Is Waiting for the Axillary Nerve to Recover after Trauma Really the Right Thing to Do?

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INTRODUCTION: Axillary nerve injuries, whether traumatic or iatrogenic, are catastrophic for patients, producing profound functional deficits and disability secondary to deltoid dysfunction. Yet, the current clinical practice is most often expectant management with intervention considered only after six months or more of conservative treatment. For patients who undergo surgical intervention, functional recovery even after optimal treatment is not always reliable. One of the many factors playing a role in determining the prognosis is the patient's age. Based on the extent of the injury, nerves undergo Wallerian degeneration with postsynaptic changes at the neuromuscular junction (NMJ) with the ensuing motor endplate (MEP) degradation. Although previous studies have emphasized the detrimental impact of increased patient age on neural regeneration, no study has focused on the effects of aging on MEP stability and target end organ innervation following peripheral nerve injury (PNI). We hypothesize that MEP degradation occurs more rapidly in the elderly population, resulting in poorer prognosis compared to their younger counterparts. Here, we use human axillary nerve injury as a model of nerve injury and present the MEP analysis of deltoid muscle samples biopsied from young and elderly patients after axillary nerve injury.

METHODS: After receiving IRB approval, denervated deltoid muscle biopsies were collected during standard-of-care surgical procedures for patients with axillary nerve injury confirmed by history, physical exam, and EMG. The muscle samples underwent tissue clearing using the reagents and protocol described by Muniterfering et al, 2018. Samples were fixed in 4% PFA and washed with PBS before immersion in CUBIC R1 solution (urea, quadrol, triton-x) at 37°C on a rotating mixer for 1.5-2 weeks. CUBIC solution was changed every 2 days until clearing was satisfactory and the tissues turned clear or light yellow. The tissues were then washed overnight in a CUBIC IHC buffer (bovine serum albumin, triton-x, sodium azide) before the immunostaining process began. Samples were stained for acetylcholine receptor-α, neurofilament, and synaptophysin to characterize motor endplates. Z-stack images of motor endplates were collected using the Keyence BZ-X810 inverted fluorescence phase contrast microscope at 20x magnification. The data was divided into young (< 60 years old) and elderly (60+ years old) groups. MEP morphology (pretzel, intermediate, plaque) was assessed according to architecture complexity, compactness, and number of perforations. Innervation status of the MEPs was also assessed.

RESULTS: Analysis of the MEPs revealed no significant difference in the percentage of healthy (pretzel) and unhealthy (plaque) morphology between the young and elderly group denervated deltoids; however, there was a significant difference in the percentage of unhealthy (intermediate) morphology MEPs. Overall, the young group showed an average of 11.77% healthy MEPs and 88.33% unhealthy MEPs while the elderly group revealed an average of 10.24% healthy MEPs and 89.76% unhealthy MEPs. Most strikingly, there was a detectable difference in MEP innervation between the two groups. Young muscle samples revealed an average of 48.78% innervated MEPs, while elderly muscle samples revealed an average of 18.82% innervated MEPs, suggesting that the initial response to injury (i.e. Wallerian degeneration) has a more profound impact on the elderly group. In fact, the elderly had a lower percentage of MEP innervation (30.77%) at the earliest timepoint of 3 months post-injury while the young group still had MEP innervation (50.0%) at 7 months post-injury. Beyond these initial differences between age groups, biopsied deltoids appeared to undergo similar rates of progressive loss of MEP innervation as the time from injury increased.

DISCUSSION AND CONCLUSION:

Current management of nerve injuries varies, with operative indications based on the assumption that irreversible MEP degeneration follows prolonged denervation. Our study reveals that while young and elderly patients demonstrate a similar rate of decline in MEP innervation as the time between nerve injury to surgery increases, elderly patients experience a much faster initial rate of MEP innervation loss. Our findings further detail that deltoid MEPs undergo a gradual loss of MEP innervation with time following axillary nerve injury. Therefore, this degenerative process has a more profound effect in elderly patients than their younger counterparts, suggesting potential benefit from earlier intervention within this age group.

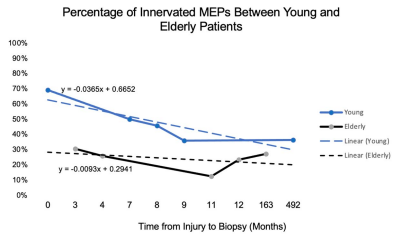

Figure 1. Percentage of innervated MEPs at various timepoints (time from injury to biopsy). Young patients (blue) demonstrated a decreasing percentage of innervated MEPs as the time between injury to biopsy increases. Elderly patients (gray) revealed a low initial percentage of innervated MEPs compared to the young group.

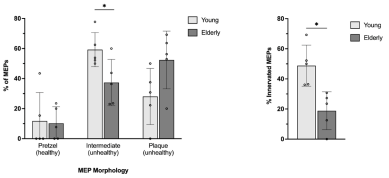

Figure 2. (A) The percentage of MEP morphology found in the deltoid biopsies of young adult and elderly patients post-axillary nerve injury. (B) The percentage of innervated MEPs between the young adult and elderly group.

Table 1. Patient demographics.

Study ID	Gender	Age (years)	Mechanism of Injury	Time from injury to biopsy (months)	Percentage of healthy MEPs (pretzel)	Percentage of unhealthy MEPs (intermediate)	Percentage of unhealthy MEPs (plaque)	Percentage of innervated MEPs
1	M	53	Stab Wound	7	0	63.50	37.50	50.00
2	F	28	MVA*	8	5.40	48.60	45.90	45.90
3	F	22	Penetrating Neck Trauma	0 (4 days)	15.38	53.85	30.77	69.23
4	M	32	Gunshot Wound	9	0	77.78	22.22	36.11
5	M	57	Gunshot Wound	492**	0	50.00	50.00	36.40
6	M	68	Grabbing > 100lbs object as it fell	163	0	36.36	63.63	27.27
7	M	77	Shoulder Dislocation	3	7.70	23.10	69.20	30.77
8	M	76	MVA*	11	0	43.75	56.25	12.5
9	M	73	Iatrogenic	12	23.50	23.53	52.94	23.53
10	M	70	Fall Off of Scooter	4	34.78	43.48	21.74	26.09

*MVA = Motor Vehicle Accident
** Patient had injury during his teenage years and decided to seek treatment at this time due to shoulder symptoms