

ORIGINAL SCIENTIFIC PAPER

Influence of PNF Therapy on the Active Range of Motion in Proximal Humerus Gunshot Injury Patients

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Abstract

The use of Proprioceptive Neuromuscular Facilitation (PNF), namely relaxation techniques, rhythmic initiation, a combination of isotonic, stabilizing and dynamic reversing, rhythmic stabilization and motor patterns, allows to improve motor function and to achieve active participations of patients with musculoskeletal injuries in the process. Therefore, the purpose of the study was to evaluate the effect of proprioceptive neuro-muscle facilitation techniques on index goniometry in individuals with gunshot lesions of the proximal humerus. The study involved 56 middle-aged men, who were randomized to two levels by group size. Investigation group (n=28) received proprioceptive neuromuscular facilitation, control group (n=28) received standard kinesiotherapy program and massage. Goniometry was performed on all participants for physical therapy on 28, 56 and 84 days of the treatment. Limits of movement amplitude were fixed in all three planes, which respond to the nature of the arthrogenic contracture. The total dynamics of increase in the amplitude of active movements in the shoulder complex in participants under the influence of proprioceptive neuromuscular facilitation, noted higher ($p=0.038$; $t=6.94$) than in control group which underwent kinesiotherapy and massage. Therefore, positive changes were observed under the influence of PNF in the active amplitude of movement in men with gunshot lesions of the proximal humerus; this in turn allowed to break the circle of pain and improve the functionality of the upper extremity.

Keywords: *proprioceptive neuromuscular facilitation, goniometry, proximal humerus, gunshot injury*

Introduction

Thousands of military personnel were injured during Joint Forces Operation in Ukraine. To date, despite attempts to peacefully resolve the situation in eastern Ukraine, the conflict has not been resolved, shelling continues, and military personnel are injured, which in turn requires rehabilitation practices to restore the health of those with gunshot wounds.

Noting the high degree of complexity of gunshot lesions, one of the consequences is a critical decrease in the amplitude of movements in the shoulder complex (SC), which necessi-

tates the development of a system of measures to normalize the amplitude indices in patients who have received gunshot injury in extreme conditions. Reduced motor activity directly decrease the activity of daily living and the ability to perform the professional duties of militaries.

The use of proprioceptive neuromuscular facilitation (PNF) techniques can restore effective motor functions by reducing pain, increasing the patient's ability to reduce muscle and increasing patient strength, increasing the amplitude of active and passive movements, improving control of motor



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function, increasing endurance patient and fatigue prevention, improving patient's ability to move and maintain stability (Shimura & Kasai, 2002; Adler, Beckers, & Buck, 2014).

PNF-therapy is a dynamic concept that is used to improve the motor function of its use in the practice of physical therapy (PT) of people with the effects of gunshot lesions (GL) will break the circle of pain, improve the amplitude of movement and achieve active participation of patients in therapy (Lee, 2015; Schmidt, Lee, Winstein, Wulf, & Zelaznik, 2018).

PNF-therapy is aimed at eliminating pathological clinical manifestations, improving functionality and includes the following techniques aimed at improving the amplitude of joint movements:

1. Soft mobilization of the shoulder joint (relaxation techniques, rhythmic initiation). Relaxation techniques include contract relax and hold relax. Restraint technique involves isometric contraction of the muscles being resisted, followed by relaxation. When applying the contraction-relaxation technique, the isotonic contraction of the resisting muscles is performed, followed by the relaxation and movement in the extended amplitude. Rhythmic initiation involves rhythmic motion over a certain amplitude, from passive movement to active resistance with added resistance (Voight, Hoogenboom, & Cook, 2008; Page et al., 2016).

2. Technique isotonic combination is characterized by concentric, eccentric and stabilizing contractions of one muscle group (agonists) without relaxation.

3. Stabilizing reverse and dynamic reverse. The stabilizing reverse technique incorporates alternating isotonic contractions with resistance to movement and enhances muscle strength, improves stability and balance. The dynamic reverse technique is used to increase the active amplitude of movement, strength and endurance of the muscles, to improve coordination by performing active movements with changing directions without interruption or relaxation.

4. Rhythmic stabilization is characterized by alternating isometric contraction with a resistance to movement, with no movement (Adler, Beckers, & Buck, 2014).

5. Motor patterns for improving the amplitude of motion in the SC: flexion - abduction - external rotation; flexion - abduction - external rotation with flexion in the elbow joint; flexion - adduction - external rotation (Yuktasir & Kaya, 2009; Ha, Han, & Sung, 2018).

Thus, the purpose of the study was to evaluate the effect of PNF techniques on goniometry in individuals with inflammatory lesions of the proximal humerus (PH).

Methods

The study involved 56 middle-aged men with GL of the PH. The exclusion criteria adopted were: chronic non-infectious illness, polytrauma (such as humerus injuries and head trauma), severe mental disorders, unconsolidated fractures, ossifying myositis. Inclusion criterion men with a diagnosis of "gunshot fracture (GF) of the PH". According to the classification of GF patients who participated in the study had the following characteristics of obtaining a GF: by type of fracture - blind; by type of wounding projectile - bullets; by the nature of the lesion - incomplete, perforated; by localization - humerus; at the level of fractures of long tubular bones - proximal; by degree of damage - open fracture, wound more than 1 cm in length without extensive soft tissue damage, which does not require secondary surgical treatment, with minor bone

damage; with concomitant injuries - without damage to large blood vessels and nerves (Burianov, Komarov, Lykhodii, Kvasa, & Zadnichenko, 2015).

All subjects who participated in the study signed an informed consent form. The research was approved by the Institutional Ethics Committee (protocol number 2/2017) and was carried out in compliance with the international principles of the Helsinki Declaration of the World Medical Association (World medical association Declaration of Helsinki, 2013), and in accordance with the Law of Ukraine "Fundamentals of Ukrainian Legislation on Healthcare" (Law of Ukraine, 1992) on ethical norms and rules for conducting medical research involving human. Prior to the clinical trial the protocol was submitted before for consideration, comments, recommendations and the results were successfully approved the ethics committees for health.

Patients were divided into two groups using the simple randomization method, which provided random distribution of patients to the investigation group (IG) and control group (CG). The general characteristics of both groups revealed their representativeness and homogeneity, which made it possible to objectively compare the results of restoration of indicators of active range of motion in these groups. The baseline of the studied parameters in patients of the two groups were not significantly different ($p=0.044$; $t=8.01$).

The IG ($n=28$) of patients after PNF-therapy PH lesions; CG ($n=28$) of patients who underwent restorative complex according to the method of a medical care, which included kinesiotherapy (KT) and massage. The study of the results, their comparison with the baseline data and the evaluation were conducted four times: before the course of therapy, on the 28th, 56th, and 84th day of the therapy.

Movement amplitude was assessed using the goniometer. The following movements were assessed: flexion, extension, abduction, adduction, external and internal rotation in the SC.

To obtain the objective results of goniometry, the following rules were followed: the patient should remove his clothing, before starting the goniometry, the initial position should be taken, precise anatomical guidelines were used to install the goniometry, and several passive joint movements should be performed before the evaluation, take measurements only in the morning, do not take measurements after considerable physical activity.

The evaluation process took place in the following sequence: the axis of the goniometry corresponded to the axis of the shoulder joint, the moving arm of the goniometry was located parallel to the moving distal segment of the body (humerus), the fixed shoulder did not change its position during evaluation. Quantitative of the volume of amplitude of movements in the SC is based on the gradation from 0 to 180° (O'Sullivan, Schmitz, & Fulk, 2014).

All statistical analyses were conducted using Statistic 6.0 (StatSoft, USA) and data were expressed in mean \pm standard deviation ($M\pm SD$). The significance of the differences, in normally distributed data was assessed by the Student's t-test for independent groups.

Results

Analysis and evaluation of the effectiveness of PNF-therapy techniques for individuals with GL of the PH, was performed with regard to the changes in the volume of active movements in the SC (Table 1).

Table 1. Dynamics of changes in the amplitude of active movements in the shoulder complex during physical therapy (M±SD)

Movements in the SC	n=56		IG (n=28)			CG (n=28)			p/t 56-day; p/t 84-day
	Before treatment	28-day	56-day	84-day	28-day	56-day	84-day		
Flexion 0-180°	100±4.2	117±2.7	147±24*	168±1.9*	117±2.5	136±2.4	146±1.9	0.02/5.5; 0.023/4.8	
Extension 0-60°	35±3.4	40±1.4	49±1.3	57±1.0*	43±1.4	48±1.4	52±1.3	0.082; 0.043/8.2	
Abduction 0-180°	97±2.8	113±2.8	139±2.4*	160±1.9*	110±2.4	125±2.4	143±2.3	0.037/7.0; 0.041/7.3	
External rotation 0-90°	42±1.7	53±1.1	68±0.8*	80±0.6*	48±0.6	57±0.6	71±0.5	0.026/8.6; 0.04/7.7	
Internal rotation 0-90°	53±2.1	66±0.6	75±0.6*	84±0.5*	67±0.1	72±0.1	81±0.1	0.041/6.2; 0.038/7.0	

Note. *Significant difference between investigation group (IG) and control group (CG) at 56, 84 days which is reflected in p/t values

Dynamics of increase in the amplitude of active movements in the SC in patients of the main group under the influence of PNF-therapy techniques, noted higher than in participants of the CG who received KT and massage.

An increase in the indicators of active flexion (AF) in the SC at all stages of the assessment of the amplitude of movements. However, significantly better values were observed in patients of IG on day 56 - 147±2.4° (p=0.02; t=5.5), and in the final examination - 168±1.9° (p=0.023; t=4.8) in contrast to patients in CG, the average values of which on day 56 they reached only 136±2.4°, and on day 84 - 146±1.9°. Thus, the average rates of AF in the SC in the IG increased by 68 degrees, and in CG patients by only 46 degrees. Significant differences in extension parameters in the SC were recorded only during the final examination in patients of IG 57±1.0° (p=0.043; t=8.2), and in patients of CG 52±1.3°. Thus, the average rates of active extension in patients of IG increased by 22 degrees, and in patients of CG by 17 degrees. The indicators of abduction in the IG at day 56 were 139±2.4° (p=0.037; t=7.0), at day 84 - 160±1.9° (p=0.041; t=7.3) and show significant differences from the indicators of patients in the CG at day 56 - 125±2.4° and day 84 - 143±2.3° days of PT. The results of the final assessment of abduction in the SC in patients of the IG showed an increase of 63 degrees, and in patients of the CG by 46 degrees. The indicators of external rotation (ER) significantly improved in the IG on day 56 - 68±0.8° (p=0.026; t=8.6) and day 84 - 80±0.6° (p=0.04; t=7.7), and in patients of the CG on day 56 - 57±0.6° and day 84 - 71±0.5°, which reflects the increase in the active amplitude of ER in the SC in patients of the IG by 38 degrees, and in patients of the CG by 29 degrees. Appropriate significant improvements were recorded in patients of the IG when assessing the internal rotation (IR) on day 56 of the course of PT 75±0.6° (p=0.041; t=6.2) and day 84 - 84±0.5° (p=0.038; t=7.0), in contrast to patients in the CG whose values on day 56 reached only 72±0.1°, and day 84 - 81±0.1°. Thus, the average rates of AF in the SC in the IG increased by 31 degrees, and in CG patients by only 28 degrees.

Thus, in percentage the rate of AF before the course of PT was 55.5% of normal, and after the course in patients IG group 93.3%, and in patients CG 81.1% of normal flexion in the SC. Before the course of PT, the rate of active extension was determined to be 58.3%, and after the course in patients of IG 95.0%, and in patients of CG 86.6% of normal. The rate of abduction before the course was 53.8%, and after the course

IG 88.8%, in patients CG 79.4% according to the norm. Evaluation of ER before the course showed 46.6%, IR - 58.8%, and after the course of PT in patients IG the rate of ER reached 88.8% and IR - 93.3%, and in patients CG ER was 78.8%, and IR - 90% of normal.

The determined percentages of active movements in the SC show limitations in all three planes in the range of 46-58% of the norm, and this, in turn, emphasizes the arthrogenic reason for the restriction of active movements.

Discussion

This study aimed to test the effect of PNF-therapy on index goniometry in individuals with GL of the proximal shoulder. The main conclusion was that the use of PNF-therapy in the process of PT of persons who received GL of the PH under extreme conditions allows to more effectively restore the amplitude of movements in the SC compared to standard KT and massage.

The study was conducted on days 28, 56 and 84 is justified by the available data, which confirm that visible improvements in functional indicators of the musculoskeletal system occur within four weeks (Shumway-Cook & Woollacott, 2016).

A significant decrease in the active amplitude (AA) of movement in the SC at the beginning of the study showed no significant difference in index goniometry at day 28 of PT between IG and CG. But there was some positive dynamics. Significant improvements in the AA of the movement in the IG were observed during the 56 and 84 days with some positive dynamics in participants CG. Therefore, it can be argued that both approaches can give a positive result in increasing the AA of motion in the SC, but four weeks of time will not be enough. This conclusion can be explained by the severity of the GL in comparison with the traumatic injury of this segment of the upper extremity. It is necessary to take into account the complexity of lesions of structures in the case of GL, the severity of the consequences, the duration of the restorative process, in contradistinction to traumatic injuries of the humerus.

Noting the high degree of complexity of GF of the proximal shoulder, it is necessary to pay special attention to the prevention and elimination of complications. Dysfunction is manifested in the form of contractures, decreased muscle strength, functional and morphological changes in the periarticular ligaments and articular cartilage, impaired blood microcirculation of the damaged limb (Burianov et al., 2015).

To build the process of PT and determine adequate tools and techniques, it is important to adequately differentiate the localization of contracture. According to the localization of the contracture, there are the main types of contractures: myogenic - occurs due to damage to muscle tissue; arthrogenic - joint damage; dermatogenic - due to scarring of the skin; desmogenic - scarring of ligaments, fascia, aponeurosis; tendogenous - due to tendon fusion, neurogenic - disorders of the nervous system (Koneremann & Gruber, 1998).

Impaired range of motion in the joints leads to limitations in the functioning of people with GL include the following activities: body care, putting on and taking off clothes, doing housework, caring for household items, paid work, sports and hobbies. This, in turn, adds to the biopsychosocial context of the PT process and purposefully corresponds to the International Classification of Functioning, Disability and Health (ICFDH) model. The ICFDH seeks to achieve greater coherence between different aspects of health and disease: biological, personal and social (McDougall, Wright, & Rosenbaum, 2010).

The ICFDH covers all aspects of human health and some components of health-related well-being, describing them in terms of health domains and health-related domains.

Limitations of life are characterized as the consequences or results of complex relationships between changes in the health of the individual, personal factors and external factors that represent the conditions in which the individual lives. As a result of these relationships, different environmental factors can cause different effects on one individual with a certain change in health.

If the main task of PT is to eliminate the consequences of illness or injury, the ultimate goal of PT is the maximum destruction of the patient's limitations, maintaining or restoring his ability to work, improving the quality of life.

And according to the biomechanical model of the practice of increasing function - the active amplitude of movements, a person automatically uses them to restore other functional skills and abilities. General functional limitations in patients with GL of the PH: difficulties with daily activity (inability to reach something above the head, inconvenience when dressing, difficulties in carrying out hygienic procedures); difficulties with professional activities (inability to keep the weight of the weapon, difficulties with motor control and with the implementation of purposeful movements, inability to adhere to the military regime of the day due to low endurance, inability to perform throwing movements).

However, one component of PNF-therapy for motor learning and neuro-muscular reorganization is internal feedback. The neurophysiological mechanism of internal feedback implies that proprioceptive disorders impair the ability to adjust, improve, and learn new movement. In such circumstances, the patient often compensates for his or her impairments by shifting attention to other sensory resources, which does not allow them to adhere to the basic principles of therapy (Singh, Nagaraj, Palikhe, & Neupane, 2017).

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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The authors confirm the positive impact of relaxation techniques, rhythmic initiation, a combination of isotonic, stabilizing and dynamic reverse, rhythmic stabilization and movement patterns on the amplitude of motion and motor control of traumatic patients (Adler et al., 2014; Page et al., 2016)

According to the authors, proprioceptive neuromuscular facilitation enhances motor function. As the primary purpose of rehabilitation is to help patients be as efficiently as possible, motor learning principles have been integrated into the PNF concept as a basic philosophical one. This can be achieved in functional tasks with different approaches (Shimura & Kasai, 2002; Ha et al., 2018).

The authors note that the concept provides stability training, improved movement coordination, improved functional activity, improved joint mobility, and increased movement volume (Olędzka & Jaczewska-Bogacka, 2017).

It is important to note that cognitive activity involves a mental process in which the patient is attuned to the activity, understands the goals of therapy and is able to make decisions and answer questions. It is necessary to draw the patient's attention to the problem and move together to solve it. Changes in motor behavior can be as simple as focusing on how a patient performs tasks and verbally controlling or showing how and what to do. Therefore, in our opinion, the use of PNF-therapy techniques for persons with severe mental disorders and cognitive deficits will not maximize the patient's potential (Hawk et al., 2017; D. Park, & S. Park, 2019).

Active participation is required for neuro-muscular rehabilitation. During the active movement, the whole system of motor control works, since in passive movement there is no efferentation and muscular activity (Schmidt et al., 2017).

Learning and restoring movement stereotypes in practice should be relevant to the context of the task. The practice of dissimilar patterns may reduce the outcome of the achievement of the respond goal (Balci, Yuruk, Zeybek, Gulsen, & Tekindal, 2016).

It is also important to consider a positive approach that assumes no pain during exercise. This is an important component of PNF-therapy, given that most people with constant pain have a fear of performing exercises because their previous experience is associated with pain. People with constant pain have the dilemma - activity may increase short-term pain, but lack of activity will lead to long-term pain (Hors, 2008).

Therefore, suggest that the use of PNF-therapy may have a positive effect on the recovery of movement amplitude in individuals with GL of other joints.

The analysis revealed significant differences between the participants group undergoing PNF-therapy and the CG participants who underwent standard KT and massage ($p=0.038$; $t=6.94$). Significant differences were noted at the third and final examination, but positive dynamics were observed throughout the course of PT. Therefore, it can be argued that the use of PNF-therapy in the process of restoring movement amplitude in the shoulder joint produces better results compared to standard KT and massage.

References

- Adler, S., Beckers, D., & Buck, M. (2014). *PNF in Practice* (5th ed.) Berlin Heidelberg: Springer-Verlag.
- Balci, N. C., Yuruk, Z. O., Zeybek, A., Gulsen, M., & Tekindal, M. A. (2016). Acute effect of scapular proprioceptive neuromuscular facilitation (PNF) techniques and classic exercises in adhesive capsulitis: a randomized controlled trial. *Journal of physical therapy science*, 28(4), 1219-1227. doi: 10.1589/jpts.28.1219

- Burianov, O. A., Komarov, M. P., Lykholdii, V.V., Kvasha, V. P., & Zadnichenko, M. O. (2015). Vognepal'ni poranennya verxnix ta ny'zhnix kincivok [Gunshot wounds of the upper and lower extremities]. *Litopys of traumatology and orthopedics*, (1-2), 204–209.
- Ha, S. Y., Han, J. H., & Sung, Y. H. (2018). Effects of ankle strengthening exercise program on an unstable supporting surface on proprioception and balance in adults with functional ankle instability. *Journal of exercise rehabilitation*, 14(2), 301–305. doi: 10.12965/jer.1836082.041
- Hawk, C., Minkalis, A. L., Khorsan, R., Daniels, C. J., Homack, D., Gliedt, J. A., ... & Bhalerao, S. (2017). Systematic Review of Nondrug, Nonsurgical Treatment of Shoulder Conditions. *Journal of manipulative and physiological therapeutics*, 40(5), 293–319. doi: 10.1016/j.jmpt.2017.04.001.
- Horst, R. (2008). *Therapiekonzepte in der Physiotherapie-PNF*. [Therapy concepts in physiotherapy PNF]. Stuttgart: Thieme [in Germany].
- Konermann, W., & Gruber, G. (1998). Sonographische Standardschnittebenen an der oberen Extremität--Schulter- und Ellenbogengelenk [Standard ultrasound sections of the upper extremity--shoulder and elbow joint]. *Ultraschall in der Medizin*, 19(3), 130–138. doi: 10.1055/s-2007-1000476 [in Germany].
- Law of Ukraine (1992) Osnovy zakonodavstva Ukrainy pro okhoronu zdorovia (2802-XII) [Fundamentals of the Ukrainian Legislation on Health Care (2802-XII)]. *Vidomosti Verkhovnoi Rady Ukrainy*. Retriever from <https://zakon.rada.gov.ua/laws/show/2801-12#Text> [in Ukrainian].
- Lee B., K. (2015). Effects of the combined PNF and deep breathing exercises on the ROM and the VAS score of a frozen shoulder patient: Single case study. *Journal of exercise rehabilitation*, 11(5), 276–281. doi:10.12965/jer.150229
- McDougall, J., Wright, V., & Rosenbaum, P. (2010). The ICF model of functioning and disability: incorporating quality of life and human development. *Developmental neurorehabilitation*, 13(3), 204–211. doi: 10.3109/17518421003620525
- Ołędzka, M., & Jaczevska-Bogacka, J. (2017). Effectiveness of Proprioceptive Neuromuscular Facilitation (PNF) in Improving Shoulder Range of Motion. A Pilot Study. *Ortopedia, traumatologia, rehabilitacja*, 19(3), 285–292.
- O'Sullivan, S. B., Schmitz, T. J., & FulkG. (2014). *Physical Rehabilitation* (6th ed.) Philadelphia: F. A. Davis.
- Page, M., Green, S., Mcbain, B., Surace, S., Deitch, J., Lyttle, N.... & Buchbinder, R. (2016). Manual therapy and exercise for rotator cuff disease. *Cochrane Database of Systematic Reviews*, 6. [Electronic version]. doi: 10.1002/14651858.CD012224
- Park, D. J., & Park, S. Y. (2019). Long-term effects of diagonal active stretching versus static stretching for cervical neuromuscular dysfunction, disability and pain: An 8 weeks follow-up study. *Journal of back and musculoskeletal rehabilitation*, 32(3), 403–410. doi: 10.3233/BMR-171107
- Schmidt, R.A., Lee, T.D., Winstein, C., Wulf, G., & Zelaznik H. N. (2018). *Motor control and learning: A Behavioral Emphasis* (6th ed.) UK: Human Kinetics.
- Singh, A., Nagaraj, S., Palikhe, R. M., & Neupane, B. (2017). Neurodynamic sliding versus PNF stretching on hamstring flexibility in collegiate students: A comparative study. *International journal of physical education, sports and health*, 4, 29–33.
- Shimura, K., & Kasai, T. (2002). Effects of proprioceptive neuromuscular facilitation on the initiation of voluntary movement and motor evoked potentials in upper limb muscles. *Human movement science*, 21(1), 101–113. doi: 10.1016/s0167-9457(01)00057-4
- Shumway-Cook, A., & Woollacott, M. (2016). *Motor Control* (5th Inter.ed.): Lippincott Williams and Wilkin.
- Singh, A., Nagaraj, S., Palikhe, R.M., & Neupane, B. (2017). Neurodynamic sliding versus PNF stretching on hamstring flexibility in collegiate students: A comparative study. *International journal of physical education, sports and health*, 4, 29–33.
- Voight, M. L., Hoogenboom, B. J., & Cook, G. (2008). The chop and lift reconsidered: integrating neuromuscular principles into orthopedic and sports rehabilitation. *North American journal of sports physical therapy*, 3(3), 151–159.
- World Medical Association (2013). World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*, 310(20), 2191–2194. doi: 10.1001/jama.2013.281053
- Yuktasir, B., & Kaya, F. (2009). Investigation into the long-term effects of static and PNF stretching exercises on range of motion and jump performance. *Journal of bodywork and movement therapies*, 13(1), 11–21. doi: 10.1016/j.jbmt.2007.10.001