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To graft or not to graft? Median to radial nerve transfer in the forearm: an alternative approach to treat proximal radial nerve injuries

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Aleksandr Gatskiy, Restorative Neurosurgery Department, Romodanov Neurosurgery Institute, 32 Platona Mayborody St., Kyiv, Ukraine, 04050, e-mail: drgatskiy@ mail.ru **Background.** Reconstruction of proximal radial nerve (RN) injuries via grafting technique brings good recovery of wrist extensors, wherein finger and thumb extensors recover to a lesser degree. The outcomes are strongly dependent on a gap length, timing of procedure, etc. An alternative approach — median (MN) to RN transfer — brings regenerating axons much closer to target muscles.

Objective. To compare the recovery of wrist and finger extensors provided by grafting versus distal neurotization technique at proximal RN injury.

Methods. Twenty-eight patients with proximal RN injuries underwent 21 reconstruction of RN continuity via grafting technique. Seven patients underwent transfer of MN branches of *m. flexor carpi radialis* (FCR) and *m. palmaris longus* (PL) to *n. posterior interosseus* (PIN) with simultaneous transfer of *m. pronator teres* (PT) to *m. extensor carpi radialis brevis* (ECRB).

Results. RN grafting brought recovery of the entire complex of thumb, fingers and wrist extensors only in 10 out of 21 cases (47.6 %). Radial wrist extensor(s) recovered in 100 % of cases, thumb extensors — in 85.7 %, finger extensors recovered in 61.9 % patients, with only 47.6 % showed recovery of thumb abductors. Eight patients required additional tendon transfers to restore fingers extension, 3 persons required thumb extension, 11 patients required thumb abduction. All 7 patients (100 %) after MN to PIN transfer received powerful and independent finger and thumb extension, as well as thumb abduction in much earlier terms. PT to ECRB transfer provided powerful and early wrist extension.

Conclusions. The outcomes of MN to RN transfer at proximal RN injuries are more predictable, cogent and time-saving compared to the outcomes received with grafting in terms of recovery of full-fledged function of fingers extensors and thumb abductors.

Keywords: nerve transfer; neurotization; nerve grafting; proximal injury; radial nerve; median nerve

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Невротизація заднього міжкісткового нерва при проксимальних ушкодженнях променевого нерва для відновлення незалежної функції розгиначів пальців кисті

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Вступ. Відновлення променевого нерва при його проксимальних ушкодженнях шляхом автологічної пластики дає змогу досягти задовільної функції розгиначів кисті. Однак відновлення функції розгиначів пальців не завжди досягає прийнятних показників. Результати відновлення значною мірою залежать від розміру дефекту променевого нерва, терміну проведення реконструктивного втручання тощо. Альтернативна методика (невротизація заднього міжкісткового нерва гілками серединного на передпліччі) значно наближує аксони, які регенерують, до цільової мускулатури передпліччя.

Мета: порівняти результати відновлення функції розгиначів кисті та пальців при реконструкції проксимальних ушкоджень променевого нерва методом автологічної нервової пластики та дистальної невротизації заднього міжкісткового нерва.

Матеріали і методи. Реконструкцію анатомічної цілісності променевого нерва методом автологічної нервової пластики проведено 21 пацієнтам, невротизацію заднього міжкісткового нерва гілками серединного нерва до променевого згинача кисті та довгого м'яза, який напружує долонний апоневроз, на передпліччі – 7.

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Результати. Автологічна нервова пластика дала змогу досягти відновлення всього комплексу розгиначів пальців лише у 10 (47,6%) пацієнтів. Відновлення функції променевих розгиначів кисті відбулося у 100% випадків, м'язів, які розгинають фаланги 1-го пальця, — у 85,7%, загальних розгиначів пальців — у 61,9%, повноцінне відновлення м'язів, котрі відводять 1-й палець, — у 47,6%. У віддалені терміни 8 пацієнтам проведено реконструктивні втручання на сухожилково-м'язовому апараті для відновлення функції розгиначів пальців, 3 — для відновлення розгинання фаланг 1-го пальця, 11 — для відновлення відведення 1-го пальця. В усіх пацієнтів після невротизації заднього міжкісткового нерва гілками серединного нерва досягнуто задовільного відновлення функції розгиначів пальців, відведення 1-го пальця в значно коротші терміни. Транспозиція м'яза круглого пронатора передпліччя на короткий променевий розгинач кисті забезпечила потужне раннє відновлення функції розгинання в променево-зап'ястковому суглобі.

Висновки. Результати невротизації заднього міжкісткового нерва гілками серединного нерва більш передбачувані, надійні, відновлення відбувається в раніші строки порівняно з автологічною нервовою пластикою при проксимальних ушкодженнях променевого нерва.

Ключові слова: невротизація; автологічна нервова пластика; проксимальне ушкодження; променевий нерв; серединний нерв

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Невротизация заднего межкостного нерва при проксимальных повреждениях лучевого нерва для восстановления независимой функции разгибателей пальцев

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Вступление. Восстановление лучевого нерва при его проксимальных повреждениях методом аутологической нейропластики позволяет достичь удовлетворительной функции разгибателей кисти. Однако восстановление функции разгибателей пальцев не всегда достигает приемлимых показателей. Результаты восстановления в значительной степени зависят от размера дефекта лучевого нерва, срока проведения реконструктивного вмешательства и т.д. Алтернативная методика (невротизация заднего межкосного нерва ветками срединного нерва на предплечьи) существенно приближает регенерирующие аксоны к целевой мускулатуре предплечья.

Цель: сравнить результаты восстановления функции разгибателей кисти и пальцев при реконструкции проксимальных повреждений лучевого нерва методом аутологической нейропластики и дистальной невротизации заднего межкостного нерва.

Материалы и методы. Реконструкцию анатомической целосности лучевого нерва методом аутологической нейропластики проведено 21 пациенту, невротизацию заднего межкостного нерва ветками срединного нерва к лучевому сгибателю кисти и длинной мышце, напрягающей ладонный апоневроз, на предплечьи – 7.

Результаты. Аутологическая нейропластика позволила достичь восстановления всего комплекса разгибателей пальцев в 10 (47,6%) случаях. Восстановление функции лучевых разгибателей кисти произошло в в 100% случав, мышц, разгибающих фаланги пальцев, - в 85,7%, общих разгибаталей пальцев - в 61,9%, полноценное восстановление мышц, отводящих 1-й палец, – в 47,6% случаев. В отдаленные сроки и 8 пациентам проведено реконструктивные вмешательства на сухожильномышечном аппарате для восстановления функции разгибателей пальцев, 3 - для восстановления разгибания фаланг 1-го пальца, 11 – для восстановления отведения 1-го пальца. У всех пациентов после невротизации заднего межкостного нерва ветками срединного нерва достигнуто удовлетварительное восстановление функции разгибателей пальцев, отведения 1-го пальца в значительно более короткие сроки. Транспозиция мышцы круглого пронатора предплечья на короткий лучевой разгибатель кисти обеспечила эффективное раннее восстановление разгибания в лучезапястном суставе.

Выводы. Результаты невротизации заднего межкостного нерва ветками срединного нерва более предсказуемы, надежны, восстановление происходит в более ранние термины по сравнению с аутологической нейропластикой при проксимальных повреждениях лучевого нерва.

Ключевые слова: невротизация; аутологическая нейропластика; проксимальное повреждение; лучевой нерв; срединный нерв

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Introduction

Proximal radial (RN) nerve injuries are most commonly associated with orthopedic trauma, whether with closed or open humeral fractures [1]; postosteosynthesis palsy of RN remains one of the severest complications of humeral osteosynthesis [2]. Clinical manifestation of proximal RN injuries produces partial or complete loss of elbow extension, as well as lack of finger, thumb and wrist extension [3]. There is no strict consensus even among surgeons, which technique provides the best outcome in terms of reanimation of fingers and wrist extension [3]. Some authors claim that nerve grafting brings satisfying results with proximal RN injuries [4], though high dependence on the gap length and timing of the procedure remain the most critical challenges [5]. Even nowadays tendon transfer remains traditional treatment for proximal RN injuries, though some authors have cited unsatisfactory results following transfer [6-8].

An alternative approach to treat proximal lesions of RN was first described by S. E. Mackinnon et al. in 1999 [8]. The technique of median to radial nerve transfer became widespread among surgical society [3], keynotes and pitfalls involved were precisely evaluated [3] and consistency of the outcomes proved the high viability of the procedure.

The **aim** of the study was to compare the recovery of wrist and finger extensors provided by grafting versus distal neurotization technique at proximal RN injury.

Methods and materials

This study presents a retrospective analysis of the consecutive series of 28 patients with proximal RN injury over a 4 year period (2013–2016). The criteria of inclusion were as follows: 1) RN injury in the region of proximal to the middle shoulder — upper shoulder — 20 patients (lateral injury)/in the spiral groove — 5 patients (posterior injury)/in the axillar groove — 3 patients (medial injury); 2) RN injury accompanied by the long gap that required autologous grafting to reconstruct nerve continuity; 3) pre-surgical absence of clinical and EMNG-based signs of reinnervation.

Patient population

Twenty-eight patients (20 men and 8 women) were enrolled into the study. All enrolled patients were examined in a standardized manner: clinical neurological examination and electroneuromyography (ENMG). The mean age of enrolled patients was 36 years (range 19–57). The mean time from occurrence of injury to surgery was 4.8 months (range 2–8). Twenty-five patients received open injury of proximal RN that was associated with humeral fracture followed by its osteosynthesis. In 3 out of 28 patients proximal injury of RN was associated with humeral fracture that required no osteosynthesis — closed injury. Eighteen patients required simultaneous re-synthesis of the humerus due to long-persisting absence of the consolidation (summarized in **Table 1**).

All enrolled patients were divided into 2 groups: Control Group and Nerve Transfer Group in accordance with utilized surgical procedure.

Table 1. Characteristics of patients enrolled into the study (D — distal stump of an injured radial nerve; P — proximal stump of an injured radial nerve; FCR — branch of median nerve to *m. flexor carpi radialis*; PL — branch of median nerve to *m. palmaris longus*; Pr — 15 months of follow-up according to study design)

Patient number (year of surgery)	Age/Sex	Time from injury to surgery (months)	Type of surgery (G-grafting, NT- nerve transfer)	Gap length (cm), N —number of nerve grafts	Follow-up period (months) (Pr + "on-demand")
#1 (2013)	40/F	5	G (D+P)	7cm, N-4	P+7
#2 (2013)	34/F	3	G	4cm, N-3	P+12
#3 (2013)	36/M	4	G (D+P)	10cm, N-4	P+3
#4 (2013)	29/M	7	G (D+P)	8cm, N-6	P+3
#5 (2014)	32/M	5	G (D+P)	12cm, N-3	P+5
#6 (2014)	35/M	8	G	5cm, N-4	P+5
#7 (2014)	42/F	2	G (D+P)	8cm, N-3	P+7
#8 (2014)	38/F	2	G (D+P)	10cm, N-3	P+9
#9 (2014)	19/M	3	G (D+P)	6cm, N-4	P+15
#10 (2014)	20/M	4	G (D+P)	8cm, N-3	P+11
#11 (2015)	28/F	4	G (D)	7cm, N-4	P+13
#12 (2015)	28/M	3	G (D)	6cm, N-3	P+11
#13 (2015)	54/F	7	G (D+P)	6cm, N-3	P+5
#14 (2015)	37/M	5	G (D+P)	8cm, N-3	P+3
#15 (2015)	42/M	5	G (D+P)	7cm, N-4	P+3
#16 (2015)	56/F	5	G (D)	5cm, N-4	P+4
#17 (2015)	33/M	6	G (D+P)	7cm, N-3	P+5
#18 before May '16	47/M	4	G (D+P)	8cm, N-4	P
#19 before May '16	19/M	2	G (D+P)	14cm, N-2	P
#20 before May '16	25/M	3	G (D+P)	10cm, N-3	P
#21 before May '16	39/M	5	G (D+P)	10cm, N-3	P+5
#22 (2015)	57/F	5	NT (FCR)	8cm	P+7
#23 (2015)	29/M	6	NT (FCR)	8cm	P+2
#24 (2015)	42/M	5	NT (FCR)	9cm	P+2
#25 (2015)	22/M	2	NT (FCR+PL)	7cm	P
#26 before May '16	45/M	4	NT (FCR+PL)	10cm	P
#27 before May '16	34/M	2	NT (FCR+PL)	11cm	P
#28 before May '16	39/M	4	NT (FCR+PL)	9cm	P

This article contains some figures that are displayed in color online but in black and white in the print edition

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Twenty-one patients with proximal RN injury (lateral injury -15, posterior injury -4, medial injury -2) were randomly selected for the autologous nerve grafting and represented the Control Group.

Seven patients with proximal RN injury (lateral injury -5, posterior injury -1, medial injury -1) were randomly selected for the median to radial nerve transfer in the forearm and represented the Nerve Transfer Group.

Surgical procedure

The initial step in all reconstruction cases was the exposure and identification of the site and severity of proximal RN injury. The exploration began on the lateral aspect of the lower/middle shoulder (presumably nondamaged region of RN) and proceeded towards posterior (spiral groove) and medial aspect of the upper shoulder. Once both stumps of RN were identified, an appropriate reconstruction technique was applied.

Twenty-one patients from the Control Group underwent autologous nerve grafting in order to reconstruct RN continuity. Mean gap length between proximal and distal stumps of RN was 7.5cm with mean number of autologous nerve grafts required -3. Sixteen cases of proximal RN injury required transfer of both stumps onto the medial aspect of the shoulder in order to shorten the gap between the stumps, 3 cases required transfer of the distal stump only (site of injury — medial injury), with only 2 requiring no pre-grafting transfer of the RN stumps. All grafts were sutured under microscopic magnification (5-8 times) with 8/0-10/0 nonabsorbable monofilament suture in a tension-free manner with 180 degrees extended forearm.

In 7 patients from Nerve Transfer Group the injury to RN that required grafting was identified during the initial stage of the surgery with mean gap length of 8.5cm between proximal and distal stumps. No reconstruction of RN main trunk was applied, and the median to radial nerve transfer was performed on the same day.

The lazy-S skin incision over the anterior surface of the forearm from the antecubital fossa to the lowerthird of the radial edge of the forearm was performed. The tendinous part of the pronator teres (PT) was identified, mobilizing towards distal forearm with radius periosteum and transected. The transsection of the deep head of PT (both muscle and tendinous arch) simplified the exposure to branching pattern of the median nerve (MN) (Fig. 1).

Anterior interosseus nerve (AIN) and main trunk of MN were identified anatomically. Lower branch of MN to PT was identified at the point of its entrance to the medial-posterior aspect of the muscle in the antecubital fossa and preserved. Distal to the lower branch of PT, the branches to the flexor carpi radialis (FCR) and palmaris longus (PL) were identified coming off medially out of the MN, confirmed by contraction of corresponding muscles with electrical stimuli. Both FCR and PL branches were mobilized distally and cut at the point of their entrance to the corresponding muscles (donor nerves). Radial sensory branch was followed proximally to the site of junction with RN common trunk. Branches to extensor carpi radialis longus and brevis (ECRL, ERB) were identified anatomically; posterior interosseus nerve (PIN) was followed distally under the arch of supinator muscle with its partial transsection; branch to supinator muscle was separated from the main PIN trunk. PIN was then followed proximally within RN towards lower shoulder and cut (recipient nerve).

FCR and PL branches of MN were sutured to PIN under microscopic magnification (5-8 times) with 9/0-10/0 nonabsorbable monofilament epiendoneural sutures in a tension-free manner anteriorly to MN trunk and AIN. Four patients received transfer of both FCR and PL branches (Fig. 2) in order to achieve "perfect sizematch" between donor and recipient nerves.

Three patients received only FCR branch transfer (Fig. 3).

We did not perform reinnervation of ECRB branches; instead tendon transfer of PT to ECRB was performed. All 7 patients required cast immobilization of the wrist joint (under 130 degrees extension) in post-op period



Fig. 1. Anterior surface of the upper-middle forearm: landmarks, key structures, transfer of MN FCR to PIN (right): $m{1}-FCR$ branch of MN; $m{2}-PIN$; $m{3}-MN$ common trunk; $m{4}-$ superficial branch of RN; $m{5}-PT$, deep head detached; 6 — BR; 7 — ECRB

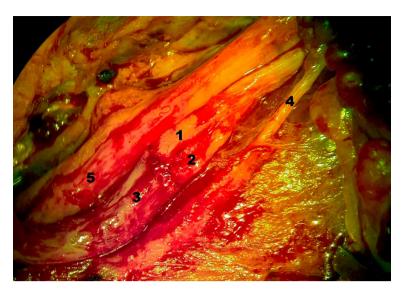


Fig. 2. MN FCR and PL transfer to PIN (right hand): 1 — FCR branch of MN; 2 — PL branch of MN; 3 — PIN; 4 — lower PT branch of MN penetrates PT; 5 — AIN (under intra-OP x8 microscopic magnification)



Fig. 3. MN FCR transfer to PIN (right hand): 1 — FCR branch of MN; 2 — PIN; 3 — MN common trunk; 4 — superficial branch of RN (under intra-OP x8 microscopic magnification)

to prevent pullout of the transferred PT tendon. We applied early mobilization of the wrist, active motions were allowed from the $1^{\rm st}$ till the $4^{\rm th}$ day post surgery in order to simplify the reeducation process, followed by 3 weeks of rigid immobilization.

In post-op period, all patients received standard therapy, which included antibiotics and painkillers. No early or delayed complications associated with surgery were observed.

The mean follow-up period was 1 year 4 months. The follow-up period included clinical neurological examination with evaluation of muscle strength with Medical Research Council Grading System (MRCS), ENMG in 6, 9, 12 and 15 months post surgery followed by ondemand examination.

A small number of patients did not allow for any meaningful statistical analysis.

Results

Control Group (RN grafting)

Younger patients (under 21 years) showed first signs of reinnervation in the 6–7th month already (deep pain in the muscles of the posterior surface of the forearm).

Mild to moderate strength in wrist extensors (mediated by ECRL and/or ECRB) was detected in the 10th month among younger patients (under 21 years). Mean terms of ECRL/ECRB recovery were 13 months. First signs of extensor digitorum communis (EDC) reinnervation did not differ among younger versus older patients, mean terms of EDC recovery were 14.5 months. The recovery of extensor pollicis longus (EPL) and abductor pollicis longus (APL) function in the majority of patients initiated simultaneously with EDC, wherein their strength reached their maximum in the 15th month and showed no tendency to improve with time.

Six patients showed recovery of ECRL only (*Fig.* 4). In 4 patients functioning of ECRL only provided proper wrist stability and extension. In 2 patients ECRL contraction was accompanied by severe (excessive) radial deviation while extending. Fifteen patients showed good recovery of both ECRB and ECRL. Simultaneous extension of the 2nd, 3rd, 4th and 5th finger was achieved in 13 patients, 3 patients showed weakened extension of the index, 5 patients experienced weakened extension of the 3rd, 4th and 5th finger with no recovery of *m. extensor digiti minimi proprius*. Three patients showed

no recovery of EPL, 4 patients demonstrated no thumb abduction.

Summarizing the outcomes of RN reconstruction via grafting technique we state: the recovery of the entire complex of thumb, fingers and wrist extensors was achieved only in 10 out of 21 cases (47.6 %). Better recovery of more proximal radial extensors (100 % of cases) was still accompanied by the lack of consistency within the outcomes, wherein 71.4 % showed recovery of both radial wrist extensors (ECRL and ECRB). Twentyeight point six percent showed recovery of ECRL only, wherein 2 of them (9.5 %) with severe radial deviation of the wrist required its transfer to ECRB (with no loss of the muscle strength) in later terms to restore proper wrist extension.

The more distally the extensors were situated, the more dramatically grew the inconsistency of recovery: 85.7 % showed sufficient recovery of thumb extension, 61.9 % showed sufficient recovery of all finger extensors, with only 47.6 % showed recovery of thumb abduction. All 8 patients (38.1 %) with incomplete EDC recovery required tenodesis of the finger extensors to provide simultaneous extension in MCP joints in later terms. Seven patients (33.3 %) required transfer of PL to EPL or APL to reanimate thumb extension or abduction, respectively. In much later terms another 7 patients (33.3 %) with incomplete (ineffective) recovery of APL of the dominant hand and no reliable improvement with time required PL transfer to restore proper thumb abduction.

The recovered functions of proximal and distal extensors in 9 out of 21 patients (42.9 %) were accompanied by simultaneous activation of thumb

and wrist extensors with complete loss of movement independency most probably due to the phenomenon of heterotopic reinnervation. Seven patients (33.3 %) required injection of botulinum toxin either into thumb or wrist extensors during the reeducation process in order to temporary turn the "parasite" movements off. Two out of 10 patients (20 %) with entirely recovered complex of wrist and finger extensors suffered from neuropathic pain and required long-term intake of painkillers.

Nerve Transfer Group

DISSN 1810-3154

PT to ECRB transfer provided powerful wrist extension in early post-op period. First signs of reinnervation of EDC, EPL and APL appeared in the 3rd month already - at that simultaneously in EDC and more distal EPL and APL. The reinnervation signs (clinical and EMG based) did not differ from any other nerve reconstruction. The co-activation of the entire forearm flexor complex with newly regenerated extensor complex was seen on EMG within 5-9 month. But as soon as voluntary finger extension was seen clinically the EMG-based signs of co-activation began to diminish to none.

All 7 patients received powerful, discrete, independent and fluid EDC, EPL and APL function in the 10th month already (Fig. 5), with only one patient showed signs of weakened thumb abduction, no improvement with time, but still required no tendon transfer (non-dominant hand). None of our patients who underwent transfer of FCR branch of MN to PIN required additional orthopedic procedures.

Discussion

According to our long-term single-center experience, we state that the exploration of the site of RN injury is a

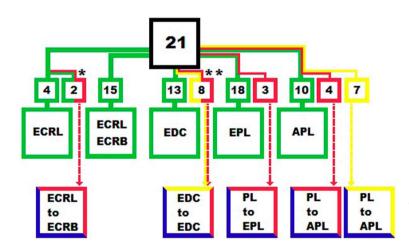


Fig. 4. Outcomes of grafting of proximal RN injuries (outlining: green — good recovery, MRCS score 4 points or higher, yellow — poor recovery, MRCS score 3 points or lower, red — no recovery, blue required tendon transfer). *recovery of ECRL, which provided no proper wrist extension due to severe radial deviation. **partial recovery of EDC, required tenodesis of individual finger extensors

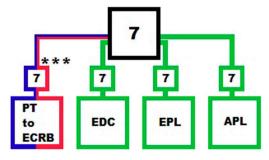


Fig. 5. Outcomes of the median to radial nerve transfer in the forearm with simultaneous transfer of PT to ECRB (outlining: good recovery, MRCS score 4 points or higher, red no recovery, blue — required tendon transfer). ***no reinnervation of ECRL and ECRB conducted, simultaneous transfer of PT to ECRB instead

must and should be considered as the initial procedure. At least 30 % of patients (not included in this study) with open- or closed-type proximal RN injury required no reconstruction of the nerve: the fascicular integrity of the nerve was preserved and the surgery ended with proper neurolysis/endoneurolysis and release of the tension. Only approximately 20 % of those patients showed no signs of spontaneous regeneration within next 12 months. In addition, the release and transfer of proximal stump of RN even with no further reconstruction dramatically ceased neuropathic pain, to a certain point facilitated humerus re-synthesis as well as the removal of the implanted osteosynthesis device in later terms.

Time spent to reanimate fingers and wrist extension with grafting technique severely debilitated patients for months, especially in dominant-hand cases. Yet still, with single RN injury, we would rather choose grafting than tendon transfer, though the debates are moving around "whether nerve grafting is equal or superior to tendon transfers" [3].

The introduction of median to radial nerve transfer in our department in 2015 partially changed our view on the process of treating proximal RN injuries. Though patients with proximal RN injury were selected for the nerve transfer predominantly randomly, the initial characteristics of the nerve injury, with presumably poor outcome (long gap, lack of grafts, humerus resynthesis, dominant hand, etc) as well as type of patient's employment pushed the decision towards the nerve transfer. During 3 initial surgeries we carefully preserved PL branch of MN in order to be able to execute an "escape" plan" — PL to EPL or APL tendon transfer if the nerve transfer failed. Simultaneous tendon transfer (PT to ECRB) provided sufficient stability, and brought the major functional advantage — enabled proper independent wrist extension in much earlier terms compared to one achieved with the grafting technique. All patients were able to return to their employment activities within 2 months post-surgery. We excluded transfer of MN m. flexor digitorum superficialis branches to ECRB branches of RN out of "classic" nerve transfer procedure [3], and it allowed a patient to learn how to extend the wrist before they needed another re-education for thumb and fingers at the time of regeneration. In general, median to radial nerve transfer brought regenerating axons closer to target motor endplates, thus reanimated fingers and thumb extension within a much shorter period of time.

Conclusions

- 1) The outcomes of MN to RN transfer are more predictable, cogent and time-saving compared to the outcomes received with grafting in terms of recovery of full-fledged function of fingers extensors and thumb abductors;
- 2) Median to radial nerve transfer provides powerful and independent functioning of the finger extensors, as well as the extensors and abductors of the thumb in much earlier terms;
- 3) Opt for median to radial nerve transfer is in advance in all cases with presumably poor outcome of reconstruction of proximal RN injuries;
- 4) Exploration of the site of RN injury is a must and should be the first step in all cases of proximal RN injuries.

Disclosure

Conflict of Interest

The authors declare no conflict of interest concerning any drugs, materials, devices or methods used in this study or the findings specified in this paper.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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