JPRAS Open 44 (2025) 162–170

Contents lists available at ScienceDirect

JPRAS Open

journal homepage: www.elsevier.com/locate/jpra

Review Article

# Advancements in biomarker techniques for precision and safety in facial nerve identification during head and neck surgeries: a comprehensive review

Cesar Raizer[a], Milanie Milan[b], Reina Ibrahim[b], Joseph El Bachour[c], Abdelrazzak Kerhani[b], Gregory Nicolas[d,*]

[a] *Department of Plastic & Reconstructive Surgery, Hospital Das Clinicas of the Faculty of Medicine, Sao Paulo, Brazil*
[b] *Faculty of Medicine, University of Balamand, Beirut, Lebanon*
[c] *Faculty of Medicine, American University of Beirut*
[d] *Department of Plastic & Reconstructive Surgery, Hospital Das Clinicas of the Faculty of Medicine of the University of Sao Paulo, Brazil*

ARTICLE INFO

*Article history:*
Received 8 May 2024
Accepted 28 July 2024
Available online 9 August 2024

*Keywords:*
Facial nerve identification
Fluorescent dye
Nerve injuries
Facial surgery

ABSTRACT

*Objective:* This review aimed to evaluate biomarkers' efficacy in enhancing facial nerve identification during head and neck surgical procedures. It summarized the literature on biomarker techniques for intraoperative visualization of the facial nerve, focusing on applications, findings, and clinical implications.
*Introduction:* Precision is paramount in head and neck surgeries due to complex anatomical structures. In particular, facial procedures demand meticulous planning to preserve facial nerve function. Iatrogenic injuries underscore the need for advancements in nerve identification techniques. This review provided insights into biomarkers' potential role in enhancing facial nerve identification.
*Methods:* A PubMed search using specific keywords yielded 45 articles, with 40 meeting the initial inclusion criteria. Selection was based on relevance to facial surgery and publication in English.

* Corresponding author.
*E-mail address:* Gregory.nicolas@ijcrcentral.com (G. Nicolas).

https://doi.org/10.1016/j.jpra.2024.07.015

After screening and conflict resolution, 12 articles underwent full-text review.

*Results:* Various biomarkers, such as fluorescent probes and retrograde tracers, have shown efficacy in improving facial nerve identification across different surgical scenarios. These techniques enhance precision in identifying nerve branches and aid in tumor resections.

*Conclusion:* Enhanced facial nerve identification prevents nerve injuries during surgery. Although fluorescent dye-based approaches show promise, further research is needed to establish safety and long-term efficacy. Adoption of these techniques could improve patient outcomes and reduce complications.



## Introduction

Surgical interventions of the head and neck demand a precision level that surpasses many other medical procedures. This region harbors delicate and crucial structures, making surgeries in this area particularly challenging. The safeguarding of the facial nerve in these procedures, such as mastoidectomies, is of paramount importance due to the potential ramifications of iatrogenic injuries, the reported incidence of which ranges between 0.6 % and 3.6 %. The repercussions of such injuries on the patient's quality of life underscore the urgency to enhance surgical techniques.

This study focused on refining the identification and protection of the facial nerve. The importance of the facial nerve, coupled with the high stakes of potential injuries, necessitates a comprehensive exploration of innovative techniques. In this context, biomarkers emerge as promising tools to elevate surgical precision, reduce complication rates, and ultimately ensure the safety and well-being of patients undergoing these procedures. Therefore, this review examined the biomarker types explored in the literature, their applications, and their potential to revolutionize the landscape of head and neck surgeries. With these cutting-edge techniques, it is essential to acknowledge their challenges and limitations to expand their applications and enhance their clinical translation.

## Methodology

A literature search was conducted on PubMed using specific keywords: (Fluorescent) AND (dye) AND (facial nerve) AND (surgery). No time limitation was specified for the search. Results were collected and managed using Rayyan, identifying and removing duplicate articles. The inclusion criteria consisted of articles written in English that specifically investigated facial nerve identification in the context of facial surgery. Exclusion criteria included articles in languages other than English, inaccessible versions, and those that did not focus on facial nerve injuries, facial nerve injury prevention, or the identification of facial nerve in facial surgery procedures.

Two independent reviewers (MM and JB) performed the initial screening of studies based on titles and abstracts to determine whether they met the inclusion criteria. Any discrepancies between the 2 reviewers were resolved by a third reviewer (RI) to avoid selection bias. The articles that passed the initial screening were subjected to full-text review. Initially, 45 articles were found and stored on Rayyan, with 5 duplicate articles detected. After manual search and screening, 12 articles were included, and 28 were excluded from the review. The selected articles were then tabulated and summarized using relevant keywords for subsections. After reading the full texts and tabulating the articles, a final total of 12 articles were included in the review (Figure 1). Each author was assigned a specific subsection for summarizing the findings, and the writing process was initiated accordingly.

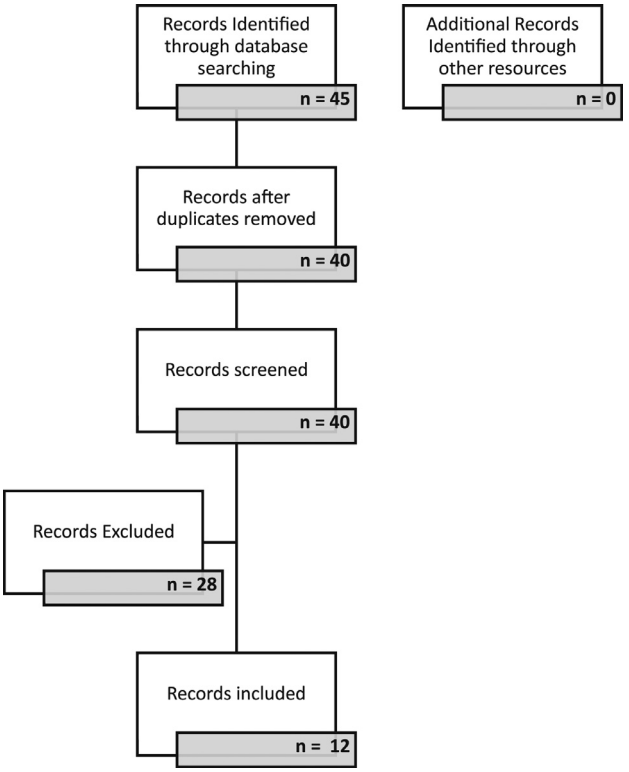


**Figure 1.** Methodology's Flow Diagram.

## Discussion

*Old techniques to prevent nerve injuries*

Preventing nerve injury has always been a top priority for surgeons performing facial plastic surgery. In the past, electromyographic (EMG) tracing was a crucial method for locating distant nerve segments after injury. However, its usefulness was limited to a window of 72 hours. After this period, the nerve endoneurium degenerates by Wallerian mechanisms, making it increasingly difficult to identify or trace nerves using EMG.

Fortunately, the perineurium and epineurium, the outer layers of nerves, do not experience the same level of degeneration. This allows for more consistent binding of fluorescent tracers even months after nerve transection.[1] However, reliance on white light visual recognition becomes necessary after this critical period. This presents significant challenges, as EMG tracing not only increases the risk of inadvertently damaging nearby healthy tissues, including neighboring nerves, but also prolongs the surgical operation, incurring additional risks and expenses.[1,2] Furthermore, innovative nerve tracers and EMG monitoring approaches depend on intact axonal pathways for successful nerve detection or labeling, making them less effective when degeneration processes begin as early as 24 hours after transection.[1]

Recognizing the limitations of existing approaches, recent developments in nerve visualization during facial plastic surgery have emerged. One major advancement is using indocyanine green (ICG), a near-infrared fluorescent tricarbocyanine dye traditionally used to test cardiologic and hepatic functioning. ICG, which was licensed by the Food and Drug Administration in 1956, has been found useful in neurovascular interventions, particularly in investigating venous drainage in brain tumors. ICG flu-

orescence imaging provides direct visualization of the vasa nervorum in the context of facial nerve trajectory, offering essential insights into regional blood flow patterns during surgery.[3] This novel approach not only overcomes the limitations of traditional methods, such as nerve stimulators (which only provided indirect identification of facial nerves and could be uncomfortable for patients), but also shows promise in improving surgical outcomes and reducing the risk of facial nerve injury during facelift procedures.[3] However, further research, possibly involving larger animal models and multiple surgeons, is needed to evaluate and refine these promising results in clinical situations.

*Biomarkers reported in the literature*

In animal experiments, neuronal tract pathway identification often involves using transgenic specimens. This model utilizes Green Fluorescent Proteins splicing upstream of peripheral neuron genes to produce *in vivo* fluorescence.[4] However, this technique is not ethically feasible in humans, necessitating alternative methods to induce peripheral neuronal fluorescence.

Various labels have been tested in animal models, but only a few have been implemented in clinical settings. The mechanisms of delivery vary depending on the molecules used to label. These methods include (1) intramuscular/transdermal injection of retrograde tracers in areas innervated by the facial nerve,[5,6] (2) intravenous injection of nerve-binding probes,[1,2,7] and (3) intravenous injection of tracers that illuminate the vascular system, specifically the vasa nervorum.[3,8,9] Other molecules with different administration modalities have also been reported in the literature, such as direct contact staining through the Nile red derivative NR5 with oxazine 4 (OX4).[10]

One evaluated label in the intramuscularly injected retrograde tracer group was the Alexa Fluor 488 cholera toxin B conjugate.[5] This label was tested in an animal study on white rabbits and specifically bound to ganglioside $G_M1$,[11] allowing nerve labeling assessment. Another dye, Fast DiO dye (3,3′-dilinoleyloxacarbocyanine),[6] was evaluated in an animal study on Wistar rats to measure its efficiency in facilitating facial nerve identification intraoperatively.

For intravenous administration, ICG was used to highlight the vasculature surrounding nervous tissue in the facial canal and the vasa nervorum surrounding the length of the nerve. One cadaveric study[9] and 2 clinical studies[3,8] evaluated the effectiveness of ICG in visualizing the facial nerve and locating the facial canal underneath bony landmarks. These visualization techniques enabled surgeons to perform tumor resections and mastoidectomies while protecting the facial nerve and its branches. ICG has previously been used in cerebrovascular surgical procedures to visualize tiny vessels, their tributaries, and potential stenoses. A supplementary video ("Intraoperative fluorescence angiography using ICG") created by one of the authors demonstrates these techniques in an intraoperative setting.

Other intravenously administered nerve-binding probes were also evaluated, specifically FAM-NP41 and FAM-HNP401. FAM-NP41 was studied in 2 animal experiments with different objectives. In a study conducted by Hussain et al.,[1] the efficiency of this fluorescent probe in aiding the identification and protection of the facial nerve during parotid cancer resection in tumor-bearing mice was evaluated. Another study by Wu et al.[2] aimed to determine the efficiency of FAM-NP41 in identifying stumps of transected facial nerve fibers in mice for re-anastomosis and assess the viability of this repair with the help of the tracer.

Alternatively, Hingorani et al.[7] conducted a comparative study of FAM-HNP401 versus FAM-NP41 in mouse models and human peripheral nerve samples taken *ex vivo*. The hypothesis was that FAM-HNP401 would serve as a more efficient label than FAM-NP41 in human tissues due to its longer washout periods and 10-fold higher signals. Results showed that FAM-HNP401 efficiently binds and distinguishes human sural, medial antebrachial cutaneous, laryngeal, and autonomic nerves from surrounding muscle tissue.

Finally, Korber et al.[10] evaluated the direct costaining of nervous tissue *in vitro* and *in vivo* with fluorophores derived from the standard Nile red. Multiple derivatives of Nile red were assessed for adipose selectivity, and the NR5 derivative, in conjunction with OX4, was evaluated as a nerve stain. Comparative analysis of NR5 + OX4 and Nile red + OX4 staining was carried out, demonstrating a higher reliability of NR5 + OX4 in discriminating between adipose-nerve and muscle-nerve *in vivo*.

Table 1 provides an overview of these tracers and the respective studies evaluating their efficiency and suitability for intraoperative use in human clinical settings.

**Table 1**
Studies supporting the use of each biomarker and its application.

| Tracer | Specimen evaluated/tracer target | Studies utilizing tracer | Application |
|---|---|---|---|
| Alexa Fluor 488-cholera toxin B subunit bioconjugate | White rabbits/ganglioside $GM_1$11 | Animal study: O'Malley et al., 2006 | Cholera toxin subunit B is a nontoxic binder of gangliosides present on the neuronal tissue surface. Bioconjugate was intramuscularly injected into the whisker pads of New Zealand white rabbits. |
| FastDiO dye (3,3'-dilinoleyloxacarbocyanine) | Wistar rats/— | Animal study: De Melo et al., 2016 | FastDiO was transdermally injected into the right face of adult Wistar rats, after which time facial nerve identification was recorded as a measure of tracer assistance in nerve identification. |
| Indocyanine green | Human cadaver/vasa nervorum illumination | Cadaveric study: Gragnaniello et al., 2010 | ICG was intraarterially injected into the major vessels of the cadavers, highlighting bony layers (these lack fluorescence) that form and protect the facial canal and semicircular canals and guiding mastoidectomy without nerve damage. |
| | Human patient/vasa nervorum illumination | Clinical study: Chen et al., 2015 | ICG was intravenously injected intraoperatively, allowing visualization of the facial nerve and facial canal during mastoidectomy, thus ensuring its protection. |
| | Human patient/vasa nervorum illumination | Clinical study: Kwon et al., 2019 | ICG was intravenously injected intraoperatively, allowing visualization of the facial nerve's vasa nervorum, appearing as a glowing network surrounding a dark tube (nerve tissue). |
| FAM-NP41 | Parotid cancer in mice/laminin 421/211 | Animal study: Hussain et al., 2016 | NP41 was injected intravenously and allowed to wash out the system before surgical resection of the tumor tissue. The binding of laminin targets preferentially highlights nervous fibers from the surrounding muscle. |
| | Transected facial nerve in mice/laminin 421/211 | Animal study: Wu et al., 2011 | NP41 was injected intravenously and allowed to wash out the system before surgical re-anastomosis of the transected nerve. The binding of laminin targets preferentially highlights nervous fibers from the surrounding muscle. |
| FAM-HNP401 | Mouse models and excised human peripheral nerves/— | Animal study: Hingorani et al., 2018 | FAM-HNP401 was studied compared to FAM-NP41 on *in vivo* animal samples through intravenous injection and *ex vivo* human nerve samples from various peripheral nerve sites. Enhanced fluorescence duration and contrast were observed in human tissue staining. |
| Nile red derivative NR5 + OX4 | Cultured cells and rodent nerve models/adipose tissue | Animal study: Korber et al., 2018 | This combination of fluorophores was administered by incubating the fluorescent label solution directly on the nerve sites. NR5 + OX4 was compared to Nile Red + OX4 staining, showing higher reliable adipose/nerve and muscle nerve discrimination *in vivo*. |
| 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI); 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO); 3,3′-dilinoleyloxacarbocyanine perchlorate (Fast DiO) | Female Sprague-Dawley rats/lipoprotein membrane | Animal study: Dogru et al., 2008 | These lipophilic stains were administered by microinjection into the whisker pad or through crystal deposition on the nerve sheaths. A comparison of the three dyes was the aim, with FastDiO being the most consistent label in results. |

## Studies that evaluated the use of biomarkers in facial surgeries: biomarker variations in facial nerve identification

Several studies have provided useful insights into the significance of biomarkers in improving facial nerve identification during surgery. Various biomarker applications, such as fluorescently labeled peptides,[1] retrograde tracers,[11] and carbocyanine dyes,[12] allow for a thorough investigation.

Hussain et al. demonstrated the effectiveness of a fluorescently labeled nerve-binding probe, FAM-NP41, in enhancing intraoperative nerve visualization during surgery. This probe can identify recently transected and intact facial nerves up to 9 months after transection.[1] For patients with facial nerve injuries, better visibility of deteriorated nerve branches may help surgeons locate and treat damaged nerves more precisely, potentially improving functional outcomes. Biomarkers such as FAM-NP41 can decrease nerve identification time, limit tissue stress, and improve surgical precision.

Building on the insights from fluorescently labeled probes, Popratiloff et al.[11] conducted a study where they labeled and tracked facial motoneurons in animals using different tracers. The study aimed to assess the accuracy of muscle reinnervation after nerve transection. Researchers compared the efficacy of 3 different tracers (Fast-Blue, Dil, and Fluoro-Gold) when injected into the rat whisker pad muscles before and after surgery. All 3 tracers successfully tagged motoneurons in the lateral facial subnucleus, indicating successful preoperative labeling. However, the study found that postoperative Dil labeling resulted in nonneuronal tagging of blood vessels and pericytes, making it difficult to determine the number of labeled neurons. Consequently, researchers concluded that preoperative Fluoro-Gold labeling and postoperative Fast-Blue labeling are the most effective tracers for evaluating post-transectional muscle reinnervation. This approach reduces counting errors caused by interindividual variability and allows for precisely measuring motoneurons' locations before and after surgery within the same animal.[11]

Expanding our understanding of biomarker applications, Dogru et al. further investigated the use of carbocyanine dyes to identify the facial nerve. They emphasized their potential as visual aids for intraoperative identification, especially in cases involving tumors.[12] In this study, 3 different carbocyanine dyes—1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO), and Fast DiO—were specifically used for the retrograde labeling of the rat facial nerve. After microinjecting the distal nerve fibers or into the face muscles, labeled nerves were observed using a dissecting stereomicroscope mounted with epifluorescence filters. Results indicated that Fast DiO is the most effective dye for easily identifying nerve fibers even under intense field illumination. This study suggested that fluorescent dyes can improve intraoperative facial nerve identification and dissection, particularly when the nerve is displaced or altered by a tumor.[12]

These studies collectively demonstrated that biomarkers, in various forms, are helpful tools for surgeons. They enhanced the precision of facial nerve identification in various surgical circumstances, particularly in head and neck procedures. Biomarkers have applications in deteriorated nerve branches, post-transectional muscle reinnervation in microsurgical nerve repair, and intraoperative identification of nerves in tumor cases. These diverse applications align with the ultimate goal of optimizing surgical precision and ensuring patient safety.

## Effectiveness of biomarker techniques in preventing nerve injury

Improved nerve identification techniques in facial surgeries are crucial in minimizing nerve injuries, ultimately enhancing patient outcomes and offering potential cost-saving benefits. The use of fluorescently labeled probes,[1] retrograde tracers,[11] and carbocyanine dyes[12] shows promise by providing surgeons with improved visualization tools and enabling the recognition of molecular and biochemical signs indicative of potential nerve injury.

Nerve damage during facial surgery can seriously affect patients' sensory and motor skills. Biomarkers such as cholera toxin B, ICG, and nerve peptide NP41 have enabled surgeons to identify and preserve nerves precisely, lowering the chance of iatrogenic injury. ICG and the nerve-binding peptide NP41 improve nerve identification. ICG is frequently used in vascular surgery and may be adapted for nerve identification due to its ability to fluoresce under suitable lighting conditions. In 1 study, 8 cadaveric mastoidectomies were performed with no injury to the facial canal and semicircular

canal innervated by the facial nerve.[9] Moreover, nerve-binding peptide NP41 improves intraoperative facial nerve identification during parotid gland cancer surgery. NP41 dramatically increases the detection of small and buried nerve branches, which are barely detectable under white light, with excellent sensitivity and specificity, potentially assisting their intraoperative identification and preservation.[1,2]

These biomarkers improve accuracy in detecting facial nerves, significantly reducing surgical adverse effects and a better postsurgery functional recovery. The ability to reliably detect deteriorated nerves[1] and follow facial nerves during surgery[11] helps reduce invasiveness, especially in anatomically dense locations such as the head and neck. The use of markers such as ICG improves the detection of degenerated facial nerves, particularly in patients with distorted facial anatomy due to tumors, growths, or radiotherapy, resulting in a more accurate surgical approach.[3] When injected intramuscularly, cholera toxin B subunit fluorescent conjugates precisely identify the distal section of the facial nerve.[5] Patients undergoing surgeries with precise nerve identification also experience improved functional recovery. One study on murine parotid cancer showed that added intraoperative fluorescence guidance enhanced postoperative facial nerve function compared to surgery performed under white light alone.[1] Patients also regain sensory and motor functions more rapidly, leading to an improved quality of life and overall satisfaction with the surgical outcome.

Furthermore, using these biomarkers reduces overall surgical time, promoting efficiency and simplifying procedures. Surgeons with improved nerve identification techniques can work with greater confidence. Reduced surgical durations reduce patients' exposure to anesthetics and surgical stress and can potentially decrease the burden of healthcare costs.[3] Using biomarkers as visual assistance[12] also minimizes reliance on traditional methods, such as electrical stimulation testing, providing a more precise and dependable alternative.

Ultimately, advances in biomarker-enhanced identification contribute to improving surgical outcomes, minimizing nerve damage, and conserving facial nerve function.

## Limitations and future research

As discussed in this review, the techniques for *in vivo* fluorescence-guided peripheral nerve surgery bring substantial promise to clinical practice, particularly in otolaryngology-head and neck surgery. These techniques can enhance surgical practice by improving tissue visualization and have been utilized in various clinical procedures, including sentinel node dissection, resection of malignant gliomas, coronary artery bypass grafting, endoscopic detection of gastric cancer, and microvascular free flap reconstruction.[1] However, it is essential to address limitations and areas for future research.

Methodological variations are evident across studies, contributing to complex results. Hussain et al. utilized a fluorescently labeled nerve-binding probe, revealing its ability to mark perineurium/epineurium even after nerve transection. However, specific changes in labeling processes and analysis methodologies were not explicitly outlined. In a study by Popratiloff et al.,[11] the comparison of various tracers demonstrated similar efficiency. Nevertheless, challenges arose with non-neuronal labeling of blood arteries and pericytes, underscoring the importance of considering tracer characteristics. Dogru et al.[12] investigated crystal implantation with microinjection methods, concluding that microinjection yielded more consistent labeling. Despite this, the potential for muscle toxicity warrants further investigation. These discrepancies underscore the necessity of meticulously considering techniques when designing studies and interpreting results.

The success of biomarker techniques in preventing nerve injury in facial surgeries is promising, with markers such as ICG, nerve peptide NP41, and cholera toxin B subunit fluorescent conjugates improving accuracy in detecting and preserving facial nerves.[1–3,5] These markers have shown improved detection of degenerated facial nerves and enhanced postoperative facial nerve function, leading to better postsurgery functional recovery.[1,3] Despite these advancements, there are challenges such as inflexibility in filter selection, concerns about systemic effects with systemic administration, and variability in specificity and sensitivity of fluorescent dyes in identifying nerves from surrounding tissues.[2,3,7,10]

In summary, while *in vivo* fluorescence techniques in peripheral nerve surgery offer numerous benefits, including improved visualization and reduced surgical complications, they are accompanied by specific challenges and limitations. Future research should focus on optimizing these techniques, ad-

dressing issues with biomarker specificity and sensitivity, and expanding their utility in various clinical scenarios.

## Ethical and safety considerations

The application of nerve visualization techniques presents a significant advancement in peripheral nerve surgery, offering benefits that encompass safety and ethical considerations. This technique is crucial in preventing surgical injuries, minimizing complications, and improving patient safety. However, this dissection carries a risk of injuring the facial nerve, with reported incidences of injury ranging from <1 % to as high as 20 %. Although buccal branch injuries are relatively common, they are typically short-lived and less clinically significant than injuries to other facial nerve branches due to their significant arborization.[3] ICG injection for nerve visualization offers the advantage of minimal adverse reactions and complications, further enhancing patient safety. In addition to minimal adverse reactions and complications, ICG for nerve visualization prioritizes patient safety. Before administration, the patient's allergy history was carefully assessed, with 1 participant excluded due to a previous allergy to ICG. The injection process involved intravenous infusion of ICG mixed with normal saline, ensuring rapid distribution within nerve vessels. Strict adherence to dosage and administration protocols was emphasized to prevent side effects. Live monitoring through a near-infrared camera also allowed real-time observation of ICG distribution, aiding in tracking facial nerve trajectory and distinguishing it from surrounding structures. Vigilant monitoring was crucial to promptly address minimal adverse effects, such as the rare occurrence of anaphylactic shock.

Acknowledging the broader context of facial nerve visualization techniques, the study emphasized awareness of the limitations and considerations, including anatomical variations impacting ICG distribution. The surgical team's expertise was highlighted, emphasizing the necessity of training in near-infrared camera usage and real-time information interpretation.

In summary, the study's safety measures, including allergy checks, precise administration protocols, live monitoring, and surgical team expertise, collectively contribute to the safety and efficacy of ICG injection for nerve visualization in surgical procedures.

## Limitations

This study presented a range of articles found in 1 database (PubMed) regarding biomarker usage and facial nerve identification in the context of facial surgery. To minimize the selection bias associated with narrative reviews, Rayyan was used to add all articles from PubMed based on the keywords selected by the authors. As mentioned in the methodology, 2 reviewers screened these articles based on inclusion and exclusion criteria. To make the selection more objective, a third reviewer was responsible for a third screening to resolve all conflicts.

## Conclusion

In conclusion, this review highlighted the importance of biomarkers in improving facial nerve identification during head and neck surgeries. The available literature sheds light on various biomarkers, including fluorescently labeled peptides, retrograde tracers, and carbocyanine dyes, showcasing their efficacy in enhancing visualization and precision during surgical procedures. The findings emphasized the potential of these biomarkers in reducing iatrogenic injuries, shortening surgical durations, and improving postoperative functional recovery. Despite notable advancements, challenges such as variations in methodology, systemic administration concerns, and limitations in adaptability still exist. The limitations underscore the need for continued research and innovation to refine and expand the application of these techniques in clinical settings, ensuring further advancements in patient care and surgical outcomes.

**Funding:** None

**Conflicts of interest:** None declared

**Ethical approval:** Not required

## Supplementary material

Supplementary material associated with this article can be found, in the online version, at 10.1016/j.jpra.2024.07.015.

## References

1. Hussain T, Mastrodimos MB, Raju SC, et al. Fluorescently labeled peptide increases identification of degenerated facial nerve branches during surgery and improves functional outcome. *PLOS ONE*. 2015;10(3):e0119600. doi:10.1371/journal.pone.0119600.
2. Wu AP, Whitney MA, Crisp JL, Friedman B, Tsien RY, Nguyen QT. Improved facial nerve identification with novel fluorescently labeled probe. *Laryngoscope*. 2011;121(4):805–810. doi:10.1002/lary.21411.
3. Kwon JG, Choi YJ, Kim SC, Hong JP, Jeong WS, Oh TS. A technique for safe deep facial tissue dissection: indocyanine green-assisted intraoperative real-time visualization of the vasa nervorum of facial nerve with a near-infrared camera. *J Craniomaxillofac Surg*. 2019;47(11):1819–1826. doi:10.1016/j.jcms.2019.07.011.
4. Green JD, Shelton C, Brackmann DE. Iatrogenic facial nerve injury during otologic surgery. *Laryngoscope*. 1994;104(8 Pt 1):922–926. doi:10.1288/00005537-199408000-00002.
5. O'Malley MR, Wittkopf JE, Cutler JL, Labadie RF, Hackett TA, Haynes DS. Fluorescent retrograde axonal tracing of the facial nerve. *Laryngoscope*. 2006;116(10):1792–1797. doi:10.1097/01.mlg.0000231344.92917.9c.
6. de Melo GM, Cervantes O, Covolan L, Baptista HA, Ferreira ES, Abrahao M. Facial nerve identification with fluorescent dye in rats. *Acta Cir Bras*. 2016;31(2):92–102. doi:10.1590/S0102-865020160020000003.
7. Hingorani DV, Whitney MA, Friedman B, et al. Nerve-targeted probes for fluorescence-guided intraoperative imaging. *Theranostics*. 2018;8(15):4226–4237. doi:10.7150/thno.23084.
8. Chen SC, Wang MC, Wang WH, et al. Fluorescence-assisted visualization of facial nerve during mastoidectomy: a novel technique for preventing iatrogenic facial paralysis. *Auris Nasus Larynx*. 2015;42(2):113–118. doi:10.1016/j.anl.2014.08.008.
9. Gragnaniello C, Kamel M, Al-Mefty O. Utilization of fluorescein for identification and preservation of the facial nerve and semicircular canals for safe mastoidectomy: a proof of concept laboratory cadaveric study. *Neurosurgery*. 2010;66(1):204–207. doi:10.1227/01.NEU.0000360567.15127.C5.
10. Korber J, Barth C, Gibbs S. Nile red derivatives enable improved ratiometric imaging for nerve-specific contrast. *J Biomed Opt*. 2018;23(7):1–13. doi:10.1117/1.JBO.23.7.076002.
11. Popratiloff AS, Neiss WF, Skouras E, Streppel M, Guntinas-Lichius O, Angelov DN. Evaluation of muscle re-innervation employing pre- and post-axotomy injections of fluorescent retrograde tracers. *Brain Res Bull*. 2001;54(1):115–123. doi:10.1016/s0361-9230(00)00411-1.
12. Dogru S, Van Daele D, Hansen MR. Retrograde labeling of the rat facial nerve with carbocyanine dyes to enhance intraoperative identification. *Ann Otol Rhinol Laryngol*. 2008;117(10):753–758. doi:10.1177/000348940811701009.