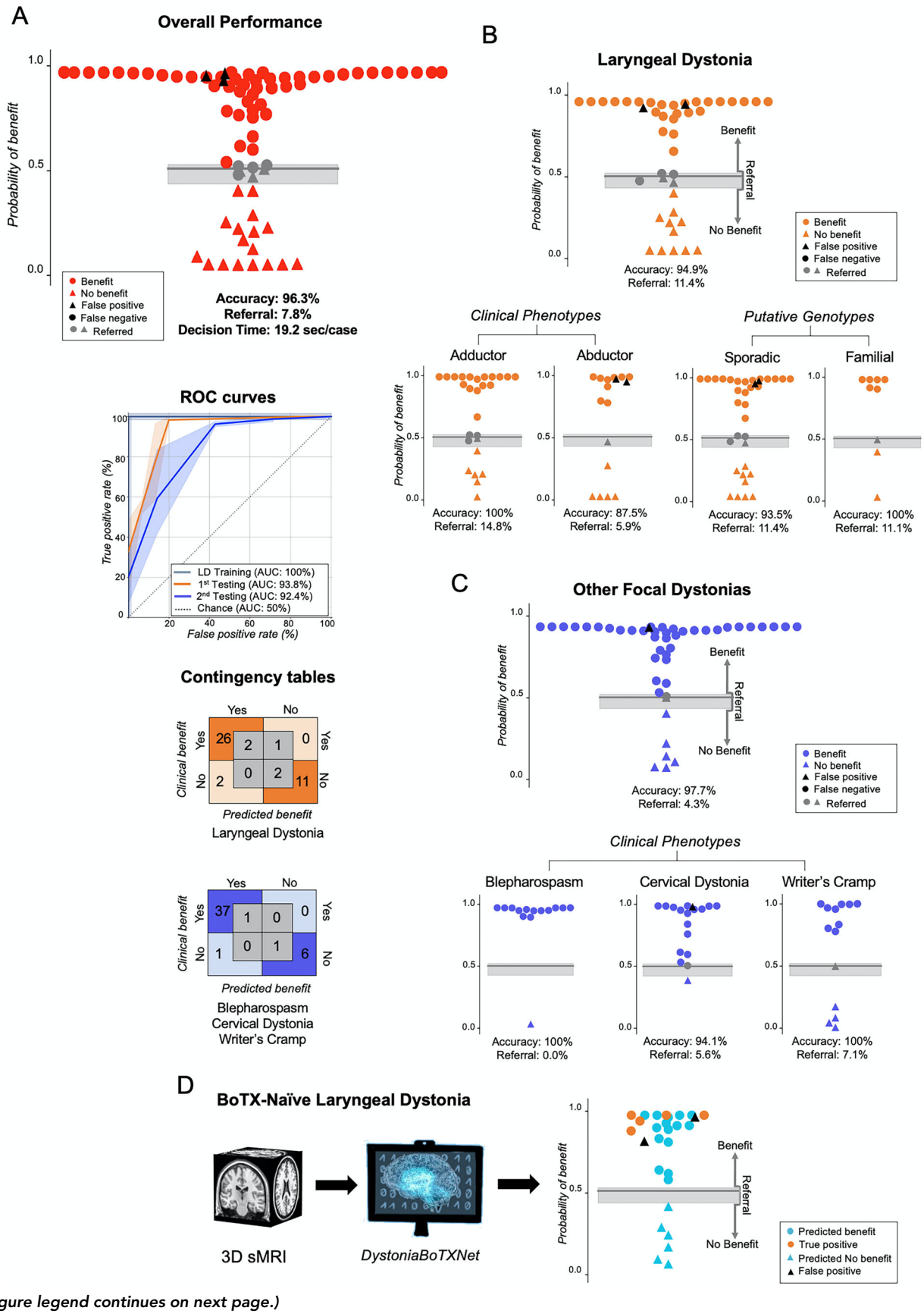
The performance of DystoniaBoTXNet and its treatment biomarker for predicting BoTX benefits in the first and second independent test sets was examined by computing the area under the receiver operating characteristic (ROC) curve (area under the curve [AUC]), accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The performance of DystoniaBoTXNet for the prospective assessment of the treatment outcome in the third independent test set of BoTX-naïve patients was evaluated by computing the probability (percentage [%]) of treatment benefit in each case.

To examine the stability of DystoniaBoTXNet performance, we stratified the patients of the first and second independent test sets based on MRI scanner vendor (GE, Siemens, and Philips), head coil (8/32 channels), and data collection site (MGB and ISMMS) to calculate the accuracy of BoTX treatment benefit in these cohorts (see Tables 2 and S2). Additionally, to assess the test–retest reliability of the DystoniaBoTXNet model, we used data from 25 patients with laryngeal dystonia (21 women and 4 men; age 56.6 ± 12.0 years) of the first independent test set who underwent 2 brain structural MRIs at 2 different time points (17.9 ± 24.7 months apart) to compute the intraclass correlation coefficient (ICC) and its 95% confident interval based on a single-measurement, absolute-agreement, 2-way mixed-effects model.[23]

Results

The training model of DystoniaBoTXNet achieved the AUC of 100% in discriminating 165 patients with laryngeal dystonia with versus without BoTX treatment benefits (Fig 2A). The model's performance was based on a fully automated, data-driven discovery of a neural biomarker of BoTX efficacy from raw brain structural MRI of these patients. The 4-layer components of the DystoniaBoTXNet biomarker included clusters in the corpus callosum (layers I–IV), left inferior fronto-occipital fasciculus (layers I–IV), bilateral inferior temporal/fusiform gyrus (left layers I–II, and right layers II–IV), bilateral superior parietal lobule (layers I–II), left inferior frontal gyrus (layer II), right middle frontal gyrus (layer II), bilateral middle orbital gyrus (layer II), and left anterior thalamic radiation (layer II; Fig 3; Table 3).

The overall performance of the DystoniaBoTXNet biomarker in predicting the BoTX treatment outcome across all forms of isolated focal dystonia was at 96.3% accuracy, with 100% sensitivity, 86.1% specificity, and



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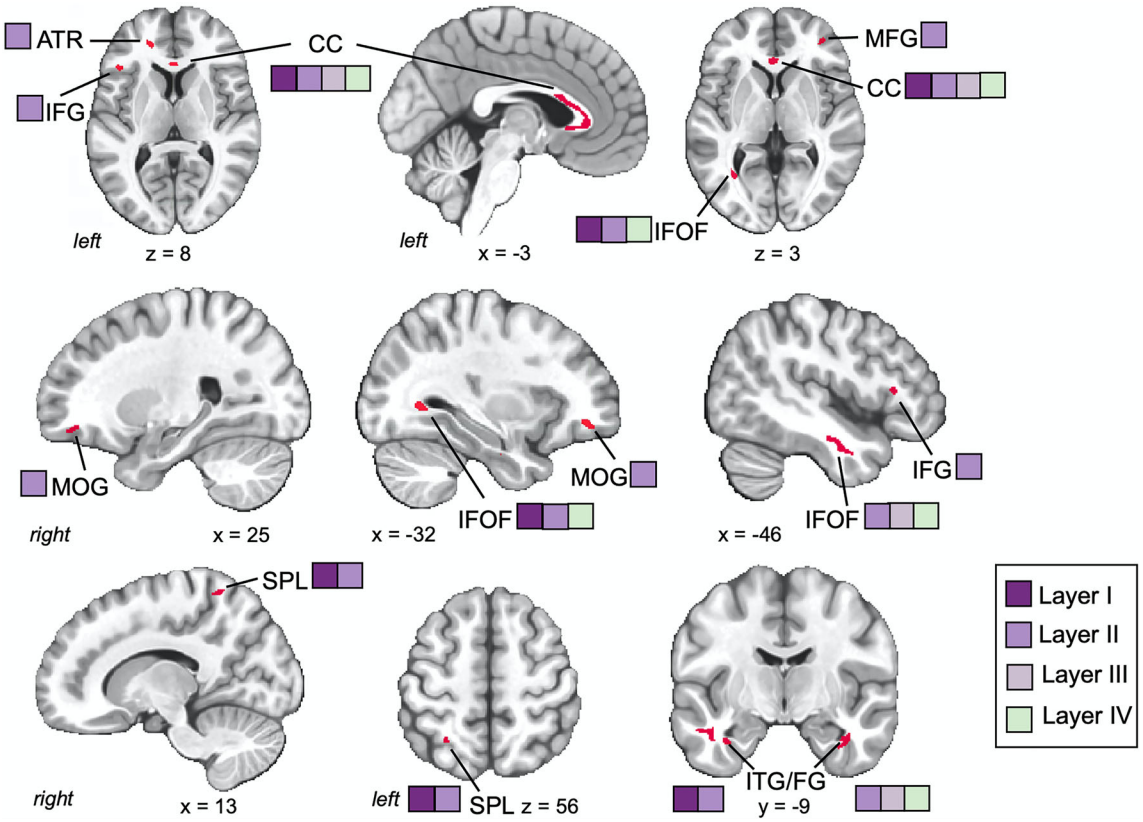

FIGURE 3: A neural network biomarker discovered by DystoniaBoTXNet for estimation of the probability of botulinum toxin (BoTX) treatment efficacy in isolated focal dystonia. A series of brain slices in the standard Montreal Neurological Institute (MNI) space depicts 2D visualization of the top 10% of voxelwise absolute maximum weight (in *red*) identified by 4 convolutional layers of DystoniaBoTXNet as components of the treatment biomarker. The affiliation of each cluster with the corresponding convolutional layer is color-coded. ATR = anterior thalamic radiation; CC = corpus callosum; IFG = inferior frontal gyrus; IFOF = inferior fronto-occipital fasciculus; ITG/FG = inferior temporal/frontal gyrus; MFG = middle frontal gyrus; MOG = middle orbital gyrus; SPL = superior parietal lobule.

FIGURE 2: Performance of DystoniaBoTXNet biomarker for predictive assessment of botulinum toxin (BoTX) efficacy in isolated focal dystonia. (A) The overall performance of DystoniaBoTXNet biomarker in the first and second independent test sets of 90 patients with laryngeal dystonia, blepharospasm, cervical dystonia, and writer's cramp. Each symbol represents a patient. Patients classified as benefiting from BoTX treatment are represented by circles; patients classified as not benefiting from BoTX treatment are represented by triangles. Colored symbols represent clinically correct treatment outcome; black symbols represent misclassifications. The *y* axis represents the probability of benefit assessed by DystoniaBoTXNet; the gray line represents the decision boundary; the gray shading represents the area of uncertainty where DystoniaBoTXNet refers the patient (*gray cross/triangle*, respectively) for further evaluation. The corresponding accuracy, referral rate, and decision time per case are reported. Receiver operating characteristic (ROC) curves for the training and first and second test sets are shown; the area under the ROC curve (area under the curve [AUC]) values for each set are reported in the key. The dotted line represents the performance of a random classifier. The corresponding contingency tables (in *orange*, for laryngeal dystonia; and in *purple*, for blepharospasm, cervical dystonia, and writer's cramp) report the number of patients who are correctly and incorrectly classified by DystoniaBoTXNet. The *gray* area of the contingency tables shows the referred patients. (B) The performance of DystoniaBoTXNet in the first independent test sets of 44 patients with laryngeal dystonia. Further stratification of patients into clinical phenotypes (adductor and abductor) and putative genotype (sporadic and familial) with the performance of DystoniaBoTXNet in each type of laryngeal dystonia is shown. Symbol and axes coding as described above. (C) The performance of DystoniaBoTXNet in the second independent test sets of 46 patients with other focal dystonia. Further stratification of patients into clinical phenotypes (blepharospasm, cervical dystonia, and writer's cramp) with the performance of DystoniaBoTXNet in each type of focal dystonia is shown. Symbol and axes coding are as described above. (D) The pipeline shows the translational use of DystoniaBoTXNet in BoTX-naïve patients with laryngeal dystonia, including the input of raw structural magnetic resonance imaging (sMRI) into the DystoniaBoTXNet platform and the output of individual probability of benefit. Symbol and axes coding as described above. *Blue* symbols represent BoTX-naïve patients at the time of study participation who did not yet receive treatment; *orange/black* symbols represent BoTX-naïve patients at the time of study participations who underwent BoTX treatment after study completion. *Orange* symbols represent clinically correct treatment outcome; *black* symbols represent misclassifications. LD = laryngeal dystonia.

TABLE 3. Components of the neural biomarker of BoTX treatment efficacy

Brain regions	Center of cluster mass *x, y, z*	Cluster size voxels	Convolutional layer
L/R corpus callosum	−2, 23, 4	957	I, II, III, IV
L inferior fronto-occipital fasciculus	−46, −6, −25	575	I, II, III, IV
L inferior temporal/fusiform gyrus	−36, −14, −26	299	I, II
R inferior temporal/fusiform gyrus	37, −11, −28	339	II, III, IV
L superior parietal lobule (area 7A)	−28, −56, 56	40	I, II
R superior parietal lobule (area 5M)	13, −49, 65	61	I, II
L inferior frontal gyrus (area 44/45)	−44, 25, 9	111	II
R middle frontal gyrus	37, 46, 3	53	II
L middle orbital gyrus	31, 46, −11	362	II
R middle orbital gyrus	−31, 46, −8	361	II
L anterior thalamic radiation	−20, 44, 8	87	II

Abbreviations: BoTX = botulinum toxin; L = left; R = right.

7.8% referral rate (see Fig 2A). The performance of the DystoniaBoTXNet treatment biomarker remained stable and independent of the MRI scanner vendor (GE, Siemens, and Philips; accuracy range = 75.0–100%), head coil (8/32 channels; accuracy range = 96.0–97.5%), or a data collection site (MGB and ISMMS; accuracy range = 92.6–100%). The test–retest reliability of DystoniaBoTXNet performance in predicting BoTX benefits was excellent at ICC = 0.93 with 95% confident interval = 0.85–0.97. The average computational time of DystoniaBoTXNet was 19.2 seconds per case, including 19.12 seconds for image processing and 0.06 seconds for the algorithmic estimation.

In the first independent test set, DystoniaBoTXNet achieved the accuracy of 94.9% in predicting the BoTX benefits in 44 patients with laryngeal dystonia and referring 5 patients (11.4%) for further evaluation (see Fig 2B). The corresponding out-of-sample AUC was 93.8%, with 100% sensitivity, 84.6% specificity, 92.9% PPV, and 100% NPV. Further stratification of patients with laryngeal dystonia based on their clinical phenotype (adductor and abductor) and putative genotype (sporadic and familial) showed that DystoniaBoTXNet had the highest predictive accuracy of treatment benefits in adductor (100.0% with 14.8% referral) and familial (100.0% with 11.1% referral) types, followed by sporadic (93.5% with 11.4% referral) and abductor (87.5% with 5.9% referral) types (see Fig 2A). Two patients with sporadic abductor laryngeal dystonia were identified as false positives (ie, having predicted but not clinically documented benefit). A review of their medical information revealed that both patients received only one unsuccessful BoTX injection.

In the second independent test set, DystoniaBoTXNet showed high algorithmic generalizability by achieving 97.7% accuracy in predicting BoTX benefits in 46 patients with blepharospasm, cervical dystonia, or writer's cramp and referring 2 patients (4.3%) for further evaluation (see Fig 2C). The corresponding out-of-sample AUC was 92.4%, with 100% sensitivity, 87.5% specificity, 97.4% PPV, and 100% NPV. The performance of DystoniaBoTXNet was highest at 100.0% accuracy in predicting the treatment outcome in patients with blepharospasm (no referral) and writer's cramp (7.1% referral), followed by 94.1% accuracy (5.6% referral) in cervical dystonia (see Fig 2C). One patient with cervical dystonia was determined to have a false positive outcome (ie, having predicted but not clinically documented benefit).

The translational use of DystoniaBoTXNet was evaluated in 29 BoTX-naïve patients with laryngeal dystonia (third independent test set). DystoniaBoTXNet predicted that there is a 94.6% median probability of BoTX treatment benefit in 23 patients (13 adductor and 10 abductor types) but only 16.9% median probability of benefit in the remaining 6 patients (2 adductor and 4 abductor types; see Fig 2D). The follow-up of 24 out of 29 patients (5 patients were lost to follow-up) found that 7 patients received BoTX treatment after they participated in this study. Among these patients, 5 patients (4 adductor and 1 abductor types) benefited and 2 patients (abductor type) did not benefit from injections. DystoniaBoTXNet was

100% accurate (median probability = 99.9%) in predicting the clinically observed treatment benefits in all 5 patients with adductor/abductor laryngeal dystonia (see Fig 2D). Conversely, DystoniaBoTXNet was false positive (median probability = 90.8%) in predicting treatment benefits in 2 patients with abductor laryngeal dystonia who did not observe a clinically meaningful response to injections.

Discussion

We demonstrated that DystoniaBoTXNet and its automatically discovered treatment biomarker provide objective and accurate estimates of BoTX efficacy from individual raw structural brain MRI of patients with isolated focal dystonia. At an overall accuracy of 96.3% and an average speed of 19.2 seconds per case, the DystoniaBoTXNet algorithm incomparably outperforms the current empirical approach of establishing treatment benefits. Hence, the DystoniaBoTXNet platform shows a high translational potential for improving clinical management of focal dystonia by aiding therapeutic decision making.

The DystoniaBoTXNet treatment biomarker includes brain regions whose alterations are commonly reported across the broad clinical spectrum of dystonia.[15–20] The identified clusters in the corpus callosum have been previously linked to abnormal interhemispheric processing, and alterations in the anterior thalamic radiation have been implicated in aberrant basal ganglia-thalamo-cortical projections to the prefrontal cortex, including the inferior and middle frontal gyri. The latter structures have also been shown to contribute to the neural endophenotypic marker of dystonia.[24–26] Abnormalities in the inferior temporal and middle orbital gyri and the underlying inferior fronto-occipital fasciculus have been reported in associations with altered heteromodal sensorimotor processing and executive control of motor behaviors in patients with dystonia.[27–32] Finally, parietal alterations have been identified in association with abnormal sensorimotor processing and subtle deficits of visuospatial attention, temporal discrimination, and spatially guided behaviors.[30,33–37] Notably, 5 out of 8 regions, including the corpus callosum, anterior thalamic radiation, inferior fronto-occipital fasciculus, inferior temporal, and orbital gyri, discovered by DystoniaBoTXNet as a BoTX treatment biomarker have been previously reported as components of the independently developed DystoniaNet diagnostic platform.[22] The reliance of both algorithmic models of DystoniaNet and DystoniaBoTXNet on similar pathophysiological features for their respective diagnostic and treatment predictive outcomes points to the significant contribution of these alterations to dystonia pathophysiology. Among the remaining components of the DystoniaBoTXNet biomarker, the superior parietal lobule and middle frontal gyrus stand out as areas involved in short- and long-term neuromodulatory response of BoTX treatment.[14] Taken together, the neural network biomarker identified by DystoniaBoTXNet is directly relevant to both dystonia pathophysiology and BoTX central effects in these patients.

It is, therefore, not surprising that the performance of the DystoniaBoTXNet treatment biomarker was highly accurate and generalizable across different forms of focal dystonia. Specifically, DystoniaBoTXNet correctly identified all patients with all types of laryngeal dystonia, blepharospasm, cervical dystonia, and writer's cramp who benefited from injections, as well as all patients with adductor and familial types of laryngeal dystonia, blepharospasm, and writer's cramp who did not benefit from injections. The 100% accuracy of DystoniaBoTXNet in predicting treatment benefits in these patients suggests the ability of the algorithm to utilize its biomarker for robust performance across different forms of the disorder. On the other hand, DystoniaBoTXNet false-positively classified as treatment-benefiting 2 patients with sporadic abductor laryngeal dystonia (95.8% and 98.2% probability of BoTX efficacy, respectively) and one patient with cervical dystonia (99.5% probability of BoTX efficacy). A review of the medical history of these misclassified patients revealed that all patients underwent only one injection cycle, which did not alleviate their dystonic symptoms. All patients declined subsequent treatment because of a lack of confidence in injection administration and efficacy.[12,38] Based on these findings, we propose that DystoniaBoTXNet may be effective in the clinical management of such false positive cases who are likely pseudo nonresponders, in whom the decision for additional injection cycles (with adjusted dose, site, or regimen) may be informed by the high algorithmic probability of BoTX efficacy.

To that end, we demonstrated the translational potential of DystoniaBoTXNet and its treatment biomarker using the exploratory cohort of patients with laryngeal dystonia who were BoTX-naïve at the time of study participation. Based on the algorithmic predictions, the majority of patients (23 out of 29, 79.3%) had a high probability of benefiting from BoTX injections, but only 24.1% of these patients (7 out of 29) eventually received injections to manage their symptoms. These data highlight a substantial underutilization of BoTX for dystonia treatment, which is particularly consequential due to generally limited therapeutic options for patients with this disorder. Among the patients who received BoTX injections after study participation, DystoniaBoTXNet correctly identified all patients with adductor or abductor laryngeal dystonia who benefited from injections. However, 2 patients with abductor

laryngeal dystonia were false positively classified as benefiting without apparent clinical effects. Again, these misclassified, likely pseudo nonresponders underwent only one injection cycle, which did not lead to their symptom improvement. Based on the high probability of BoTX efficacy (98.8% and 82.8%, respectively) in these patients, they are highly likely to benefit from injections if appropriately treated.

In conclusion, we developed and tested a novel deep learning algorithm, DystoniaBoTXNet, which uses its automatically discovered treatment biomarker and raw structural brain MRI to provide an objective, accurate, and fast estimate of BoTX efficacy in patients with isolated focal dystonia. Notably, because the DystoniaBoTXNet treatment biomarker was identified on fully symptomatic patients outside their treatment cycle, it can be utilized to estimate BoTX benefits both in patients who received prior BoTX injections and those who are treatment naïve. Thus, the DystoniaBoTXNet treatment biomarker may aid clinical decisions for objective candidate selection before BoTX treatment initiation. Likewise, it may help recalibrate the use of BoTX injections in those who failed initial treatment attempts. Such a novel clinical-algorithmic approach to BoTX treatment is expected to enhance the therapeutic management of patients with isolated dystonia while balancing the associated costs.

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Author Contributions

K.S. and D.Y. contributed to the conception and design of the study. L.C.O., D.Y., and K.S. contributed to the acquisition and analysis of data. D.Y., L.C.O., and K.S. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

The authors declared no conflict of interest.

Data Availability

All data relevant to clinical and research information of the datasets used in this study are included in the manuscript. The dataset used to train and test DystoniaBoTXNet is administered by Mass General Brigham. The dataset in its entirety is not currently publicly available, but a subset may be requested from the corresponding author, subject to the data use agreement and approval by the Mass General Brigham Data and Tissue Sharing Committee.

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