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ORIGINAL ARTICLE

Gender-Affirming Surgery

Donor and Recipient Nerve Axon Counts in Gender-affirming Radial Forearm Phalloplasty: Informing Choice of Nerve Coaptations

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Background: A key component of success of a nerve transfer is the innervation density, which is directly affected by the donor nerve axonal density and donor-to-recipient (D:R) axon ratio. Optimal D:R axon ratio for a nerve transfer is quoted at 0.7:1 or greater. In phalloplasty surgery, there are currently minimal data available to help inform selection of donor and recipient nerves, including unavailability of axon counts.

Methods: Five transmasculine people who underwent gender-affirming radial forearm phalloplasty had nerve specimens processed with histomorphometric evaluation to determine axon counts and approximate donor-to-recipient axon ratios.

Results: Mean axon counts for recipient nerves were 6957 ± 1098 [the lateral antebrachial (LABC)], 1866 ± 590 [medial antebrachial (MABC)], and 1712 ± 121 [posterior antebrachial cutaneous (PABC)]. Mean axon counts for donor nerves were 2301 ± 551 [ilioinguinal (IL)] and 5140 ± 218 [dorsal nerve of the clitoris (DNC)]. D:R axon ratios using mean axon counts were DNC:LABC 0.739 (0.61–1.03), DNC:MABC 2.754 (1.83–5.91), DNC:PABC 3.002 (2.71–3.53), IL:LABC 0.331 (0.24–0.46), IL:MABC 1.233 (0.86–1.17), and IL:PABC 1.344 (0.85–1.82).

Conclusions: The DNC is the more powerful donor nerve with greater than two times the axon count of the IL. The IL nerve may be under-powered to re-innervate the LABC based on an axon ratio consistently less than 0.7:1. All other mean D:R are more than 0.7:1. DNC axon counts may be excessive for re-innervation of the MABC or PABC alone with D:R of more than 2.5:1, potentially increasing risk of neuroma formation at the coaptation site. *(Plast Reconstr Surg Glob Open 2023; 11:e4971; doi: 10.1097/GOX.0000000000004971; Published online 10 May 2023.)*

INTRODUCTION

Phalloplasty is rapidly increasing in frequency in both the United States and globally due to improving access to gender-affirming surgical procedures. One of the main tenets of an "ideal" phalloplasty includes the return of tactile and erogenous sensation.[1] In the modern era of phalloplasty, sensation remains a top priority for many transmasculine individuals.[2,3] Despite this consistent emphasis on the importance of sensation, much of the literature has focused on outcomes related to flap-specific complications, urologic outcomes, and erectile devices.[4–6] Comparatively little attention has been paid to sensory outcomes.[7]

Current evidence regarding the sensory experience of transmasculine individuals who underwent phalloplasty demonstrates substantial differences in subjective and objectively measured sensation.[3] Reported outcomes range widely with some individuals reporting sensory outcomes that do not meet expectations. Not surprisingly, there is currently a complete lack of consensus on the optimal technique and strategies to maximize phallic sensation. This general lack of consensus includes which nerves are coapted in surgery.[8] Nerve coaptation strategies have included single versus multiple, different combinations of donor and recipient nerves and end-to-end versus end-to-side coaptations. Although data support improved sensory outcomes when nerve coaptations are performed, there has not been nerve histomorphometric data available to help inform the selection of donor and recipient nerves used in phalloplasty.[8]

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It has also been under-recognized that the nerve coaptations performed in phalloplasty surgery are actually sensory nerve transfers. Our current understanding of nerve transfers suggests that a core principle for a successful nerve transfer is a sufficient donor-to-recipient axon ratio.[9,10] Recent reviews on phalloplasty sensation discuss the critical role of innervation density on sensory outcomes and the role of the donor nerve axon density as a key factor in ultimate sensory recovery of the phallus.[8] Despite this, the axon counts and potential donor-to-recipient axon ratios in phalloplasty have not yet been reported.

With regard to nerve physiology, a minimum number of 20%–30% of nerve fibers (axons) are needed to maintain function, due to the ability of nerve fibers to collaterally sprout.[11,12] This explains why nerve transfers with donor:recipient (D:R) axon ratios as low as 0.3:1 have shown clinical success.[13,14] However, nerve transfers with low D:R axon ratios demonstrate greater variability in their outcomes and a higher rate of failure. In scenarios with a low D:R axon ratio, achieving the necessary 30% of nerve fibers reaching and re-innervating target relies on near perfect nerve regeneration. Not surprisingly, nerve transfers with a higher ratio (typically a D:R of >0.7:1) have demonstrated superior and more reliable results. A sufficient D:R ratio (ideally 0.7:1 or greater) is now understood to be a key factor determining the success of a nerve transfer. One strategy to increase the number of axons that make it across the coaptation site includes increasing the axon count of the proximal donor nerve, in other words, increasing the ratio of proximal:distal or D:R axons.[15] However, increasing the donor axon count excessively may result in neuroma formation at the coaptation site, with D:R ratios of greater than 2.5:1 potentially increasing this risk.[15] The majority of our current understanding of how nerve histomorphometric data correlate with clinical results of nerve transfers have been gained through motor nerve transfers.[13,14] Commonly accepted methods to evaluate potential results of nerve transfers include donor-to-recipient comparisons of histomorphometric nerve characteristics such as axon counts with the assumption that the current methods of donor-to-recipient comparison may also be applied to inform selection of sensory nerve transfers.[9,10] Current selection of nerve coaptations in phalloplasty is not yet data-driven and is based mainly on surgeon opinion. Objective nerve histomorphometric data will provide one additional data point to inform surgeon selection of nerve coaptations in phalloplasty surgery.

The radial forearm is the most common donor site used in gender-affirming phalloplasty. At present, axon counts of the nerves used in radial forearm phalloplasty are not available. Therefore, donor-to-recipient axon ratios have yet to be used in the decision-making process regarding nerve selection in phalloplasty cases. As there is likely to be variability in the donor and recipient nerve axon counts, there may be more favorable nerves to use in combination to achieve more ideal D:R axon ratios. Higher D:R ratios (>0.7:1) should increase the chance of a critical number of axons innervating the phallus, while minimizing risk of neuroma formation at the coaptation sites (<2.5:1). Therefore, the specific aims of our study are twofold. First, to obtain axon counts of the donor and recipient nerves used in gender-affirming radial forearm phalloplasty. Second, to utilize those axon counts to determine the D:R axon ratios of available nerve transfers.

Takeaways

Question: What are the axon counts and ratios of nerve coaptations in gender-affirming radial forearm phalloplasty?

Findings: The dorsal nerve of the clitoris is the most powerful donor nerve available. The ilioinguinal nerve may be under-powered to re-innervate the lateral antebrachial nerve based on a low donor-to-recipient axon ratio. All other donor-to-recipient axon ratios in radial forearm phalloplasty are equal to or greater than an ideal of 0.7:1.

Meaning: Axon counts can be used along with other considerations such as territory of innervation and size match to better inform selection of nerve coaptations in phalloplasty beyond surgeon opinion alone.

METHODS

This study was approved by our institutional review board and funded by a grant awarded internally via a faculty initiative pool. Following informed consent, samples of each of the five sensory nerves that are routinely transected during radial forearm phalloplasty surgery at our institution were collected from five transmasculine individuals undergoing surgery: the anterior branch of the medial antebrachial cutaneous (MABC), lateral antebrachial cutaneous (LABC), posterior antebrachial cutaneous (PABC), ilioinguinal (IL), and dorsal nerve of the clitoris (DNC).

In our institutional phalloplasty program, it is our current practice to coapt one DNC to the LABC and one IL to the PABC. The LABC and PABC are chosen as recipients as they directly innervate the zone of the flap that becomes the phallic shaft. The LABC is a good size match for the DNC and the PABC is a good size match for the IL. The MABC is not used as a recipient for nerve at our center as it innervates the tissue used to create the phallic urethra.[7] However, the anterior branch of the MABC is transected during surgery as the tissue it innervates is harvested as part of the flap. The posterior branch of the MABC is spared and left innervating the remaining skin bridge on the ulnar aspect of the forearm. The DNC and IL represent the donor nerves, and the LABC, MABC and PABC represent the recipient nerves in this study.

The LABC, MABC, and PABC were sampled at the level of the antecubital fossa. The IL was sampled several centimeters distal to its emergence through the external inguinal ring, before arborization, and the DNC was dissected at the base of the clitoral body (level of the pubic symphysis) and followed distally to a point just before its branching, where it was transected and sampled (Fig. 1). The small excess of each sensory nerve that is trimmed away to freshen the nerve ends before microsurgical coaptation is what was collected for analysis. Specimens were each approximately 5 mm in size.

Nerve samples were immediately placed in 3% glutaraldehyde fixative. Specimens were post-fixed in 1% osmium tetroxide and serially dehydrated in ethanol and toluene, then embedded in araldite, sectioned on an ultramicrotome into 1-μm cross sections, and counter-stained with 1% toluidine blue. Histomorphometric analysis was performed at 1000× magnification using a Leitz Laborlux S microscopic and image analysis software (Clemex Vision Professional, Longueuil, Quebec) to obtain axon counts for each specimen[16] (Figs. 2 and 3).

Descriptive statistics were used to determine mean, range, and standard deviation for the number of axons in each sensory nerve. D:R axon ratios were calculated based on mean axon counts for each possible coaptation of donor to recipient nerve.

RESULTS

Twenty-five specimens (five from each nerve) were collected from five transmasculine patients undergoing gender-affirming radial forearm phalloplasty. Three of the 25 specimens were found to be too small at the time of analysis and lacked sufficient tissue to perform axon counts. These were excluded.

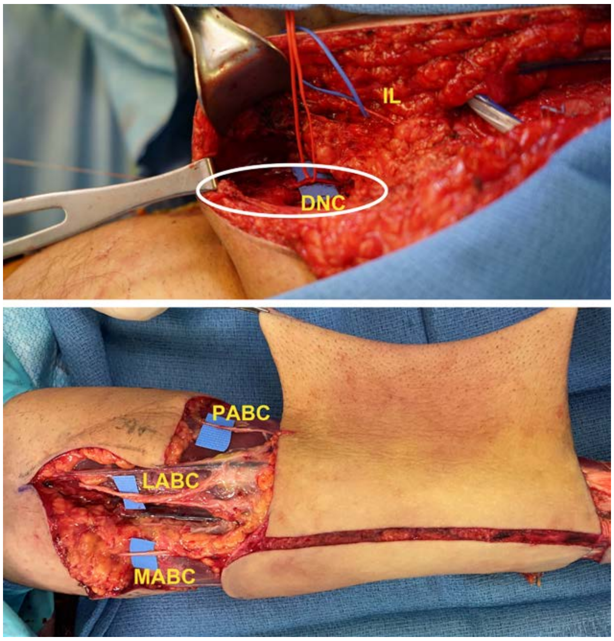

Fig. 1. Surgical anatomy of the donor and recipient nerves used in gender-affirming radial forearm phalloplasty. The LABC, MABC, and PABC were sampled at the level of the antecubital fossa. The IL was sampled several centimeters distal to its emergence through the external inguinal ring, before arborization, and the DNC was sampled at the base of the clitoral body at the level of the pubic symphysis. White oval demarcates location of the clitoral body.

Mean axon counts of donor nerves (Table 1) were 5140 ± 218 (range 5021–5543) for the DNC, and 2301 ± 551 (range 1558–2875) for the IL. Mean recipient axon counts were 6957 ± 1098 (range 5401–8238) for LABC, 1866 ± 590 (range 850–2667) for MABC and 1712 ± 121 (range 1577–1851) for PABC.

Mean D:R axon ratios with the DNC as the donor were DNC:LABC (0.739), DNC:MABC (2.754) and DNC:PABC (3.002). D:R axon ratios with the IL as the donor were IL:LABC (0.331), IL:MABC (1.233) and IL:PABC (1.344). See Table 2.

DISCUSSION

Axon counts and donor-to-recipient axon ratios are predictive of outcomes after nerve transfer. Sufficient regeneration of axons from the donor nerve across the coaptation and into the target is necessary for both sensory and motor recovery. As sensation has historically been overlooked in lieu of focusing on restoration of motor function in the setting of nerve injury, the majority of our understanding of the correlation of nerve histomorphometric axonal data with clinical outcomes of nerve transfers comes from the study of motor nerves.[9,13–15] Although significantly less attention has been paid to sensory nerve transfers and sensory outcomes, the predictive value of donor-to-recipient axon ratios is based on our current understanding of nerve physiology and presumed to also apply to sensory nerve transfers.[10]

The literature supports an ideal D:R axon ratio of 0.7:1 or greater, showing optimal clinical recovery. D:R axon ratios that are excessively high (>2.5:1) may increase the risk of neuroma formation at the coaptation site.[15] The availability of histomorphometric data of the nerves used in radial forearm phalloplasty will allow surgeon selection of nerve coaptations with favorable D:R axon ratios while minimizing the risk of neuroma-in-continuity. Notably, the donor-to-recipient axon ratios in this study demonstrate large variability, with the lowest ratio being 0.331 (IL:LABC) and the highest 3.002 (DNC:PABC). This variability may partially explain some of the range of sensory outcomes reported in the phalloplasty literature.

Although most of the D:R axon ratios in this study are favorable, there are some nerve coaptations with excessively low or high ratios. The power of the DNC as a donor is worth drawing attention to. The DNC on average has more than 2.2 times the number of axons as the IL nerve. Consideration should be given to using the DNC as a primary donor in gender-affirming phalloplasty cases if optimal sensation is desired. The D:R axon ratio of IL:LABC was consistently much lower than optimal, indicating there may be a higher risk of an inadequate number of axons reaching target. For this reason, the IL may not be the best choice to re-innervate the LABC alone. However, D:R axon ratios that are excessively high may also be an issue. D:R axon ratios for the DNC:MABC (mean 2.754) and DNC:PABC (mean 3.002) frequently had an excess number of donor axons available for a more limited number of recipient pathways with D:R more than 2.5:1, potentially increasing

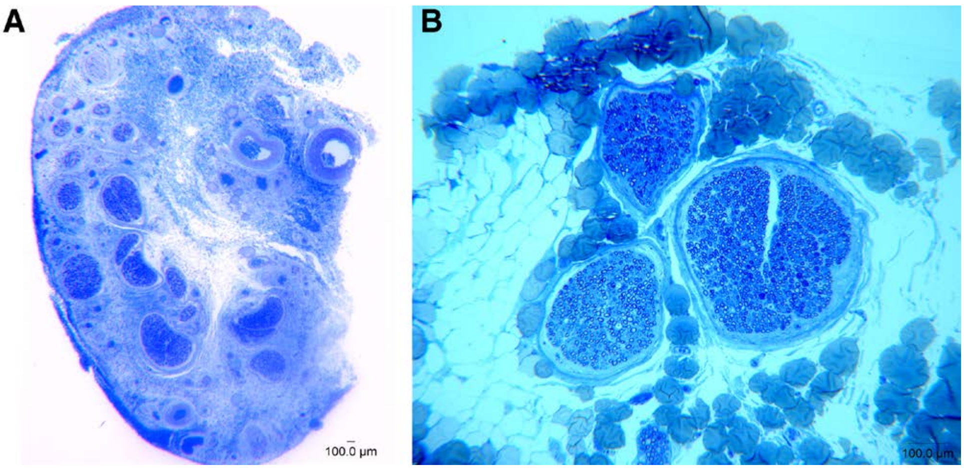

Fig. 2. Representative cross-sections of the donor nerves [dorsal nerve of the clitoris (A) and ilioinguinal nerve (B)] used for histomorphometric analysis to obtain axon counts.

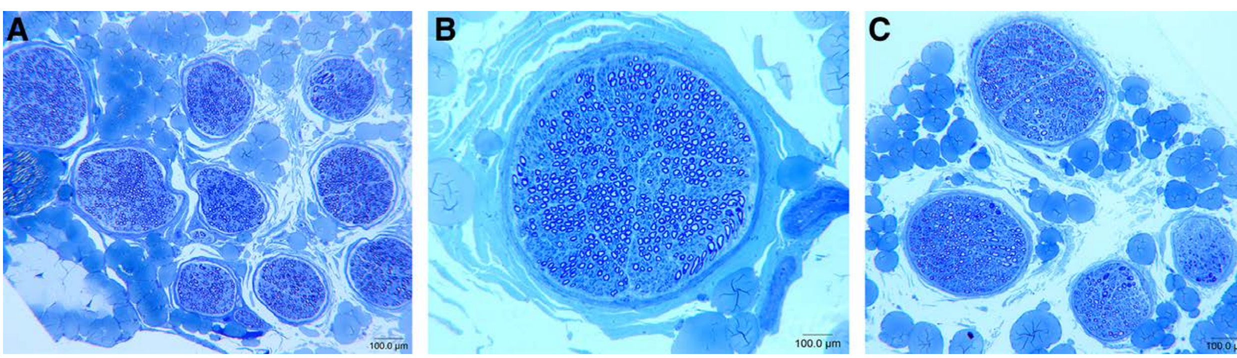

Fig. 3. Representative cross-sections of the recipient nerves [lateral antebrachial cutaneous (A); anterior medial antebrachial cutaneous (B); center and posterior antebrachial cutaneous nerve (C)] used for histomorphometric analysis to obtain axon counts.

Table 1. Axon Counts of the Donor and Recipient Nerves in Gender-affirming Radial Forearm Phalloplasty

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Mean
DNC	5543	5189	5023	4926	5021	5140 ± 218
IL	2470	1558	NR	NR	2875	2301 ± 551
LABC	5401	6494	8238	7696	NR	6957 ± 1098
MABC	2120	1807	850	1887	2667	1866 ± 590
Posterior	1569	1834	1851	1730	1577	1712 ± 121

The DNC is the most powerful donor nerve with 2.2× the number of axons available compared with the IL nerve. The LABC has the highest recipient axon counts with 3.5–4× the number of recipient pathways available for nerve regeneration compared with the MABC or PABC.

Table 2. D:R Axon Ratios in Gender-affirming Radial Forearm Phalloplasty

	LABC	MABC	PABC
DNC	0.739	2.754	3.002
IL	0.331	1.233	1.344

Ideal D:R include DNC:LABC (0.739) and IL:MABC (1.233) and IL:PABC (1.344). DNC:MABC and DNC:PABC may be excessive with ratios or 2.754 and 3.002 respectively, increasing risk of neuroma-in-continuity at the coaptation site.

risk of formation of a neuroma-in-continuity at the coaptation site. Additionally, denervating the territory of an entire DNC (hemi-clitoris) may be unnecessary in some cases in order to have an adequate number of donor axons available to innervate the territory of the PABC or the MABC. Therefore, it may be possible to use a single DNC to re-innervate the PABC and MABC together (with a mean summative D:R axon ratio of 1.44) or using a portion of a DNC (50%–60%) to innervate either the PABC or MABC alone. One additional consideration regarding the clinical application of the axon counts obtained in this study is the impact of nerve branching. The donor nerves in this study were both isolated at a known anatomic site (the external inguinal ring for the IL and the base of the clitoral body at the pubic symphysis for the DNC). They were then followed distally for a short distance and transected just prior to branching. This is the practice at our institution in order to ensure we are capturing the maximal number of axons from each donor nerve used in surgery. Some centers may prefer to coapt the recipient nerves of the flap to a more distal branch of the DNC or the IL nerve. In these scenarios, the axon counts of a branch will certainly be lower than the axon counts of the proximal nerves prior to any branching. This should be taken into consideration when determining location of nerve coaptation into the DNC or IL nerves.

Findings in this study support our current clinical practice coapting the DNC:LABC (D:R 0.739) and IL:PABC (D:R 1.344). In addition to favorable D:R axon ratios, we find these nerve coaptations are a good clinical size match for microsurgical coaptations and preferentially innervate the tissue of the phallic shaft. It is the LABC and PABC that directly innervate the penile shaft, whereas the anterior branch of the MABC innervates the phallic urethra. It is our preference to prioritize shaft over urethral innervation.[7] There remains a lack of consensus on optimal nerve coaptations in radial forearm phalloplasty with a wide range of surgeon practices. Beyond the coaptations we described above, alternative innervation strategies have included a single nerve coaptation in lieu of multiple, DNC:MABC, IL:LABC and end-to-side coaptations instead of end-to-end. Other groups report using a single DNC to transfer into multiple antebrachial cutaneous nerves. Although this clinical strategy is certainly viable, this will lower the D:R axon ratio to below a theoretical ideal of 0.7:1. This does not necessarily mean inferior clinical outcomes, as it is known that only 25%–30% of the normal number of innervating fibers are needed to maintain function. However, decreasing the number of donor axons relative to recipient pathways will decrease the "buffer" of excess axons available, potentially decreasing the frequency which an optimal number of axons innervate target. Ultimately, there are multiple successful strategies that have been reported to provide for phallic sensation, and the data here should be used to further refine individual surgeon and patient decision-making.

It must be noted that variability exists in anatomy of the donor and recipient nerves used in radial forearm phalloplasty. In our experience the greatest variability in nerve anatomy exists in the ilioinguinal nerve and the antebrachial cutaneous nerves of the forearm. As far as nerve donors, the DNC anatomy and size is very consistent, as are the mean axon counts. The ilioinguinal nerve clinically varies in size with accompanying variability seen in its axon counts. Regarding the recipient nerves, the LABC demonstrates consistent axon counts but varies in regards to its arborization. Although the LABC most commonly divides into an anterior and posterior branch, it occasionally branches very proximal with extensive arborization which can make dissection and inclusion of all branches and their ultimate coaptation more difficult. The PABC demonstrates consistent axon counts, anatomic location and size but can demonstrate variability in regards to its branching as well. Most commonly there is a single PABC present, but there may be a second branch present in the proximal forearm in the setting of a high branch point of the PABC proper.[7] However, the greatest variability of the antebrachial cutaneous nerves of the forearm exists in the MABC. The MABC proper branches into anterior and posterior branches above the elbow. There can be significant variation in the relative size of anterior versus posterior branches. The variability seen in axon counts of the IL and the MABC in this study correlates with our experience of the clinical variation in nerve anatomy seen with the IL and MABC. Intraoperative decision-making regarding nerve coaptations should be based on more than just the axon counts and D:R axon ratios described here. Other factors should include clinical size match, anatomy, and territory of flap innervation in addition to patient goals. The histomorphometric data provided in this study are just one additional data point to inform nerve selection.

There are several limitations to this study that warrant discussion, including a small sample size. All individuals from whom samples were collected are transmasculine people on testosterone therapy. This should not affect axon counts, but there were no control samples from individuals in the absence of testosterone therapy. Most importantly, there are limitations to the nerve count techniques used in this study. With current methodology, only myelinated axons are counted when performing typical axon count studies.[17] Myelinated axons are the largest sensory fibers and are involved in proprioception and mechanical sensation. They are arguably the most important sensory fibers. Typical axon count studies are performed only on myelinated fibers as current techniques are limited in their ability to count free nerve endings or unmyelinated fibers without significant time, difficulty, and expense, mostly limited to electron microscopy.[16] Therefore, the contributions of unmyelinated fibers are not fully appreciated in this work. The small sample size also means the study is under-powered to control for variability in D:R axon counts. However, there are several key points that can be gleamed from this data. The DNC is the more powerful donor nerve available in gender-affirming phalloplasty procedures, and the IL nerve alone is likely underpowered to reliably innervate the LABC. Other nerve coaptations, based on D:R axon ratios seem favorable for nerve regeneration, suggesting that variability in sensory outcomes reported in the literature may be due to more than just variation in nerve coaptations performed. Sensation after phalloplasty is likely dependent on many factors such as nerve regeneration, sensory rehabilitation, and central processes such as cortical mapping and plasticity.[18]

Despite these limitations, this is the first report of the axon counts and the donor-to-recipient axon ratios used in gender-affirming radial forearm phalloplasty. Future directions should include repeating this work when reliable methods are available that can also quantify and capture unmyelinated axon counts. This study does not report long-term sensory outcomes. There exists a need for large, long-term outcome studies on phalloplasty sensation, in addition to a standardized method of measuring sensory outcomes such as erogenous sensation. The data reported here cannot in isolation determine which nerve coaptations are best. However, this study does provide objective data to better inform surgical decision-making, that has not yet been previously available. Lastly, the axon counts reported in this article have clinical relevance to potential future directions in transmasculine genital gender-affirming surgery. There is ongoing discussion around feasibility of penile transplantation in the transmasculine population. The axon count of the DNC is an important data point to inform the prognosis of sensory recovery after nerve transfer of the DNC to the dorsal nerve of the penis in the setting of penile transplantation. These data may ultimately aid in the future exploration of this field.[19,20]

CONCLUSIONS

In radial forearm phalloplasty, the DNC is the most powerful donor nerve available with approximately 2.2 times the axon count of the IL. The IL nerve is likely under-powered to re-innervate the LABC based on a D:R consistently less than or equal to 0.7:1 or less. All other D:R axon ratios of available nerve transfers in radial forearm phalloplasty are greater than 0.7:1. Based on nerve histomorphometry and D:R axon ratios, favorable nerve coaptations may include DNC:LABC and IL:PABC or IL:MABC. In some cases, the DNC axon count may be excessive for re-innervation of the MABC or PABC alone with D:R in excess of more than 2.5:1, risking neuroma formation at the coaptation site.

Notably, the donor-to-recipient axon ratios reported in this study do not replace the need for long-term sensory outcomes. However, they should be used in combination with other pertinent considerations such as territory of flap innervation and clinical size match in order to better inform selection of nerve coaptations in phalloplasty cases beyond surgeon opinion alone.

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DISCLOSURE

The authors have no financial interest to declare in relation to the content of this article.

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