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Cover Image: "Wedding Picture" by Jessica Wohl.

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What's In This Issue?

Letter from the Founding Facilitator for Volume 3, Issue 1

Richard J. Barohn, MD

This is the first issue of volume 3! An exciting milestone in the history and life of a new periodical. We have packed a lot of great content in this issue. Along with this piece in the *What's on Your Mind?* category is another wonderful article by Dr. Josh Freeman called *Brooklyn Nostalgia* from his blog. It is a beautiful piece of writing. Then, in honor of Black History Month (February 2022), I wrote about Dr. Charles Richard Drew, a true pioneer in the field of blood product transfusions. This first came out in one of my "Messages from the Executive Vice Chancellor for Health Affairs" at the University of Missouri in a shorter format.

In the New Stuff category, there are a number of articles on myasthenia gravis. Dr. Gloria Ortiz-Guerrero and colleagues at KUMC (and myself from my time at KUMC neurology) analyzed the results of repetitive stimulation testing on a cohort of MG patients. Dr. Ortiz-Guerrero did this study as a resident and fellow. Dr. Bakri Elsheikh and THE Ohio State University neuromuscular team (my neuromuscular alma mater!) performed a depression screening study in a group of MG patients.

Also from THE Ohio State University, Dr. Jonathan Morena reported on thirty zero-negative MG patients and their clinical features. Dr. Mukaish Kumar, along with Dr. Raghav Govindarajan and Wei Huang (all at the time at the University of Missouri) developed an Empathetic Neurological Care-structured evaluation and they then measured empathy in both American medical graduates and international medical graduates.

Also in the New Stuff category is an article that is first authored by Dr. Beladakere, a neurology resident at the University of Missouri, describing the effect of 3,4-Diaminopyridine phosphate (DAAP) in SOD ALS mice. The experiments were performed by our colleague Dr. John Stanford at the University of Kansas Medical Center and were meant to support our team's efforts to do a randomized control trial of DAAP in ALS patients. To cut to the punchline, unfortunately the DAAP in SOD mice study did not show a benefit compared to placebo. To compliment this article,

In the Proposed Stuff section Drs. Raghav Govindarajan, Stanley Iyadurai, and the KUMC/MU team are publishing a valiant attempt to get an NIH-funded DAAP in ALS study. And not only was the proposed use of DAAP in ALS novel, we also proposed a novel trial design named Efficacy and Effectiveness Too (EE2). In EE2 studies, a phase three efficacy randomized control trial is nested in a larger effectiveness study. In the effectiveness portion of the study, ALS patients are enrolled who do not meet the stricter entry criteria for the efficacy study are randomized to DAAP versus placebo as well. The idea is to see how the drug works in a larger population. So even if the efficacy study meets statistical significance, the study also asks whether that is also the case for a larger group of ALS patients, and if not, is there a trend? This is a very interesting concept I learned from Dr. Harry Selkar at Tufts several years ago at one of the CTSA (NIH clinical translational science award) meetings we attended. He helped me understand the concept and set up brainstorming groups through the NCATS CTSA program for our team in Boston, which was very enlightening. So we put together this proposal for a EE2 study for DAAP in ALS to NCATS, and it did not get funded. Since the SOD DAAP study was negative we did not feel quite as terrible compared to our other grant rejections! Nevertheless, just because an animal study is negative, or positive, of course does not mean a human study will be negative, or positive! But they often do inform one another. And to my knowledge, the NIH has still not funded a true EE2 study. In essence an EE2 study is designed to not only answer a question about whether drug is effective in a homogenous study population (FDA Phase 3) but also in the wider population, the latter being an FDA Phase 4 study approach.

In the Clinic Stuff section there are a number of fascinating cases. Dr. Tim Fullum just finished his neuromuscular fellowship at KUMC and that group wrote up an interesting case of mononeuropathy multiplex due to lymphatoid granulomatosis. Dr. Ramin Khodabandehlou from the Amman Clinic in Tehran, Iran sent us a case of leg amyotrophic diplegia and reviewed the LAD literature. Dr. Kumar and the University of Missouri team wrote up a case of Guillian-Barre syndrome in the setting of COVID-19 infection and reviewed the literature. And Drs. Helena Hwang and Jeffrey Elliot at University of Texas Southwestern in Dallas describe an interesting and rare paraneoplastic syndrome with weakness and a low phosphorous that is tumor-induced or oncogenic osteomalacia.

In the Other Stuff section, we are publishing several beautiful poems. One called *Boots* is by Michael Stanley, MD, a neurology resident at the Massachusetts General/Brigham Neurology program. Dr. Stanley sent this poem to the journal last year in hopes it could come out for Veteran's Day. I am sorry we did not get it published in time for Veteran's Day but it is a lovely poem that can be appreciated

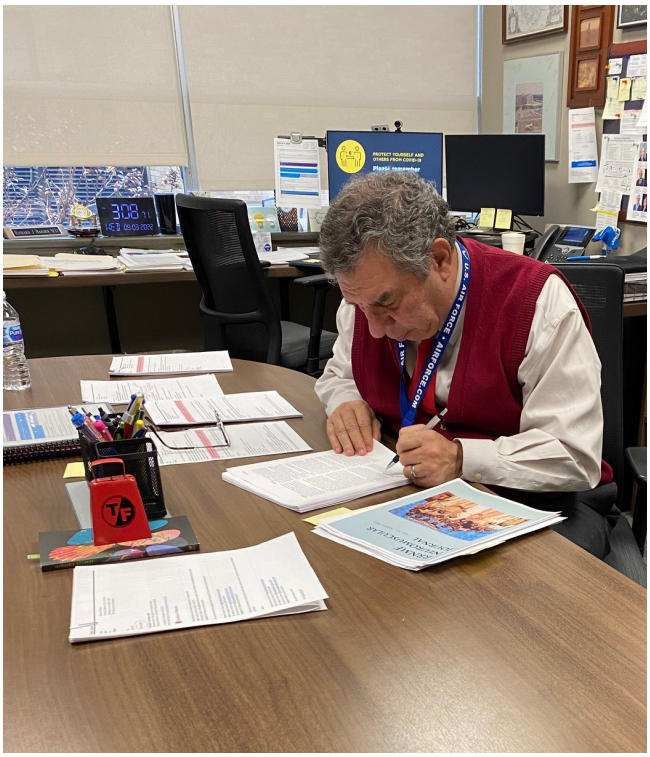
at any time. When Dr. Stanley submitted the poem he wrote us the following:

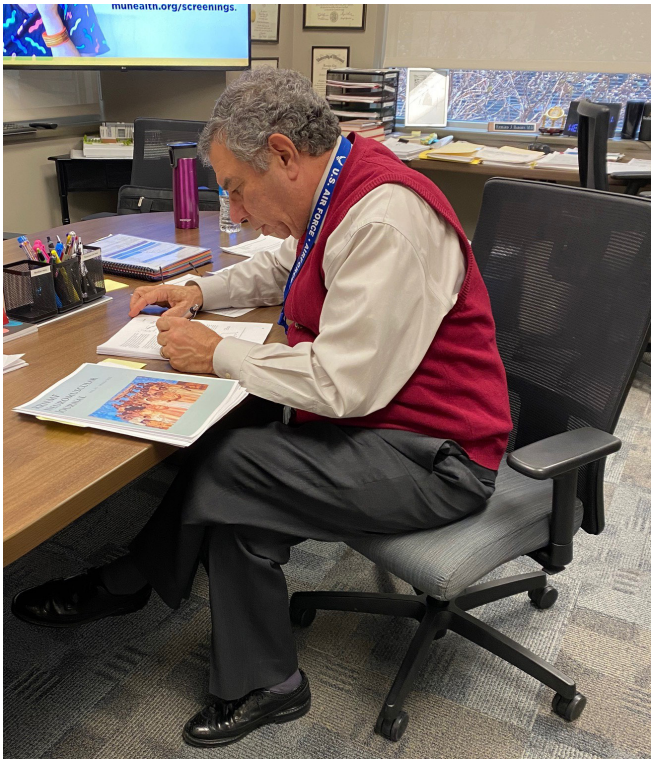
"I'm offering for submission to your journal a narrative poem about a neurological injury (among them phantom limb) suffered by a veteran explored through the telling of his beloved cat. I thought it would be a good fit for the November edition of your journal, considering Veterans Day falls in November, and after a year of the COVID campaign it is time to cherish the heroes of wars medical as well as military". Thank you, Dr. Stanley for submitting this poem.

Two veteran poets and neuroscientists, Elizabeth Rowe, PhD and Vernon Rowe, MD have once again sent us poems of theirs for this issue. And they have two more they sent me for an upcoming issue, so stay tuned.

The cover is another amazing painting by Jessica Wohl who has been featured on the cover of Issues 4 and 5 in Volume 2. This piece is called "Wedding Picture". As I said when I showed her paintings on the cover of Issue 5 from 2021, she had photo albums of her family's wedding pictures from the 1960's and 1970's. I think the paintings from Issue 5 may have been 1960's and the current very large work of art is from a 1970's wedding, from the fashion and hairstyles! This is a very large work consisting of six canvas paintings, each roughly three by four feet so the composite work is about nine by eight feet. When we moved into our home in Columbia, Missouri, we had a wall in the vaulted living room that I knew would be a perfect fit for this painting. I had remembered seeing it at a Crossroads gallery in Kansas City at a First Friday art walk many years ago. I emailed Jessica if she had ever sold it and she had not. I guess finding a home for a piece this large can be a challenge. I told Jessica "I will buy it!" Jessica shipped the painting to Kansas City and my friend and artist Jorge Garcia cleaned it up (it had been in storage for over a decade) and he then brought the painting(s) to Columbia along with a scaffold and he and a colleague expertly hung this beautiful and complex work of art in our living room. The wedding party is on one big wall, and they are looking at paintings on the opposite wall that were on the cover of Vol2/Issue 5: "Smile for Grandma" and "Erwin at the Buffet Table". Thank you, Jessica, for allowing us to publish your paintings for the covers of the journal.

I would like the readership of the journal to know that we are now the official journal of the Muscle Study Group. This was approved by the MSG Executive Committee last fall. We are also of course the official journal of Rick's Real Neuromuscular Friends! Therefore, we have now indicated this on the page where we list all the facilitators and other information about the journal.





Rick editing Volume 3, Issue 1.

Brooklyn Nostalgia

Joshua Freeman, MD

The web is full of sites where people reminisce about the places they are from, especially when they don't live there anymore and so can romanticize it a bit. Brooklyn, New York, where I am from, seems to be particularly a focus of such memories. One of them, complete with pictures, can be found at http://www.screanews.us/NewYork/BrooklynOld.htm.

This site has a lot of good pictures, and some very good memories. Of course, most of the text is hallucinatory (few or no one-parent families), often projecting ("this was my life, so I imagine it was that of others'"), and frequently stupid. The "good old days" were not always so good. There is even a book by Otto L. Bettmann called "The Good Old Days – They Were Terrible!" (Random House, 1974), reviewed here nearly 30 years after it was published. Another site compared various "good old days", going back generation by generation showing what seemed better in memory was not really a very good time. The conclusion on that site is that "the good old days" were never a time period but rather when you were a kid. It is not the time -- it is that things seemed simpler, and often better, and we repress the bad memories from -- when we were kids.

That said, our memories are our memories; we accept the things that are better and regret the things that we miss. However, it is fun to look back at our past, and at our youth, the good and the bad. As far as the Brooklyn described on the website above, at least the pictures are good. While the memories range from the 30's to the 60's, they seem to focus largely on the 50's, and there is a report card from Kindergarten (1a) in 1949, so you can guess his current age.

Many of these things were still around in my memory, although the prices were up. Subways were 15 cents when I started riding them; for a quarter, the newsstand guy at the Neck Rd station would give you the NY Times and a token in change. Phil Ochs wrote a song in the 60s about the "Daily News" with the lyric "7 little pennies in the newsboy's hand and you ride right along to never-neverland!", and I remember that it was a big deal when it went up from a nickel. There is an early picture of me wearing a Daily News "Strike" body placard, having gone with Uncle Benny (who worked there) on a picket line. And Uncle Benny would bring the Sunday News comics home on Saturday -- magic!

I remember pizza slices at 15 cents also, but the real treat was walking to Roma's Pizzeria on Avenue U just east (but if you had asked me I would have said "north" probably, since in that part of Brooklyn the "East" numbered streets increased from west to east; the numbers of the streets were going UP, so of course it would be north!) of Nostrand Ave to get a WHOLE pizza for dinner. And walk back with it 5 blocks. Toss-up as to whether this beat Brennan and Carr, hot roast beef sandwiches, on the opposite corner. Which I think is still there. (On the map, the area where I lived is the northern part of "Sheepshead Bay", bleeding into southern part of what is labeled "Midwood"; the angled street that crosses the border SW to NE is Kings Highway, which is what most people called that area; Avenue U is a few blocks to the south. Often the whole area north of Sheepshead Bay up to nearly Prospect Park was simply referred to as "Flatbush". For another take, see the 1974 movie "The Lords of Flatbush", which featured young Sylvester Stallone, Henry Winkler, and Perry King!).

I remember the last of the straw-seat subway cars, the oldest on the Franklin Avenue Shuttle which ran for 3 stops and connected the Prospect Park station on the Brighton train (ours; then the Q, now the D) with the Independent train at Franklin Avenue (or so it said on the maps). Mostly I rode the one stop to the Botanic Gardens station in order to walk the long block down Eastern Parkway to the Brooklyn Museum; a couple of times to the second stop, Park Place, where the Brooklyn Children's Museum was (and, I am told, Brooklyn Jewish Hospital, where I was born, and now part of "Interfaith Hospital" having merged with St. John's).

I certainly remember the crowded beaches of Brighton and Coney Island, and Mrs. Stahl's (under the Brighton Beach subway station on Coney Island Ave and Brighton Beach Ave) and Shatzkin's (on the Boardwark in Coney Island around W. 38th St.? The same guys with the paper shopping bags with knishes in one hand were hefting a huge metal cooler on their other shoulder, picking their way

among the jam-packed beaches (I can't even describe these -- ENORMOUS beaches, matched in size hardly anywhere in the world, hundreds of yards from boardwalk to ocean -- SOLIDLY packed with people, a couple of inches between their blankets!) shouting "Hot knishes! Cold drinks!"

We did play a little stickball, and more "Catch a fly is up" (later I heard it referred to as "One-o-Cat") in the street, as well as an odd version of baseball at the spot between our house and the Constantinos' where the narrow driveway on our side was home plate and lined up about midway across from the double driveway of the "rich" (read "attached single family, probably costing about $8000) houses across the street, which were 1st and 2nd bases on the corners. Pitcher stood in the middle, no bat but slapped the red rubber ball with your hand. That ball, a "spaldeen" (I understand because they were the discarded cores of Spalding tennis balls that were without hairy covers) were the mainstay of every game we played. In addition to the above, and a little hopscotch or potsy (is there a difference?), we had "two-box" (= "hit the penny"), "3-box" (also called "box baseball"), and "5-box" (a complex game involving bouncing the ball in each box). Also "off the wall" and Chinese handball (why "Chinese"?). And, of course, the mainstay because you could play it alone or with friends: stoop-ball (catch it on one bounce, 5 points; on the fly 10 points; off a "point", 50 points – it comes at you fast – and off the top step point, 100 points! Or something like that.)

Movies were a quarter for a double-feature, at least on Saturday afternoons (take your sister!). And they were preceded by cartoons. And a nickel bought a candy bar, which went fast. Necco Wafers lasted forever, but had minimal taste. A compromise might be Jujyfruits, Jujubes, or Turkish Taffy, which stuck to your teeth and could be licked out for hours. Maybe that's why I had so many cavities – or maybe it was because, as a dentist pointed out many years later, that NYC fluoridated its water sometime between the eruption of my "6-year molars" (full of cavities) and my "12-year molars" (very few). I do remember the unanesthetized pain of getting them filled, though!

I never had report cards like the guy on the website (I think he went to Catholic school). First of all, they were not A-F. We had (U)nsatisfactory, (N)eeds improvement, (S)atisfactory, and SO (really S^o, with a superscripted degree mark) that meant superior. I got a lot of S's, some SO's, and scarcely ever better than "N" in my two bete noirs, handwriting and Conduct (sometimes called "self-control")! It seems to me that in Albany, when I was younger, we got graded in both "Ability" and "Effort". I will allow readers to decide which of these I received lower grades in.

I think those high schools in the pictures are Boys, Brooklyn Tech, and Erasmus Hall. I went to none of them.

Bedford Ave was "HS row", being home to (south to north), James Madison (my alma mater, and that of 3 current or recent Senators and a Supreme Court justice, several sports figures, and Carol King – then Klein – and Chris Rock -- ok, this picture is from before my time!), Midwood, and Erasmus. Most of the newer HS (not Boys or Erasmus) were "U-shaped"; Tech was a full rectangle, and so big (8 floors!) on each floor than instead of numbering the rooms like 401-435 (as at Madison), they were numbered by corridor (e.g., 4W20).

As far as Coney Island amusements were concerned, when I was a kid Luna Park was gone, but Steeplechase was still there. I never liked the fast or scary or high rides, although decades later my son Matt managed to get himself really sick by riding (alone) the Cyclone several times. I did like Skee-Ball. And shooting. And another big thing was that there were Tuesday night fireworks in Coney Island all summer long, and we mostly watched from the roof of Grandma's building on E. 22d St. I remember when I first discovered somewhere, with pity, that there were places where there were only fireworks once a summer, on July 4.

And, while the website's observation that most people in Brooklyn were Italian, Irish or Jewish might not have been completely true, I remember being quite surprised at some point in adolescence when I discovered there were White Protestants (Christians, but not Catholic and, unlike the non-Catholic Christians I knew, were not Black) -- an idea also expressed (obviously not stolen from me, but I had it way before I read it there) by the mayor-loosely-based-on-Ed-Koch in Tom Wolfe's "Bonfire of the Vanities".

The image of Brooklyn in the popular consciousness, and the memories of those who left it, change with the generations. The "Kid from Brooklyn" was a staple of World War II movies, tough, smart-alecky, and ethnic, often with a Jewish or Italian name. And relatively small, certainly smaller than the big Southern farmboy, another stock character. In the 1950s, when TV was new and based mostly in New York, both the writers and characters were often New Yorkers, and particularly Brooklynites. Shows

like Jackie Gleason's The Honeymooners (Irish working class folks living in an apartment that was sparse by any standard) and The Goldbergs (Jewish, and a little higher level working class) introduced (stereotypical) Brooklyn folks to the rest of the country. There were a lot of smart kids from Brooklyn, many in important jobs all over the country.

And now Brooklyn is hip. Having transformed the winter hotels and apartments of Brooklyn retirees in Miami's South Beach in the last 20 years, young "new" (="often moved from elsewhere") New Yorkers are filling the places those retirees came from, moving from the exorbitant rents of Manhattan and long-gentrified Park Slope to the slightly-less exorbitant rents of Brooklyn, filling the formerly Hasidic area of Williamsburg and the asbestos-shingled houses of Greenpoint and Red Hook with yuppies, and the leading edge now moving into – Bushwick! Of course, nostalgia ain't what it used to be!

Black History Month: Remembering a pioneer in medicine: Dr. Charles Richard Drew

Richard J. Barohn, MD

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Every day, blood transfusions are performed worldwide for patients in need of blood products. They are so routine nowadays that we do not give much thought to the pioneering scientists who made these modern blood transfusions possible; this was not always the case.

One of the scientists who made modern transfusions possible was Dr. Charles Richard Drew (1904 - 1950). Born in Washington DC to a carpet layer and schoolteacher, young Charles Drew was a high school athlete and was also very bright. He was able to obtain a partial scholarship to Amherst College in Massachusetts where he again excelled at sports. However, at a track meet at Brown University in Rhode Island, he and three other black athletes on the Amherst team were not allowed to eat alongside the white athletes in the hotel where they were all housed. Drew never forgot this incident.

After college, he did not have enough money for medical school, so he taught biology and worked as an athletic director at Morgan State College. Eventually he was accepted to McGill University Medical School in Canada where he came under the influence of John Beattie, a British doctor who was interested in the problems of blood transfusions. Drew found Canada very welcoming to African Americans; he excelled both academically and again in sports, leading McGill to several national track championships and graduating near the top of his class. He ultimately became an intern at Montreal General Hospital where he focused clinically in the areas of surgery and emergency room medicine; in the 1930s in the United States, African Americans could only do internships at black hospitals.

While Drew was in medical school, the Nobel prize was awarded to Karl Landsteiner who determined that all persons have one of four different types of blood: A, B, AB and O. Even with this discovery, blood preservation and storage were issues. Even if the right blood type could be found for a patient, by the time the blood typing was done often the blood could no longer be used. The blood would spoil by forming clots. He and Dr. Beattie worked on the blood preservation problem in Canada, and then Drew

Dr. Charles Richard Drew (Image credit: Associated Photographic Services, Inc - National Library of Medicine)

obtained a research fellowship to train at Columbia Medical School in New York City. There, he not only worked on the problem of blood preservation but he also obtained an Sc.D. (Doctor of Science) degree, the first granted to an African American in the United States, and was certified as a surgeon. He became an authority on solving all the technical problems of collecting blood in "blood banks."

In 1942, he patented a device that improved the process of preserving blood; he also discovered that if the blood that was collected from donations was stored in the form of plasma, it could be kept refrigerated for extended periods of time. This discovery became crucial as World War II broke out and more and more blood products were required to keep injured soldiers alive and treat shock from blood loss. At the request of his mentor, Dr. Beattie, who was serving in England as that country was being attacked, the American Red Cross put Drew in charge of a nationwide program to collect and dispatch blood products, particularly plasma, from the United States to Europe. He standardized procedures at all participating hospitals for collecting and processing blood products to avoid contamination.

However, as the project became successful from a technical standpoint, the armed services informed the American Red Cross that "colored" blood would not be acceptable. This resulted in many who protested this policy, but the armed services initial solution was to allow African American blood to be collected but it would be segregated. Drew resigned. A Newspaper headline at the time said: "Negro surgeon, World Plasma Expert, Derides Red Cross Blood Segregation" and printed the following:

"No Negro blood accepted but- When terrible blitz raids of London in September 1940 killed and wounded thousands and an emergency call went out to America for dried blood for transfusions, it was an American Negro surgeon to whom English medical men appealed to organize and send U.S blood plasma overseas."

Drew was quoted at the time in a newspaper to say: "The question arises- is there a difference between blood of different races? Is it possible to transmit the traits and characteristics of one race to a member of another race by means of blood transfusion?... One can say without hesitation that no difficulties have been shown to exist between the bloods of different races which would in any way counter-medicate the use of the blood from one individual of one race to an individual of another race for the purpose of transfusion providing the bloods were of the same group. There are many who have a real fear born of ignorance that the blood of a Negro carries with it the possibility of their offspring having dark skin and other characteristics of the Negro race. Only extensive education, continued wise government and an increasing fight on our part to disseminate the scientific facts and raise our levels of achievement can overcome this prejudice which to a large extent is founded on ignorance."

Eventually the armed services and the American Red Cross cancelled the policy. But the experience discouraged Drew and he ultimately returned to full time surgical practice. After the war, Drew practiced and taught at Howard University where he trained many black surgeons. He also continued to fight for equal treatment in the field of medicine for African Americans. He fought the American Medical Association policy to not accept African Americans into that society and he created a separate group, the National Medical Association to which most black physicians eventually joined. The AMA never allowed black people to enter during Drew's lifetime.

Today, Dr. Drew is universally deemed the "Father of the Blood Bank." (Image credit: National Portrait Gallery, Smithsonian Institution; gift of the Harmon Foundation)

Charles Richard Drew unfortunately had an early and untimely death. He was invited to attend a medical conference at the Tuskegee Institute in Alabama. He took three young black surgical residents as he wanted to experience the conference, but the residents could not afford the train fair so Drew drove them in his car. Driving late at night, Drew's car veered off the road and crashed. Drew was killed but fortunately the three residents were unharmed. So, the next time you see or hear about a patient getting a blood transfusion, think of Dr. Charles Richard Drew.

Timing of Decremental Response During Repetitive Nerve Stimulation in Myasthenia Gravis

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ABSTRACT

Introduction: A decrement >10% detected during repetitive nerve stimulation (RNS) is supportive of considering a diagnosis of myasthenia gravis (MG). Several studies have found that most of this decrement is seen between 4 to 6 min post-exercise. However, there is limited literature looking at whether shorter timing and lower cuttoff would be sufficient. This study aimed to evaluate if RNS up to 2 min post-exercise is adequate to detect a decrement response > 10% as well as calculate sensitivities and specificities using different cutoff values (>9%, >8%, >7%, and >6%) for abnormal decrement. **Methods:** A retrospective review of RNS and serology data between 2013 to 2017 in patients with and without MG was performed at The University of Kansas Medical Center. According to positive serology and electrodiagnostic testing, patients were divided into control and MG groups. **Results:** 76 patients with MG and 100 controls were identified. An abnormal decrement was detected in 95% of MG patients within 2 min post-exercise. Also, using cutoff values ≥ 9% on facial and accessory nerves and ≥ 7% on the ulnar nerve maintained specificities ≥95%, and sensitivities increased from 30% to 37%, 36% to 62%, and 21% to 41%, respectively. *Conclusions:* RNS up to 2 min post-exercise might be sufficient to detect a significant decrement in MG patients. Also, lowering cutoff values increases RNS sensitivity, maintaining or slightly decreasing specificity.

Keywords: *Myasthenia gravis, Decrement, Repetitive nerve stimulation, Sensitivity, Specificity*

Introduction

Repetitive nerve stimulation (RNS) and single-fiber electromyography (SFEMG) are electrodiagnostic testings used for the evaluation of myasthenia gravis (MG) (1). SFEMG is the most sensitive test for the assessment of suspected MG cases, but RNS is used more widely due to its availability, rapidity, non-invasive nature, and high specificity (>95%) (2, 3).

RNS comprises 5 to 10 trains of supramaximal stimuli at low frequencies (2–3 Hz) during the recording of compound muscle action potentials (CMAPs) at baseline, immediately after 60 s of exercise, and at 1, 2, 4, and 6 min post-exercise. A decrement >10% at any stimuli has been considered positive to diagnose neuromuscular-junction disorders, including MG. Several studies have suggested an evaluation of up to 6 min because the most significant decrement is seen between 2 to 4 min after exercise (4). However, studies looking at whether shorter timing would provide identical information are lacking.

The sensitivity of RNS varies according to MG severity, muscle tested, and cutoff values used (5, 6). Some studies have been performed to propose "ideal" cutoff values. Still, many of them have failed due to small sample sizes or technical issues that have restricted the analyses of electrodiagnostic data (7). Historically, most neuromuscular teams have set up a decrement >10% as a cutoff for MG (3). Nevertheless, a lower cutoff could be used due to the precision of modern equipment, according to other studies (8, 9).

This study aimed to ascertain if RNS up to 2 min post-exercise is sufficient to detect an abnormal decrement in MG patients. We also aimed to determine different sensitivities and specificities when the cutoff is lowered (9% to 6%) in commonly tested nerves.

Methods

After obtaining IRB approval, a retrospective chart review of patients referred for evaluation of symptoms suggestive of MG was carried out at the University of Kansas Medical Center from January 2013 to September 2017. Patients were identified from a database of neuromuscular diseases using the tenth revision of the International Statistical Classification of Diseases and MUS-codes. Demographic, clinical, serologic, and electrodiagnostic information was extracted. RNS was obtained in all these patients and was performed by either two of our EMG technicians. Also, some of them underwent SFEMG according to each clinician's criteria and were performed by the same neuromuscular specialist, including

the interpretation of the results. Patients with symptoms suggestive of MG with either + AChRAb, + muscle-specific kinase - MuSK, or + low-density lipoprotein receptor-related protein 4 - LRP4, + RNS, or + SFEMG were assigned to the MG group. These patients were further divided into ocular MG (OMG) and generalized MG (GMG) if weakness involved only ocular muscles or other muscle groups. Patients with both negative serology and electrodiagnostic testing (RNS and SFEMG) were included in the control group.

Standard testing methods were used during RNS evaluation in our center (4). Five to ten trains of supramaximal stimuli at a rate of 3 Hz were applied to facial, ulnar, and accessory nerves after placing an active surface electrode (E1) on the belly of the assessed muscle and referential electrode (E2) over the tendon of the same muscle. Stimulation was applied while recording CMAPs at rest, immediately after 60 s of exercise, and then at 1, 2, 4, and 6 min post-exercise (4, 10). An abnormal RNS result was considered when the amplitude between the fifth stimulus compared with the first stimulus exhibited a decrement greater than 10% (4).

We analyzed the timing of significant decrement after RNS overall and across facial, ulnar, and accessory nerves. In addition, we calculated the sensitivities and specificities using abnormal decrement cutoff values of 9%, 8%, 7%, and 6% for facial, ulnar, and accessory nerves in MG, OMG, and GMG groups at baseline and pre/post-exercise.

Statistical analysis was performed using Microsoft Excel 2016 and MedCalc software 2018. Frequencies and percentages represented gender, clinical, serologic, and electrodiagnostic information in non-MG, OMG, and GMG groups. For ages, mean ± standard deviation (SD) was used. T-test and chi-square test were used to compare numerical and categorical variables, respectively, between OMG and GMG patients.

Results

One hundred and seventy-six patients were identified, of which 76 were diagnosed with MG, and 100 were assigned to the control group. Demographic data, serologic status, electrodiagnostic testing in the MG, MG subtypes, and control groups are shown in table 1.

All patients in the control group were tested for AChRAb and RNS. Sixty percent were tested for MuskAb, and 40% had SFEMG performed. The highest decrement response was ten seen in 1 patient when the accessory nerve was stimulated at 2- and 6-min post-exercise; however, SFEMG was not performed. In addition, 17 patients had a decrement response of 9, which was more prevalent when the accessory nerve was stimulated, with the earliest response at baseline in 12 patients.

Overall for MG patients, 71% (54 patients) were positive for AChRAb, 51% (39 patients) had abnormal RNS, and 17 out of 19 had abnormal SFEMG. In the patients with abnormal SFEMG, 5 had abnormal RNS. One was seropositive with decremental response <7% in the facial nerve. The remaining patients, eleven, were seronegative with normal RNS. Conversely, the two patients with normal SFEMG had decremental response >10%, and one of them was also seropositive. Twenty patients with OMG and 48 with GMG were identified. Age (56 ± 14.4 *vs.* 63 ± 13 years) and gender (57% *vs.* 32% women; 43% *vs.* 67% men) differences were seen in the MG vs. control group. The presence of AChRAb between OMG and GMG was statistically significant (54% *vs.* 81%; p=0.0129).

For OMG patients, 54% had abnormal RNS and 54% were positive for AChRAb, with overlapping results in 36%. One patient was positive for LRP4 with negative RNS and SFEMG. SFEMG was abnormal in 10 out of 11 patients. For GMG patients, 50% had abnormal RNS, and 81% had positive AChRAb with overlapping results in 40%. One patient who was positive for MuSK had abnormal RNS. Seven out of 8 patients had abnormal SFEMG, and two had

Table 1. Demographic, clinical, serologic, and electrodiagnostic information of MG, MG subtypes, and control groups

	Control (n=100)	MG (n=76)	OMG *(n = 28)*	GMG *(n = 48)*	*P*-value
Age	51.85 ± 14	61.44 ± 15	63 ± 13	61 ± 16	0.521
Women	56 (56%)	25 (33%)	6 (21%)	19 (40%)	0.0914
Men	44 (44%)	51 (67%)	22 (79%)	29 (60%)	0.0914
Ocular Symptoms	NA	NA	28 (100%)	44 (92%)	0.1271
Generalized Symptoms	NA	NA	NA	48 (100%)	NA
AChRAb	0/100	54/76 (71%)	15/28(54%)	39/48 (81%)	0.0129
RNS	0/100	39/76 (51%)	15/28 (54%)	24/48 (50%)	0.7381
SFEMG	0/39	17/19 (89%)	10/11 (91%)	7/8 (88%)	0.8360

Note: NA = Not Assessed

abnormal RNS. Abnormal RNS, AChRAb, and SFEMG were not seen simultaneously in OMG and GMG groups.

The prevalence of significant decrement obtained for stimulation of facial, ulnar, and accessory nerves was 24%, 25%, 28% for OMG patients and 34%, 20%, 41% for GMG patients, respectively. The first significant decrement response was detected within 2 min post-exercise in 95% (37 patients) of MG patients, and 62% (24 patients) were observed at baseline. In two patients, an abnormal response was detected after 2 min post-exercise; both cases were positive for AChRAb. The prevalence of the first significant decrement detected in MG, OMG, and GMG groups at various time points is shown in Table 2.

RNS sensitivity increased, and specificity decreased as cutoff values decreased in all the MG and MG subgroups. Using cutoff values >9% on facial and accessory nerves and >7% on the ulnar nerve maintained specificities ≥95%, and sensitivities increased from 30% to 37%, 36% to 62%, and 21% to 41%, respectively. The different sensitivities and specificities for the MG, OMG, and GMG groups using cutoff values between >10% to >6% are shown in Table 3.

Discussion

Different authors have suggested an assessment of up to 6 min during RNS based on maximal decrement as this may be seen between 2 to 4 min after exercise. This effect is called "post-exercise exhaustion" and consists of a depression in end-plate excitability after maximum voluntary contraction or tetanic stimulation (4).

Our study found that 95% of MG patients had a decremental response >10% within 2 min post-exercise. Significantly, 62% of them had this decrement pre-exercise. Only two patients had a significant decrement after 2 min post-exercise, and they both were positive for AChRAb.

Table 2. Frequencies of the first decremental response >10% seen in MG, OMG, and GMG patients

Time	Baseline	Imm. post	1 min	2 min	4 min	6 min
MG (n=39)	24(62%)	2(5%)	6(15%)	5(13%)	1(2.5%)	1 (2.5%)
OMG (n=15)						
Facial (n=6)	4 (80%)	0%	0%	2 (33%)	0 %	0%
Ulnar (n=4)	2 (50%)	0%	1 (25%)	0%	0%	1 (25%)
Accessory (n=5)	2 (40%)	0%	2 (40%)	1 (20%)	0%	0%
GMG (n=24)						
Facial (n=12)	9 (75%)	0%	2 (17%)	0%	1 (8%)	0%
Ulnar (n=8)	7 (88%)	1 (12%)	0%	0%	0%	0%
Accessory (*n=12*)	7 (58%)	1 (8%)	2 (17%)	2 (17%)	0%	0%

Table 3. RNS sensitivity and specificity using different decrement cutoff values in MG (*n* = 48), OMG (n=15), and GMG (n=33) patients

	Facial nerve		**Ulnar nerve**		**Accessory nerve**	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
>10%						
MG	30%	100%	21%	100%	36%	100%
OMG	24%	100%	25%	100%	28%	100%
GMG	34%	100%	20%	100%	41%	100%
>9%						
MG	33%	100%	25%	100%	43%	99%
OMG	32%	100%	30%	100%	33%	99%
GMG	34%	100%	22%	100%	48%	99%
>8%						
MG	37%	89%	31%	98%	62%	81%
OMG	36%	89%	35%	98%	56%	81%
GMG	37%	89%	29%	98%	66%	81%
>7%						
MG	43%	82%	33%	95%	70%	74%
OMG	40%	81%	35%	95%	67%	74%
GMG	46%	82%	32%	95%	72%	74%
>6%						
MG	52%	74%	41%	89%	77%	66%
OMG	48%	74%	40%	89%	67%	66%
GMG	54%	75%	41%	89%	83%	66%

Decrement was also seen immediately post-exercise in two patients, which is unusual. However, after reviewing the waveforms, low recruitment was noted at baseline, and decrement >10% was also present at various time points immediately post-exercise. Our findings are inconsistent with the available data in the literature, which states that the most significant decrement is detected after 2 min post-exercise (4).

In OMG patients, 100% had abnormal responses within 2 min postexercise in the facial nerve, which correlates with the involvement of ocular muscles. The prevalence of significant decrement >10% obtained for stimulation of facial, ulnar, and accessory nerves were 24%, 25%, and 28% for OMG patients, and 34%, 20%, 41% for GMG patients, with not much difference between these two MG subgroups. It is also impressive that decremental response in the ulnar nerve was higher in OMG than GMG. Unfortunately, we were unable to find studies with similar findings to compare with. We assumed that some OMG patients probably had involvement of other muscles different from the ocular ones. Still, clinical involvement may have been so mild that it was imperceptible to them, or it could be related to subtle variabilities in the RNS technique by our EMG techinicias. In addition, it is unclear why some patients with positive serology and RNS underwent SFEGM as this was decided per each clinician in our neuromuscular department.

We also calculated the sensitivities and specificities of RNS using different cutoff values. Overall, sensitivity between 30% to 80% and specificity from 90% to 100% when using a cutoff value >10% have been previously reported (5)(9). Similar findings were seen in our study except for sensitivity in the ulnar nerve, which was lower than usual, 21%. We found that sensitivity increased and specificity maintained or slightly decreased as cutoff values decreased from 10% to 6%. Similar findings were seen by *Lamb et al.* (12). The sensitivity of RNS can vary according to the distribution or severity of MG (7), and using lower cutoff values may be feasible due to better equipment precision nowadays (8). Abraham *et al.* (9) recommended an optimal cutoff value between 7% to 8% for facial stimulation because sensitivity increased, and specificity remained >90%. Similar findings were seen in our study. In MG patients, cutoff values >9% for facial and accessory nerves and >7% for ulnar nerve were associated with specificities ≥95% with increased sensitivities at these points. Looking at OMG and GMG subgroups separately, similar results were seen.

Our study has several limitations. Sampling bias may be seen due to our study's retrospective nature. Also, we did not include the severity of MG in our cohort, which might influence the sensitivities seen across the different nerves tested. A positive correlation between RNS testing and MG severity was reported previously (11). Also, the size of our cohort with abnormal RNS was small. Finally, we did not evaluate RNS according to muscle groups. Sensitivity and specificity may change according to the muscles assessed.

In conclusion, we found that 2 min of post-exercise testing could be sufficient to detect a significant decrement for MG diagnosis. We propose shorter timing for RNS testing during MG evaluation. In addition, a cutoff <10% could be used for MG evaluation. Accepting a cutoff >9% for facial and accessory nerves and >7% for the ulnar nerve maintain specificities >95% accompanied by an increment in sensitivities for MG. However, further prospective studies will be necessary to confirm our findings.

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Screening for Depression in Myasthenia Gravis

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ABSTRACT

Introduction: There are conflicting reports of depression prevalence in myasthenia gravis (MG), and the influence of somatic symptoms on screening assessments is not clear. We investigated the frequency of somatic and non-somatic symptoms of depression in MG. We also explored the relationship between depression and MG using disease severity and quality of life measures.

Methods*:* Three cohorts of participants (MG, autoimmune disease, and healthy controls) were prospectively assessed with the Beck Depression Inventory 2 (BDI-II) and BDI-Primary Care (BDI-PC) surveys, modified Rankin Scale, MGFA classification, MG-MMT, MG-ADL and MG-QOL15.

Results: A total of 31 MG, 29 disease controls, and 30 healthy controls were enrolled. Depression frequency indicated by BDI-II in MG [48% (15/31)] and disease control [31% (9/29)] was not significantly different [p=0.1968]. However, we found a significantly higher frequency than healthy controls 10% (3/30) [p=0.0016]. In contrast, depression frequency indicated by BDI-PC was similar in the MG 29% (9/31) and disease controls MG, 24% (7/29) [p=0.7737] as well as the healthy controls 10% (3/30) [p=0.1056]. Scores on BDI-II and BDI-PC were strongly correlated (Spearman r=0.8728, p<0.0001).

Using the BDI-II scale, participants with MG who were depressed had higher scores on MG-ADL, and MG-QOL15 than those who were not depressed. The difference in MG-ADL and MG-QOL15 scores remained significant using the BDI-PC score.

Discussion: These findings suggest depression screening assessments that include physical symptoms could overestimate depression in MG and chronic autoimmune neuromuscular disorders. Yet, a higher frequency of self-reported depression is associated with increased disease severity and lower quality of life even when somatic symptoms were excluded (BDI-PC).

Keywords: *Myasthenia gravis, Depression, Beck Depression Inventory-II (BDI-II), Beck Depression Inventory-Primary Care (BDI-PC)*

Introduction

Myasthenia gravis (MG) is a chronic autoimmune disorder characterized by fatigable muscle weakness [1]. MG typically presents with weakness of specific muscle groups including eyelids, extra-ocular muscles, bulbar and limb muscles. Fatigue, tiredness, and lack of energy are common complaints in patients with MG that may be mistaken for depression. Psychological symptoms in MG have been described since 1966 [2]. It has been reported that as many as 20-30% of individuals with MG are initially misdiagnosed with a psychiatric illness [3].

Identification of patients with MG and co-existing depression is important for optimal management. This recognition is particularly important to help avoid unnecessary use of immunosuppressive medications. Several studies have evaluated the prevalence of depression in patients with MG; however, different screening tools were used to establish the diagnosis of depression in each of these studies [4-10]. The various scales used differed in ability to capture and differentiate the affective or mood-related symptoms of depression from physical symptoms of depression. Several scales include questions on fatigue, tiredness, exhaustion, sluggishness, loss of energy and sleep problems, resulting in conflicting conclusions about the prevalence of depression in patients with MG.

Despite some of the studies that reported no increase in depression compared to the general population [6], the majority of the available data support an increased frequency of depression in myasthenia [5, 7, 8, 10, 11]. However, it is unclear if this is independent of somatic symptoms that may be attributable to MG disease activity.

In clinical practice, there is a need for a tool that is valid and easily administered to screen MG patients for the presence of depression. Exhaustive and lengthy psychological tests are unlikely to be performed. Furthermore, understanding whether there is an association between psychiatric symptoms and severity of MG may inform disease management. There are conflicting results regarding whether patients with more severe disease and those treated with immunosuppressive medications are more likely to have mood disorders [12, 13].

The primary aim of this study was to address whether there are differences in the frequency of depression and physical symptoms of depression in patients with MG compared to other chronic autoimmune neuromuscular disorders and healthy controls. We used two screening depression assessments, the Beck Depression Inventory

2 (BDI-II) and Beck Depression Inventory for Primary Care (BDI-PC) self-reported tools. The BDI-PC is a short screening scale independent of physical function that contains a subset of 7 questions from the standard BDI-II, which assess only non-somatic complaints of depression [14]. BDI-PC, also referred to as Beck Depression Inventory—Fast Screen (BDI—FS), is validated against depression diagnosis based on DSM-IV criteria obtained using the Mood Module from the Primary Care Evaluation of Mental Disorders, self-reported depression, and other instruments such as the Patient Health Questionnaire and Neuropsychiatric Inventory. BDI-PC was shown to be reliable and valid in multiple medical illnesses such as end-stage renal disease, pain clinic populations, chronic fatigue syndrome, Parkinson's disease and multiple sclerosis [15-18]. A secondary aim was to explore the relationship between depression and disease severity and quality of life in patients with MG.

Materials and Methods

Study Overview

This was a prospective cross-sectional study performed at The Ohio State University Wexner Medical Center Neuromuscular Clinics. This study was approved by The Ohio State Wexner Medical Center Institutional Review Board, and all participants signed a written consent form. Patients were recruited from the Ohio State University Wexner Medical Center Neuromuscular Clinics. Participants enrolled included adult patients age 18 or older in three different cohorts (MG, disease controls and healthy controls). MG participants were required to have a diagnosis of MG established based on typical clinical picture and positive serological tests and/or decrement on repetitive nerve stimulation or increased jitter on single fiber EMG. Disease control participants had a chronic autoimmune neuromuscular disorder specifically immune-mediated neuropathy or inflammatory myopathy. MG and disease-control participants were recruited from patients seen in the outpatient clinic setting. Individuals with no history of neuromuscular or neurological disease were enrolled as healthy controls. Controls were recruited from the families of potential subjects. Participants were excluded if they were cognitively impaired as judged by the neurologist, unable to provide consent, or had a known psychiatric disorder other than depression.

Participant Assessments

Participant demographics, immunomodulatory treatments, and disease duration were recorded. To assess for the presence of depression, participants completed two screening surveys, the BDI-II and BDI-PC. BDI-II is a self-reported inventory with 21 items, each scored from 0 to 3 [7, 19]. The total score was subdivided to indicate whether the depression was mild (score of 14-19), moderate (score of 20-28) or severe (score of 29—63) [20]. BDI-PC contains a subset of 7 questions from the standard BDI-II, which assess for non-somatic complaints of depression [14]. Items include symptoms of sadness, pessimism, past failure, loss of pleasure, self-dislike, self-criticalness and suicidal thoughts or wishes. BDI-PC score > 4 is considered positive.

We used modified Rankin scores to stratify patients in terms of overall disability.

The modified Rankin score measures the degree of disability and dependence in patients with neurological disorders. Participants with MG were classified using the Myasthenia Gravis Foundation of America (MGFA) classification, and all participants completed manual muscle testing, modified Rankin Scale, MG-Activities of Daily Living (MG-ADL) scale, and MG-Quality of Life (MG-QOL15) scale.

Statistical Analyses

Statistical analyses were performed using Prism version 8.4.2 (Graphpad, San Diego, CA). Descriptive statistics including mean and standard deviation were determined and shown as mean ± standard deviation (SD). Unpaired t-test was for two group comparisons (MG vs Disease controls, MG vs Healthy controls, and Depressed vs Non-depressed MG participants). Frequencies were compared between groups (MG vs Disease controls, MG vs Healthy controls, and Depressed vs Non-depressed MG participants) using Fisher's exact test. Similarly, frequencies of positive screening for depression on the BDI-II and BDI-PC in MG patients, when stratified by sex, were compared using Fisher's exact test. Spearman correlation coefficient was calculated to investigate the relationships between the BDI-II and BDI-PC scores in patients with MG. A p-value <0.05 was considered significant.

Results

A total of 90 participants were enrolled: 31 MG, 29 disease controls, and 30 healthy controls. The study was conducted over a 10-month period. Demographics are detailed in (**Table 1**). MG participants included 17 males and 14 females with a mean age of 56±17 years. MGFA classification at the time of evaluation was as follows: grade 1=8 participants, grade 2=20 participants, and grade 3=3 participants. Average disease duration at the time of evaluation was 10 years, and the majority (93%) of MG participants were on immunosuppressant or immunomodulatory treatment. These included prednisone,

Table 1. Characteristics of enrolled patients.

	MG N=31	Disease-control N=29	MG vs. Disease-control (p value)	Healthy Control N=30	MG vs. Healthy Control (p value)
Age (years± SD)	56±17	51±15	0.2314	42±13	0.0010
Sex (women/men)	17/14	21/8	0.0.0396	22/8	0.0374
Disability (mRS)	1.7±1.1	1.9±1.1	0.4394	0.03±0.18	<0.0001
Disease duration (years ± SD)	10.45±9.7	6.7±8.3	0.1168	0/30 (0%)	
Percentage on immunomodulatory meds	28/31 (93%)	29/29 (100%)	0.2381	0/30 (0%)	
Percentage on prednisone	24/31 (77%)	11/29 (37%)	0.0109	0/30 (0%)	
Percent with depression based on BDI-II scores	15/31 (48%)	9/29 (31%)	0.1968	3/30 (10%)	0.0016
Percent with mild depression based on BDI-II score	6/31 (19%)	6/29 (21%)	>0.9999	3/30 (10%)	04729
Percent with moderate depression based on BDI-II score	6/31 (19%)	2/29 (7%)	0.2566	0/30 (0%)	0.0240
Percent with severe depression based on BDI-II score	3/31 (10%)	1/29 (3%)	0.6128	0/30 (0%)	0.2377
Percent with depression based on BDI-PC score	9/31 (29%)	7/29 (24%)	0.7737	3/30 (10%)	0.1056
Patients on prednisone with depression based on BDI-II score	12/24 (50%)	4/11 (36%)	0.4928		
Patients on prednisone with depression based on BDI-PC score	6/24 (25%)	3/11 (27%)	>0.9999		

azathioprine, mycophenolate, cyclosporine, tacrolimus, intravenous immunoglobulins, and plasmapheresis. The disease controls included 13 patients with inflammatory muscle disease, 11 with CIDP, 4 with multifocal motor neuropathy and 1 with idiopathic neuropathy. All were using an immunosuppressive or immunomodulatory treatment.

There were no significant differences between the MG and disease control participants for age, overall disability using the modified Rankin scale, and disease duration, but there was a higher frequency of women in the disease control group and more patients with MG were on prednisone. Comparing the MG and healthy participants, in the healthy control group there was a higher frequency of women and the participants were younger.

Using the BDI-II scale, depression frequency was similar in MG patients 48% (15/31) and disease control 31% (9/29) but higher than normal controls [p=0.001] (Table 1). In contrast, using the BDI-PC scale, which assesses the affective component of depression, there was no significant difference in MG patient depression frequency compared to disease or healthy controls (**Table 1**). The scores on the BDI-II and BDI-PC showed strong correlation (Spearman r=0.8728, p<0.0001). The majority of MG (80%) and disease control (57%) patients were on prednisone, and at the time of this study, more MG patients were on prednisone as compared with disease controls. When comparing only the MG and disease control patients that were treated with prednisone, the rates of depression in MG were slightly higher, though not significantly, on BDI-II and were similar on BDI-PC.

The scores of the BDI-II defined depression suggests a severity in the moderate to severe range in 60% (9/15) of MG patients, compared to 33% (3/9) of disease controls. Of the MG patients who screened positive for depression using BDI-II, (8/15) 53% were on antidepressant medications at the time of enrollment compared to (3/7) 42% of the disease control. We also explored depression screening when MG patients were stratified by sex to determine the frequencies of depression on the BDI-II and BDI-PC between men and women. On the BDI-II, 6 of 14 (43%) men screened positive for depression and 9 of 17 (53%) of women screened positive for depression (Fisher's exact test, p= 0.7224). On the BDI-PC, 3 of 14 (21%) screened positive for depression and 6 of 17 (35%) of women screened positive for depression (Fisher's exact test, p= 0.4564).

In the MG cohort, clinical characteristics were compared between participants with depression and

Table 2. The differences between depressed and non-depressed patients with myasthenia gravis using the Beck Depression Inventory-II (BDI-II) screening tool.

	MG depressed BDI-II N=15	MG non-depressed BDI-II N=16	p-value
Age (mean ± SD) years	57±12	55±21	0.7086
MG-MMT (range 0-120)	9.6±10.3	4±5.3	0.0608
MG-QOL 15 (range 0-60)	24.3±12.6	12±9.8	0.0046
MG-ADL (range 0-24)	7.5±3.8	4.12±3.3	0.0117
Disease duration (years)	11.9±10.5	9.2±8.6	0.4367
Functional status (mRS)	2.1±0.8	1.1±1.0	0.0042

Table 3. The differences between depressed and non-depressed patients with myasthenia gravis using Beck Depression Inventory-Primary Care (BDI-PC) as screening tool.

	MG depressed BDI-PC N=9	MG non-depressed BDI-PC N=22	p-value
Age (Mean ± SD) years	56±12	56±19	0.9469
MG-MMT (range 0-120)	11.1±12.3	5.3±6.3	0.0953
MG-QOL 15 (range 0-60)	27.6±11.1	15.1±14	0.0095
MG-ADL (range 0-24)	8.5±3.2	4.9±3.7	0.0179
Disease duration (years)	13.2±12.4	9.3±8.4	0.3176
Functional status	2.2±0.8	1.4±1.1	0.0793

Table 4. Comparison of MG patients with depression on BDI-II and BDI-PC versus BDI-PC only

	MG depressed BDI-II and BDI-PC N=9	MG depressed on BDI-PC only N=6	p-value
Age (Mean ± SD) years	56±12	60±14	0.5593
MG-MMT (range 0-120)	10±13	9±8	0.8674
MG-QOL 15 (range 0-60)	27.7±11.1	23.33±12.4	0.4917
MG-ADL (range 0-24)	8.6±3.2	7.2±4.3	0.4854
Disease duration (years)	13.2±12.4	9.5±8.57	0.5361
Functional status	2.2±0.8	2.3±0.8	0.8027

without depression per the BDI-II (**Table 2**) and the BDI-PC (**Table 3**) surveys. When stratified by the BDI-II, age and disease duration were similar in the depressed and non-depressed MG participants, but participants with MG depression had worse scores on MG-ADL, MG-QOL15, and modified Rankin score compared to those who were not depressed (**Table 2**). Also, participants with depression on the BDI-PC showed worse scores for MG-ADL and MG-QOL15, but modified Rankin scores were not significantly different (**Table 3**). Furthermore, we compared clinical characteristics in MG patients with depression on both the BDI-II and BDI-PC versus the group of MG patients with depression on the BDI-PC only which demonstrated no significant differences between the two groups (Table 4).

Discussion

The signs and symptoms of neuromuscular disorders, including MG, may overlap with somatic symptoms of depression. Thus, we hypothesized that such symptoms could be misconstrued as symptoms of depression and could impact depression screening tools. Therefore, we investigated two formats of the BDI, the BDI-II and the BDI-PC.

We found an increased frequency of depression in patients with MG and autoimmune neuromuscular disease controls compared to healthy controls using a BDI-II scale that combines affective and somatic symptoms of depression. In contrast, using the BDI-PC, a tool that exclusively assesses the affective (non-somatic) symptoms of depression, depression frequencies in MG and disease controls, while still higher were not statistically significant.

Most prior studies have suggested that there is an increased frequency of depression in patients with MG as compared with the general population [5, 7, 8, 10, 11].

In our study, we found similar frequencies of depression in participants with MG as compared with other autoimmune neuromuscular disorders. This is aligned with a prior study by Stewart and colleagues [8]. In this study, the frequency of depression in patients with MG was compared to a control group that included mainly non-immune mediated neuromuscular disorders [8]. Our study included control participants with chronic autoimmune neuromuscular diseases requiring the use of immune based therapies. Similar to our study, the study by Stewart et al. showed a lack of difference in physical symptoms between MG and neuromuscular disease control, but comparison to a control group was not performed.

In our study, a higher percent of MG patients were on corticosteroids as compared with the autoimmune control group. There are several reports of increased incidence of mood disorders in the setting of long-term steroids [21, 22]. When comparing frequencies of depression in patients with MG and autoimmune disorders on prednisone, we found no significant differences, but the percentage of patients treated with prednisone and having depression was higher compared with autoimmune controls on BDI-II. Because of the relatively small sample size of patients treated with prednisone in the MG and autoimmune disease controls groups, an effect of steroids cannot be excluded and requires further study.

Over-representation of somatic symptoms in MG and chronic autoimmune neuromuscular disorders is likely captured by BDI-II scale items, such as lack of energy, tiredness and fatigue. Despite that, we noted that almost half of the patients defined as depressed using BDI-II were not on antidepressant medications at enrollment. This underscores the need for increased vigilance in screening for depression in patients with myasthenia and other chronic neuromuscular diseases. There was no evidence that the disease severity by modified Rankin Scale, a measure of overall degree of disability and independence in daily activities, was different between the MG and disease controls.

Our study also demonstrated association between depression and disease severity but not age or disease duration. In patients with depression on the BDI-II, there were more severe findings on measures of disease severity and disease impairment of activities of daily living and quality of life as well as functional status. Interestingly, on the BDI-PC, similar findings were seen for impact on measures of disease severity and disease impairment of activities of daily living and quality of life, but functional status was similar between depressed and non-depressed participants. The findings are complex and difficult to disentangle in regard to cause and effect. It is likely that worse disease severity is associated with worsening depression, but it is also possible that depression could negatively impact disease activity and outcomes. Thus, these findings deserve further attention in future studies. A future study that investigates an interventional treatment for depression in conjunction with disease-specific outcome measures for function and quality of life of MG could provide important insight.

We conclude that BDI-II and BDI-C are easily administered and valid tools suitable for use in the clinical setting to screen for depression in patients with MG. The finding of significantly high frequency of depression in a cohort of patients with predominately mild to moderate disease severity calls for regular screening for depression in patients with MG. Nonetheless, these results must be interpreted with caution given the limitation related to the small sample size and the need for future investigation for convergent validity in this patient population. Larger

prospective trials are needed to address the effect of treatment of depression in MG on overall measures of motor function and quality of life.

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Abbreviations
Myasthenia Gravis (MG)
Beck Depression Inventory 2 (BDI-II)
Beck Depression Inventory - Primary Care (BDI-PC)
Myasthenia Gravis Foundation of America (MGFA)
Myasthenia Gravis Manual Muscle Testing (MG-MMT)
Myasthenia Gravis Activities of Daily Living (MG-ADL)
Myasthenia Gravis Quality of Life-15 (MG-QOL15)

Conflicts of Interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Characteristics of Triple Seronegative Myasthenia Gravis: A Single Center Experience

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ABSTRACT

Background: There is variability in the literature regarding the characteristics of triple seronegative myasthenia gravis (SNMG) patients. Most studies were performed before LRP4 antibodies were discovered, and characterizations of triple seronegative patients are lacking in the literature.

Methods: We retrospectively investigated patients diagnosed with myasthenia gravis (MG) at Ohio State University from 2009 to 2019. Triple SNMG was defined by a history and examination that was consistent with MG and positive SFEMG, RNS or edrophonium testing, but negative serology for AChR, MUSK, and LRP4 antibodies.

Results: A total of 210 AChR+, 9 MuSK+, 6 LRP4+, 9 double SNMG, and 21 triple SNMG patients were reviewed. Triple SNMG patients required significantly fewer immunosuppressive agents compared with AChR+ patients (p=0.0001) and a trend towards a less frequent history of hospitalizations, myasthenic crises and intubations compared to all antibody positive groups. Triple SNMG patients had a significantly higher frequency of ocular disease (33%) compared to AChR+ patients (13%) (p=0.0250). One triple and one double SNMG patient had thymic hyperplasia and improved after thymectomy. 11 triple SNMG patients had negative genetic testing for CMS.

Conclusion: Our results further elucidate the clinical characteristics of triple SNMG, which include the predominance for ocular disease and a less severe disease course. Although likely rare, investigation for thymic pathology should be a consideration even in SNMG, and thymectomy should be considered when there are thymic abnormalities on imaging. We did not find alternate diagnoses in SNMG patients and thus ancillary testing should be considered in carefully selected patients for cost-effective care.

Introduction

Myasthenia Gravis (MG) is an autoimmune neuromuscular junction (NMJ) disorder affecting roughly 14-40 per 100,000 individuals in the United States. Acetylcholine receptor (AChR) autoantibodies were discovered in 1973 and are found in about 80% of MG patients [1,2]. In 2001, muscle-specific kinase (MuSK) antibodies were described, accounting for another 7-15% of generalized MG patients [3]. Lipoprotein receptor-related protein (LRP4) antibodies were found to be likely pathogenic in 2011 and recently became available for commercial testing [4]. The percentage of patients with double (i.e. negative testing for AChR and MuSK antibodies) seronegative myasthenia gravis (SNMG) with elevated titers of LRP4 antibodies has varied depending on the population studied, ranging from 2-50% [4-6]. The remaining population that is negative for AChR, MuSK and LRP4 autoantibodies is referred to as triple SNMG. For some of these triple SNMG patients, it may be that either the assay is not sensitive enough to identify the antibody or other disease-causing antibodies have not yet been identified [7-9].

In comparison to AChR or MuSK antibody positive patients, those with SNMG have been found to have overall less severe disease, sometimes making diagnosis more challenging [8,10]. Bulbar and respiratory muscle involvement are less frequent, and a thymoma is rarely found [11]. Electrophysiological abnormalities of NMJ transmission have been found to be more severe in seropositive compared to seronegative patients [12,13]. Further compounding the diagnostic challenge, congenital myasthenic syndromes (CMS) can also closely mimic SNMG [14,15].

The majority of studies that have evaluated the characteristics of patients with SNMG were performed prior to routine testing of LRP4 antibodies. Therefore, in this retrospective study, we sought to compare seropositive MG versus triple SNMG patients seen at The Ohio State University Wexner Medical Center (OSUWMC) in a ten-year span to advance the understanding of triple SNMG.

Methods

All patients seen at OSUWMC with an ICD-10 code diagnosis of MG from March 1, 2009 to June 30, 2019 were retrospectively reviewed. Cases were included if the patient was 18 years of age or older and had at least one of the following: positive antibodies (AChR, MuSK, or LRP4), a positive single fiber electromyogram (SFEMG),

a positive repetitive nerve stimulation (RNS), or a positive edrophonium test. Antibody positive MG was defined on the basis of elevated AChR, MuSK or LRP4 antibody titers. Triple SNMG was defined by a history and examination that was consistent with MG, positive NMJ testing (SFEMG, RNS or edrophonium testing), and negative testing for AChR, MUSK, and LRP4 antibodies. Data was collected through Research Electronic Data Capture (REDCap) and included patient demographics, clinical characteristics, and assessments. The clinical characteristics included age of symptom onset, time from onset to diagnosis, muscles affected, presenting symptoms, history of hospitalizations, history of myasthenic crisis, history of intubations, immunosuppressive agents used, history of thymectomy, and history of autoimmune disease. Results of physical examination, antibody testing, chest computed tomography (CT), electromyogram (EMG), RNS, SFEMG, and congenital myasthenia panel testing were reviewed. The congenital myasthenia panel included 26 mutations known to cause CMS. Ohio State's review board IRB approved this study. The review committee waived the requirement for written informed consent.

Graphpad Prism (ADD version) was used for all analyses. Descriptive statistics were calculated to summarize the groups (AChR, MuSK, LRP4, and SNMG). Continuous data were compared between groups using one-way ANOVA with Dunnett's multiple comparisons tests to compare the mean of the AChR, MuSK, and LRP4 versus the mean of the SNMG. To compare frequency data, Fisher's exact test was used to compare SNMG versus each seropositive group (AChR, MuSK, and LRP4).

Results

A total of 210 AChR+, 9 MuSK+, 6 LRP4+, 9 double SNMG, and 21 triple SNMG patients were identified and reviewed. A total of 16 patients were excluded due to negative antibody testing and either negative NMJ testing or having a clinical presentation that was atypical for a NMJ disorder. There were four patients with positive SFEMG testing that were excluded because the clinical presentation was either more consistent with an alternate diagnosis or was unclear. In these four patients, the alternate diagnoses included steroid myopathy, dropped head syndrome and CMS. One patient had an unclear diagnosis. Table 1 summarizes the demographics and characteristics of the included patient cohorts. The mean age of onset for triple SNMG patients was 52 and did not differ significantly from antibody positive patients. The average time from symptom onset to diagnosis was significantly longer in triple SNMG compared to AChR+ and MuSK+ patients, but did not differ from LRP4+ patients. Triple SNMG patients had a similar female to male ratio compared with antibody positive patients. SNMG patients had a significantly higher percentage of ocular MG compared to AChR+ patients.

Triple SNMG patients required significantly fewer immunosuppressive agents compared with AChR+ patients. SNMG patients also showed a trend towards a less frequent history of hospitalizations, myasthenic crises and intubations compared to all antibody positive groups. MuSK+ patients had the highest rates of hospitalizations, myasthenic crises, and intubations. Bulbar weakness was seen most frequently in MuSK+ patients. Decrement on RNS was most commonly seen in MuSK+ patients, and was seen significantly more often than in patients with triple SNMG.

One triple and one double SNMG patient had thymic hyperplasia. No SNMG patients had a thymoma. One LRP4+ patient had a thymoma. The most commonly associated autoimmune disorder in all patients was Grave's disease (8/255 patients). Seven of these patients were AChR+ and one was double seronegative. Double and triple seronegative patients did not differ significantly in regards to any of the categories listed in Table 1.

Of the 21 triple SNMG patients, eight had AChR antibodies retested and were all negative on repeat testing. 11 triple SNMG patients had negative genetic testing for CMS. No patients had a family history of CMS and most responded well to immunotherapy if they were on immunosuppressive agents. Seven triple SNMG patients

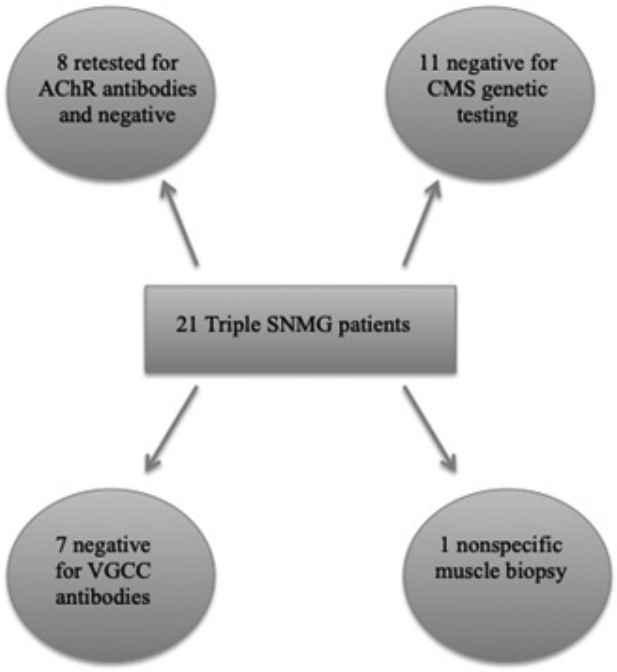

Figure 1. Diagnostic work-up performed in triple SNMG patients.

Table 1. Triple SNMG patients compared to antibody positive patients.

	SNMG (n=21)	AChR+ (n=210)	p-value	MuSK+ (n=9)	p-value	LRP4+ (n=6)	p-value	p-value (all groups)
Age of onset (years)	52	53	0.9743	45	0.7591	32	0.1519	0.1072
Time to diagnosis (years)	7.8	**2.1**	**0.0029***	**0.7**	**0.0433***	8.6	0.9933	**0.0026***
Female	11 (52%)	106 (51%)	0.9999	5 (56%)	0.9999	5 (83%)	0.3497	0.4598
Ocular MG	7 (33%)	**28 (13%)**	**0.0250***	1 (11%)	0.3742	0	0.1548	0.0666
History of MG related hospitalizations	7 (33%)	97 (46%)	0.3581	6 (67%)	0.1232	2 (33%)	0.9999	0.3507
History of myasthenic crisis	5 (24%)	69 (33%)	0.4702	5 (56%)	0.1155	2 (33%)	0.6334	0.4108
History of intubation	3 (14%)	42 (20%)	0.7730	2 (22%)	0.6220	1 (17%)	0.9999	0.9254
# of IS agents currently being used	0.24	**1.03**	**0.0001***	0.44	0.8444	0.83	0.2264	**0.0001***
MGFA classification at last evaluation	1.93	1.98	0.9931	2.29	0.6378	1.75	0.9549	0.6783
Bulbar weakness	2 (9.5%)	30 (14%)	0.7466	3 (33%)	0.1432	0	0.9999	0.2541
Decrement on RNS	7 (37%)	46 (54%)	0.2139	**8 (89%)**	**0.0157***	2 (40%)	0.9999	0.0722
Thymoma	0	28 (13%)	0.0854	0	0.9999	1 (17%)	0.2222	0.2011
Thymic hyperplasia	1 (4.8%)	16 (7.6%)	0.9999	0	0.9999	0	0.9999	0.6990
Race Caucasian African American Asian Hispanic	 20 (95%) 1 (5%) 0 0	 166 (82%) 28 (14%) 7 (3.5%) 1 (0.05%)		 5 (56%) 3 (33%) 0 1 (11%)		 4 (67%) 2 (33%) 0 0		

* $p < 0.05$

IS= Immunosuppressive, MGFA= Myasthenia Gravis Foundation of America

were negative for voltage gated calcium channel (VGCC) antibodies. One triple SNMG patient had a muscle biopsy for further evaluation, and this showed only nonspecific muscle fiber size variability. Figure 1 summarizes the diagnostic work up in our triple SNMG patients.

Discussion

It is important to better characterize and understand the SNMG population to prevent delay in diagnosis, misdiagnosis, and unnecessary testing. The average time from symptom onset to diagnosis in our triple SNMG population was 7.8 years, significantly longer than AChR+ and MuSK+ patients. This can lead to delay in treatment and potentially misguided treatments that can lead to side effects. There have been many theories on why the SNMG population is antibody negative. The leading hypotheses are that the sensitivity of the clinically available assays may be unable to detect the presence of AChR antibodies or that there are antibodies involved in the pathogenesis that are yet to be identified. Radioimmunoprecipitation (RIA) or enzyme-linked immunoassay (ELISA) is the predominant antibody test used to diagnose MG. However, these tests have limited sensitivity of antibody detection due to the antibodies binding poorly to recombinant or soluble antigens [7]. More recently, cell-based assays (CBA) have been developed and have an increased test sensitivity. However, compared to the RIA or ELISA, CBA can be costly and time consuming. CBA also requires access to

tissue culture facilities and staff with expertise in the assays, limiting their use to specialized research centers [7].

In addition to the currently available antibodies, it is likely that additional pathogenic antibodies will be discovered. Recently, antibodies to cortactin have been investigated for their potential role in MG and have been identified in 23.7% of patients with double SNMG [16]. Agrin antibodies inhibit MuSK phosphorylation and AChR clustering in vitro and have been found in both double and triple SNMG patients [6,17].

Our triple SNMG patients overall had less severe disease compared to seropositive patients, similar to what has been previously reported [6,8,10,18,19]. We found that SNMG patients required fewer immunosuppressive agents and trended towards a lower rate of hospitalizations, myasthenic crises, and intubations. Our MuSK+ population had the highest rates of hospitalizations, myasthenic crises, intubations, and highest mean MGFA classification, indicating they had the most severe disease course. This is consistent with prior reports [20].Our results indicate that triple SNMG patients more commonly had isolated ocular MG compared to seropositive patients. Previous literature has reported a wide range (16-55%) of ocular MG in seronegative patients, likely due to studies defining seronegative patients differently in terms of which antibodies were tested [10,21,22]. Our triple SNMG population fell within this range, having 33% ocular MG.

Our triple SNMG patients had a similar age of onset compared to AChR+ patients. There was a bimodal distribution in the AChR+ group but a normal distribution in the SNMG group. The SNMG mean age of onset was 52, similar to what has been reported [6,7,23]. There was a similar female to male ratio. Previous literature has reported an equal female to male ratio [19], while others have reported a slight female predominance [6,8,9].

One double and one triple SNMG patient had thymic hyperplasia. There is a wide range of reported prevalence of thymic hyperplasia in SNMG patients in the literature, ranging from 6-71% [11,24,25]. However, these studies defined SNMG as only AChR-. Despite this wide range, AChR+ patients have been shown to have similar rates of thymic hyperplasia to SNMG patients [24].

Both of our SNMG patients with thymic hyperplasia had improvement in symptoms and a reduction in MG medications after thymectomy. The triple SNMG patient had sustained improvement, whereas the double SNMG eventually had worsening symptoms years later and had to be restarted on pyridostigmine and prednisone. Thymectomy in SNMG patients has generally resulted in similar results to seropositive patients, however, most studies have been performed on only AChR- patients (not tested for MuSK or LRP4) and there are no large studies on triple seronegative patients [11,24-26]. Our two cases emphasize the importance that thymic pathology can be present even in SNMG, and thymectomy can provide benefit at least when there are thymic abnormalities present on imaging and thymic hyperplasia found through biopsy.

Ancillary testing for patients with SNMG should be considered when other diagnoses are plausible. However, there should also be a balance between finding the correct diagnosis and cost-effective care in this patient population. CMS patients may be misdiagnosed as SNMG. Certain characteristics to prompt congenital myasthenia panels include: positive family history, early onset, slow progressive symptoms, and lack of response to immunotherapy. RNS characteristics may include afterdischarges or decrement that is brought out by prolonged exercise. In our population, the 11 triple SNMG patients tested for CMS all had negative genetic testing. However, these patients did not have the above-described characteristics to prompt testing. Our results indicate that if patients do not have the above-mentioned characteristics concerning for CMS, congenital myasthenia testing is low yield. When any of the aforementioned clinical characteristics favoring CMS are present though, including lack of response to immunotherapy, we would recommend pursuing CMS testing at this time.

Conclusion

Our results further elucidate the clinical characteristics of triple SNMG and the predominance for ocular disease and a less severe disease course. Although likely rare, investigation for thymic pathology should be a consideration in SNMG, and thymectomy should be considered when there are thymic abnormalities on imaging. In our population, we did not tend to find alternative diagnoses in SNMG patients and thus ancillary testing should be considered in carefully selected patients for cost-effective care.

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A Pilot Study to Compare the Standardized Patient's Perception of Empathy Among the American Medical Graduates and International Medical Graduates Applying for Residency Training in the United States

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ABSTRACT

Background: Empathy is an essential ingredient of patient-centered care. Traditional neurology clerkships do not provide a structured way to teach, evaluate and inculcate the virtue of empathy among our medical students while dealing with various complex neurological conditions. We designed an innovative Objective Structured Clinical Evaluation (OSCE) entitled as Empathetic Neurological Care (ENC) OSCE as a part of clerkship evaluation to assess empathy among American Medical Graduates (A.M.G.s) and International Medical Graduates (I.M.G.s). We aim to illustrate the model of Empathetic Neurological Care (ENC) OSCE and to report the comparative analysis of empathy scores among the A.M.G.s and I.M.G.s.

Methods: This is a pilot study comparing empathy among A.M.G.s and I.M.G.s, measured by the Standardized Patients (SPs) using the Jefferson Scale of Patient Perceptions of Physician Empathy (5-Point Likert Type Scale) during pre-designed ENC-OSCEs. The proposed curriculum included complex neurological cases involving breaking bad news (e.g., delivering the diagnosis of A.L.S.), opioid addiction counseling, disclosing medical error (stroke), and explaining the diagnosis of non-epileptic seizures.

Results: 12 students (6 A.M.G.s, 3 Males/3 Females and 6 I.M.G.s, 4 Males/2 Females) completed the OSCE. A.M.G.s scored higher in empathy scores graded by SPs than I.M.G.s (P=0.0004).

Conclusion: A.M.G.s scored higher empathy as compared to I.M.G.s by the SPs during ENC-OSCEs. We highlight the importance of focused empathy training with assessment during neurology clerkship rotations for A.M.G.s and the need of formal curricular training as a part of the orientation program at the beginning of residency training for I.M.G.s.

Keywords: *Empathy, American Medical Graduates, International Medical Graduates, Jefferson Scale of Patient Perceptions of Physician Empathy, Neurology*

Background

Empathy is an essential ingredient of patient-centered care, the cornerstone of medical practice.[1] Physician's empathy positively impacts patient safety by providing a better patient outcome, stronger physician-patient relationship, greater patient satisfaction, and better quality of healthcare.[2,3,4] Empathy in the clinical context is defined as "a predominantly cognitive attribute that involves an understanding of experiences, concerns, and perspectives of the patient, combined with a capacity to communicate this understanding and an intention to help.[5]

Realizing the importance of the cultivation of professional attitude and essential communication skill of expressing empathy in tomorrow's physicians, the medical schools and health institutions across the world are increasingly focusing on developing a curricular communication training.[6,7,8] 'Being compassionate' is one of the seven learning objectives proposed by the association of American medical college as a part of the Medical School Objectives Project (M.S.O.P.) while training the medical graduates in the United States.[9]

The physicians who receive medical education from the medical schools outside the United States and Canada, commonly referred to as International medical graduates (I.M.G.s) are an integral part of healthcare in the U.S.A. I.M.G.s form around one-quarter of physicians in training and practicing physicians in the United States.[10] I.M.G.s play a vital role in the United States healthcare as well as graduate medical education (G.M.E.) by adding diversity to the healthcare system, contributing to the physician workforce and by serving in underserved areas.[11] With the increased number of residency positions of primary care-specialties in recent years, there is increasing opportunity for I.M.G.s to pursue their career in the United States. Neurology is one of the specialties with the most significant percentage (37%) of I.M.G.s.[11]

To be eligible to apply for the residency training in the United States, both A.M.G.s and I.M.G.s undergo the same evaluation process (U.S.M.L.E.) where communication and interpersonal skills (C.I.S.) are an integral part of clinical skill evaluation. Even after succeeding in these evaluation steps, I.M.G.s still face challenges during cross-cultural communication in practice.[12] Although there is little known about empathy and the pertinent barriers I.M.G.s face during the cultural transition, we believe that focused training of I.M.G.s as they enter residency training will be a great initiative to enhance and sustain empathy in tomorrow's physician-workforce of the United States.

Traditional neurology clerkships do not provide a structured way for us to teach, evaluate and inculcate the virtue of empathy among our medical students while dealing

with various complex neurological conditions. To establish the need of training future professionals regarding empathy skills, while providing wholesome patient-centered care, we designed an innovative Objective Structured Clinical Evaluations (OSCEs) model as a part of neurology clerkship entitled as Empathetic Neurological Care (ENC) OSCEs, and we conducted a pilot study to compare empathy among A.M.G.s and I.M.G.s.

Methods

This study was approved by the Institutional Review Board (IRB) at the University of Missouri, Columbia, MO. A pilot study was conducted between six A.M.G.s and six I.M.G.s.

The ENC-OSCEs were developed by the clerkship director in consultation with experts in each neurological subspecialty. Four pilot OSCEs focusing on delivering bad news (A.L.S. diagnosis), counseling of non-epileptic seizure patients, counseling opioid use in headache patients, and disclosing medical errors (stroke) were chosen based on the variety and complexity of cases. Cases were built from real-life clinical encounters. The ENC OSCEs cases have been offered to both third (M3) and fourth (M4) year medical students (A.M.G.s) as a part of routine clerkship evaluation. We encouraged I.M.G.s who were working as clinical researchers in our department with a plan of residency training application to participate in the OSCEs to practice their clinical skills. Both A.M.G.s and I.M.G.s were blinded about the empathy evaluation to minimize the social desirability bias. Characteristics of the A.M.G.s and I.M.G.s are included in Supplementary file Table:1. All the participants were given handouts about the patient scenario and the expected checklist at the beginning of the clerkship. A didactic session focusing on patient-centered care was done, and the OSCEs were typically held in the middle of the clerkship just before the mid-block feedback. Participants were encouraged to practice with their peers by exchanging the roles as physicians/SP. and get the evaluation with constructive feedback from each other using the assessor checklists, including the empathetic statements and nonverbal empathetic expressions before actual OSCEs. Our emphasis with the OSCEs was for students to focus on demonstrating empathy and not on history and neurological exams, which were being taught and assessed by the traditional means. Once the OSCEs were built, the clerkship director trained the standardized patients (SPs) in different OSCE scenarios. Patients/families with these conditions were invited to talk with SPs to help with their role play. We trained four different standardized patients in all the OSCE scenarios and have used the four SPs throughout our OSCE encounters. SPs encountered and evaluated three students each. Each session lasted for 15 min. Before the OSCE, both the A.M.G.s and I.M.G.s were given a checklist of items to discuss and a brief review of medical literature on their ENC-OSCE cases. The evaluations were collected from the Standardized patients (SPs) immediately after each encounter which were then compared for A.M.G.s v/s I.M.G.s.

Implementation Method

The Standardized patients (SPs) evaluated participants using the Jefferson Scale of Patient Perceptions of Physician Empathy (JSPPPE). The JSPPPE is a validated tool to measure empathy in the setting of medical education and patient-centered care. Each item answered on a 5-point likert scale (1 indicating "strongly disagree" and 5 indicating "strongly agree").

The evaluation by SPs during the OSCEs was used for both formative and summative evaluation. The feedback from SPs was done immediately after the OSCE, and the comments from SPs were included in the faculty evaluation of the student. These comments have been a part of the student's M.S.P.E.

Statistical Analysis

We performed this analysis to see the difference in Jefferson empathy score between A.M.G.s and I.M.G.s. We performed chi-square method for categorical variables and 2 sample t-test for continuous variables. All the analyses were done using Statistical Analysis Software (SAS) (Release: 3.8, Enterprise Edition).

Results

In our study, 12 students completed the OSCE. There were 6 students in the A.M.G. group and 6 in the I.M.G. group. The results are shown in Table: 1. There was not a statistically significant difference seen for age (29.7±5.5 vs 24.5±1.4) nor gender (50.0% vs 66.7%) between A.M.G.s and I.M.G.s The A.M.G.s' scores were significantly higher in empathy expression than I.M.G.s' (110±8.9 vs 81.7±9.8; P=0.0004) graded by SPs using Jefferson Scale of Patient Perceptions of Physician Empathy (JSPPPE).

Discussion

Empathy in the clinical context is referred to as 'clinical empathy,' the term which expands beyond merely 'being in someone's shoes' or 'acknowledging feelings of others', involving cognitive, emotive, behavioral, and moral domains.[14,15] The cognitive attribute of empathy in contrast to sympathy (intense emotions) makes it a skill that can be learned, taught, and assessed.[16] In a six-year longitudinal study of medical students in Japan, the targeted medical

Table 1: (Age, Gender and Empathy Comparison among AMG and IMG)

Variables	AMG	IMG	P-value
Total	6	6	
Age - mean ± SD	29.7±5.5	24.5±1.4	0.0687
Male - number (%)	3(50.0%)	4(66.7%)	0.5582
Empathy -mean ± SD	110±8.9	81.7±9.8	0.0004

education program with the emphasis on communication skills training for empathy development has been proven to improve the quality of patient care significantly.[17] Engaging medical students in focused communication skills training has enhanced empathy in several other research studies.[18] Medical professionalism will be fragmentary without empathy training.

Empathy in medical students represents the future of doctor-patient relationships and compassionate care during residency training and clinical practice.[19] The curricular activity of the art of expressing clinical empathy towards the patients has been undervalued in medical education.[20] The primary emphasis of clerkship training used to be only on developing clinical thinking by achieving expertise in history taking and physical examination. Unfortunately, empathetic communication and rapport building are perceived as less important skills to learn than the medical students' clinical skills as they progress to the higher level of clinical training during medical school.[21]

The American Board of Internal Medicine (A.B.I.M.) has recommended the training of empathy and humanistic qualities as an integral part of graduate medical education during the first and second year of medical school.[22] Most of the students swore by the Hippocratic Oath as they enter this noble profession of medicine with the goal to serve and ease human sufferings. In an empiric study conducted at the Jefferson medical school, Hojat et al. have reported that empathy among the medical students gradually declined as they proceeded to a higher level of medical education during medical school.[23] Results of the systemic review of 11 studies by Neumann et al.; to evaluate empathy in medical students has also demonstrated a significant decrease in empathy during medical school in 3 longitudinal and six cross-sectional studies.[24] Ironically, the need of empathy from physicians in training is crucial at the point of bed-to-bench transition during the third year when they interact with patients and their families. Redesigning the curriculum to change the focus of this training from the first two years to the last two years of training has been focused on the recent years. Also, understanding the underlying causes of the decline in medical student's empathy and addressing these issues is a crucial part of medical education. There is limited insight available regarding the factors responsible for this conflicting behavioral change during the transition from pre-clinical years to clinical clerkships. As reported in the literature, distress in the form of burnout due to study and work overload, depression, low self-esteem, etc., is considered as a major cause of the empathy decline in medical students.[25,26,27,28] Other reasons for lack of empathy in medical professionals include biographical experiences, personality traits, lack of role-models, improper learning environment, ill-treatment by superiors, and social support, etc.[29, 30,31,32,33] A survey was done to understand the medical student's perception of factors affecting empathy during medical education. The results showed that professional growth-promoting clinical experiences and mentoring were the topmost important medical students' factors rated by the medical students.[34]

Medical students can thrive and learn to achieve clinical expertise but creating a strong and trustworthy doctor-patient relationship is an art every medical student must learn. As with the clinical skills, the 'use it or lose it' principle is also applicable to the medical professionalism attributes such as empathy. Medical students can be trained via lectures, simulations, discussions of video clips, reviews of their clinical encounters, experiential learning, focused empathy training, role-playing, creative arts, poetry writing, literature reviews, and dramas.[23] Some of the approaches tried by medical educators to reinforce and maintain the practice of empathy among undergraduate medical students have been found to be significantly effective.[35] Training and evaluating the medical students in providing holistic, patient-centered care during clerkships assures that what is learned in the classrooms will be translated into compassionate bedside care. In addition to succeeding in providing patient-centered care, physicians can also reap the benefits of practicing empathetic skills. Studies have shown a significant reduction in burnout, increased professional satisfaction, and well-being among those who express empathy in their practice.[35, 36, 37]

With globalization in the modern era, physicians and patients from diverse ethnocultural backgrounds encounter with each other more frequently. Approximately 24% of residents and 26% of physicians from various specialties practicing in the United States are I.M.G.s.[38] International medical graduates face several communication barriers as they switch between different cultural environments. In a qualitative analysis of communication barriers perceived

by I.M.G.s, Dorgan et al. have reported two broad categories - education-related barriers and interpersonal barriers[39]. Educational barriers include science-focused training without emphasizing the physician-patient communication and a lack of formal communication training. Interpersonal barriers entail unfamiliar dialects, new power dynamics, and different rapport-building expectations[39]. Forming a substantial part of the U.S. physicians' workforce, I.M.G.s' training concerning empathy should be equally emphasized while adopting empathy enhancing interventions in the graduate medical education (G.M.E.) curriculum to optimize patient-centered care.

Several complex neurological conditions affect the quality of life of patients. Patients and their families rely on physicians to exchange information, decision-making, and emotional support during these tough periods of their lives. Breaking bad news such as diagnosing fatal diseases such as Amyotrophic Lateral Sclerosis, educating patients about their addiction to opioids, declaring the diagnosis of non-epileptic seizures, and disclosing medical errors, affect patients and families psychosocially. These challenging issues can significantly influence the doctor-patient relationship in either way. However, accepting these challenges as opportunities to express empathy can strengthen the doctor-patient relationships while providing patient-centered care. Learning and practicing how and when to provide empathetic care by communicating effectively, medical students can deal comfortably with real-life complex clinical scenarios in daily practice. With this philosophy, we designed the ENC-OSCEs specifically for complex neurological conditions.

The OSCEs are used for both formative and summative evaluation of the students. The feedback from the OSCEs (including feedback from standardized patients) will be a part of the medical school performance evaluation (M.S.P.E.). Our pilot study is a primer to show the impact of educational intervention during the neurology clerkship on SPs' perception of physician's empathy and the need of focused intervention strategies for I.M.G.s as they enter into neurology residency training in our institute.

We found that both M4s and M3s performed better than international graduates in terms of empathy scores as perceived by standardized patients. Student feedback on the ENC OSCE was highly positive, ranging from 'humbling experience' to 'highlight of clerkship.' Randomly chosen videos of different OSCE encounters were reviewed with patients and their families who have repeatedly validated its authenticity and its reflection of a real-life encounter, with some saying that they felt it was them as the SPs in the video. The school of medicine highlighted the opioid counseling OSCE as one of the educational tools being developed/taught to students in combating opioid addiction/epidemic in its L.C.M.E. report. To our knowledge, our study is unique in the comparative evaluation of empathy among the A.M.G.s and I.M.G.s as perceived by standardized patients and scored on JSPPPE utilizing an innovative ENC-OSCEs model.

We believe that sustaining the skill of empathy or regaining the lost art of medicine during the practice is a challenging but certainly not impossible task for medical educators. The metamorphosis of medical students into compassionate physicians/caregivers can be achieved through focused training during their clerkship rotation.

Conclusion

As seen in our pilot study, A.M.G.s scored higher than I.M.G.s in empathy evaluation by SPs. We wish to highlight the importance of a designed curriculum to train and evaluate medical students during their clerkships to inculcate empathy as a professional skill while dealing with complex neurological conditions during neurology clerkship rotations. We also wish to highlight the need of empathy-building training as a part of the orientation program/residency curriculum explicitly addressing the needs of international medical graduates from diverse ethnocultural backgrounds.

Assessment of empathy by the standardized patients can serve as constructive feedback to the medical students and reinforce the practice of empathy as a second nature. Our ENC-OSCEs model may serve as a promising tool to improve the neurology patient experience.

Future Direction

Future large-scale studies are needed to validate Jefferson Scale of Patient Perceptions of Physician Empathy (JSPPPE) as a tool to evaluate empathy during medical encounters of complex neurological conditions. We intend to do a large-scale study to direct our attempts to enhance empathy among both A.M.G.s and I.M.G.s as they enter residency training and eventually the US-physician workforce. At our institute, the ENC-OSCEs will be offered to incoming neurology residents with constructive feedback as a part of their orientation. New OSCEs involving an end-of-life discussion with a patient who suffered a massive stroke, a telemedicine encounter with a chronic migraine patient, and counseling of a patient with Huntington's disease will be added to the ENC-OSCEs.

Limitations of the Study

We have conducted this pilot study with a small number of participants, and to establish the effectiveness of our intervention, we intend to do a large-scale study in

the future with more A.M.G.s and I.M.G.s. The drawback of empathy assessment during OSCE, as reported by Chen et al., may be a social desirability bias as the participants may exhibit the desired behavior pattern during these OSCEs. However, we tried to minimize this bias to some extent by blinding the participants about their empathy evaluation by SPs. We encouraged them to perform as in their routine clerkship OSCEs. There are several other factors other than formal training during medical school that attributes to low empathy among the I.M.G.s, such as language proficiency, personality traits, cultural upbringing, attitude, cultural competence, prior clinical and professional experiences in the United States etc. These factors are not addressed in our pilot study. Further study to investigate the factors affecting empathy among I.M.G.s is needed before future empathy training interventions.

List of abbreviations: Objective Structured Clinical Evaluations (OSCEs), Empathetic Neurological Care (ENC), American Medical Graduates (A.M.G.s), International Medical Graduates (I.M.G.s), Standardized Patients (SPs), *Amyotrophic Lateral Sclerosis (A.L.S.),* Medical School Objectives Project (M.S.O.P.), Graduate Medical Education (G.M.E.), Jefferson Scale of Patient Perceptions of Physician Empathy (JSPPPE).

Declarations

- Ethics approval and consent to participate:

This study was performed under the relevant guidelines and regulations of ethical principles and approved by the local ethics committee of the University of Missouri I.R.B.

- Consent for publication:

Consent was obtained from the participants before submission for publication.

- Availability of data and materials:

The datasets used during the current study are available from Mukaish Kumar (M.K) and Raghav Govindarajan (R.G) on reasonable request.

- Competing interests:

The authors have no conflict of interest to report.

- Funding

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- Authors' contributions:

M.K and R.G involved in designing concepts, literature search, writing and in drafting the article. W.E performed the analytical calculations. All authors contributed to the final manuscript.

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Supplemental Material

Table S1: Baseline characteristics of AMG and IMG

No:	Age (Years)	Gender	IMG/AMG
1	23	F	IMG
2	24	F	IMG
3	24	M	IMG
4	27	M	IMG
5	25	M	IMG
6	24	M	IMG
7	28	M	AMG
8	25	M	AMG
9	40	M	AMG
10	26	F	AMG
11	31	F	AMG
12	28	F	AMG

Abbreviations:
F=Female, M=Male,
IMG= International Medical Graduates
AMG=AMG American Medical Graduates

3,4-diaminopyridine Phosphate in Symptomatic SOD1-G93A Mice

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ABSTRACT

Objective: To study the effect of 3,4-diaminopyridine phosphate (3,4-DAP) on body weight, grip strength, neurological score and survival in symptomatic SOD1-G93A mice.

Method*:* We administered 3,4-diaminopyridine phosphate (3,4-DAP) at 0, 8, and 16 mg/kg to SOD1-G93A mice 5 days/week beginning at 90 days of age. We measured body weight, grip strength, neurological score and survival in this model of ALS.

Results*:* 3,4-DAP had no influence on body weight, grip strength, neurological score or survival in this transgenic mouse model.

Conclusion*:* Our study showed that 3,4-DAP administration had no effects on survival, bodyweight, grip strength and neurological score of mice with SOD1 G93A mutation with intervention starting at 90 days of age. We believe that larger animal studies, longer treatment times and/or earlier in life treatment are required to further investigate the utility of 3,4 DAP in ALS patients.

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal and rapidly progressive motor neuron disease characterized by the selective loss of corticospinal neurons within the motor cortex and of a-motor neurons of the spinal cord and brainstem.[1] There is progressive muscle weakness, atrophy, and spasticity, which reflect the degeneration and death of upper and lower motor neurons and muscle denervation.[1] There is some experimental evidence that supports the "dying back hypothesis" that postulates that ALS begins with the degeneration of the neuromuscular junction (NMJ), which results in axonal degeneration and, finally, motor neuron loss.[1,2] Both pre- and postsynaptic alterations interact with synaptic components of the NMJ and contribute to the progression of ALS.[1,2]

3,4-diaminopyridine (3,4-DAP) is a quaternary ammonium compound, which increases the release of acetylcholine in the neuromuscular synapse by prolonging the activation of calcium influx at the nerve terminal.[3] It selectively blocks potassium channels in nerve membranes owing to an enhanced influx of calcium ions by potassium blockade and the resulting in prolongation of the nerve terminal action potential.[3,4] By this mechanism 3,4-DAP increases impulse-evoked transmitter release from motor nerve terminals.[3-5]

3,4 DAP has shown to improve motor weakness for a short period of time by enhancing peripheral synaptic efficiency and has been used for symptomatic treatment of motor impairment due to multiple sclerosis and Lambert Eaton myasthenia syndrome (LEMS).[5,6] A couple of double blinded studies in the past have noted functional motor status improvement with 3,4 DAP along with rehabilitation in ALS patients.[7,8]

We used 3,4-DAP to observe if increasing neurotransmitter availability in the neuromuscular junction would have any effect on grip strength, body weight, neurological score and survival in symptomatic SOD1-G93A mice.

Materials and Methods

Animals and dosing: Thirty-six male SOD1-G93A mice were acquired from Jackson Laboratories. Firdapse (3,4-DAP) was obtained from Catalyst Pharmaceuticals, Inc. Mice were divided into three groups: a 3,4-DAP 8 mg/kg group, a 3,4-DAP 16 mg/kg group, and a saline vehicle group. After collecting baseline body weight and grip strength data (see below), we administered 3,4-DAP or saline vehicle (10 ml/kg, ip) 5 days/week beginning at 90 days of age. Drugs were administered following grip strength tests. Procedures were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee and adhered to the Guide for the Care and Use of Laboratory Animals.

Grip strength testing: Mice were tested for grip strength using an inverted wire screen. Specifically, mice were placed on the screen and then the screen was inverted and held 2 feet above a cushioned surface. The duration that the mice were able to remain on the screen before releasing was recorded across two trials. The mean duration of the two trials was used as the measure of grip strength for each mouse on each day. Mice were tested until they exhibited loss of righting reflex for 30 seconds. At this point they were

euthanized. Some mice were found dead in their cage. The day in age for either of these events was recorded as day of death and used for survival analysis (see below).

Neurological score: Both hind legs were assessed daily for each mouse from 50 days of age and neurological score was calculated using a scale 0 to 4 that was developed by observation at ALSTDI. Criteria used to assign each score level were:

0- Full extension of hind legs away from lateral midline when mouse is suspended by its tail, and mouse can hold this for 2 seconds, suspended 2-3 times.
1- Collapse or partial collapse of leg extension towards lateral midline (weakness) or trembling of hind legs during tail suspension.
2- Toes curl under at least twice during walking of 12 inches, or any part of foot is dragging along cage bottom/table.
3- Rigid paralysis or minimal joint movement, foot not being used for forward motion.
4- Mouse cannot right itself within 30 seconds from either side.

Data Analysis: Data for body weight, grip strength and neurological score were expressed as percentage of pre-drug baseline and analyzed using a 2-way Analysis of Variance (ANOVA) with group assignment (vehicle vs 8 mg/kg vs 16 mg/kg) as the between-subjects variable and testing day (every 7 days) as the within-subjects repeating variable (Systat 13). Survival analyses were performed using each mouse's day of death (GraphPad Prism). We also compared latencies between the day each mouse lost at least 20% body weight and the day the mouse was euthanized or found dead in its cage using a one-way ANOVA.

Results

On analysis of the variables, we found that there was no significant change in the variables throughout the study period in the mice with SOD1 mutation.

Although there was no significant association noted between 3,4-DAP and body weight, mice who were administered 16mg/kg of 3,4 DAP showed slightly higher body weight through the study period as compared to those who were administered the 8mg/kg dose. A similar trend is noted for the grip strength, neuro score and survival as well. These results are depicted in figure 1A, 1B, 1C and figure 2.

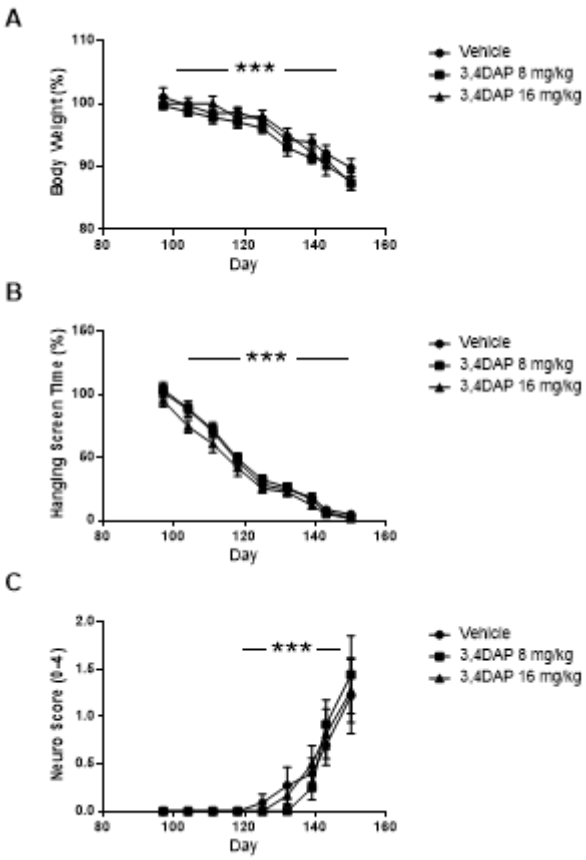

Figure 1. (A) Body weight, (B) Grip strength (measured by hang time), and (C) Neurological score (0 = normal, 1-3 = progressively abnormal hindlimb behavior during tail suspension, 4 = loss of righting reflex; see Neurological Scoring System at the end of this document). None of these measures were affected by 3,4-DAP. Asterisks indicate significant main effect of Day (disease) on mice in the three groups.

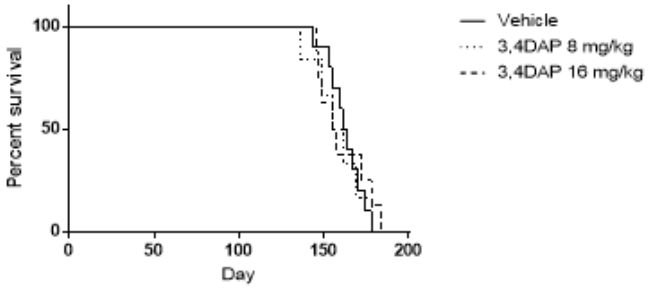

Figure 2. Survival was not affected by the administration of 3,4-DAP.

Discussion

3,4-DAP increases the release of acetylcholine in the neuromuscular synapse by prolonging the activation of voltage gated calcium channels at the nerve.[3,4] By this mechanism, it enhances neuronal excitability and improves neuromuscular and central synaptic transmission.[3,4] It has been successfully used to improve muscle strength and fatigue in Lambert-Eaton myasthenic syndrome and multiple sclerosis patients.[5,6]

NMJ integrity tightly depends on the presynaptic release of acetylcholine and on the clustering of acetylcholine receptors on the muscle plasma membrane to trigger muscle action potentials.[2] Immunofluorescence studies have shown that the density of synaptic vesicles in motor axon terminals from mutant SOD1 mice was significantly reduced compared to the wild-type.[2] In a study by Clark et al,[9] distal axonal and NMJ alterations were noted in muscles of SOD1 G93A mice even before the onset of the clinical symptoms of ALS.[9]

Although there is no current translational application, a couple of double blinded studies in the past have noted functional motor status improvement with 3,4 DAP along with rehabilitation in ALS patients.[7,8] In the recent years there has been discussion regarding how NMJ pathology has relatively received little attention in ALS and it has been proposed that additional research should focus on the potential of preserving NMJs in order to delay or prevent disease progression.[10] The above study was an attempt in that direction.

The conclusion of the above study is that 3,4-DAP administration had no effects on survival, bodyweight, grip strength and neurological score of mice with SOD1 G93A mutation with intervention starting at 90 days of age. We believe that larger animal studies, longer treatment times and/or earlier in life treatment are required to further investigate the utility of 3,4 DAP in ALS patients.

Acknowledgements

This work was supported by the Kansas Intellectual & Developmental Disabilities Research Center (NIH U54 HD 090216). We thank Catalyst Pharmaceuticals Inc., for providing the investigational product-3.4 DAP needed for the study.

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EE2: 3,4-Diaminopyridine Phosphate for AAL—The EEDAPP-ALS Trial

Raghav Govindarajan, MD; Stanley Iyadurai, MD; Alex Karenevich, PhD; Laura Herbelin, BS; Jeffrey Statland, MD; Richard J. Barohn, MD

Submitted to: National Center for Advancing Translational Sciences/NIH
In response to RFA/PA: PAR 19-099

The genesis of this unfunded NIH proposal was two-fold. First Dr. Raghav Govindarajan (at University of Missouri at the time), and Dr. Stanley Iyadurai, a neuromuscular neurologist at Catalyst Pharma, had the idea that 3,4-diaminopyridine phosphate (DAPP) might be beneficial for ALS. The theory was that DAPP, working at the presynaptic terminal of the neuromuscular junction (NMJ) might enhance function by increasing the release of acetylcholine vesicles. One of our colleagues at the University of Kansas Medical Center (KUMC), Dr. Hiroshi Nishimune, had been developing data that the NMJ was critical in ALS and that ways to preserve function at the NMJ could prolong survival time in SOD mice.

The second factor going on at this time was that I was learning about efficacy to effectiveness (E2E) studies by Dr. Harry Selkar, the principal investigator of the clinical and translational science program at Tufts University. Dr Selkar had been telling me about how a subtype of E2E studies called efficacy and effectiveness too (EE2) made a lot of sense in trial design. The overall concept of EE2 trials is that a phase 3 efficacy study is nested in a larger effectiveness study. The idea is to simultaneously prove efficacy in a narrow more homogenous population of subject, and at the same time enroll additional patients who do not meet the criteria for the efficacy study to get a sense on how the intervention has an effect in a larger population.

For enrollment criteria for the efficacy portion of the study, we planned to use a slight modification of the fairly rigid entry guidelines used in the edavarone pivotal study for ALS. For the effectiveness portion of the study, we allowed ALS patients to be randomized who did not meet these criteria. We proposed to enroll 200 study participants into the efficacy component and an additional 100 study participants into the effectiveness component and planned to use 24 sites that were part of the CTSA consortium or the IDeA-CTR consortium of trial sites. In the months leading up to the submission we had utilized the NCATS CTSA Trials innovation network (TIN) consultation process to vet and refine their proposal.

We had applied and were accepted to present the proposal to experts at an in-person TIN meeting in Boston in April 2019. At this meeting there were experts from the NIH, FDA, pharma and a number of clinical trial experts that provided useful feedback. What happened to this valiant effort to repurpose a new drug for ALS? Two things. As the grant was being reviewed, we got data back from a study that one of our other colleagues, John Stanford, PhD, was performing for us at KUMC. He did a controlled trial of DAPP in SOD mice. The results are reported in this issue of the RRNMF NM Journal.

Unfortunately, DAPP did not have any benefit in the animal model of ALS. Then we got the critiques back from NCATS/NIH which are attached. We were not funded to do this innovative EE2 trial. The reviewers seemed to be uncomfortable funding this unconventional trial design.

To date, we do not believe the NIH has funded a EE2 trial. There also was some hesitancy about the DAAP hypothesis for ALS. We wanted to publish the proposal and the critiques in the RRNMF Neuromuscular Journal under "Proposed Stuff" as the grant outlines what an EE2 trial design is, and this may be a new concept for many readers of the journal. We also wanted to have readers understand the thought process on why we believed DAPP should be studied in ALS.

Project Summary/Abstract
The overall goal of this application is to perform an innovative Efficacy Effectiveness -Too trial design (EE2) in Amyotrophic Lateral Sclerosis (ALS) in which we can simultaneously enroll a homogenous population to determine efficacy and a wider population to determine effectiveness in a broader population. ALS is a rare, relentlessly progressive and fatal neurodegenerative disease affecting cortical and spinal motor neurons. The exact mechanism of ALS is unknown. This clinical trial will study the efficacy and effectiveness of 3,4-Diaminopyridine Phosphate (3,4-DAPP) in patients with ALS. The mechanism of action of 3,4-DAPP is at the presynaptic terminal of the neuromuscular junction (NMJ) to enhance function by producing an increase in the release of acetylcholine vesicles. This drug was recently approved by the FDA for the treatment of the Lambert -Eaton myasthenic syndrome and may improve the function at the NMJ in ALS patients the same way exercise does. This proposal would be the first time an EE2 trial is done in a rare disease and will include 20 CTSA sites and 4 IDeA State CTR sites dispersed across the United States. There are five sites (Kansas, Missouri, Nebraska, California-Irvine, and Florida-Gainesville) that are designated as lead sites for the study. The specific aims for this study are as follows: **1.** Perform an EE2 study in ALS at 20 CTSA sites and 4 IDeA CTR sites and simultaneously enroll a cohort to determine efficacy and a more heterogenous cohort which combined with the efficacy cohort will determine effectiveness in a broader population. This will serve as a blueprint for the CTSA consortium to perform EE2 studies on rare diseases. **2.** Determine if 3,4-DAPP can alter the course of the disease in ALS patients. **2a.** Assess the efficacy of 3,4-DAPP by measuring changes in the slope of ALSFRS-R in a well-defined progressing cohort of ALS as previously defined in the edavarone study. We hypothesize that 3,4-DAPP will slow down the progression of ALS by 30% as measured by the slope of the ALSFRS-R at the end of 6 months in this well-defined narrow cohort. The dose of 3,4-DAPP will be 80mg/day or the highest tolerated dose up to that level. **2b.** Simultaneously recruit ALS patients with a more heterogenous entry criteria to more likely reflect a general ALS population and determine effectiveness. The aim is to determine if there are trends when looking at a more heterogenous population that suggest 3,4-DAPP may have a benefit **2c.** Measure secondary outcome measures in both populations: survival, the slope of decline of FVC, the change in an ALS specific quality of life measure (ALSAQ-40) and a patient reported ALS outcome measure, PADL ALS. At the conclusion of the study, there will be an open-label extension study which will allow all ALS patients who consented to participate in the study to have access to the active research drug. This will be funded by a different mechanism through a partnership with Catalyst Pharmaceuticals.

Contact PD/PI: Barohn, Richard Jude

Project Narrative

We will test an innovative trial diesing in amyotrophic lateral sclerosis (ALS), a rare, relentlessly progressive, fatal disease, by conducting a clinical trial, **EE**2: 3,4-**Dia**mino**p**yridine **P**hosphate for **ALS** - The EEDAPP-ALS Trial to determine 3,4-Diaminopyridine Phosphate versus placebo benefits patients with ALS by slowing down disease progression. In addition to performing a Phase III efficacy study in ALS with narrow inclusion criteria, we will simultaneously enroll a more heterogenous ALS group to determine effectiveness in a more generalizable population. 20 CTSA and 4 IDeA State CTR sites dispersed throughout the USA will be leveraged for this unique proposal.

Contact PD/PI: Baronn, Richard Joel

EE2: 3,4-Diaminopyridine Phosphate for ALS - The EEDAPP-ALS Trial

Amyotrophic lateral sclerosis (ALS) is a rare relentlessly progressive and fatal neurodegenerative disease affecting cortical and spinal motor neurons. The exact mechanism of ALS is unknown. The prevailing theory is that ALS is a dying forward phenomenon, in which primary damage occurs in the motor neurons and then extends in an anterograde fashion. Alternatively, multiple animal studies (such as SOD1 mice, drosophila and zebra fish ALS models) have demonstrated a distal axonopathy in which motor neuron degeneration starts at the nerve endings and progresses toward the cell bodies in a dying back manner leading to muscle denervation. Clinical and electrophysiologic correlates of muscle fatiguability suggest and element of neuromuscular junction transmission (NMJ) transmission dysfunction. In SOD mice there is loss of laminin beta2 which is believed to produce the dying back phenomenon. There is an interaction between laminin beta2 and the P/Q type VGCC that causes NMJ denervation and cause a decreased number of active zones. Adult ALS patients show decreased active zone size in spinal cord synapses. Therefore, the loss of laminin beta 2 can cause NJM denervation in SOD1 mice. Exercise as an intervention for ALS has been performed in SOD mice and ALS patients and recovers laminin beta 2 levels at NMJs and ameliorates NMJ denervation in SOD1 mice and rats.

The mechanism of action of 3,4-Diaminopyridine Phosphate (DAPP) is at the presynaptic terminal of the NMJ to enhance function by producing an increase in the release of acetylcholine vesicles. This drug was recently approved by the FDA for the treatment of the Lambert -Eaton myasthenia syndrome, Firdapse® may improve the function at the NMJ in ALS patients the same way exercise does. Several small studies of 3,4-DAPP in ALS included a placebo controlled cross over study and two open label studies. Overall, small but significant improvements in function were seen.

Many phase 3 ALS trials have failed to show efficacy and one of the possible explanations is that the populations enrolled are too heterogenous. This problem was solved recently in the study of edavarone for ALS in which they had a narrow inclusion criterion in order to enroll a relatively homogenous population. Edavarone (Radicava®) was efficacious in slowing ALS Functional Rating Scale by 30% and the drug was approved by the FDA in 2017.

While we now have a pathway to perform efficacy studies in ALS, patients and families want the option to be part of the drug research process and it is important to obtain knowledge of the effectiveness of the drugs in a larger more generalized population of ALS patients. One way to address this seeming dichotomy of crossed purposes is to use the innovative Efficacy Effectiveness -Too trial design (EE2) in which we can simultaneously enroll a homogenous population to determine efficacy and a wider population to determine effectiveness in the broader population. The EE2 trial design has never been attempted in a rare disease population.

AIMS:

1. Perform an EE2 study in ALS at 20 CTSA sites and 4 IDeA CTR hubs and simultaneously enroll a cohort to determine efficacy and a more heterogenous cohort which combined with the efficacy cohort will determine effectiveness in a broader population. This will serve as a blueprint for the CTSA consortium to perform EE2 studies on rare diseases.

2. Determine if 3,4-DAPP can alter the course of the disease in ALS patients.

2a. Assess the efficacy of 3,4-DAPP by measuring changes in the slope of ALSFRS-R in a well-defined progressing cohort of ALS as previously defined in the edavarone study. We hypothesize that 3,4-DAPP will slow down the progression of ALS by 30% as measured by the slope of the ALSFRS-R at the end of 6 months in this well -defined narrow cohort. The dose of 3,4-DAPP will be 80mg/day or the highest tolerated dose up to that level.

2b. Simultaneously recruit ALS patients with a more heterogenous entry criteria to more likely reflect a general ALS population and determine effectiveness. The aim is to determine if there are trends when looking at a more heterogenous population that suggest 3,4-DAPP may have a benefit

2c. Measure secondary outcome measures in both populations: survival, the slope of decline of FVC, the change in an ALS specific quality of life measure (ALSAQ-40) and a patient reported ALS outcome measure, PADL ALS

RESEARCH STRATEGY

A. Statement of the Problem and its Significance to Translational Science:

The overall goal of this application is to employ novel trial methodology through an Efficacy and Effectiveness Too (EE2) design in a rare disease, amyotrophic lateral sclerosis (ALS). Using the EE2 design will allow a heterogenous group of patients to be on the study medication but also allowing the measurement of drug efficacy in a narrow subgroup of participants that is typical of many standard clinical trial designs.[1] This novel trial design can serve as a blueprint for similar studies in other rare diseases and it has the potential to improve enrollment and accelerate therapy development. Importantly, this trial design responds precisely to the priorities of ALS patients and caregivers by addressing their concern over the slow pace of generalizable treatments that can impact quality of life and slow progression. To our knowledge our EE2 trial has never been performed in a rare disease. This multi-center study will be performed exclusively at CTSA and IDeA State CTR sites. These are sites with expertise to accomplish this innovative rare disease trial design. In addition, we are testing a novel mechanism of action for treating ALS through the use of 3,4-Diaminopyridine Phosphate (3,4-DAPP) which works at the neuromuscular junction (NMJ). The investigators are partnering with a pharmaceutical company, Catalyst Pharmaceuticals, which will provide the drug recently approved by the FDA for Lambert-Eaton Myasthenic Syndrome, another rare disease indication. This will be a unique partnership of ALS clinics at CTSA/CTR sites, the National Institutes of Health, and industry to complete a combined phase 3 (efficacy) and phase 4 (effectiveness – in generalizability) in a rare disease that could have an immediate impact to benefit ALS patients if the drug can be shown to slow progression of this rare and fatal disease.

Challenges for rare diseases: A rare disease is defined in the US as having < 200,000 people affected. There are approximately 7000 rare diseases in the US, so taken together that represents 25-30 million people.[2-5] Rare diseases are complex, chronic, and often have inadequate or no treatment options available. Therefore, rare diseases represent a major unmet medical need and can result in a large share of US health care spending.[6] Barriers to developing new therapies for rare diseases include: 1) needing to use multiple sites to recruit sufficient numbers of patients for statistical rigor; 2) difficulties with regulatory oversight for large multicenter studies causing delays in start-up and increasing study costs; 3) identifying and contacting eligible participants; 4) repeated and often lengthy study visits; 5) lack of patient and caregiver input into study design and conduct; and 6) geographic distance or medical infirmity.[7] The National Clinical and Translation Science Award (CTSA) and Institutional Development Award (IDeA) - CTR programs gives us an opportunity to overcome these barriers and enhance our existing national ALS infrastructure by adapting existing CTSA models for IRB reliance and leveraging the Trial Innovation Network (TIN) Vanderbilt University Recruitment Innovation Center. This NCATS Innovation award initiative allows to pursue a phase 3 and 4 trial in the CTSA/CTR consortiums for a rare disease.

Challenges for ALS: Riluzole, an oral drug, was approved as a treatment of ALS in 1993 because it has shown a modest benefit in survival, with most studies showing a three-month survival benefit.[8] Edaravone was shown to reduce rate of decline in function by 30% (as measured by ALSFRS-R)[9]; however, it is expensive, requires intravenous therapy 14 days a month, and is often denied by insurance companies due to a lack of evidence showing effectiveness studies in a more general ALS population. Our own experience shows only about 15% of patients are receiving both medications, despite over 70% being on riluzole during the edaravone clinical trial. Many phase 3 ALS trials have failed to show efficacy and one of the possible explanations is that the populations enrolled are too heterogenous.[10] This problem was solved recently in the edaravone study in which they used a very narrow inclusion criterion in order to enroll a relatively homogenous population (see below).[11-12]

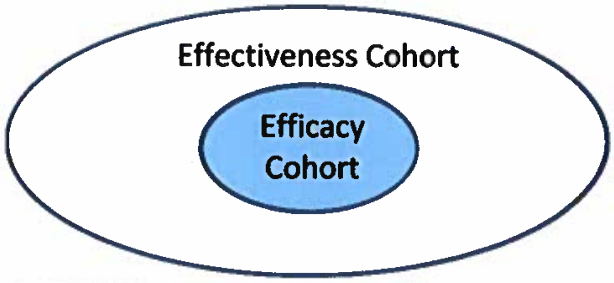

Figure 1: Reprinted from published article - Selker HP, Eichler H, Stockbridge NL, McElwee NE, Dere WH, et al. Efficacy and effectiveness Too Trials: Clinical Trial Designs to Generate Evidence on Efficacy and on Effectiveness in Wide Practice. Clin Pharm & Therapeutics. 2019; 105(4):857-866.

In the Efficacy-Effectiveness Too Trial of 3,4-Diaminopyridine Phosphate (3,4-DAPP) in Amyotrophic Lateral Sclerosis (EEDAPP-ALS) study we will be measuring the drug's efficacy in a narrow subgroup

and will simultaneously include a heterogenous population which will allow us to study the effects of the drug on a more diverse group of ALS patients (Figure 1). This will be measuring the drug's effectiveness in a more generalized ALS population. This will be responsive to the patients and families with ALS who have reportedly told researchers that they do not want to be excluded from trials.

B. Rationale:
There is increasing interest in determining both efficacy of a drug in a Phase 3 study as well as effectiveness of the drug in a more generalizable, real world, diverse population. However, effectiveness studies, while often contemplated, are seldom performed after a positive Phase 3 efficacy study when the drug is FDA approved and on the market. Thus, patients and payers, two key stakeholders, never have additional information on how the drug will perform in patients in a general population who may not have met the original entry criteria in the Phase 3 labeling study.

At least two approaches have been suggested to a prior plan for an efficacy and effectiveness study when a phase 3 study is first designed. One is to perform an Efficacy to Effectiveness study (E2E) (Figure 2) when an effectiveness study is designed to begin immediately after enrollment is complete or after the final patient has finished a phase 3 trials. The effectiveness study, in this case, is launched before the final results of the phase 3 study are available.[1] An even more expeditious design is the EE2 study (Figure 3). In an EE2 study, the patients for the phase 3 study and a more generalizable population of patients with the disorder are enrolled simultaneously.[1] Therefore, at the conclusion, there is an immediate answer to both the efficacy question as well as the effectiveness question. This is beneficial to multiple stakeholders on the research and health care spectrum – patients, caregivers, clinicians, and payers. All would like to know the answer to both clinical research questions as quickly as possible. Pharmaceutical stakeholders on the other hand may see an EE2 approach as risky for two reasons – they are investing financially in the effectiveness study before an answer to efficacy is known and the results could show the drug is efficacious in the narrow group of patients who meet study entry criteria but did not have the same effect in the wider more heterogenous patient group. In this case it is possible insurers may elect not to reimburse for the drug unless the strict efficacy criteria are met.

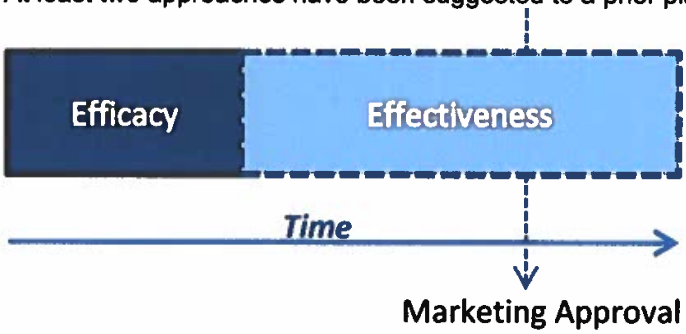

Figure 2: Example of E2E design: Reprinted from published article – Selker HP, et al. Efficacy and effectiveness Too Trials: Clinical Trial Designs to generate Evidence on Efficacy and Effectiveness in Wide Practice. Clin Pharm & Therapeutics. 2019; 105(4): 857-866.

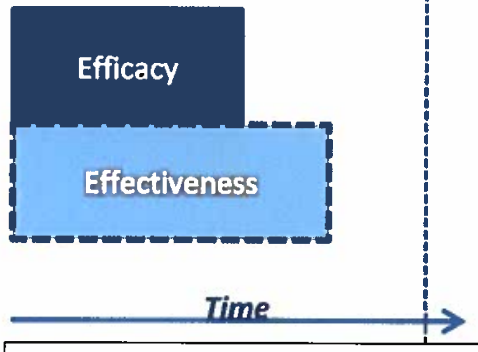

Figure 3: Example of EE2 design: Reported from published article – Selker HP, et al. Efficacy and Effectiveness Too Trials: Clinical Trial Designs to Generate Evidence on Efficacy and Effectiveness in Wide Practice. Clin Pharm & Therapeutics. 2019; 105(4): 857-866.

Innovation Opportunity for Trial Design in ALS:
Many phase 3 ALS trials have failed to show efficacy, and one of the possible explanations is that the populations enrolled are too heterogeneous. This problem was solved recently in the study of edavarone for ALS in which they used a narrow inclusion criterion to enroll a homogenous population. The cohort that was enrolled showing efficacy consisted of patients aged 20 to 80 years; ALS -Functional Rating Scale-Revised (ALSFRS-R) of at least 2 points on all 12 items; forced vital capacity (FVC) of 80% or more; definite or probable ALS according to the revised El Escorial criteria; disease duration of 2 years or less; and who had a 1 to 4 point decrease in ALSFRS-R during a 12 week lead-in period before randomization.[11] In this narrow ALS population, edavarone was efficacious in slowing ALSFRS-R by 30% and the drug was approved by the FDA

in 2017. This makes edavarone only the second drug approved for ALS since 1993 (riluzole) which has only a minimal effect on ALS survival. Currently only about 30% of patients seen in the clinic meet these selective criteria and many ALS trials have similar strict criteria. Therefore, many ALS patients that do not meet the strict inclusion criteria have minimal to no options in terms of treatment (in the case of edaravone) or participation in a clinical trial.[12]

While we now have a pathway to perform efficacy studies in ALS, patients and families want the option to be part of the drug research process and it is important to obtain knowledge of the effectiveness of the drugs in a larger more generalized population of ALS patients. One way to address this seeming dichotomy of crossed purposes is to use the innovative Efficacy Effectiveness -Too trial design (EE2) in which we can simultaneously enroll a homogenous population to determine efficacy and a wider population to determine effectiveness in the broader population.[1] The EE2 trial design has never been attempted in a rare disease population.

C. Strategy and Methodology

Aim 1: Perform an EE2 study in ALS at 20 CTSA sites and 4 IDeA CTR hubs and simultaneously enroll a cohort to determine efficacy and a more heterogenous cohort which combined with the efficacy cohort will determine effectiveness in a broader population. This will serve as a blueprint for the CTSA consortium to perform EE2 studies on rare diseases.

Collaboration:

We will leverage our existing ALS patient-centered clinic and clinical trial research infrastructure and national CTSA and IDeA-CTR programs and collaborating with the TIN (see letters of support). For this study, the University of Kansas Medical Center is the sponsoring institution and will serve as the data coordinating center (DCC) as well as the clinical coordinating center (CCC): responsible for managing implementation of the central IRB, site management, data quality assurance, and project management. We applied for and obtained initial TIN consultation and then we obtained full TIN support. We used the Recruitment Innovation Center (RIC) resources and our engaged ALS patient experience to design the study and to identify potential sites. Our organizational structure will include a national network of 24 ALS specialty clinic sites—all at existing CTSA/CTR hubs. The University of Kansas Medical Center (KUMC) has experience running large multi-site studies—most recently completing a 40-site study in small fiber neuropathy and 2 national phase II studies of rasagiline in ALS, and with an ongoing dose ranging study of ranolazine in ALS, and a national multi-center phase II study of memantine in ALS. To maintain efficient communications and coordination, we will have four regional lead sites to accomplish the study. KUMC has primary responsibility as the study sponsor and will co-lead the central U.S. hubs with the University of Missouri-Columbia. The University of Nebraska Medical Center will

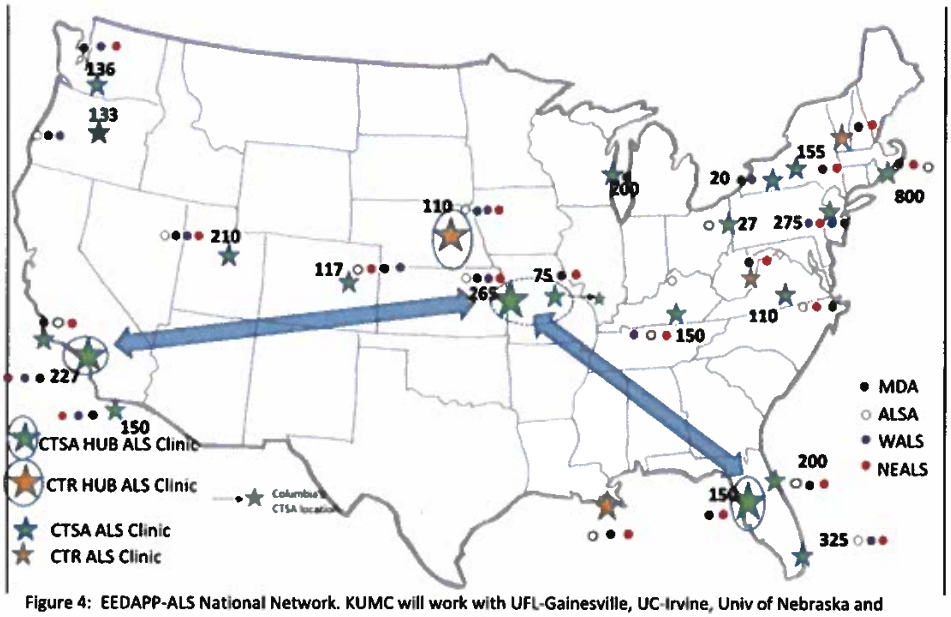

Figure 4: EEDAPP-ALS National Network. KUMC will work with UFL-Gainesville, UC-Irvine, Univ of Nebraska and Oklahoma University to manage network recruitment and implementation. Numbers represent estimated ALS participants. Green star=CTSA ALS Clinics, Brown star=CTR ALS Clinics; MDA = Muscular Dystrophy Association Clinic, ALSA = Amyotrophic Lateral Sclerosis Association Clinic; WALS = the Western ALS Study Group; NEALS = the Northeast ALS Consortium. CReATe = member of the NCATS Rare Disease Clinical Research Network for ALS

serve as the IDeA-CTR lead and the University of Florida-Gainesville and University of California-Irvine will serve as CTSA leads for the east and west coast regions, respectively (see Figure 5). A prior analysis of ALS clinical trials showed the most successful sites in recruitment, retention, and protocol adherence were sites that were experienced in clinical trials, with improved efficiencies with each trial and full-time coordinator.[13] We will use multiple existing ALS infrastructures to conduct this study. First is the national network of ALS multi-disciplinary clinics funded by the Muscular Dystrophy Association and ALS Association. Second, most of these sites are members of existing ALS research consortia, which requires baseline level of clinical trial preparedness and includes NEALS and WALS. Together the 24 sites we preselected have over 5,000 ALS patients. We estimate that 30% would qualify for the efficacy component and about 40-50% would qualify for the effectiveness component.

EEDAPP-ALS is a multi-PI project led by Dr. Richard Barohn (Contact PI- University of Kansas Medical Center) and Dr. Raghav Govindarajan (PI- University of Missouri Medical Center). Other ALS lead Co-investigators are Dr. Miguel Chuquilin (CTSA site investigator – University of Florida - Gainesville), Dr. Tahseen Mozzafar (CTSA site investigator- University of California-Irvine), and Dr. Americo Fernandes (CTR site investigator- University of Nebraska). This team will oversee all aspects of trial implementation and has over 85 years of demonstrated, collaborative research across their institutions. Andrew Heim (Project Lead, KUMC) will report to the PI's and will oversee all operations of the project as it relates to the trial responsibilities while ensuring smooth integration with the other sites. Leveraging these existing relationships with the TIN resources greatly enhances this proposed national multi-site study, and improves clinical trial processes, such as regulatory oversight, patient identification and recruitment, and study retention.

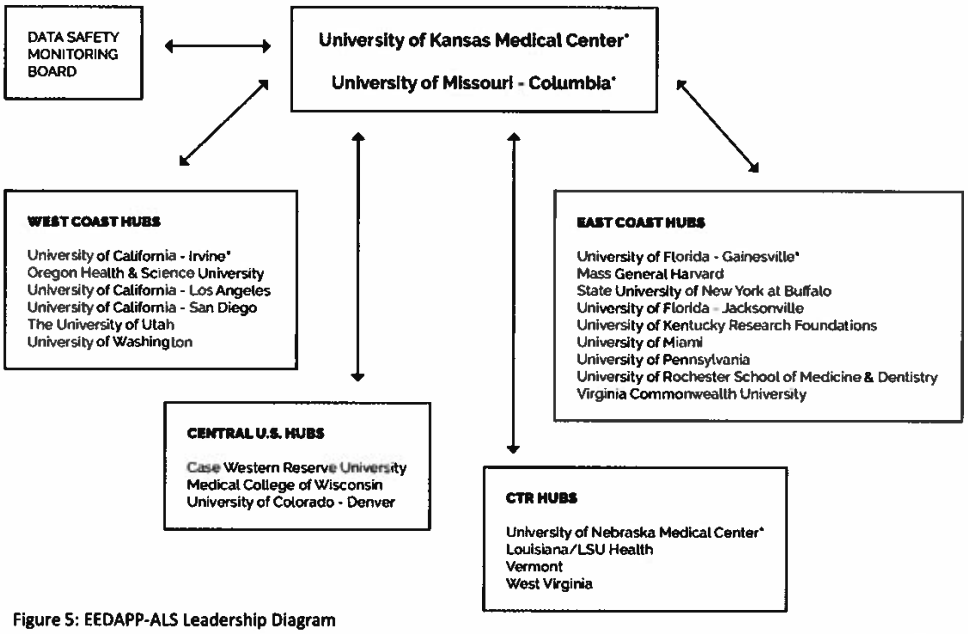

Figure 5: EEDAPP-ALS Leadership Diagram

* lead site

The proposal went through a rigorous consultation through the Johns Hopkins University (JHU)-Tufts TIC and Vanderbilt RIC of the TIN network, including a 2-day live meeting at the JHU-Tufts TIC Design Lab held in April 2019. Dr. Barohn and his team (Drs Govindarajan, Karanevich, Statland, and Ms Herbelin) were invited by the TIN to present their EE2 concept and proposal. Representatives from industry, payers, NIH, FDA and a number of clinical trial experts participated and provided useful advice and recommendations. The research team made significant revisions to their protocol resulting in the current proposal. We obtained advice from experts in the EE2 design field. We will leverage the TIN's design studio experience in conducting a first of its kind EE2 study in a rare disease. After the design studio event, we applied for an initial TIN consultation which occurred over several calls. We then asked for a comprehensive consultation which was granted (see letters of support).

Our industry partner, Catalyst Pharmaceuticals, was also essential in the development of this proposal. Stanley Iyadurai, Vice President of Clinical Affairs at Catalyst Pharmaceuticals, played a vital role in this collaboration by supporting our proposal and assisting us in the development of the specific aims, trial

design, and study medication dose limits and titration schedules.

Our comprehensive framework for patient, caregiver and family collaboration This study is responsive to the input received from patients and families over the course of ongoing patient engagement at KUMC. Figure 6 displays the operating model of engagement that guides the activities to ensure patient- and family/caregiver-centeredness for ALS research. Each engagement element depicted informs the other, and each makes a unique contribution. The model is a visual tool that ensures investigators fully use the unique contributions of non-academic team members. It also helps address organizational and representational issues for decisions at all levels for the design and execution phases of the study. To maintain engagement throughout this study, our Patient and Family Advisory Council (PFAC) will be reconfigured to include participants from all of the collaborating sites. Our track record for maintaining an active group using only phone and video connections (vs. in person meetings) is strong, and using an online communication platform (i.e., Zoom) lets the group build trust and familiarity with one another and with the research team as the study progresses. The PFAC is facilitated by a patient engagement expert, Kim Kimminau, PhD, who ensures the group stays focused on the research challenges and progress of the study over time.

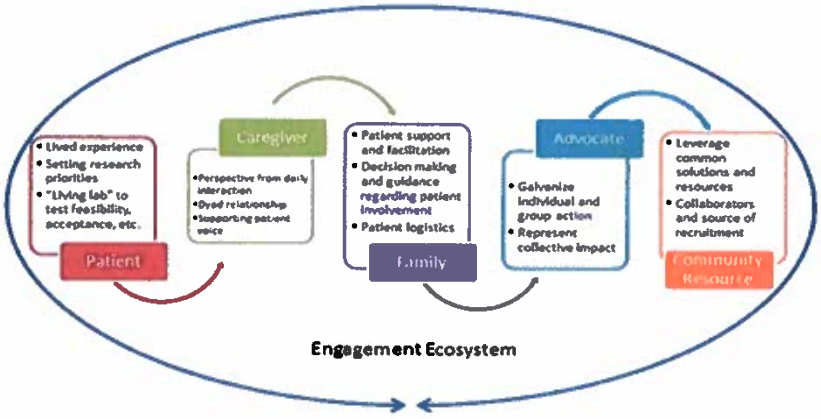

Figure 6: ALS Engagement Ecosystem

The innovative trial design for this project stemmed from ongoing dialogue with patients, families and the industry given the desperate need for more treatments options for ALS. While edaravone has shown to slow the progression, it is expensive, has a cumbersome infusion protocol, and is not widely used.[14] Riluzole has shown to prolong survival by three months without any significant effect on function. Data from two sources show that there is a high variability in the use of these medications.[2] A report was ran in March 2019 in the Mid-America ALS Chapter's Patient Reported Database which showed that out of 515 total patients, 74 patients (14%) are on riluzole alone, 66 (13%) are on edaravone alone, 124 (24%) are on both, and 251 (49%) are on neither.[3]

The Greater Plains Collaborative, PCORNet is based at the University of Kansas Medical Center and has 11 partner sites. We queried the electronic medical records of 10 sites (Medical College of Wisconsin, Marshfield Clinic, University of Utah, University of Nebraska Medical Center, University of Kansas Medical Center, University of Iowa, University of Texas Southwestern Medical Center, University of Indiana, University of Missouri, and University of Texas Health Sciences Center at San Antonio) and found that out of 2160 active ALS patients, 998 are on riluzole alone (46%), 11 on edaravone alone (5.2%), 146 on both (6.7%). The 11th site was a pediatric site. The reasons for this wide variability in use of two FDA approved drugs remains unclear. Using this information, we convened a patient and patient/caregiver dyad focus group. Two key findings from this helped shape the approach for this study. First, patients shared enthusiastic support for a new drug study targeting a novel mechanism of action. Second, patients and caregivers were passionate about ensuring the study would be available to as many patients as possible—a topic discussed at length. They understood opening inclusion criteria produces broader patient participation and potentially generalizability but at the same time might reduce the efficacy of the trial. Thus, having a trial design that can allow a heterogenous group of patients to participate without compromising the efficacy was an ideal solution and responsive to their priorities.

Innovation:
The key innovations for Aim 1 are: 1) exploring the EE2 model in a rare disease like ALS, a first of its kind trial in ALS and any rare disease; 2) building an EE2 trial experienced CTSA/CTR network which can be

used for other rare diseases and non-rare diseases. Aside from being the first randomized trial of 3,4-DAPP in ALS, this trial allows studying the effects of 3,4-DAPP in a homogenous patient population similar to what was used in other phase 3 studies and led to FDA approval in those drugs. The use of an efficacy and effectiveness too (EE2) trial design will ensure that 3,4-DAPP will also be tested under conditions relevant to usual clinical care.

Efficacy trials, typically designed to gain regulatory marketing approval, evaluate drugs in optimally selected patients under advantageous conditions. Effectiveness trials, designed to evaluate use in usual practice, assesses treatments among more typical patients in real-world conditions. However, this risks that the data collected on the narrower more homogenous group of patients will not be realized when implemented in real-world care. The innovative "efficacy and effectiveness too (EE2) trials," which simultaneously satisfy the requirements of both efficacy and effectiveness trials, would be used for the first time in a rare disease condition. Thereby, this trial's design addresses the problem that most new treatments are tested in highly selected samples that are not representative of how it would be used in widespread practice. Thus, this trial will include the broader group of patients with ALS who wouldn't qualify for the efficacy cohort because they don't meet certain inclusion criteria.

An EE2 trial is an ideal innovation for the CTSA and IDeA CTR consortium to embark on as it is a novel and unique trial design concept. At this time, the design has not been widely adopted in any disease area. Our exploration to take on these types of projects has met with some hesitancy and reluctance from potential funders. One NIH Institute Director, when approached on whether that Institute would be open to funding an EE2 trial, responded "We like to fund one trial at a time". Clearly, there is some equipoise regarding EE2 trials. Our contention is that the NCATS Innovation Awards RFA is the ideal mechanism to fund the early attempts at innovative EE2 trial and that this example is of particular interest as it involves a rare disease. We are able to perform this innovative Phase 3 trial in a CTSA/CTR consortium with NCATS funding because we are targeting a rare disease.

Translation:
Applying new innovative trial design in the conduct of clinical trials in rare diseases has the potential to speed the translation of trial results into practice by appealing to a larger group of patients with limited options for participating in clinical trials, and therefore encouraging faster enrollment. Patients and families with ALS are desperate for new therapies to be translated quickly from research trials. Since 3,4-DAPP is FDA approved already for another disease, if we can show efficacy in ALS, the drug can quickly become available to ALS patients.

Statistical considerations:
As described in the paper by Selker et al [1], an EE2 trial is designed to test the primary efficacy hypothesis/es in the efficacy cohort according to a plan prespecified in the protocol and statistical analysis plan. The results of these pre-specified analyses will determine the success of the study and are used for regulatory decision. The effectiveness cohort may have prespecified statistically powered endpoints or may be considered exploratory. The effectiveness cohort will also be the main population used for safety analyses.

Recruitment:
The study is a collaboration of 20 CTSA and 4 IDeA-CTR ALS centers that care for more than 5000 patients with ALS. The University of Kansas Medical Center will work with sites to establish recruitment strategies that will work for each local setting. Each site investigator will submit a recruitment plan and agree to screen every eligible participant referred to or seen at the site. The University of Kansas Medical Center will review recruitment performance metrics, and screening logs will explicitly identify the key criteria for enrollment. In addition, trial metrics reports from the University of Kansas Medical Center will be inspected regularly to look for trends and abnormalities within the data. The reports will include: monthly screened, consented, and enrolled participants including a ratio of hospital/registry statistics vs. number screened. Most solutions to poor recruitment performance are local and depend on the efforts of motivated, capable personnel who understand the protocol and have adequate resources to recruit participants into the trial. Richard Barohn, Raghav Govindarajan, and staff will maintain close working relationships with site investigators and coordinators to understand local problems and help ensure site

investigators quickly implement local solutions. This will be carried by having monthly site investigator and study coordinator calls. Scheduled contact with the study Patient and Family Advisory Council (PFAC) will ensure connectivity and relevance of patient and family input to the PI and scientific leads on the study.

Data Management:
Data Management will be overseen by the Department of Biostatistics and Data Science Department. For this study, we will be using the VELOS/CRIS database system that is a 21 CFR Part 11 Compliance database. Data Management will ensure site database training, will produce site level metrics on data quality and queries, and will create reports for the Data Safety Monitoring Board (DSMB) and for EE2 randomization in Aim 2.

Barriers:
Rolling out a new trial design such EE2 across multiple CTSAs and CTRs will be challenging as many investigators are not familiar with this design and IRBs will be scrutinizing the protocol closely. However, we will be leveraging TIN experience of designing and conducting EE2 trials in this study, particularly the expertise of Drs Cohen and Selker. Recruitment difficulties also may be challenging in this rare disease, but our combined networks, covering ~5000 ALS patients, can be used to disseminate study information.

Defining Success:
We will demonstrate the ultimate clinical success of this project if we: 1) demonstrate the feasibility of doing EE2 trial in a rare disease like ALS 2) meet our enrollment and study completion timeline 3) build a CTSA/CTR network that can do EE2 trial in rare diseases. This will be discussed in our Aim 2 section below.

We will disseminate the results of this study using our CTSA and IDeA infrastructure, patient engagement networks, and relationships with advocacy groups. If we are able to successfully complete the trial it will provide a blue print for EE2 trial for other rare diseases.

Aim 2:
We will determine if 3,4-DAPP can alter the course of the disease in ALS patients. In this aim, we will assess the efficacy of 3,4-DAPP by measuring changes in the slope of ALSFRS-R in a well-defined progressing cohort of ALS as previously defined in the edavarone study (Aim 2a). We hypothesize that 3,4-DAPP will slow down the progression of ALS by 30% as measured by the slope of the ALSFRS-R at the end of 6 months in this well -defined narrow cohort. The dose of 3,4-DAPP will be 80mg/day or the highest tolerated dose up to that level (Aim 2a). Simultaneously, we will recruit ALS patients with a more heterogenous disease status to more likely reflect a general ALS population and determine effectiveness (Aim 2B). The aim 2B is to determine if there are trends when looking at a more heterogenous population that suggest 3,4-DAPP may have a benefit (Aim 2b). We will also measure secondary outcome measures in both populations: survival, the slope of decline of forced vital capacity (FVC), the change in an ALS specific quality of life measure (ALSAQ-40) and a patient-reported ALS outcome measure, PADL ALS (Aim 2c). We will use our Patient and Family Advisory Council (PFAC) to inform and interpret the patient outcomes associated with this aim.

Rationale:
Neuromuscular Junction Pathology in ALS: The exact mechanism of ALS is unknown. The prevailing theory is that ALS is a dying forward phenomenon, in which primary damage occurs in the motor neurons and then extends in an anterograde fashion. Alternatively, multiple animal studies (such as SOD1 mice, drosophila and zebra fish ALS models) have demonstrated a distal axonopathy in which motor neuron degeneration starts at the nerve endings and progresses toward the cell bodies in a dying back manner leading to muscle denervation.[15-18] Clinical and electrophysiologic correlates of muscle fatiguability in ALS patients also suggest an element of neuromuscular junction (NMJ) transmission dysfunction.[19-20] Several studies of muscle biopsies from ALS patients suggest muscle denervation develops prior to significant motor neuron loss indicating early NMJ involvement.[19-20] In SOD1 mice, there is loss of laminin β2 which is believed to produce the dying back phenomenon. In SOD1 mice, the loss of an interaction between laminin β2 and the P/Q type voltage gated calcium channel (VGCC) causes NMJ denervation. This is because the interaction between laminin β2 and P/Q type VGCC anchors this channel at presynaptic terminals and organizes NMJ presynaptic active zones, the synaptic

vehicle release sites. Decreased levels of laminin β2 and P/Q type VGCC at ALS NMJs cause a decreased number of active zones.[21]

NMJ denervation in SOD1 mice:
ALS patients show decreased active zone size in spinal cord synapses.[22-23] Chronic inhibition of laminin β2 causes decreased number of active zones and NMJ denervation in wild type mice. Therefore, the loss of laminin β2 can cause NMJ denervation in SOD1 mice. Exercise as an intervention for ALS has been performed in SOD1 mice and ALS patients. Exercise recovers laminin β2 levels at NMJs and ameliorates NMJ denervation in SOD1 mice and rats. When SOD1 mice are crossed with transgenic mice expressing laminin β2 in muscle, transgenic expression of laminin β2 without exercise ameliorated NMJ denervation in SOD1 mice. Thus, laminin β2 ameliorates dying back neuropathy in ALS can improve the NMJ structure and function.[21]

Why could 3,4-DAPP benefit patients with ALS?
The mechanism of action of 3,4-DAPP is at the presynaptic terminal (at the voltage gated potassium channel) of the NMJ to enhance function by producing an increase in the release of acetylcholine vesicles.[24] This drug was recently approved by the FDA for the treatment of the Lambert-Eaton myasthenia syndrome, a disorder of the presynaptic terminal in which antibodies are directed against the P/Q voltage gated calcium channel.[25] 3,4-DAPP may improve the function at the NMJ in ALS patients the same way exercise does.[26] Benefit of exercise for ALS patients is an increase in laminin β2. We think this is a *druggable phenomena* and that 3,4-DAPP may produce this. There have been several small clinical studies of 3,4-DAPP in ALS. Aisen **et al.** demonstrated that 3,4-DAPP was well tolerated in all ALS patients, but limited by gastrointestinal side effects.[27-28] They also found a statistically significant improvement in Functional Independence Measure and speech assessment scores in addition to providing data on the pharmacokinetic properties of 3,4-DAPP in ALS patients. The standard outcome of ALS clinical trial namely ALSFR-R was **not** measured in this study.[27-28] Bertorini et al. in a double-blind, crossover design of 17 ALS patients, demonstrated that 3,4-DAPP was well tolerated with only four subjects reporting tingling of lips and fingers during the active drug period.[29] The subjective scores for fatigue and weakness showed a mild improvement after 4 weeks on DAP compared with placebo. A significant benefit of **3,4-DAPP** was also demonstrated in the timed verbal scores. The study was underpowered to demonstrate a measurable change in ALSFRS-R.[29]

We believe we have sufficient justification with the current information to move directly to a Phase 3/4 EE2 trial. The justification is as follows: 1) **3,4-DAPP** has been FDA approved for another severe neuromuscular disorder and have been shown to be safe and efficacious and **there is extensive clinical experience with this drug even prior to FDA approval as an off-label use. There is also strong pre-clinical and clinical evidence of neuromuscular junction pathology in ALS** 2) there have been three prior small phase 2 ALS studies with 3,4-DAPP as noted above; 3) patients and families and the ALS community are anxious to show that another drug (in addition to riluzole and edaravone) can slow the progression of ALS. Repeating another phase 2 trial is an unneeded delay; 4) Industry (Catalyst Pharmaceuticals) is very supportive and interested in the current phase 3/4 EE2 trial. They may use this data to go to the FDA to obtain a labeling indication for ALS if the study is positive.

Collaboration:
TIN, TIC, and RIC collaborations as well as the PFAC were previously described. Using a national infrastructure of 20 CTSA sites and 4 IDeA CTR sites, we will enroll ALS participants in a prospective 6-month placebo-controlled trial. All sites have ALS specialty clinics sponsored by MDA or ALSA, and experience with ALS clinical trials, being affiliated with WALS and NEALS. ALS patients seen in these clinics represent the full spectrum of disease, both sexes, all races/ethnicity, urban and rural dwelling, and diverse socioeconomic status. To ensure all eligible patients have a chance to participate, we will also reach out to patients directly using national registries, which include the advocacy organizations.

Innovation:
The key innovations for Aim 2 are: 1) exploring a novel mechanism of action for ALS at the neuromuscular junction in a large trial 2) conducting a drug trial with new inclusion criteria for ALS that was put forth in edaravone study and has not been reproduced in any other clinical trial 3) exploring the correlation between

patient reported PADL-ALS with ALSFRS-R in a real world clinical trial, with input and augmented interpretation of findings by patients and caregivers.

Translation:
Methodological issues for conducting the first of its kind EE2 study in a rare disease was discussed in Aim 1. Here we discuss implementation of the EE2 study design with 3,4-DAPP through a multi-center, double blind, prospective placebo controlled 6-month trial. We will enroll patients to both efficacy component and effectiveness component simultaneously. If positive, the result of this study will have enormous implications for ALS patients and their families.

Trial design: This will be a multi-center, double blind, prospective placebo controlled 9-month trial. Patients will be randomized to medication vs. placebo in a 1:1 ratio stratified by cohort (efficacy and effectiveness) and edaravone (yes/no). We will enroll patients to both efficacy component and effectiveness component simultaneously. Participants will be seen in clinic every 3 months to coincide with their standard of care visits and participants are allowed to be on the two FDA approved disease modifying medications (riluzole and edaravone). The drug will be provided in 20 mg tablets that is scored. Patients will start at 1 tablet 4 times a day for 1 week (total of 40 mg week). They will increase the dose to 1 ½ tablet (15 mg) four times a day for 1 week (total of 60 mg week). The last dose will be ramped up to 20 mg four times a day of the active drug or placebo (for a total of 80 mg). Following completion of the blinded portion of the study, all interested participates will have an opportunity to participate in an open label extension study (for patients in both efficacy and effectiveness components) for at least 6 months which will provide more longitudinal data of 3,4-DAPP in the ALS population and will allow all ALS patients who consented to participate in the study to have access to the active research drug. A full description of the trial design is located in the HUMAN SUBJECTS section of the grant.

Patient characteristics: 200 study participants will be enrolled into the efficacy component and 100 study participants will be enrolled into the effectiveness component.

Inclusion criteria:
Efficacy Cohort (Specific Aim 2a)
18 years of age who meet the diagnosis of "definite" or "probable" ALS according to the El Escorial revised Airlie House diagnostic criteria, forced vital capacity (%FVC) of at least 80% or more, duration of disease from the first symptom (any ALS symptom) within two years or less, scores of at least 2 points on all 12 items of ALSFRS-R bilaterally, change in revised ALS functional rating scale (ALSFRS-R) score during a 12-week observation period of –1 to –4 points. These are a slight deviation from the pivotal labelling indication trial for edaravone, that we dropped the Japanese ALS rating system as a criterion.

Effectiveness Cohort (Specific Aim 2b)
18 years of age who meet the diagnosis of "definite" or "probable" or "lab supported" ALS according to the El Escorial revised Airlie House diagnostic criteria, forced vital capacity (%FVC) of at least 70% or more, duration of disease from the first symptom (any ALS symptom) within three years or less, subjects who initially attempted to get into the efficacy phase, but failed the lead-in drop of the ALSFRS-R over the 12 -week period.

Exclusion criteria:
Hypersensitivity to any component of this medication, history of past or current seizures, history of asthma, evidence of prolonged QT syndrome. There is no absolute upper limit of normal for the QTc interval, family history of prolonged QTc syndrome, history of unexplained syncope, seizures or cardiac arrest.

Recruitment:
See the recruitment plan in Aim 1 and Human Subjects section. A search of local CTSA electronic health records identified >5000 ALS patients covered by the 24 CTSA sites and 4 IDeA CTR sites chosen for this study. We will leverage our relationships with advocacy organizations,

Interventions: While neuromuscular pathology has been implicated in ALS, they have not systematically been explored in a large multicenter trial. 3, 4-DAP acts at the presynaptic terminal (at the voltage gated potassium channel) of the NMJ to enhance function by producing an increase in the release of acetylcholine vesicles. This drug was recently approved by the FDA for the treatment of the Lambert-Eaton myasthenia syndrome, a disorder of the presynaptic terminal in which antibodies are directed against the P/Q voltage gated calcium channel. 3,4-DAPP may improve the function at the NMJ in ALS patients the same way exercise does.

Primary outcome:
We hypothesize that 3,4-DAPP will slow down the progression of ALS by 30% as measured by the slope of the ALSFRS-R at the end of 6 months in the efficacy cohort. The primary outcome of this study is the slope of decline in ALSFRS-R a validated patient reported 12-item survey that measures impairment of limb, bulbar and respiratory functions. The scale is measured as a part of routine clinical care across centers in the study. The slope of decline of each arm will be compared at baseline at randomization, 3 months and at the end of 6 months. The Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) is a validated questionnaire-based scale that assesses the ability of ALS patients to perform physical tasks across four main domains: gross motor activity, fine motor activity, respiratory function, and nutrition. The scale has been designed to be a clinical rating instrument that could be readily administered for monitoring the progression of patients in routine clinical practice as well as serve as an outcome measure in clinical trials. The ALSFRS-R is composed of 12 questions regarding aspects of daily functioning, and the answers given on a 5-point scale (0-4). The spheres measured are: 1) speech, 2) salivation, 3) swallowing, 4) handwriting, 5) cutting and handling utensils (with two subtypes depending on gastrostomy status), 6) dressing and hygiene, 7) turning in bed and adjusting bed clothes), 8) walking, 9) climbing stairs, 10) dyspnea, 11) orthopnea, and 12) respiratory insufficiency. ALSFRS-R is a strong predictor of survival, declining with disease progression at a rate that is quite consistent across clinical trials- 0.92 units per month with a relatively small variance (standard error of 0.08). Further, values of Cronbach's alpha for ALSFRS-R were greater than 0.67 for all individual ratings and the association between the ALSFRS-R and the Sickness Impact Profile (SIP), a well-accepted quality of life measurement, was strong, with Spearman coefficient of r= -0.71.[30]

Secondary Outcomes (Specific Aim 2c):
The secondary measures include assessing the survival, the slope of decline of forced vital capacity, the change in an ALS specific quality of life measure (ALSAQ-40) and a patient reported ALS outcome measure, PADL ALS. Amyotrophic Lateral Sclerosis Assessment Questionnaire-40 is a disease-specific measure, designed specifically to assess health related quality of life. The content of the measure was designed on the basis of patient self-report. The instrument contains 40 questions that measure five areas of health state: physical mobility, activities of daily living and independence; eating and drinking; communication; and emotional functioning.[31] The questionnaire addresses experiences of importance to individuals with ALS in such diverse areas as fear of falling when walking, difficulties cutting and eating food, participating in conversations, feelings of isolation, social embarrassment, as well as measuring feelings of fear and hopelessness about the future, that are all quite distinctively associated with it. Dimension scores are coded on a scale from 0 (perfect health as assessed by the measure) to 100 (worst health as assessed by the measure). Cronbach's alpha for ALSAQ-40 exceeded 0.9 for all individual ratings and the ALSAQ-40 total score to item correlation ranged from 0.61 to 0.92 (Spearman's r, $P<0.001$). PADL-ALS is a patient-centric revision of the ALSFRS designed specifically to conduct large pragmatic trials in ALS using the EMR patient-portal which has added questions about pseudobulbar affect, pain, and faith.[32]

Statistical analysis:
Power and Sample Size: Sample size was derived assuming a t-test with equal variance across treatment arms. We expect patients who don't receive any treatment to progress at roughly -1.25 ALSFRS-R per month, and that those on edaravone will decrease at 70% of this rate, with a standard deviation of 5 (as was seen in the 6-month edaravone pivotal phase 3 trial). We further assume that 3/4 DAP will slow progression by an additional 30%, and that the common standard deviation between baseline and 12 months will stay roughly 5. Assuming 45% of patients in the efficacy cohort will be on edaravone, using a weighted average to obtain the average six-month decline in the placebo arm (-6.5 ALSFRS-R) and the treatment arm (-4.55 ALSFRS-R), and estimate the six-month standard deviation of 5, which results in needing N = 210 patients per treatment group with 80% power at alpha = 0.05 via a two-sided test.

Reporting: Categorical measurements will be summarized by raw number observed and percent. Continuous measurements will be summarized by mean, median, standard deviation, minimum and maximum. For efficacy measurements (including coefficients associated with statistical modelling), 95% confidence intervals will be provided for the mean.

Primary Analysis: The null hypothesis that in the efficacy cohort there is no difference in change of six-month slope in the ALSFRS-R, from baseline, between treatment groups. The alternative is that the change of six-month slope in the ALSFRS-R, from baseline, is smaller in the treatment group compared to the placebo group.

The difference in change of six-month slope in the ALSFRS-R, from baseline, between treatment groups will be evaluated via a linear mixed effects model. Time, treatment group, and edaravone use will be included as fixed effects, and time and intercept will be included as random effects. An unstructured covariance structure will be utilized. In the event of convergence issues, a first-order autoregressive covariance structure will be utilized instead.

The null hypothesis will be rejected if the coefficient associated with treatment group (the indicator of being treated 3,4-DAPP) is statistically significantly greater than 0 with a type I error rate of 0.05.
Secondary Analyses: Survival from baseline will be analyzed using Kaplan-Meier across treatment arms. Analysis of the six-month slope in change of forced vital capacity (FVC) from baseline will be performed in the same fashion as that of the primary analysis. Six-month change in ALSAQ-40 from baseline will be evaluated using ANCOVA, with treatment group and edaravone use included as covariates.

Missing data: Observed cases only will be used, with no imputation.

Sensitivity Analyses: If modelling assumptions are violated, all six-month slopes may instead be evaluated via a Wilcoxon Rank-Sum test which solely looks at the raw difference between the six-month endpoint or last-observed endpoint and the baseline value. Similarly, the Six-month change in ALSAQ-40 from baseline may also be evaluated via Wilcoxon Rank-Sum test.

Safety monitoring and Safety analysis:
Drug Safety: All drugs used in this study are FDA-approved and have considerable safety data available for the use in humans. Possible drug interactions for this study would include the study medications and existing standard of care medications, which includes riluzole, and edaravone. Drug interactions were reviewed with the pharmaceutical company and no major interactions were noted. The study doses chosen are within the FDA labeling dose ranges for both drugs (the FDA safety labels for each drug are available at drugs@FDA.gov).

For this study we propose real-time safety monitoring which will include AE reporting monthly during the run-in phase, then weekly as the dose is ramped up and then monthly. Participants safety will be monitored by our investigators and coordinators, Medical Safety Monitor and by our DSMB. The Medical Safety Monitor (MSM) and DSMB will receive periodic safety reports of all adverse events including serious adverse events (SAEs) if necessary. All clinical safety endpoints and SAEs will be summarized by AE code in terms of frequency of the event, number of subjects having the event, severity, and relatedness to the study treatment.

Partnership:
This study is a partnership among the ALS research teams, and the patients and caregivers who have had, and will have, an active role in study design, conduct, and dissemination of results and industry. We are partnering with Catalyst Pharmaceuticals to provide the drug and placebo and allowing us to cross file on their IND for 3,4-DAPP. We will continue to use a Patient and Family Advisory Council during the conduct of the study to help give feedback on recruitment, retention, results interpretation and dissemination; and we will collaborate with the advocacy organization (MDA, ALSA) to help with recruitment and study results dissemination.

Barriers:

Rolling out a new trial design such EE2 across multiple CTSAs and IDeA CTRs will be challenging. However, we will be leveraging TIN experience of designing and conducting EE2 trials in this study. **The inclusion criteria for the efficacy cohort has never been tested in a clinical trial outside Japanese clinical trial. Patients fulfilling that inclusion criteria might be hard to recruit but our combined networks, covering** ~5000 ALS patients, can be used to disseminate study information. The MDA and ALSA and Catalyst Pharmaceuticals will assist us in getting the word out about the study to patients and families. **The absence of good biomarkers to monitor treatment response and disease progression has plagued ALS clinical trials. In particular to this study the lack of suitable biomarkers for monitoring neuromuscular junction pathology might prevent us from measuring treatment responders and non-responders. This is a known barrier in all ALS clinical trials and ALSFRS-R continues to be the universally accepted biomarker of treatment response.**

Defining Success:
We will demonstrate the ultimate clinical success of this project if we: 1) are able to show a slowing down of ALSFRS-R slope by 30% in the efficacy component; 2) to determine if there are trends of efficacy (either with primary end point or secondary end points) when looking at a more heterogenous population which provides data for generalizability which traditional trial designs lack; 3) demonstrate correlation between patient reported ALSFRS-R(PADL-ALS) with ALSFRS-R in a real world clinical trial. We will disseminate the results of this study using our CTSA and CTR infrastructure, patient engagement networks, and relationships with advocacy groups. If the drugs tested prove efficacious, it will have an immediate impact on patients with ALS and their family members and will become only the second drug to have a disease modifying effect on ALS. If the trial is positive, our partner, Catalyst Pharmaceuticals, may elect to file with the FDA for labeling indication for 3,4-DAPP for ALS.

SUMMARY STATEMENT

PROGRAM CONTACT:
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(Privileged Communication)

Release Date: **04/25/2020**
Revised Date:

Application Number: **1 U01 TR003420-01**

Principal Investigators (Listed Alphabetically):
BAROHN, RICHARD J. (Contact)
GOVINDARAJAN, RANGASWAMY

Applicant Organization: UNIVERSITY OF KANSAS MEDICAL CENTER

Review Group: **ZTR1 CI-4 (01)**
National Center for Advancing Translational Sciences Special Emphasis Panel
CTSA Collaborative Innovation Awards Review Meeting

Meeting Date: **02/20/2020**
Council: **MAY 2020**
Requested Start: **07/01/2020**

RFA/PA: **PAR19-099**
PCC: **1CCIA12**

Project Title: **EE2: 3,4-Diaminopyridine Phosphate for ALS - The EEDAPP-ALS Trial**

SRG Action: **++**
Next Steps: **Visit https://grants.nih.gov/grants/next_steps.htm**
Human Subjects: 48-At time of award, restrictions will apply
Animal Subjects: 10-No live vertebrate animals involved for competing appl.
Gender: 1A-Both genders, scientifically acceptable
Minority: 1A-Minorities and non-minorities, scientifically acceptable
Age: 3A-No children included, scientifically acceptable

Project Year	Direct Costs Requested
1	749,957
2	749,288
3	749,977
4	749,898
TOTAL	2,999,120

++NOTE TO APPLICANT: Members of the Scientific Review Group (SRG) were asked to identify those applications with the highest scientific merit, generally the top half. Written comments, criterion scores, and preliminary impact scores were submitted by the assigned reviewers prior to the SRG meeting. At the meeting, the more meritorious applications were discussed and given final impact scores; by concurrence of the full SRG, the remaining applications, including this application, were not discussed or scored. The reviewers' comments (largely unedited by NIH staff) and criterion scores for this application are provided below. Because applications deemed by the SRG to have the highest scientific merit generally are considered for funding first, it is highly unlikely that an application with an ND recommendation will be funded. Each applicant should read the written critiques carefully and, if there are questions about the review or future options for the project, discuss them with the Program Contact listed above.

ADMINISTRATIVE NOTE
1U01TR003420-01 Barohn, Richard
ADMINISTRATIVE NOTE – RESOURCE SHARING
PROTECTION OF HUMAN SUBJECTS UNACCEPTABLE

DESCRIPTION (provided by applicant): The overall goal of this application is to perform an innovative Efficacy Effectiveness – Tool trial design (EE2) in Amyotrophic Lateral Sclerosis (ALS) in which we can simultaneously enroll a homogeneous population to determine efficacy and a wider population to determine effectiveness in a broader population. ALS is a rare, relentlessly progressive and fatal neurodegenerative disease affecting cortical and spinal motor neurons. The exact mechanism of ALS is unknown. This clinical trial will study the efficacy and effectiveness of 3,4-Diaminopyridine Phosphate (3,4-DAPP) in patients with ALS. The mechanism of action of 3,4-DAPP is at the presynaptic terminal of the neuromuscular junction (NMJ) to enhance function by producing an increase in the release of acetylcholine vesicles. This drug was recently approved by the FDA for the treatment of the Lambert -Eaton myasthenic syndrome and may improve the function at the NMJ in ALS patients the same way exercise does. This proposal would be the first time an EE2 trial is done in a rare disease and will include 20 CTSA sites and 4 IDeA State CTR sites dispersed across the United States. There are five sites (Kansas, Missouri, Nebraska, California-Irvine, and Florida-Gainesville) that
are designated as lead sites for the study. The specific aims for this study are as follows: 1. Perform an EE2 study in ALS at 20 CTSA sites and 4 IDeA CTR sites and simultaneously enroll a cohort to determine efficacy and a more heterogenous cohort which combined with the efficacy cohort will determine effectiveness in a broader population. This will serve as a blueprint for the CTSA consortium to perform EE2 studies on rare diseases. 2. Determine if 3,4-DAPP can alter the course of the disease in ALS patients. 2a. Assess the efficacy of 3,4-DAPP by measuring changes in the slope of ALSFRS-R in a well-defined progressing cohort of ALS as previously defined in the edavarone study. We hypothesize that 3,4-DAPP will slow down the progression of ALS by 30% as measured by the slope of the ALSFRS-R at the end of 6 months in this well-defined narrow cohort. The dose of 3,4-DAPP will be 80mg/day or the highest tolerated dose up to that level. 2b. Simultaneously recruit ALS patients with a more heterogenous entry criteria to more likely reflect a general ALS population and determine effectiveness. The aim is to determine if there are trends when looking at a more heterogenous population that suggest 3,4-DAPP may have a benefit 2c. Measure secondary outcome measures in both populations: survival, the slope of decline of FVC, the change in an ALS specific quality of life measure (ALSAQ-40) and a patient reported ALS outcome measure, PADL ALS. At the conclusion of the study, there will be an open-label extension study which will allow all ALS patients who consented to participate in the study to have access to the active research drug. This will be funded by a different mechanism through a partnership with Catalyst Pharmaceuticals.

PUBLIC HEALTH RELEVANCE (provided by applicant): We will test an innovative trial design in amyotrophic lateral sclerosis (ALS), a rare, relentlessly progressive, fatal disease, by conducting a clinical trial, EE2: 3,4-Diaminopyridine Phosphate for ALS - The EEDAPP-ALS Trial to determine 3,4-Diaminopyridine Phosphate versus placebo benefits patients with ALS by slowing down disease progression. In addition to performing a Phase III efficacy study

in ALS with narrow inclusion criteria, we will simultaneously enroll a more heterogenous ALS group to determine effectiveness in a more generalizable population. 20 CTSA and 4 IDeA State CTR sites dispersed throughout the USA will be leveraged for this unique proposal.

CRITIQUES

Critique 1
Significance: 6
Investigator(s): 3
Innovation: 4
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BAROHN, R
Approach: 6
Environment: 3

Overall Impact: This is an interesting application in terms of a new trial design, which seems to
address the issue of whether a drug that "works" in a restricted group of patients will also work in a larger, more generalized population. However, the data supporting the testing of this drug, 3,4-Diaminopyridine Phosphate (DAPP), are quite weak. Though there are data arguing for a primary pathology at the neuromuscular junction (NMJ) in Amyotrophic Lateral Sclerosis (ALS), the rationale for the use of this particular drug is not encouraging. Two previous small trials of 3,4-DAPP did not show any significant positive effect, and the underlying science provided by the Principal Investigator (PI), Richard Barohn, M.D., regarding laminin beta 2 is unreferenced other than a single abstract that can be found as a seminar title at Queensland University, Australia. Other than the selection of the drug to be used in the trial, there are also problems with the statistical arguments and inconsistencies with the power analyses. In summary, the overall impact is low.

Significance
Strengths
- This is a novel approach to a clinical trial in ALS that may be informative for future trials in ALS and other rare disorders.

Weaknesses
- The proposed hypothesis is not well supported by preliminary data, preclinical studies, or the literature.
- The expected outcome, given previous experience with this drug, will not lead to significant improvement in the lives of ALS patients.
- The argument that the success of edaravone makes a good target for efficacy for an ALS drug places a very low bar on the definition of success for patients.

Investigator(s)
Strengths
- The Contact Principal Investigator (PI), Richard Barohn, M.D., is a leader in the field of neuromuscular disease and clinical trials. His administrative experience will certainly be a positive for this project.

• The roles of other consortia participants are those of a typical multicenter clinical trial group, and the skillsets involved are likely adequate.

Weaknesses

• The Multiple PI (MPI), Raghav Govindarajan, M.D., is much less experienced, has few publications and no track record for this level of leadership in such a large consortium.

Innovation

Strengths

• The Efficacy Effectiveness-Too (EE2) design is certainly innovative, as it has not previously been used in ALS or other neurodegenerative diseases.

Weaknesses

• The outcome measures are standard; no innovative outcomes specific to the proposed mechanism of action of the drug are presented.

• The description of the statistical basis for the EE2 design is unclear.

Approach

Strengths

• A strength is the use of the CTSA hubs and established ALS clinical sites to form an integrated consortium that can work together to provide the numbers of patients necessary to support a clinical trial of a rare disease with very restrictive entry criteria.

Weaknesses

• The choice of 3,4-DAPP for this EE2 trial is weak, due to a lack of preclinical data supporting this drug in ALS and the previous negative (but clearly small) trials of 3,4-DAPP in ALS.

• The EE2 design, as described, seems a bit counterintuitive. One would expect that if the drug works in the less restrictive trial population (effectiveness cohort), then it will necessarily work in the more restrictive cohort (efficacy), unless one believes that these represent different disease mechanisms.

• From a power analysis perspective, typically the number of patients needed for a cohort with less restrictive inclusion criteria would be more than that for a cohort with more restrictive entry criteria. However, the trial design states 200 participants in efficacy and 100 in effectiveness (page 228). This seems backwards.

• The section on power and sample size states that N=210 patients/treatment group. This is not consistent with the previous statement of 300 total participants and makes the statistical plan suspect.

• The patients are being separated on and off edaravone, but they are not being stratified for riluzole. Given that any clinical effect of 3,4-DAPP is unlikely to be better than either of these two approved drugs, multiple groups would need to be compared: placebo only, DAPP only, DAPP + riluzole, DAPP + edaravone, DAPP + both, and possibly even placebo plus each of the other drugs. This is not addressed in the statistical discussion.

• The doses and dose escalation schemes are confusing. 3,4-DAPP will be provided in 20 mg tablets and started at one tablet 4x/day for one week. This is 80 mg not “40mg week” (page 228). Similarly, the escalated doses of one and a half tablets 4x/day is 120 mg, not 80 mg. This may be a typographical error, repeated in the statistics section.

• Scant data are presented demonstrating that the numbers of patients fitting the entry criteria will be recruited and that those numbers might range from 200 to >500. Stating that there are 5,000 ALS patients in their region is fine, but it does not provide data on how many patients are at each center, what each center's population looks like (early vs. late disease, slow vs. fast disease) and what the other centers have done previously in ALS clinical trials. This is not a trivial problem, since the majority of patients will not meet the entry criteria and many patients will choose not to participate.

Environment

Strengths

• The CTSA hub infrastructure at the University of Kansas Medical Center (UKMC) is impressive.

• The collaborative network is in place.

Weaknesses

• More data are needed to assure that the adequate numbers of patients will be recruited.

Study Timeline

Strengths

• A reasonable clinical trial timeline is included.

Weaknesses

• Assurance is needed that enough patients with appropriate inclusion criteria can be recruited.

Protections for Human Subjects: No issues.

Data and Safety Monitoring Plan: Adequate.

Inclusion of Women, Minorities, and Individuals Across the Lifespan:

• Sex/Gender: Distribution justified scientifically.

• Race/Ethnicity: Distribution justified scientifically.

• For NIH-Defined Phase III trials, Plans for valid design and analysis: Scientifically acceptable.

• Inclusion/Exclusion Based on Age: Distribution justified scientifically.

Adequate.

Vertebrate Animals: [No reviewer comments].

Biohazards: [No reviewer comments].

Select Agents: [No reviewer comments].

Resource Sharing Plans: Adequate.

Authentication of Key Biological and/or Chemical Resources: [No reviewer comments].

Budget and Period of Support Recommend as Requested.

Critique 2
Significance: 4
Investigator(s): 2
Innovation: 3
Approach: 5
Environment: 2

Overall Impact: ALS is a fatal disease for which limited disease-modifying therapies are presently available. This proposed national consortium will test the medication 3,4-DAPP as a potential diseaseslowing agent, employing the EE2 trial design. The medication 3,4-DAPP, through its action at the presynapsis of the NMJ, is approved for use in Lambert-Eaton myasthenic syndrome, which is physiologically centered at the presynaptic terminal of the NMJ; it has been tested in limited ways in ALS over the past several decades. The EE2 design will use highly constrained criteria for enrollment into the efficacy arm, emulating what was performed for the Japanese edaravone trial, while also including research participants representing the broader real world spectrum of ALS in the effectiveness arm of the study. The Contact PI at KUMC is a well-recognized leader of multisite trials, the various consortium sites chosen are appropriate, and the lead site at KUMC has a strong track record in directing multicenter clinical trials in neuromuscular disease. Strengths include the clear need for disease-modifying therapies in this fatal disease; the first such EE2 clinical trial design in a rare disease population; appropriately chosen primary and secondary outcome measurements; and the fact that there will be eventual access to the drug for all participants in the clinical trial. Weaknesses include the limited preclinical data to support 3,4-DAPP as potentially beneficial in ALS; concern that while the efficacy component of the study is effectively powered, the effectiveness component may not be; and the absence of a well-delineated plan for how the University of Florida and the University of California Irvine will lead the East and West coast sites, respectively, for this study.

Significance
Strengths

- ALS is a fatal disease for which very limited disease-modifying therapies are presently available, thus there is a clear need for disease-modifying therapies.
- Success in the field demands multi-center studies like this and the idea of an EE2 trial via a multi-center consortium has merit.

Weaknesses

- While the trial medication 3,4-DAPP is approved for use in Lambert-Eaton myasthenic syndrome, which is pathophysiological centered at a site of action of the medicine, the presynaptic terminal of the NMJ, the scientific premise for its use in ALS is not nearly as well developed or justified.

Investigator(s)
Strengths

- Dr. Barohn is an established clinician scientist with a strong track record in clinical research and human clinical trials in neuromuscular disease.

- As Director of the CTSA hub at the University of Kansas, the PI is well-positioned to coordinate activities that are related to this.
- The University of Kansas team has a solid track record working with multisite consortia in ALS.
- MPI of the Kansas Missouri leadership team is Dr. Govindarajan, a neuromuscular specialist who recently was promoted to Associate Professor at the University of Missouri.
- Co-investigator Theodora Cohen, Ph.D., at Tufts University, who will provide statistical input to the EE2 study proposed, has appropriate experience in clinical trial design, analysis and reporting.
- The leadership plan makes it clear that the Contact PI will be mentoring the MPI in the conduct of large multicenter clinical trials.
- Participating sites have over 5,000 ALS patients, which should readily fill enrollment needs.

Weaknesses

- The 0.6 calendar months requested effort for Dr. Govindarajan may not be sufficient for the work required since the application states on p. 157 that the MPI "will provide oversight of the entire project and development implementation of all policies, procedures and processes."
- There is some concern that little is specifically described about both the ALS clinical efforts and clinical research at the University of Missouri, which is a lead institute in this application.
- Details are missing regarding how the University of Florida and UC-Irvine sites will be the lead sites for the East and West Coast institutions, respectively.

Innovation

Strengths

- This is the first such EE2 clinical trial design in a rare disease population. It is likely appropriate that an EE2 design be used in rare neurodegenerative diseases that have significant clinical heterogeneity, like ALS.

Weaknesses

- [No reviewer comments].

Approach

Strengths

- Incorporation of the Great Plains Institution for Clinical Translational Research spanning the North Central states is an encouraging step in collaboration across CTSA hubs and similar IDeA entities.
- The preparatory work to explore the Greater Plains collaborative electronic medical records (EMRs) to assess use of riluzole and edaravone by current ALS patients gives some confidence for the collaborative nature of the study.
- The EE2 design is appealing. While efficacy may be established for a narrow subset of ALS patients in the efficacy study, there may be supportive data for the broader ALS community through the combined study. In that sense, the impact of a positive result would be much higher.
- Appropriately chosen primary and secondary outcome measurements.

• KUMC will serve as the single IRB of record for the study; KUMC has a track record serving as a single IRB for collaborative initiatives like this one.
• Data safety and monitoring plan is adequate and includes remote monitoring through the KUMC quality assurance department.
• Data safety monitoring board will meet three times yearly with appropriate inclusion of a member of the ALS community.
• The University of Florida and the University of California Irvine will serve as regional leads for the east and west coast respectively, and the University of Nebraska will serve as lead regionally for the IDeA Centers.
• Primary endpoint will be measured using, appropriately, a linear mixed effects model to estimate the slope of the ALS Functional Rating Scale-Revised (ALSFRS-R).
• The drug, 3,4-DAPP is available. It is manufactured by Catalyst pharmaceuticals (letter indicates their support) and the IND application was submitted last fall.
• The Patients and Family Advisory Council will be kept informed at least every four months of the status of the study and as needed when pertinent information becomes available.
• Study results will be disseminated in the ALS Association and Muscular Dystrophy Association newsletters and posted on the web to broadcast to a wider community.
• Appropriate letters are in place from the various collaborating sites and coinvestigators to document involvement.
• Eventual access to the drug for all participants in clinical trial.
• Timeline is feasible.

Weaknesses

• Quite limited preclinical/human subject data to support 3,4-DAPP as potentially beneficial in ALS.
• Concern that while the efficacy component of the study is effectively powered, the effectiveness component may not be, thereby undercutting the goal of the EE2 trial.
• Absence of a well-delineated plan for how the University of Florida and UC-Irvine will lead the east and west coast sites, respectively, raises some concern for connectedness across sites.
• The following sentence in exclusion criteria on p. 228 is difficult to discern: “There is no absolute upper limit of normal for the QTC interval, family history of prolonged QTC syndrome, history of unexplained syncope, seizures or cardiac arrest.”

Environment

Strengths

• The neuromuscular research clinical trials unit at KUMC is highly ranked and has strong track record of success.
• The various collaborating sites are all established in clinical research neuromuscular diseases including ALS. Most participate in existing regional or national ALS consortia and are expected to be able to easily recruit participants for the EE2 design study.
• Appropriate use of the CTSA network and builds on existing strengths within the network.

Weaknesses

• Details are not explicitly provided regarding how the University of Florida and UC-Irvine sites will be the lead sites for the east and west coast institutions.

Study Timeline: Adequate description of timeline.

Protections for Human Subjects: Acceptable.

Data and Safety Monitoring Plan: Acceptable.

Inclusion of Women, Minorities, and Individuals Across the Lifespan:

- Sex/Gender: Distribution justified scientifically.
- Race/Ethnicity: Distribution justified scientifically.
- For NIH-Defined Phase III trials, Plans for valid design and analysis: Scientifically acceptable.
- Inclusion/Exclusion Based on Age: Distribution justified scientifically.

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Vertebrate Animals: Not Applicable (No Vertebrate Animals).

Biohazards: Not Applicable (No Biohazards).

Select Agents: [No reviewer comments].

Resource Sharing Plans: Acceptable.

Authentication of Key Biological and/or Chemical Resources: [No reviewer comments].

Budget and Period of Support: [No reviewer comments].

Critique 3
Significance: 2
Investigator(s): 4
Innovation: 5
Approach: 5
Environment: 3

Overall Impact: This is a comprehensive collaborative effort spanning 20 CTSA hubs and four IDeA centers to rapidly study a novel treatment for ALS. The clinical trial is a major undertaking and having leadership distributed across multiple CTSA hubs with regional leadership roles, particularly given the limited experience collaborating on such a complex trial, the likelihood of success is questioned. The use of placebo and risks with the study design do not appear objectively discussed. The sample size appears like it is larger than required had a design effect been applied accounting for repeated measures. Overall, the treatment approach to ALS is considered significant; this approach to get to that point is not as well received.

Significance

Strengths

- ALS is a critical, neurodegenerative disease that warrants rapid, structured testing of new treatments.
- Leverages some unique CTSA hubs with existing infrastructure.

Weaknesses

- The EE2 design appears premature for this treatment in this population. There appears to be limited pilot data and the likelihood of success is uncertain.

Investigator(s)

Strengths

- The senior leadership and the co-investigators are well trained and bring broad expertise to the trial.
- The idea of regional hubs helps logistics of the study, but more details would have enhanced this plan.

Weaknesses

- Beyond PI-level investigators, it is unclear if all of the sites will have the resources needed to manage the study.
- Investigator effort in the consortium is too low to have viable engagement.

Innovation

Strengths

- None noted.

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Weaknesses

- The whole application is built around the EE2 approach. As the investigators likely are aware, this is a bit of a polarizing approach in that some will love it and others will be more guarded. The inclusion and exclusion study does not define an effectiveness subgroup; this appears as a treatment failure. Given some subjects could be on placebo, this is a significant design flaw.

Approach

Strengths

- Potential for diverse, representative enrollment into the ALS study. Accelerates the testing of a novel indication for an existing compound in ALS.
- Data management plans using the VELOS database; central IRB plans are in place. The approach is described very briefly. More details are required, but the basic framework should meet the study's needs.

Weaknesses

- The application is unbalanced in technical details and promoting the EE2 study design.
- The EE2 study design is not likely the panacea being suggested. There are ethical considerations about interactions with standard of care, withholding standard of care, what level of evidence is needed before expanding use, etc. that warrant more attention. While there needs to be an acceleration of treatments and testing in rare diseases, there are also important methodological and resource considerations that warrant more

attention. The study design still closely resembles a post hoc subgroup analysis of any clinical trial. Planned or unplanned, it is still basically the same approach.
• On page 228 the effectiveness cohort is defined and importantly it is not an effectiveness cohort. This would almost appear as non-responders vs. broad inclusion criteria.
• It is unclear if there is any dissemination product available for this study. This is a large simple trial. This is very well established. Pragmatic trials are also well established. What specific attributes of the CTSA hubs are being leveraged for this award?
• The methods speak of obtaining the full Trial Innovation Network (TIN) support for this application. It is unclear what this means objectively. A summary of some of the discussions and how this protocol design has been chosen relative to alternatives would have increased the scientific rigor of the application.
• A more objective recruitment feasibility assessment is expected. This would have included justification for the individual sites selected as well as discussion of incident cases. It is expected that moving treated patients to this trial may not be a certainty; the calculations do not address this concern.
• Preliminary data for Specific Aim 2 are non-quantitative and lacking in figures and tables. Important questions about dosing are unresolved. It is not clear why the "extensive off label use" data is not presented directly in the application. If this data is so extensive, does this raise concerns for the need of the EE2 study design?
• Sample size calculations do not appear to account for the longitudinal data being available. The repeated measures and comparisons of slopes over time could have increased the "effective sample size" and reduced the overall number of participants studied. Given the cost and complexity of this first randomized trial in a rare disease, optimization of the sample size is expected.
• The analysis plan specifies a mixed model, which would be a strength. The investigators state, however, that the primary test is the treatment parameter. There would still be a treatment by time interaction terms that would be needed to be tested to summarize the differential slope relative to placebo. Likewise, model-based contrasts comparing the final timepoints estimated means is expected.

Environment

Strengths

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• The CTSA consortium, builds on an existing Central IRB and data management center expertise.

Weaknesses

• There is limited collaboration of these sites to date. The geographic spread will make the trial more difficult to coordinate due to time zone and travel demands.

Study Timeline

Strengths

• There is an attempt to mine I2B2 records across many of the sites. There is some indication of a prevalent pool of participants.

Weaknesses

• The recruitment still feels ambitious and hard to manage across the sites. Each site is expected to enroll, on average, about three participants per year. This does not seem to be enough volume to maintain much visibility and consistency of the site investigative teams.
• A more detailed accrual feasibility section that accounts for a study enrollment fraction (say 1/10 of all newly diagnosed patients) may have provided a more objective assessment of accrual feasibility.

Protections for Human Subjects: Unacceptable.
Overall, the human subject plan is repetitive and unfocused. There is not enough discussion on the consent process and consideration for starting standard of care in newly diagnosed patients.
The use of pure placebo is not justified. More information on the risks of placebo in a degenerative disease should have been addressed in the protocol. It is unclear if this protocol, as written, would pass the IRB process.

Data and Safety Monitoring Plan: Acceptable.
Basic DSMB structured in the application. More details on the charter, particularly around early monitoring of the study and evaluation of the "effectiveness" arm is warranted going forward.

Inclusion of Women, Minorities, and Individuals Across the Lifespan:
• Sex/Gender: Distribution justified scientifically.
• Race/Ethnicity: Distribution justified scientifically.
• For NIH-Defined Phase III trials, Plans for valid design and analysis: Scientifically acceptable.
• Inclusion/Exclusion Based on Age: Distribution justified scientifically.
Statistical plan is basic, but coverage of key elements is generally acceptable. More details are included in the trial protocol, which is welcomed, but this extended the length of the application considerably.
Vertebrate Animals: Not Applicable (No Vertebrate Animals).
Biohazards: Not Applicable (No Biohazards).
Select Agents: Not Applicable (No Select Agents).
Resource Sharing Plans: Unacceptable.
The primary concern is the general premise of disseminating the EE2 study model. This is viewed as a
weak alignment to the PAR.
Authentication of Key Biological and/or Chemical Resources: Not Applicable
(No Relevant Resources).
Budget and Period of Support: Recommend as Requested.
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THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:
PROTECTION OF HUMAN SUBJECTS: UNACCEPTABLE
Overall, the human subject plan is repetitive and unfocused. There is not enough discussion on the consent process and consideration for starting standard of care in newly diagnosed patients.
The use of pure placebo is not justified. More information on the risks of placebo in a

degenerative disease should have been addressed in the protocol. It is unclear if this protocol, as written, would pass the IRB process.

ADMINISTRATIVE NOTE – Resource Sharing Plans: UNACCEPTABLE.
The primary concern is the general premise of disseminating the EE2 study model. This is viewed as a weak alignment to the PAR.
Footnotes for 1 U01 TR003420-01; PI Name: Barohn, Richard J.
NIH has modified its policy regarding the receipt of resubmissions (amended applications).See Guide Notice NOT-OD-18-197 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-197.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER
The roster for this review meeting is displayed as an aggregated roster that includes reviewers from multiple TR Special Emphasis Panels Meetings
for the 2020/05 council round.
This roster for TR is available at:
http://public.era.nih.gov/pubroster/Reports?DOCTYPE=SEP&DESFORMAT=PDF&AGENDA_SEQ_NUM_P=387632

A Rare Potential Cause of Mononeuropathy Multiplex

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Introduction

The clinical presentation of multifocal involvement of two or more noncontiguous peripheral or cranial nerves which impairs the motor, sensory, sensorimotor, or somatic function defines mononeuropathy multiplex (MNM).[1] If the underlying pathology is progressive in nature, patients may evolve to have overlapping multifocal neuropathies in contiguous areas (overlapping multifocal neuropathy) which may be extensive enough to obscure the original anatomical pattern of multifocal features. While the differential for such presentations is broad and includes various inflammatory, autoimmune (i.e. multifocal acquired demyelinating sensory and motor neuropathy), infectious, drug-induced, genetic, mechanical, or neoplastic etiologies; the syndrome is often concerning for an underlying vasculitic neuropathy, especially when other systemic signs and features are present.[1,2] In this clinical scenario, consideration of potential mimics of vasculitic neuropathy is important as the underlying treatment may differ and affect clinical outcomes.[3,4] Here we present a case of mononeuropathy multiplex associated with a rare entity in a 58-year-old male in whom standard treatment with immunosuppression is contraindicated.

Case Report

A 58-year-old male reported symptom onset 4 months prior to neuromuscular consultation with numbness on his right foot and shin as well as left arm hypersensitivity and discomfort. He had no preceding illnesses but did report receiving the flu vaccine approximately 3 weeks prior to the onset of his initial symptoms. Symptoms began to progress over the course of 1-2 weeks with ascending numbness in his right leg as well as a knot-like sensation and pain in the back of the calf for which he presented to the ED with a negative workup for DVT. He continued to have progression of symptoms over the following weeks to include his right leg feeling heavier with exercise or longer drives as well as a similar knot-like sensation/pain in his left calf. His hypersensitivity also progressed to include his right arm. Throughout the second month of symptoms, he had a repeat emergency room visit as well as an appointment with his primary care manager with additional workup demonstrating a negative lumbar X-ray and brain MRI. Notable lab findings were a negative Lyme antibody titer as well as a positive ANA (titer 1:320) and borderline low B12 level (278 ng/mL) for which he was started on oral supplementation.

His numbness and hypersensitivity progressed to include his bilateral thumbs and forearms. Ultimately, he was referred to an outside neurologist two months after symptom onset where he was documented to have asymmetric distal sensory loss in his right greater than left lower extremities. A C-Spine MRI and EMG were ordered. He developed the acute onset of right foot drop two weeks after his initial neurology consultation (2.5 months after symptom onset). An EMG showed an asymmetric neuropathy, prompting an urgent neuromuscular evaluation at the University of Kansas Medical Center due to concern for mononeuropathy multiplex.

His initial exam demonstrated mild lower facial weakness (4/5) as well as left upper extremity elbow extensor weakness (4+/5), right ankle dorsiflexion/plantar flexion weakness (dorsiflexion 0/5, plantar flexion 3/5), and mild left dorsiflexion weakness (4/5). Reflexes were symmetric in the upper extremities and at the knees; however, his right Achille's reflex was diminished (1/4) as compared to the left (2/4). He had a multifocal pattern of decreased pinprick sensation including over the bilateral thumbs (left worse than right), bilateral lateral forearms, lateral thighs, and diffusely below the knees with the exception of some sensation in the lateral feet, bilaterally, and the left sole of the foot. Vibration and proprioception were diminished at the toes, and his gait was steppage.

EMG performed on the day of presentation was consistent with an asymmetric sensorimotor axonal peripheral neuropathy (Table 1). Given his history, examination, and electrophysiologic findings, he was admitted for expedited workup for a possible vasculitic neuropathy. Laboratory workup was notable for an elevated ANA at 1:160, Vascular Endothelial Growth Factor (VEGF) of 99.6 pg/mL (reference <=96.2 pg/mL), and Kappa/Lambda free light chain ratio of 1.81 (reference

0.26-1.65). Additional studies, including other typical vasculitic labs, were negative (Table 1). CSF was evaluated and unremarkable and an abdominal fat pad aspirate, obtained due to the slightly elevated Kappa/Lambda ratio, was normal (Table 1).

A PET CT, obtained to rule out malignancy in the setting of the unexpected kappa/lambda ratio, demonstrated hypermetabolic lesions in the lung left upper and lower lobes and a pleural lesion adjacent to the right lower lobe, concerning for metastatic disease (Figure 1). These findings prompted a biopsy of the hypermetabolic left lower lobe lung lesion in addition to his previously planned right sural nerve and peroneus brevis muscle biopsies (biopsy sites chosen based on surgeon preference). He was started on empiric high dose steroids and mycophenolate mofetil while biopsy results were pending. However, he tolerated this treatment poorly, and it was stopped within 30 days with no additional immunosuppression started. He remained on symptomatic treatment for his neuropathic pain while initial biopsy results were pending.

The right peroneus brevis muscle biopsy demonstrated an inflammatory neuropathy with perivascular and endomysial chronic inflammation and increased sarcolemmal MHC class I expression (Figure 2) while the right sural nerve biopsy demonstrated an inflammatory neuropathy with vascular and parenchymal inflammation and one poorly formed granuloma. A Fite stain was negative

Table 1. Initial electrodiagnostic findings and summary of hospital laboratory workup.
Abbreviations: Lat. Latency, Amp. Amplitude, Dur. Duration, Dist. Distance, CV Conduction Velocity. R. Right, L. Left, EDB extensor digitorum brevis, TA tibialis anterior, FH fibular head, APB abductor pollicis brevis, ADM abductor digit minimi, PL peak latency, MAC median antebrachial cutaneous nerve, PSW positive sharp waves, Fibs fibrillation potentials, Poly polyphasia, NML normal, MUAPs motor unit action potentials

Initial EMG							
Motor NCS							
Nerve/Sites	Lat. (ms)	Amp. (mV)	Dur. (ms)	Dist. (mm)	CV (m/s)		
R. Peroneal-EDB	**NR**	**NR**	**NR**	**NR**	**NR**		
L. Peroneal-EDB	4.6	**0.4**	6.0	80	**30** (FH-ankle) **45** (Knee-FH)		
L. Peroneal-TA	3.9	3.1	16.5		45		
R. Peroneal-TA	**5.0**	**0.5**	17.5		**23**		
R. Tibial-AH	4.9	**0.9**	8.3	80	45		
L. Tibial-AH	4.2	**2.2**	8.0	80	48		
Normal Studies	R. Median-APB, R. Ulnar-ADM						
Sensory NCS-all antidromic unless otherwise specified							
Nerve/Sites	PL (ms)	Amp (μV)		Segment			
R. MAC	2.7	5.9		Elbow-forearm			
L. MAC	3.1	**2.9**		Elbow-forearm			
L. Sural	4.4	**2.4**		Lower leg-ankle			
R. Sural	**4.8**	**1.9**		Lower leg-ankle			
R. Sup. Peroneal	4.64	**1.8**		Lateral leg-ankle			
L. Sup. Peroneal	**NR**	**NR**		Lateral leg-ankle			
Normal Studies	R. Median (wrist-digit II), R. Ulnar (wrist-digit V), R. Radial (forearm-snuffbox)						
Needle Electrode Examination							
Muscle	Insertional Activity	Spontaneous Activity		Volitional Activity			
		PSWs	Fibs	Poly	Amp	Dur	Recruit
R. Tibialis Anterior	NML	4+	4+	No MUAPs			
R. Gastrocnemius	NML	3+	3+	No MUAPs			
R. Vastus Lateralis	NML	None	None	None	NML	NML	Mild
Normal Muscles	R. Semitendinosus, R. Gluteus medius, R. mid and low paraspinals, R. first dorsal interosseous, R. Biceps brachii, R. Deltoid						
Additional Workup (obtained as part of initial hospitalization)							
Blood Tests	**Abnormal:** ANA 1:160, VEGF 99.6 pg/mL, Kappa/Lambda FLC 1.81						
	Negative/Normal: ACE, SSA/SSB, B12, Cryoglobulins, Anti-DS-DNA, Copper, Hepatitis A/B/C serology, HIV 1 and 2 Ag Ab, SIFE, RF, Anti-Smith, Anti-RNP, MPO/PR3, Syphilis, T Spot, LDH, CEA, AFP						
Cerebrospinal Fluid	Protein: 39, Glucose: 63, WBC: 2, Cytology: negative						
Other	Abdominal Fat Pad Biopsy: negative for amyloid deposition						

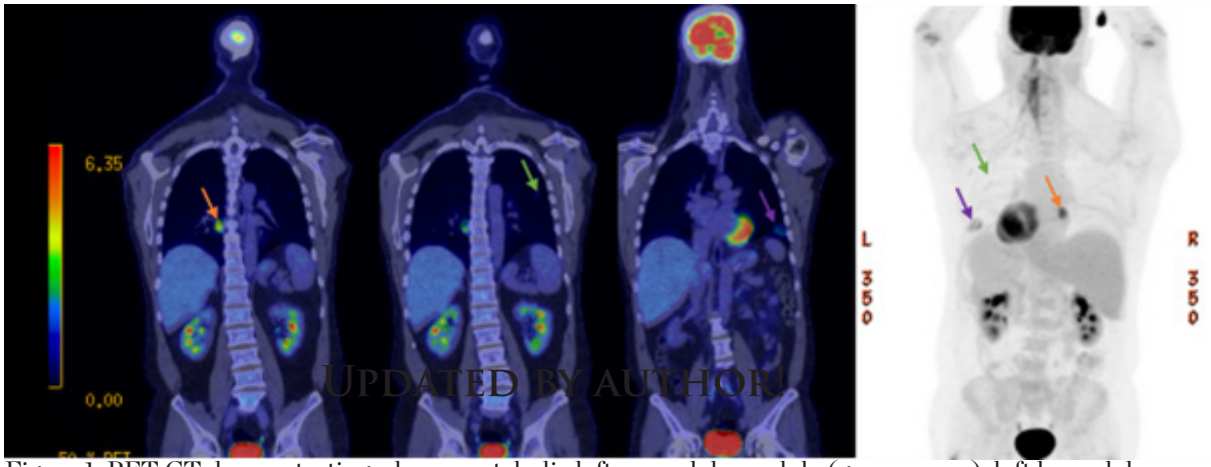

Figure 1. PET CT demonstrating a hypermetabolic left upper lobe nodule (green arrow), left lower lobe mass (purple arrow) and right lower lobe pleural-based mass (orange arrow).

for Mycobacterium leprae. No vasculitis was observed. The inflammatory infiltrates in both the nerve and muscle consisted predominantly of mature T cells, rare mature B cells, and macrophages (Figure 3). A Ki-67 antibody demonstrated rare mitotically active mononuclear cells in the endomysial and perivascular infiltrates. No Epstein-Barr virus encoding region (EBER) positive cells were seen in the nerve or muscle. Though these findings showed no neoplasm in the nerve or muscle, his lung biopsy showed an atypical angiocentric lymphoid infiltrate with large atypical B cells, some of which were EBV positive, diagnostic of lymphomatoid granulomatosis, grade 2.

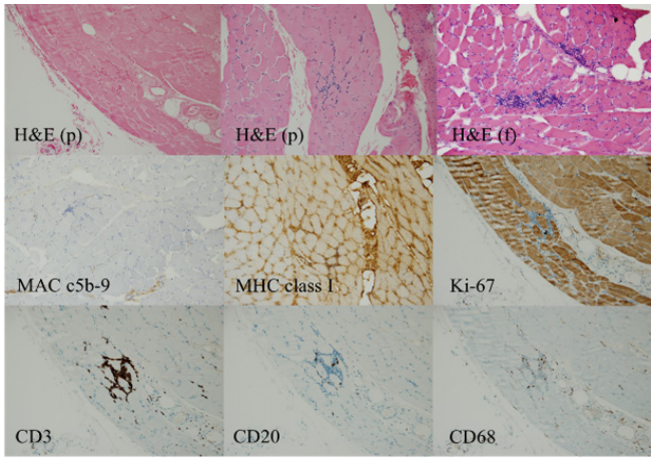

Figure 2. Right peroneus brevis muscle biopsy. H&E: focal perivascular and endomysial inflammation. MAC C5b-9: no endomysial capillary staining. MHC Class I: increased sarcolemmal and weak sarcoplasmic expression. Ki-67: rare endomysial/perivascular positive nuclei. CD3: numerous endomysial/perivascular aggregates of small lymphocytes. CD20: rare endomysial/perivascular small lymphocytes. CD68: few endomysial/perivascular macrophages. EBER-ISH staining: negative (not shown). P – paraffin section. F – frozen section.

Given the lung biopsy results, he was referred to the NIH after consultation with Hematology/Oncology for discussion of treatment options and enrollment in a clinical trial. Unfortunately, he developed side effects to the experimental therapy which prompted cessation, however, he achieved remission of his lymphomatoid granulomatosis at the time his therapeutic drug trial was discontinued and remains under watch for a recurrence. At last follow up, approximately 16 months after onset of symptoms, he had regained right lower extremity function, no longer with a complete foot drop, only needing a soft brace for support rather than a carbon fiber ankle foot orthotic. He is able to drive. He still had some bilateral foot numbness, though improved, and only had some mild intermittent pains in his feet, mostly in the evenings. He was back to driving, including his motorcycle, and was regularly walking or riding a bike for exercise.

Discussion

Lymphomatoid granulomatosis (LYG) was initially described in 1972 and mistaken for a T-cell disorder because of the predominance of T-cells on pathologic examination.[5] Subsequent evaluations determined that LYG is a rare Epstein-Barr (EBV) associated B-cell lymphoproliferative disorder whose histology and clinical features distinguish it from other EBV associated B-cell lymphoproliferative disorders, post-transplant lymphoproliferative disorders, and lymphomas.[6-9]

The disorder typically affects adults in the fourth to sixth decades with a male predominance of approximately 2:1.[8-10] While there is near universal lung involvement at diagnosis, greater than 90%, only one- to two-thirds of patients present with pulmonary symptoms, such as cough or shortness of breath. Non-specific systemic symptoms such as fever, weight loss, and fatigue often lead to initial

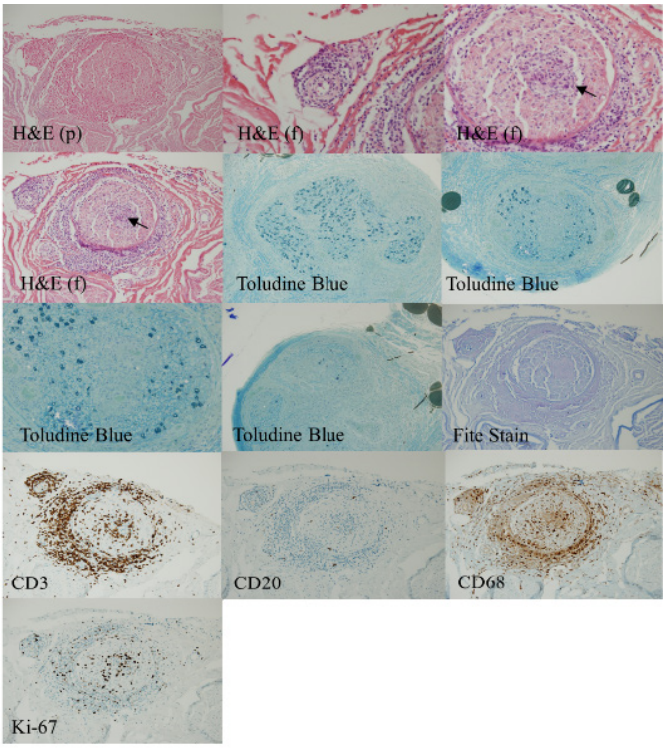

Figure 3. Right sural nerve biopsy. H&E stain: epineurial, perineurial, and endoneurial blood vessels with perivascular lymphocytic infiltrates and a poorly formed granuloma (arrow). Toludine blue: variable loss of myelinated axons within fascicles. Fite Stain: negative for organisms. CD3: many endoneurial/perineurial/epineurial/perivascular lymphocytes. CD20: rare endoneurial/perineurial/epineurial lymphocytes. CD68: many perineurial/epineural/perivascular macrophages; no well-formed granulomas. Ki-67: few positive nuclei.

medical evaluation.[9,11,12] Extrapulmonary manifestations are common and include cutaneous findings such as erythematous papules and/or subcutaneous nodules (25-50%). Central nervous system involvement (26-40%) can include various symptoms depending on the site affected with imaging demonstrating intraparenchymal lesions with or without linear enhancement or enhancement of the cranial nerves/leptomeninges. Other potential disease sites include renal (19-40%), hepatic (17-29%), and rarely the peripheral nervous system/cranial nerves (0-15%).[9,10,13-15]

Disease pathogenesis is secondary to defective immune surveillance of EBV-infected B cells with a functional defect in $CD8^+$ cytotoxic T cells thought to be the inciting event.[9] The pathology of LYG demonstrates angiocentric, destructive infiltrates of neoplastic and reactive lymphocytes with variable degrees of parenchymal necrosis. The predominant infiltrate consists of CD3 positive T cells with CD4 positive cells representing the majority subtype and a variable population of large, atypical B cells.[7,9,10] Due to the angiocentric nature of the pathology and the multi-system involvement, LYG can be difficult to distinguish from other forms of primary or secondary vasculitis, notably granulomatosis with polyangiitis (GPA), eosinophilic granulomatosis with polyangiitis, and sarcoidosis.[3,4] Key distinguishing features of LYG include areas of necrosis sparing the blood vessel wall as well as the presence of large, atypical B-cells which are EBV positive. Despite the presence of macrophage and histocytes, well-formed granulomas are absent.[3,16] Histologic grading of lymphomatoid granulomatosis is based on the number of EBV+ atypical B cells present and the degree of coagulative necrosis. Lower grades are characterized by rare EBER+ atypical B cells and focal or absent coagulative necrosis while higher grades have an increased number and size of EBER+ atypical B cells and often-extensive coagulative necrosis. An increased frequency of monoclonality by molecular analysis of EBV-infected B cells is also seen with higher grades.[9,10]

While the case presented above lacked EBV positive B cells in both the muscle and nerve biopsy, it remains unclear whether his MNM reflects direct LYG involvement of the peripheral nervous system or an epiphenomenon/paraneoplastic process. While no published studies confirm neoplastic infiltrates in peripheral nerves in LYG, direct involvement remains a possibility, similar to the skin. Cutaneous lesions of LYG, though common, show neoplastic cells only in a subset of cases.[10,17] Prior case series reviewing skin biopsies from patients with cutaneous manifestations of LYG demonstrated that while all biopsies consisted of a subcutaneous infiltrate composed of small T-cells and histiocytes with variable degrees of necrosis, only 19 – 37.5% had large, atypical B cells that were EBER positive, compared to greater than 75% in comparable lung biopsies. This discrepancy has been attributed to either sampling error or the possibility that some cutaneous manifestations may be an epiphenomenon related to the upregulation of cytokines and chemokines in relation to the immune response to EBV. Prior studies have linked the vasculitis of LYG to this phenomenon.[10,17,18] Outside of the skin having a similar inflammatory infiltrate but lacking EBER positive atypical B-cells, there is a single case report of a muscle biopsy with similar findings in a patient who presented with a nodular rash and cutaneous ulcerations, significant proximal weakness, and weight loss who was ultimately diagnosed with LYG with presumed paraneoplastic polymyositis.[19]

Given the lack of definitive findings described in peripheral nerves, pathologic evaluation of sites outside of the peripheral nervous system remain the definitive diagnostic standard for LYG, even if the primary symptoms are in the peripheral nerves. There are only two additional

case reports describing mononeuropathy multiplex in patients ultimately diagnosed with LYG, with both being diagnosed post-mortem.[4,13] One of the cases was treated presumptively for vasculitis prior to death based on perivascular and intramural lymphocytic inflammation of small vessels on the muscle and nerve biopsy.[4] Given its multiorgan involvement and histological features of angiocentric inflammation, LYG is a potential mimic for vasculitis.[1-4]

As the underlying pathology is that of dysregulated immune surveillance and immunosuppression, accurate diagnosis is important as treatment varies from other systemic vasculitities. As LYG is related to dysregulated immune surveillance of EBV infected B cells, immunotherapy such as corticosteroids or single-agent chemotherapy, which have been trialed in the past, fail to provide disease control and lead to a high rate of progression to higher grade disease or lymphoma.[3,9] Treatment of low-grade LYG consists of withdrawal of any immunosuppressants, interferon alpha or intravenous gamma globulin to enhance the immune response, and possibly immune checkpoint inhibitors. While observation and immunosuppression withdrawal has been trialed for those with iatrogenic immune suppression, most eventually require disease-directed therapy.[9] High grade disease is less likely to respond to augmentation of the immune system and often requires therapies akin to those used for other aggressive EBV-associated malignancies such as diffuse large B-cell lymphoma. Immunochemotherapy such as dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (DA-EPOCH-R) as well as high dose chemotherapy and stem cell transplantation have been used for high grade disease.[9] Even with immune modulation or immunochemotherapy, relapse with low-grade disease or progression to high grade disease remains common due to the defective immune surveillance of EBV-infected cells in LYG cases. In these cases, crossover treatment to a treatment available but not previously received for LYG has been shown to be effective.[9] Prognosis is variable with a more favorable prognosis for lower grade disease. With the advent of pathobiological based therapies over the past 20 years, survival has improved from the historical median overall survival of 2 years to half of treated patients now living at least 10 years.[9]

In conclusion, LYG is an extremely rare angiodestructive lymphoproliferative disease secondary to EBV associated B-cell lymphoproliferation. Given its propensity for multi-organ involvement and the angiocentric nature of its pathology, it is a potential mimic of both primary and secondary vasculitides. Evaluation for subclinical sites of involvement, particularly pulmonary, may be required to secure a definitive diagnosis given that cutaneous, muscle, and peripheral nerve pathology may not allow for a definitive diagnosis of LYG and prove misleading. A pathological clue to the LYG diagnosis in our case is the absence of vessel wall fibrinoid necrosis. Appropriate diagnosis is highlighted by the fact that treatment for LYG does not include immunosuppression as typically prescribed for other causes of MNM.

This case has been presented previously at the virtual 43rd Carrell-Krusen Neuromuscular Symposium held from February 18-19, 2021 and at the Peripheral Nerve Society (PNS) Virtually Anywhere Conference from June 13, 2021.

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Asymmetrical Onset of Leg Amyotrophic Diplegia (LAD): A Case Report

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Case Presentation

Progressive muscular atrophy (PMA) comprises approximately 10% of patients with motor neuron disease (MND). Some of the patients initially presenting with PMA will develop, when followed over time, upper motor neuron findings leading to the diagnosis of ALS. True PMA cases represent a pure lower motor neuron presentation of sporadic motor neuron disease and are in a spectrum with ALS and PLS. While PMA typically affects both the arms and legs, some patients have predominantly upper extremity involvement and others may have selective leg weakness referred to as brachial amyotrophic diplegia (BAD)[1,2] and leg amyotrophic diplegia (LAD).[3] These cases of progressive muscular atrophy remain restricted to a body region for extended periods and are considered slow regional variants of motor neuron disease. LAD is a variant of progressive muscular atrophy (PMA) where weakness is confined to the legs for at least two years, and there are no upper motor neuron signs.[4] An LMN syndrome confined to the leg was first described by Pierre Marie and his student Patrikios in 1918 and was known as the pseudopolyneuritic variant of ALS, the Marie-Patrikios form, or the peroneal form of ALS. Here we present another case of LAD for the neuromuscular literature.

A 42-year-old right-handed female who presented with low back pain and right leg weakness had difficulty walking which slowly progressed to bilateral leg weakness over thirty months. She stated that her weakness had been getting worse after recovering from COVID-19 infection about nine months prior. The patient initially noticed right leg weakness about 30 months ago with catching of the toes on uneven surfaces and difficulties in climbing up stairs, moreso than descending stairs. The patient denied dysphagia, dyspnea, dysarthria, trouble with balance, bowel/bladder incontinence, or sensory abnormalities. She denied any past medical history aside from her history of mild-moderate COVID-19 infection and had not noticed any long-term sequelae from that illness. The patient denied taking any medications.

On physical exam, the patient was observed to be using bilateral crutches for walking. Cranial nerve examination was normal and upper extremity muscle strength was full. On manual muscle testing, right hip flexion -4/5; and left hip flexion 4/5; Right hip abduction -4/5; left hip abduction was 4/5; Ankle dorsiflexion (ADF) -4/5 right and 4/5 left; ankle inversion and eversion-4/5 right and 4/5 left; Bilateral ankle plantar flexion (APF), knee flexion and extension were normal. In summary, there was an asymmetric pelviperoneal pattern involvement, with relative sparing of knee extension and/or ankle plantar flexion with bilateral distal leg atrophy. Sensory examination was normal to pinprick, vibration, and proprioception. The upper extremities had normal deep tendon reflexes (DTRs); however, the lower extremity DTRs were unobtainable bilaterally with absent Babinski sign, jaw jerk and Hoffmann reflexes.

All metabolic, toxic, infectious, inflammatory, vasculitis profiles, and CSF examinations (including oligoclonal band and CSF IgG index) were normal. Brain, cervical, thoracic and lumbosacral MRIs were normal as well. On electrodiagnostic study (EDX), there was mild reduction in amplitude of lower limb compound motor action potentials (CMAPs) with right leg more than left without sensory abnormalities. There were also reductions in amplitude of both lower limb F-waves, H-reflexes, neurogenic MUAPs, and recruitment motor unit action potentials (MUAPs) on lower limb muscles with rare positive sharp waves only on distal leg muscles without fasciculation. Upper limbs, cervical and thoracic paraspinal and bulbar muscles EDX study were normal. She was diagnosed with the LAD (leg amyotrophy diplegia) variant of progressive muscular atrophy.

Discussion

LAD is a leg onset variant of progressive muscular atrophy (PMA). LAD weakness is confined to the legs for at least two years without upper motor neuron signs. The natural history of LAD differs from typical forms of ALS and PMA. LAD is a slowly progressive disorder. An asymmetric pelviperoneal pattern of weakness should heighten the suspicion for LAD.[4] Recent descriptions of regional variants suggest some patients have part isolated to a single spinal region for many years, including leg amyotrophic diplegia.[5] Leg weakness is a presenting symptom in about one third of ALS cases. Therefore, it is impossible to predict if there will be a slow clinical course during the early stages. However, LAD should be a consideration when weakness remains restricted to the legs for two or more years in the absence of upper motor neuron signs thereby failing to fulfill El Escorial criteria for ALS.[6,7]

In 2009, Wijesekera *et al* described the flail leg syndrome (FLS) which is characterized by progressive distal leg weakness and atrophy.[5] While FLS weakness allows for leg restriction for one year and pathologic tendon reflexes, LAD is defined as the absence of spread to other myotomes

or upper motor neuron signs for at least the first two years.[8] Lower extremity regional motor neuron disorder can be manifested by Flail Leg Syndrome (FLS) with more distal and symmetrical lower extremity atrophy and weakness (S1-S3 involvement) or Leg Amyotrophic Disorder (LAD) with more asymmetrical pelviperoneal pattern of weakness and atrophy (L4-L5 involvement).

There are multiple questions that remain regarding MND. Further research is required to elucidate whether there are any differences between upper motor neuron cell body and lower motor neuron cell body involvement in MND. It is also poorly understood what leads to the asymmetric vs symmetric pattern in MND and what factors may contribute to the susceptibility of lower or upper motor neuron cell bodies.

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Guillain-Barre Syndrome Secondary to COVID-19: A case report and short review of other published cases

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ABSTRACT

Background: COVID-19 related Guillain-Barré syndrome has a broad spectrum of presentation. In most reported cases, respiratory symptoms preceded neurological deficits by one to two weeks, suggesting that the clinical course is mostly post-infectious. In this case report, we present a para-infectious case of GBS with COVID-19.

Case presentation: A 37-year-old male patient presented with fever, chills, myalgia, cough, and anosmia. COVID-19 test came positive. He was managed conservatively. On the 7th day of follow-up, he recovered except for a persistent loss of smell and taste. Two weeks after his initial presentation, he reported low back pain and bilateral lower extremity weakness and had a repeat COVID-19 test, which returned positive. His history, physical exam, CSF analysis, nerve conduction, and electromyography test revealed Guillain-Barre Syndrome. We managed GBS with supportive treatment in the hospital, and on follow-up of three months, he recovered fully.

Conclusion: In our case, we report a para-infectious case of GBS with C0VID-19, and we managed this case without intravenous immunoglobulin or plasmapheresis. The decision to treat a COVID-19 related GBS case with a traditional GBS treatment option (intravenous immunoglobulin or plasmapheresis) should be taken in conjunction with co-morbidities and a tailored case by case basis.

Keywords: *COVID-19, Guillain Barre Syndrome, Acute inflammatory demyelinating polyradiculopathy, Anosmia*

Introduction

Guillain Barre Syndrome (GBS) is an acute immune-mediated disease of the peripheral nerves and nerve roots (polyradiculoneuropathy) that is usually elicited by various infections.[1] Classically, GBS presents as progressive, ascending, symmetrical limb weakness, along with areflexia or hyporeflexia and with or without cranial nerve involvement, which can progress over days to several weeks.[1,2] In the majority, an antecedent respiratory or gastrointestinal infection history was found two to four weeks before the onset of the neurological syndrome of GBS.[1] COVID-19 manifestations can range from mild flu-like illness to severe pneumonia or acute respiratory distress syndrome. Neurological, cardiac and thromboembolic complications are widely reported from across the globe.[3,4,5] From a neurological point of interest, SARs-CoV-2 infection can cause encephalopathy, encephalitis, myelitis, meningitis, acute cerebrovascular disease, Guillain Barré Syndrome (GBS), and exacerbation of myasthenia gravis.[6,7] There are very few cases of para-infectious GBS with COVID-19 reported in the literature. Here we report a case of GBS during the active phase of COVID-19 infection.

Case Report

A 37-year-old Hispanic gentleman with a past medical history of diabetes mellitus type-2, asthma, and hypertension initially presented with fever, chills, myalgia, cough, and anosmia. He is a poultry farm worker, and many of his co-workers had come down with similar symptoms. Given the symptoms and outbreak at his work, the nasopharyngeal swab was tested for COVID-19 with CDC Real-Time- Polymerase Chain Reaction primers (RT-PCR) came positive. The patient was managed conservatively. He did not require hospitalization. On the 7th day of follow-up, the patient had recovered, except for the persistence of loss of smell and taste. Two weeks later, to his initial presentation, the patient presented to ER with three days history of low back pain and progressively worsening bilateral lower extremity weakness. In those three days at home, weakness progressed to the point where the patient could not walk without holding support with both hands. Neurology was consulted to evaluate for weakness. Repeat Nasopharyngeal swab tested positive for COVID-19 (RT-PCR). The patient was asymptomatic from a respiratory viewpoint; his oxygen saturation was 99%, he was comfortable on room air. On neurological exam, the patient was awake, alert, and oriented. Strength was MRC 5/5 (Medical Research Council scale) in both upper extremities and MRC 2/5 in both lower extremities distally and MRC 3/5 proximally. The patient was areflexic in the lower extremity, and upper extremity reflexes were diminished at 1+. There was paresthesia involving both lower extremities to pinprick and fine touch in a patchy distribution. Vibratory sense at the first metatarsal head was reduced symmetrically (5 seconds), while normal

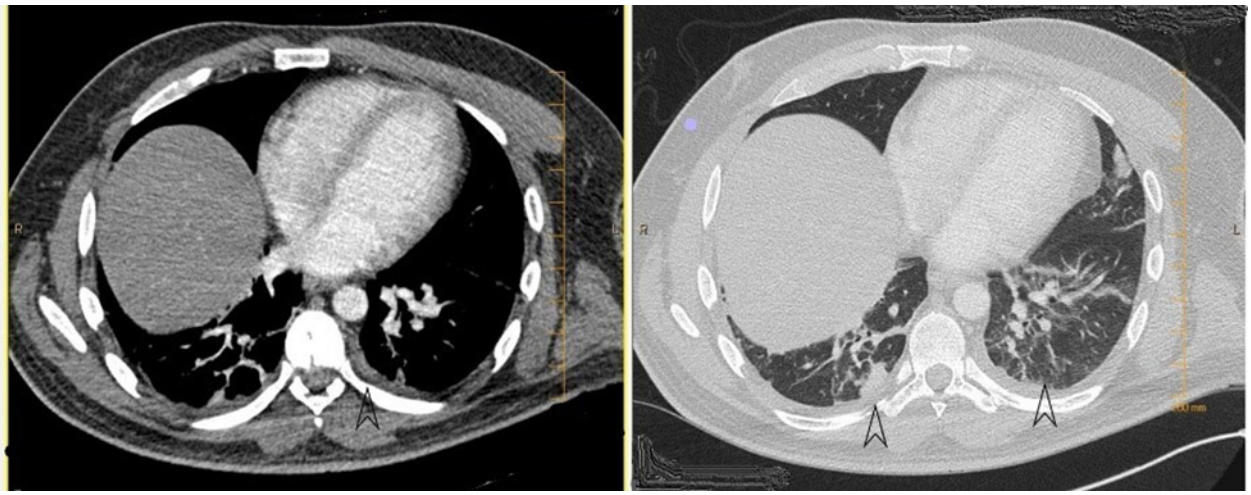

Figure 1: CT chest showing consolidation of lung bases

in both upper limbs at the base of the thumb (more than 14 seconds); there was no joint position loss sensation in upper or lower limbs. His rectal tone and perianal sensation were intact, and plantar reflexes were mute. There was no extraocular weakness, facial palsy, hypophonia, orthopnea, or respiratory distress. His NIF (negative inspiratory force) was -65 cm of water (normal). Other cranial nerve examination was normal. Based on history, exam findings, Guillain-Barré syndrome was suspected. We recommended MRI (with and without contrast) of the cervical, thoracic, and lumbar spine, which was unremarkable. His CT chest showed ground-glass opacity in bilateral lung bases at day 19 of his initial symptoms (Figure 1). Blood work showed; ESR 67 mm/hour (normal value 0-22 mm/hour), CRP 15.16 mg/dL (normal value 0.00-0.50 mg/dL), D-dimer 5.40 mcg/ml (normal value <0.50), Fibrinogen 560 mg/dl (normal value <50 mg/dl) LDH 797 units/L (normal value 140-280 U/L), CK 31 units/L (normal value 22-198 U/L), HbA1c 13.4% (normal value 4%-5.6%). CSF study showed protein of 107 mg/dl (normal value 8-43 mg/dl), 0 WBC, glucose of 166 mg/dl (normal value 50-80 mg/100 mL or greater than 2/3 of blood sugar). CSF analysis for Epstein Barr virus, Cytomegalovirus, Varicella, Herpes simplex type I & II were negative. We performed NCV-EMG (Nerve Conduction Velocity- Electromyography) to confirm Guillain-Barré syndrome and look for a loss pattern, either axonal or demyelination. Nerve conduction study summarized in *Table 1*. Sensory nerve conduction studies of the left radial and both sural nerves showed low evoked response amplitude and slow conduction velocity. Motor nerve conduction studies of the left median and left peroneal nerves showed normal distal motor latency, normal evoked response amplitude, and slow conduction velocity. The left tibial nerve shows normal distal motor latency, low evoked response amplitude, and slow conduction velocity in demyelinating range (laboratory criteria for tibial nerve: CMAP amplitude more than 3.2 milli-volts and conduction velocity less than 32 meter/second). There were no temporal dispersions. The minimum F wave latencies obtained were prolonged on the left median, peroneal and tibial nerves. Needle electromyography (though limited) showed reduced recruitment in tibialis anterior, peroneus tertius, and vastus medialis with neurogenic motor unit potentials in peroneus tertius. Based on the history of rapidly progressive lower extremity weakness, albumin-cytological dissociation on the CSF study, and electrodiagnostic findings, the patient was diagnosed with a acute inflammatory demyelinating polyradiculopathy (AIDP). Based on electrodiagnostic criteria for AIDP, published by Al-Shekhlee et al.,[8] we categorized our patient electro-diagnostically as a suggestive case of AIDP. However, H reflexes were not checked. Also, we suspect the patient had an underlying chronic sensorimotor axonal neuropathy likely from long-standing poorly controlled type-II diabetes mellitus. Throughout the hospital course, he was managed conservatively and did not require additional respiratory support. He was getting physiotherapy while in hospital and after discharge. He was discharged upon the improvement of motor strength. At six weeks of follow-up, he was able to walk with a cane; his sense of taste and smell had returned as well. At three months of telehealth follow-up, he was walking without any support. The patient's clinical syndrome of acute flaccid paralysis and demyelination on electrodiagnostic studies was not secondary to uncontrolled diabetes, as the patient improved in a relatively shorter duration, unlike diabetic neuropathy. Follow-up EMG-NCV was not planned as the patient was clinically improved.

Discussion

Guillain-Barré syndrome can occur secondary to an autoimmune response to a bacterial or viral epitope that leads to the formation of antibodies that cross-react with

Table 1: Nerve Conduction Study-Electromyography

Sensory Nerve Conduction Study				
Nerve/Site	Segment	Peak ms.	Amp. uV	Vel m/s
Left Radial- Forearm	Snuffbox	2.7 (<2.9)	7.0 (>15)	46.3
Left Sural- Calf	Lateral Ankle	5.2 (<4.4)	1.6 (>6)	34.1
Right Sural- calf	Lateral Ankle	4.6 (<4.4)	1.6 (>6)	36.5
Motor Nerve conduction study				
Nerve/Site	Recording site	Latency ms	Amp. mV	Vel m/s
Left Median- Wrist	APB	4.1 (<4.4)	4.8 (>4)	
Left Median- Elbow	APB	9.6	4.4	45 (>49)
Left Peroneal- Ankle	EDB	4.8 (<6.5)	4.6 (>2.5)	
Left Peroneal- Fibular head	EDB	14.4	3.8	33.4 (>44)
Left Peroneal- Popliteal fossa	EDB	16.9	3.5	37.3 (>44)
Left Tibial- Ankle	AH	4.5 (<5.8)	6.9 (>4)	
Left Tibial- Popliteal fossa	AH	18.2	3.7	30.7
F wave				
Recording site	Value. mS			
Left- Median	32.6 (<31)			
Left- Paroneal	60.5 (<57)			
Left Tibial	59.1 (<58)			

ganglioside, i.e., molecular mimicry.[20] In earlier epidemics, MERS (Middle Eastern respiratory syndrome) and Zika virus were reported to cause Guillain-Barré syndrome.[21] *Table 2* describes the clinical courses of reported COVID-19 related GBS cases. The spectrum of presentation is broad; outcomes are variable with different interventions. In most cases, respiratory symptoms preceded neurological deficits by one to two weeks, suggesting the clinical course is mostly post-infectious, like other commonly known GBS triggers. Abolmali M. et al. reported three cases of COVID-19-related GBS which the author describes as para-infectious as all three patients developed neurological manifestations during the active phase of COVID-19.[22] None of these three cases were reported to get tested for SARs-CoV-2 PCR in CSF.

Generally, post-infectious cases are caused by an autoimmune reaction to the infectious agent, which cross-react with neural antigen. In such cases, neuropathy develops several days to weeks later after the initial infection. On the other hand, following COVID-19 illness, if hyperimmune response ensues, the appearance of neuropathic symptoms earlier is logical. The para-infectious course of neuropathy develops relatively faster within a few days due to infectious agents, like neuroborreliosis, neuro brucellosis, and West Nile Virus-related acute flaccid paralysis, etc. None of the reported GBS cases were found to have COVID-19 RT-PCR positive on CSF. COVID-19 cases may remain clinically silent at the beginning or asymptomatic throughout.[23] Some can develop aggressive diseases relatively later than others. We hypothesize the timeline of initial respiratory symptoms in relation to onset of the neurological syndrome might not accurately indicate (rather underestimate) the window period between actual infection and onset of neurological syndrome in some cases. The incubation period also varies from few days to two weeks. While suffering from asymptomatic or mild symptomatic COVID-19 infection, the patient can still trigger an immune response. We could not conclude any correlation between the severity of COVID-19 disease and Guillain Barre syndrome. Relatively milder to severe, both patterns of cases were reported to cause GBS.

In our case, the patient presented after two weeks of COVID-19 diagnosis; his respiratory symptoms had resolved by then. But we still could find ground-glass

opacity on chest CT. Among the eighteen cases described (including ours), eight cases were diagnosed as acute inflammatory demyelinating polyneuropathy (ours was a paraparetic variant), five cases as acute motor-sensory axonal neuropathy (AMSAN), two cases as Miller Fisher variant, two cases as facial diplegic variant, one case as acute motor axonal neuropathy (AMAN), and one case as polyneuritis cranialis variant. One case was reported as a severe autonomic failure, and one with dysautonomia. CSF protein was high in eleven cases. Four cases had normal CSF protein. COVID-19 RT-PCR in CSF was negative in all tested cases. We did not test CSF for COVID-19 (RT-PCR) in our case, as it's not a reliable marker for neurological injury. COVID-19 related neurological issues seem to be caused by indirect mechanisms.[24] Thirteen cases were treated with IVIG, one case with IVIG and plasmapheresis, two cases (including our case) were not treated with IVIG or plasmapheresis. We did not choose to treat our case with IVIG, as COVID-19 illness is widely reported to cause thromboembolic events,[25] and thrombotic events are a known side effect of IVIG (likely related to hyper-viscosity).[26,27] Plasmapheresis was not chosen, because the patient was thrombocytopenic with platelet count of 23,000/microliter (normal range 150,000-450,000/microliter), and anemic, hemoglobin of 6.9 gm/dL (normal 13.5-17.5 gm/dL). Plasmapheresis can deplete coagulation factors and antithrombin III, leading to bleeding complications.[28] Our patient did not have any signs of impending respiratory failure, nor did his weakness progress further. Two of the total eighteen cases who did not get IVIG or plasmapheresis had a good recovery. Our patient also had fair motor recovery at six weeks, and at three months, was walking without support. Of the eight cases treated with IVIG, five had a good recovery, two did not improve, and one progressed to neuromuscular respiratory failure.

Overall, we believe GBS related to COVID-19 disease can present with a large spectrum of neurologic syndrome. Neuromuscular respiratory failure, severe dysautonomia secondary to GBS would make hospital courses in ICU even more complicated. Early recognition of GBS manifestation and early decision on plasmapheresis and intravenous immunoglobulin is mandatory. At the same time, co-morbidities, clinical course, and the severity of the clinical situation should be gazed meticulously before deciding on a treatment plan.

Declarations

1. Ethics approval and consent to participate:
This study is approved by the University of Missouri I.R.B.

2. Ethics Statement:
Written consent was obtained from the patient before submission for publication.

4. Competing Interest:
The authors have no conflict of interest to report.

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6. Author Contributions:
Biswajit Banik (BB) and Mukaish Kumar (MK) wrote the manuscript of the article. Raghav Govindarajan (RG), BB, and MK were involved in designing concepts, literature search and involved in drafting the article. All authors contributed to the manuscript, and RG approved the final version of the manuscript.

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Table 2: Summary of COVID-19 associated GBS cases

Author	Pertinent clinical information and timeline of neurological and COVID symptoms.	Investigations	Electrodiagnostics	Diagnosis	Treatment	Outcome
(Zhao, Shen et al. 2020)[9]	61-year female, presented with fatigue and acute weakness of both lower extremities progressive to upper extremities. On exam, symmetric Lower extremity strength 3/5 (MRC scale) and upper extremity strength 4/5 (MRC scale), areflexia in both legs and feet, also distal decreased sensation to pin prick and light touch. Patient initially presented with neurological symptoms, on 8th day of presentation with neurological symptoms developed dry cough, fever. CT showed ground glass opacity. Oropharyngeal swab tested positive for COVID.	-CSF protein: 124 mg/dl. (Day 4) -CSF WBC-Normal	Delayed distal latencies Absent F waves in early course.	AIDP	-IVIG -Ropinavir -Arbidol -Ritonavir	Total recovery at day 30 with normal reflexes.
(Padroni, Mastrangelo et al. 2020)[10]	70-year old female, presented with acute onset of asthenia, hand-feet paresthesia, gait difficulty. On exam, B/L UE and LE extremity strength 4/5 (MRC scale), absent deep tendon reflexes, preserved light touch and pinprick sensation. Patient had developed fever (38.5 C) and dry cough 24 days prior to her presentation with neurological symptoms. She tested positive on nasopharyngeal swab (RT-PCR) on 2nd day of respiratory symptoms.	-CSF protein 48 mg/dl. (Day 4) -WBC: $1x10^6$/L, normal (0-0.8 x 10^6/L)	Conduction block, Temporal dispersion, Soleus H reflex absent.	AIDP	-IVIG	Patient intubated on day 5.
(Ottaviani, Boso et al. 2020)[11]	66-year female, presented with 3 days of walking difficulty and fatigue progressing to paraparesis. On exam, paraparetic, distal UE strength 4/5 (MRC scale), diffuse areflexia, no clear sensory deficit. Patient had mild fever and cough 10 days prior neurological symptoms. Initial nasopharyngeal swab was negative for COVID, repeat swab positive. CT chest showed ground glass appearance.	-CSF protein 180 mg/dl. -WBC 0. -Ganglioside antibody negative. -CSF SARS CoV-2 not detected.	Absent F wave. Diffuse prolong distal motor latencies. Reduced distal CMAP, slight reduction in conduction velocity.	AIDP with axonal loss.	IVIG Lopinavir Ritonavir Hydroxychloroquine	Patient worsened. Needed Mechanical intubation, developed unilateral facial palsy
(Assini, Benedetti et al. 2020)[12]	55-year male, initially developed anosmia, ageusia; hospitalized for COVID-19 (Oropharyngeal swab positive) related respiratory issues. On 3rd day pt. required intubation for respiratory failure secondary to COVID pneumonia. Later, on day 20, developed Neurological syndrome of eyelid ptosis, dysphagia and dysphonia. On exam, bilateral masseter weakness, tongue protrusion deficit due to CN XII palsy, deficit of soft palate elevation due to CN X palsy, diffuse hyporeflexia, no extremity weakness. Respiratory symptoms onset at least 20 days prior to onset of neurological symptoms.	CSF protein: Normal. Oligoclonal band both in CSF and serum with increased IgG/Albumin ratio (233)	Symmetric demyelination with sural sparing pattern. Repetitive nerve stimulation negative. Anti-ganglioside antibody negative.	AIDP with Miller fisher overlap	-IVIG -Idrossichlorochine -Arbidol -Ritonavir -Lopinavir	Very rapid clinical recovery in swallowing, speech, tongue motility and strength, ptosis.

(Assini, Benedetti et al. 2020)[12]	60-year male, hospitalized for COVID-19 respiratory symptoms, requiring tracheostomy and assisted ventilation; at day 20 of hospitalization, developed acute weakness in lower limb with distal distribution and foot drop on right side. Gastroparesis, paralytic ileus, loss of BP control noticed. On exam, deep tendon reflexes absent, distal weakness of all four limbs noticed. Respiratory symptoms onset 20 days prior (or more) to onset of neurological symptoms.	CSF protein: Normal. Oligoclonal band in CSF and serum with increased ratio. CSF IgG/albumin 170.	Severe sensory-motor axonal polyneuropathy with relative sparing of conduction velocity. Loss of amplitude in sensory and motor axon potential.	AMSAN- with severe autonomic neuropathy	-IVIG -Hydroxychloroquine -Tocilizumab	Symptomology improved except gastroparesis. Paralytic ileus improved. Hyporeflexia and foot drop persisted.
(Toscano, Palmerini et al. 2020)[13]	77-year female, presented with paresthesia in limbs and hands, over next 36 hours became flaccid areflexic, tetraparetic. Later during IVIG developed dysphagia, tongue weakness. COVID-19 symptoms 7 days prior to onset of neurological symptoms.	Day 2: CSF- Normal Day 10: CSF Protein: 101 mg/dl. (Normal 23-43 mg/dl). CSF WBC: 4/mm^3 Spine MRI- Caudal nerve root enhancement	Axonal loss Sural sparing pattern	AMSAN	-IVIG, two cycles	Poor outcome after 2 cycle of IVIG. Persistence of severe UE weakness, LE paraplegia Dysphagia
(Toscano, Palmerini et al. 2020)[13]	23-year male, presented to ER, with facial weakness that progressed to total LMN type facial paresis in 2 days. Also had loss of taste, lower limb paresthesia, generalized areflexia, sensory ataxia. COVID-19 related respiratory symptoms 10 days prior to presentation.	Day 3: Protein 123 mg/dl, no cells.	-Axonal sensory motor changes involving both limb, sural sparing pattern. -Decreased facial nerve CMAP amplitude. -MRI head- Facial nerve enhancement bilateral	AMSAN with facial diplegia	-IVIG	Improved ataxia, paresthesia, mild improvement of facial weakness
(Toscano, Palmerini et al. 2020)[13]	55-year male, presented with neck pain, paresthesia in four limbs and lower limb weakness. In two days, he was flaccid areflexic tertraparetic. COVID-19 related symptoms 10 days prior to onset of neurological symptoms.	Day 3, CSF Protein: 193 mg/dl, no cells. CSF: negative PCR for SARs-CoV-2	Severe axonal neuropathy MRI spine: Enhancement of caudal nerve root	AMAN	-IVIG- 2 cycles	Poor outcome; Neuromuscular respiratory failure, facial diplegia
(Toscano, Palmerini et al. 2020)[13]	76-year male, presented with lumbar pain, lower extremity weakness and loss of smell. In four days become flaccid areflexic tetraparetic. COVID-19 related symptoms 5 days prior to onset of neurological symptoms.	CSF protein: Normal, no cell. CSF: negative PCR for SARs-CoV-2	No electrodiagnostic data.	AIDP	-IVIG	Some motor improvement, unable to stand after 1 month

(Toscano, Palmerini et al. 2020)[13]	61-year male, presented with difficulty in climbing stairs, lower limb paresthesia, over one day pt. unable to stand. On exam, generalized areflexic, paraparetic. On 2nd day of IVIG developed tetraparesis, dysphagia, and facial weakness. On 3rd day, Neuromuscular respiratory failure. COVID-19 related symptoms 7 days prior onset of neurological symptoms .	Day 3: -Protein 40 mg/dl. -CSF WBC: 3/mm^3 CSF: negative PCR for SARs-CoV-2	Day 4: conduction block, demyelination.	AIDP	-IVIG -Plasmapheresis	Poor outcome: Developed bacterial pneumonia during IVIG, delayed Plasmapheresis
(Sedaghat and Karimi 2020)[14]	65-year male, presented with 5 days of acute progressive ascending quadriparesis. On exam, pt. was quadriplegic, facial diplegic, areflexic. Sensory loss to vibration and fine touch. COVID-19 related symptoms roughly 10 days prior to onset of neurological symptoms.	No CSF study available.	Decreased amplitude of CMAP, no sensory nerve action potential.	AMSAN	-IVIG -Hydroxychorloquine -Ritonavir, Lopinavir -Azithormycin	Unknown.
(Caamano and Beato 2020)[15]	61-year male, presented with liquid dipping on his right facial commissure which progressed to bifacial weakness. On exam, facial nerve palsy, absent blink reflex, good motor strength. COVID -19 symptoms 10 days prior to onset of neurological symptoms.	CSF protein: 44 mg/dl CSF WBC: 0. CSF: negative PCR for SARs-CoV-2	Electrodiagnostic not done	Facial diplegic variant	-Prednisone for two weeks.	No improvement.
(Gutierrez-Ortiz, Mendez et al. 2020)[16]	50-year male, presented with vertical diplopia, paresthesia around mouth. On exam, deep tendon reflex absent, planter flexor, right eye hypertropia, R eye intranuclear ophthalmoplegia, L eye nystagmus on L gaze. COVID-19 respiratory symptoms, along with ageusia, anosmia started 5 days prior to neurological symptoms.	CSF protein: 80 mg/dl GD1b-IgG positive CSF: negative PCR for SARs-CoV-2	Electrodiagnostic not done	Miller fisher variant	-IVIG	Ataxia and cranial nerve weakness improved, except ageusia and anosmia.
(Guierrez- Ortiz, Mendez et al. 2020)[16]	39-year male, presented with acute onset of diplopia. On exam, pt. had esotropia, severe abduction deficit in both eyes, fixation nystagmus, upper gaze more impaired, bilateral abducens palsy, absent deep tendon reflexes. Good motor strength, no sensory loss, no ataxia. COVID-19 related symptoms onset 3 days prior to onset of neurological symptoms.	CSF protein: 62 mg/dl CSF: negative PCR for SARs-CoV-2	Electrodiagnostic not done	Polyneuritis cranialis Varitant	No treatment, sent home.	Two weeks later complete recovery.

(Otmani, Moutawakil et al. 2020)[17]	70-year female, presented with progressive bilateral weakness and tingling resulting in total functional disability within 48 hours. On exam, by day 10, quadriplegic, hypotonic, areflexic. COVID-19 symptoms onset 13 days prior to onset of neurological symptoms.	CSF protein: 100mg/dL CSF: negative PCR for SARs-CoV-2	Marked reduction or absent of electrical potential in both sensory and motor nerve, little or no abnormality in conduction velocity.	AMSAN	IVIG Azithromycin Hydroxychloroquine	No improvement after one week.
(Camdessanche, Morel, et al.2020)[18]	64-year male, presented with paresthesia of hand and feet, over next 3 days developed flaccid tetraparesis. On exam, patient was MRC 2/5 in the legs, 2/5 arms, 3/5 in forearm, 4/5 in hands. Deep tendon reflexes absent, loss of vibration in lower limbs. COVID-19 symptoms onset 11 days prior to onset of neurological symptoms	CSF: Protein 166mg/dl.	Demyelination.	AIDP	-Lopinavir -Ritonavir	Not known
(Brooks, Megan 2020) [19]	54-year male, presented with ascending limb weakness and numbness. On exam deficit was quadriparesis and areflexia, burning dysesthesia, mild opthalmoparesis. COVID-19 related symptoms onset 2 weeks earlier to onset of neurological symptoms.	CSF study: Not reported	Demyelination	AIDP Dysautonomia	-IVIG	Was intubated for short period Motor strength outcome not known.

A rare paraneoplastic syndrome causing weakness, pain and low serum phosphorous

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Keywords: *FGF-23, paraneoplastic, hypophosphatemia, myalgia, myopathy*

Introduction

Muscle pain is a common complaint often prompting neuromuscular referral. The differential for this clinical problem is broad, with causes ranging from inflammatory, toxic, metabolic, hereditary, or idiopathic. The presence of accompanying weakness, elevations in creatine kinase (CK) or abnormal EMG findings often triggers more extensive investigations into etiology. Here, we report an unusual case of musculoskeletal pain and weakness resulting from an FGF-23 related paraneoplastic syndrome and briefly review this rare but treatable condition that may be encountered within neuromuscular practice.

Case Report

A 25-year-old man was referred to neuromuscular clinic for evaluation of musculoskeletal pain, spasms and weakness. Symptoms had begun 4 years earlier with pain located primarily in low back, bilateral shoulders and hip but extending into arms and thighs. Pain was described as sharp, deep, becoming more constant over time, and accompanied by axial muscle spasms. Two years prior, he noted onset of bilateral leg weakness, more proximal then distal. He had difficulty lifting his legs at the hip and began to have frequent falls. He also developed weakness in both arms although to a lesser degree than his legs. He struggled lifting objects overhead, but hand movements/dexterity were largely unaffected. He lost muscle bulk throughout and experienced a 20-pound unintentional weight loss. He had developed chest pain over the past 2-3 months noting periods of dyspnea. He also began using a wheelchair for mobility due to pain, weakness and falls. He denied fasciculations, paresthesia, urinary incontinence, and bulbar or ocular symptoms.

His past medical history was notable for asthma, and a right inguinal lump which he was told was a lymph node. He was diagnosed with avascular necrosis of the head of the left hip 9 months earlier, after several episodes of acute weakness in the hip causing him to fall. He also had history of a foot fracture. He had been seen by his PCP and several orthopedists without establishing a clear diagnosis. Overall, there was no significant family or social history.

On exam, he had normal mental status and cranial nerves. His muscle bulk and tone were normal. There was proximal weakness in hip flexors and shoulder abductors, although proximal muscle testing was limited by pain. Distal muscle strength appeared normal. Sensory exam was normal and reflexes were preserved. Gait assessment was pain limited. On general physical exam, he had chest wall deformity with pectus excavatum. No scoliosis was noted.

Electrophysiological evaluation demonstrated normal motor and sensory nerve conductions in both upper and lower extremities. EMG exam found increased insertional activity in the deltoid with a rare fasciculation. Motor unit morphology and recruitment were unremarkable.

Table 1: Significant Laboratory Values

Lab	Result	Normal
CK	55 U/L	39-308 U/ml
PTH	50.8 pg/ml	15-65 pg/ml
TSH	1.3 mclU/ml	0.4-4.5 mcIU/ml
K	4.4 mmole/L	3.6-5.0 mmole/L
Ca	10 mg/dl	8.4-10.2 mg/dl
Magnesium	2.2 mg/dl	1.6-2.6 mg/dl
Vitamin d,25-OH Vitamin d 1,2 d-OH	50.4 ng/ml **13 pg/ml**	30-80 ng/ml 18-64 pg/ml
Alkaline phosphatase	**464 U/L- total** **358 IU/ml-bone**	40-129 U/L 12-43 IU/ml
P04	**1.2 mg/dl**	2.4-4.5 mg/dl
FGF-23	**7150 RU/ml**	<180 RU/ml

Significant laboratory evaluation is shown in Table 1. Glutamic decarboxylase (GAD) and Caspr2 antibodies in serum were negative. Aggressive Neutraphos supplementation was initiated, but normal phosphorous levels could not be achieved. A diagnosis of FGF-23 related osteomalacia and hypophosphatemia was made and a search for neoplasm began. Given the history of a right inguinal lymph node, the patient underwent abdominal/pelvis/hip imaging by CT and MRI modalities which

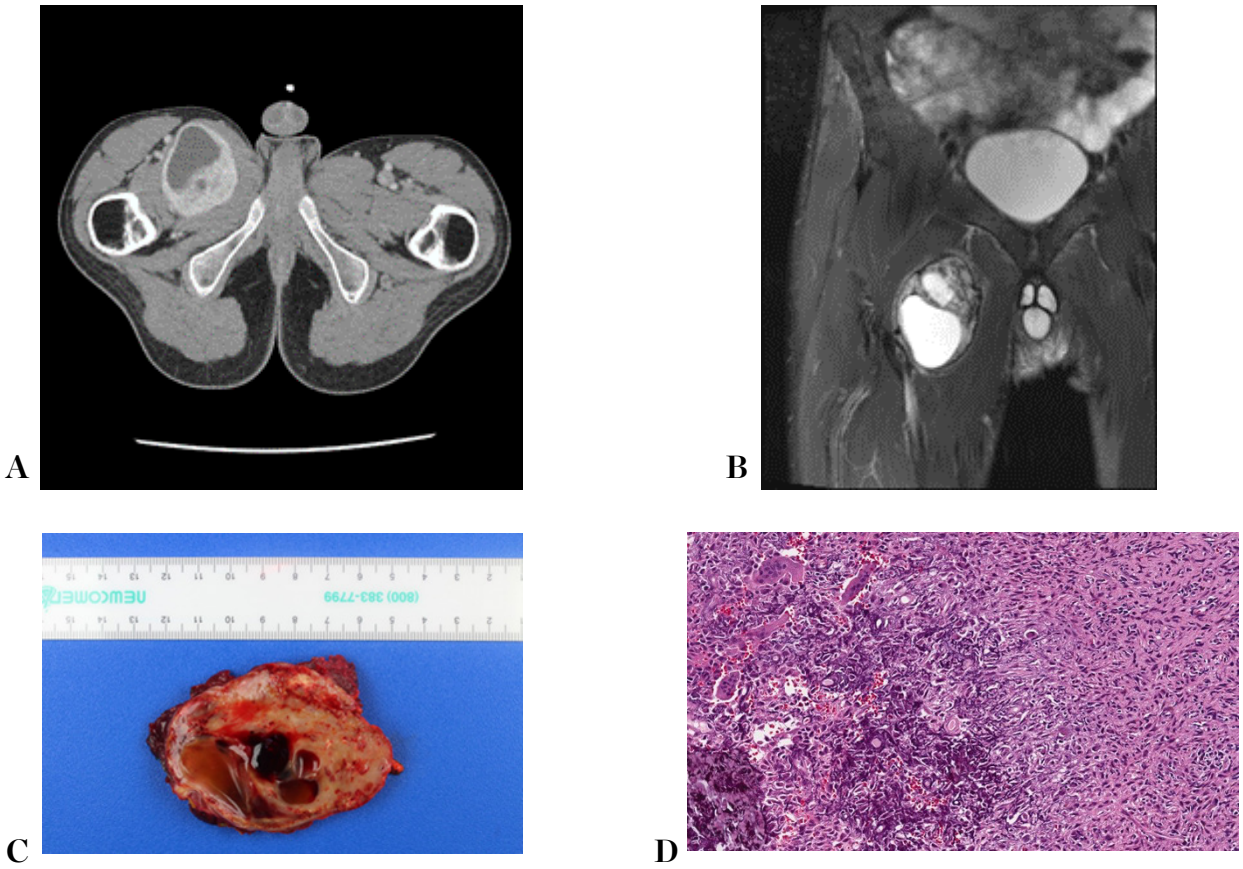

Figure 1. A) Contrasted CT scan of the pelvis showing a 5.5 x 7.4 cm mass anteriorly centered at right adductor brevis muscle. B) T2 fat suppressed MR image of pelvis/thigh. C) Gross photograph of phosphaturic mesenchymal tumor showing mass with cystic change and hemorrhage. D) Tumor consists of spindled cells with areas of calcification. Osteoclast-like giant cells are also present.

showed an enhancing mass with apparent areas of cystic change centered at the adductor brevis muscle of the right thigh (Figure 1A-1B). Tissue from a CT-guided biopsy revealed a phosphaturic mesenchymal tumor. The tumor was surgically excised, but the right obturator nerve was sacrificed due to the large portion of tumor involving the adductor muscles. Final pathology confirmed a phosphaturic mesenchymal tumor (Figure 1C-1D) with surrounding lymph nodes and adjacent muscle, free of tumor. Within 6 days of tumor resection, serum phosphorous normalized at 3.5 mg/dl and remained stable thereafter. Proximal weakness also improved. At 1 month follow-up, patient had noted improved strength and function with reduced pain as well.

Discussion

Tumor induced or oncogenic osteomalacia (TIO) is a rare paraneoplastic disorder characterized by the triad of muscle weakness, musculoskeletal pain and bony fractures in the setting of persistent low serum phosphate.[1] The syndrome has been reported in wide range of ages, from childhood well into adulthood (mean age in early 5th decade) with no male/female predilection.[2,3] It results from production of FGF-23, a key hormonal regulator of phosphate and vitamin D metabolism by phosphaturic mesenychmal tumors (PMTs), a usually benign spindle shaped cell neoplasm derived from skeletal stem cells.[4,5] Rarely, paraneoplastic TIO phenotypes with FGF-23 expression have also been reported with small cell carcinoma of the lung, breast cancer and ovarian neoplasms.[6-8] FGF-23, one of three sub-members of the fibroblast growth factor metabolic subclass, acts primarily by suppressing expression of NPT2a and NPT2c sodium-phosphate cotransporters in the renal proximal tubule serving to decrease phosphate reabsorption and promote wasting.[4] In addition, FGF-23 may impair hydroxy vitamin d synthesis which is necessary for optimum phosphate absorption from the small intestine.[9] The prolonged low serum phosphate and vitamin d abnormalities result in significant osteomalacia which accounts for the clinically observed bony pain and multiple fractures seen with TIO.

As FGF-23 is not recognized to have direct effects on skeletal muscle function, the proximal weakness associated with TIO derives in part from the effects of seriously low and prolonged hypophosphatemia on skeletal muscle.[10] Low serum phosphate can lead to muscle breakdown

and rhabdomyolysis as levels of phosphate critical for adequate ATP production are compromised.[11,12] In animal models, prolonged hypophosphatemia can directly lead to a myopathy that itself may also increase the probability of rhabdomyolysis.[10] Intramuscular inorganic phosphate levels are also critical for myofibrillar performance and maintaining adequate Ca2+ release from the sarcoplasmic reticulum (SR) for muscle fiber contraction. Such alterations can produce skeletal muscle weakness as well as enhanced muscle fatigue.[13] The significant weight loss or as yet other unknown factors secreted by the tumor might also contribute to the proximal weakness observed in TIO.

Primary treatment for TIO-related musculoskeletal pain, weakness and hypophosphatemia consists of recognizing the disorder, then identifying and resecting the tumor. In the rare cases where complete tumor excision is not possible, adjunctive radiation therapy and potential use of anti-FGF 23 monoclonal antibodies (burosamab, Crysvita) may be considered.[14] Although rarely encountered in neuromuscular practice, TIO diagnosis should not be missed, as it is a treatable and reversible paraneoplastic syndrome. This case emphasizes the importance of assessing routine comprehensive metabolic panels in cases of musculoskeletal pain or weakness, as it was the abnormal alkaline phosphatase and serum phosphorous levels which initiated the proper course in making this rare diagnosis.

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Boots

Michael Stanley, MD

What's lost and what's found
in our boots on the ground
when Johnny came hobbling home?

I followed him home one cold winter's day
bounding after his footprints along their way
right up to his home, and there I meowed
'til his hearth and his home I was allowed.

I spied his new boots warming up by the stove,
and into those boots I soon snugly dove.
So he called me 'Boots' because of that:
a funny name for a tortoise-shell cat!

My Johnny grew up, and, well, I grew old,
my heart fluttered worried, but his beat bold.
He marched off to guard us, to flex youthful might,
to trample the wrongful, to champion the right.

Years later while hunting one night in the snow,
I remembered the footprints of long, long ago.
When I crept through the cat-door, I suddenly knew
that—bless my nine lives—my wish had come true!

Straight into his lap I leapt with a purr,
but things seemed so different from what they once were.
I made for the boots by the fire...still chilled.
But one wasn't quite empty; it wasn't quite filled.

At breakfast I'd brush my tail over that leg
in the usual manner a cat tries to beg,
but that leg it was thin. It was cold. It was steel.
And all of my pleadings Johnny just couldn't feel.

At times there's an itch haunting him like a ghost,
and I'll use that strange leg like my own scratching post,
but it does him no good, so we'll go for a walk,
and he'll tell me what ails him, for he knows I won't talk.

I wish I could trade-in the lives I still hold—
risky it is, as I'm getting quite old.
I'd buy what he gave us. I'd give what he lost
just to follow both footprints once more through the frost.

Poems

Elizabeth Snow Rowe

Sometimes poems just come,
but sometimes not for a while.

Maybe they are like paintings
or spectacular photographs.

They are everywhere.
All around us.
We just need to choose one
and write it down.

Quick before the light changes.

Published in ***Sailing Downwind*** by Elizabeth Snow Rowe.

Time Heals All Wounds—But One

Vernon Rowe, MD

He was a huge
hulk of a man
but the blade cut his belly
like it was a melon.
He was cheerful at first
but as weeks wore on
like his cheap shoes
and time spun out
with miles of gauze packing,
his wound stank
and Leroy shrank
shriveled nearly
to skin and skeleton.
One day, barely
conscious, he whispered:
"Let me go, Doc,"
and I did.