

[DOI]10.12016/j.issn.2096-1456.2023.06.004

· 临床研究 ·

吲哚菁绿近红外荧光成像技术在颌骨放射性骨坏死术中坏死骨质界定中的应用

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【摘要】 目的 探讨吲哚菁绿近红外荧光成像技术在颌骨放射性骨坏死术中界定颌骨切除范围的可行性, 为临床医生提供参考。方法 下颌骨放射性骨坏死患者8例, 术中截骨前10 min经肘静脉注射吲哚菁绿, 术中以影像学结果为主要依据对坏死骨病灶行保守性切除后, 再以0.3 cm为初始距离逐步扩大低荧光强度区域的潜在死骨切除范围, 扩大切除范围前后行骨截面近红外荧光成像和荧光强度测定, 分析统计学差异。所有骨坏死患者术后均进行定期随访以评估疗效。结果 8例颌骨放射性骨坏死患者均获得清晰的吲哚菁绿近红外荧光图像, 扩大下颌骨切除范围(0.95 ± 0.14) cm的新鲜骨创面荧光强度为(226.2 ± 15.8) au, 高于基于术中肉眼观察和影像学结果的切除创面(108.8 ± 3.4) au ($t = 20.718, P < 0.001$)。8例患者术后随访好转率达87.5%。结论 吲哚菁绿近红外荧光成像技术可辅助加强清除病变颌骨直到颌骨创面新鲜出血, 对颌骨放射性骨坏死患者术中界定切除范围具有重要的临床价值。

【关键词】 放射性骨坏死; 坏死骨; 截骨术; 吲哚菁绿; 近红外荧光成像; 荧光强度; 新鲜骨创面

【中图分类号】 R78 **【文献标志码】** A **【文章编号】** 2096-1456(2023)06-0408-06

【引用著录格式】 康梓钦, 王岳鹏, 何奕霖, 等. 吲哚菁绿近红外荧光成像技术在颌骨放射性骨坏死术中坏死骨质界定中的应用[J]. 口腔疾病防治, 2023, 31(6): 408-413. doi:10.12016/j.issn.2096-1456.2023.06.004.

Application of indocyanine green in near-infrared fluorescence imaging to detect necrotic bone associated with osteoradionecrosis of the jaws KANG Ziqin, WANG Yuepeng, HE Yilin, CAI Yongkang, HUANG Zhiqian. Department of Oral and Maxillofacial Surgery, Sun Yat-sen Memorial Hospital, Sun Yatsen University, Guangzhou 510120, China

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【Abstract】 **Objective** To investigate the application of indocyanine green in near-infrared fluorescence imaging to determine the scope of necrotic bone resection in osteoradionecrosis of the jaw and to provide a reference for clinicians. **Methods** Eight patients with osteoradionecrosis of the jaws were enrolled. Indocyanine green was intravenously injected through the elbow vein 10 minutes before osteotomy. After conservative resection of necrotic bone lesions based on imaging results, the scope of potential dead bone resection in the area of low fluorescence intensity was gradually expanded at an initial distance of 0.3 cm. Near-infrared fluorescence imaging and fluorescence intensity determination of bone cross-section were performed before and after extended resection. Statistical differences were analyzed. All patients with osteonecrosis underwent regular follow-up to evaluate the postoperative efficacy. **Results** Indocyanine green was injected into all 8 patients with osteoradionecrosis for near-infrared fluorescence imaging and the scans were clear; the fluorescence intensity of fresh bone wounds with an expanded mandibular resection range of (0.95 ± 0.14) cm was (226.2 ± 15.8) au, which was higher than that based on intraoperative macroscopic observation and radiological results (108.8 ± 3.4) au, ($t = 20.718, P < 0.001$). The postoperative follow-up improvement rate of 8 patients was 87.5%.



微信公众号

【收稿日期】 2022-09-22; **【修回日期】** 2022-11-12

【基金项目】 广东省自然科学基金面上项目(2019A1515011932); 广州市科技计划项目(202103000093)

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Conclusion Near-infrared fluorescence imaging with indocyanine green can assist in the successful removal of necrotic bone until fresh bleeding of the jaw wound occurs, which has important clinical value in defining the resection range of osteoradionecrosis of the jaw.

【Key words】 osteoradionecrosis; necrotic bone; osteotomy; indocyanine green; near-infrared fluorescence imaging; fluorescence intensity; fresh bone wounds

J Prev Treat Stomatol Dis, 2023, 31(6): 408-413.

【Competing interests】 The authors declare no competing interests.

This study was supported by the grants from Natural Science Foundation of Guangdong Province (No. 2019A1515011932); Guangzhou Science and Technology Program Project (No. 202103000093).

颌骨坏死 (osteonecrosis of the jaws, ONJ) 于 2003 年在牙科文献中首次报道, 该术语的创造是为了描述在用高剂量静脉注射双膦酸盐治疗的癌症患者中出现的一系列牙齿问题^[1-2]。颌骨放射性骨坏死 (osteoradionecrosis of the jaws, ORNJ) 是头颈部恶性肿瘤放疗后的严重并发症之一。随着放疗方式从常规 2D 放疗到 3D 适形放疗再到调强放疗 (intensity modulated radiation therapy, IMRT) 的演变, 目前 ORNJ 发生率降至 4% ~ 8%^[3-4]。其临床症状包括: 疼痛、软组织肿胀或溃疡、局部化脓、口内外窦道、脓肿、神经功能障碍及病理性骨折。颌骨坏死患者术中如何确定坏死骨边界, 一直是治疗难题。近年来, 吲哚菁绿 (indocyanine green, ICG) 的近红外荧光 (near-infrared fluorescence, NIF) 成像技术逐渐用于外科领域, 基于吲哚菁绿增强的渗透性和滞留效应 (enhanced permeability and retention effect, EPR)^[5], 本研究中, 将吲哚菁绿 NIF 技术应用于 ORNJ 患者术中坏死骨质界定, 取得良好效果, 现将治疗结果和经验回顾性报道如下。

1 资料和方法

1.1 临床资料

选取 2020 年 10 月至 2021 年 6 月在中山大学孙逸仙纪念医院口腔颌面外科就诊的 8 例下颌骨 ORNJ 患者。所有患者均无严重肝、肾功能障碍。男 6 例, 女 2 例; 年龄 45 ~ 69 岁, 平均 (58.1 ± 10.2) 岁。根据《ORNJ 的临床诊疗专家共识和临床实践指南》中新的临床分期^[6], ORNJ 患者 II 期 6 例, III 期 2 例。术前患者的软组织损伤以及张口受限等一般情况均已记录以备和术后随访疗效进行比较。本研究已获中山大学孙逸仙纪念医院伦理委员会批准, 所有患者或家属对治疗知情同意, 并签署知情同意书将他们的匿名数据用于研究。

1.2 术中坏死骨病灶的吲哚菁绿 NIF 成像

注射用吲哚菁绿 (国药准字 H20073073, 丹东医创药业有限责任公司), 使用剂量为 0.75 mg/kg。将吲哚菁绿以 10 mL 灭菌注射用水稀释后, 于术中病灶骨截除前 10 min, 经肘静脉在 30 min 内缓慢泵入。术中使用 REAL-IGS FLI-10A 型 NIF 设备 (南京诺源医疗器械有限公司, 中国) 对坏死骨病灶行 NIF 成像。

1.3 坏死骨病灶扩大切除范围前后骨截面的荧光强度测定

首先在术中暴露坏死灶清晰视野后, 在融合影像模式下, 第一次记录死骨及周围组织荧光强度; 然后以影像学结果为主要依据, 合并对坏死骨结构、颜色、质地等的主观印象进行初步保守性坏死骨病灶切除, 每个点测 3 次, 取 10 个相近似的点的均值作为坏死骨保守性切除后新鲜创面的荧光强度; 随后在呈阶梯样起伏的荧光较弱端对应的截骨创缘面上以 0.3 cm 为初始距离逐渐扩大潜在死骨切除边界, 直到所有骨截面的荧光强度波动趋于稳定, 记录扩大切除距离以及最终新鲜创面的平均荧光强度。

1.4 随访

所有骨坏死患者术后均需定期复查随访, 术后 3、6、12 个月进行疗效评价。通过疼痛、开口度、创口愈合以及影像学 (X 线片、CT) 四个指标进行评估。治愈: 无疼痛、开口度改善 ≥ 1 cm、创口完全愈合、无死骨和 (或) 骨质愈合良好, 影像学表现骨密度增高或无骨密度降低; 有效: 疼痛缓解、开口度改善为 0.5 ~ 1 cm、创口基本愈合、无死骨和 (或) 骨质基本愈合, X 线片表现为骨质稀疏、缺损、呈不规则疏松, CT 表现为局限性骨质密度降低、骨小梁稀疏; 无效: 疼痛未缓解、开口度改善 < 0.5 cm、创口未愈、有死骨和 (或) 骨质愈合不良, X 线片显

示为融合性斑片状骨质破坏,CT可见斑点状、斑片状、条片状死骨,呈高密度,边界清楚。好转率=(治愈人数_{术后12个月}+有效人数_{术后12个月})/随访总人数_{术后12个月}×100%。

1.5 统计学方法

应用SPSS 25.0软件进行数据分析,两组荧光强度比较采用Student *t* 检验, $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 ORNJ患者坏死骨质扩大切除范围前后的吡啶菁绿NIF成像情况

8例患者临床资料如表1所示。8例下颌骨ORNJ患者均在术中获得的清晰的吡啶菁绿NIF图像,影像结果显示无论是扩大切除前还是扩大切除后骨截面均可观察到新鲜血液流出,但扩大切除后的骨截面血流量较扩大切除前更多,肉眼可见骨截面绿色荧光强度普遍更强并较稳定,未观察到呈断崖式下降的荧光位点。图1为1例示范病例的术中吡啶菁绿NIF成像情况。

2.2 坏死骨质扩大切除范围前后骨截面荧光强度的差异

8例ORNJ患者基于影像学和术者的主观印象行保守性死骨切除后的下颌骨骨截面荧光强度为 (108.8 ± 3.4) au,在此基础上扩大切除的范围为

(0.95 ± 0.14) cm,扩大切除后骨截面的荧光强度为 (226.2 ± 15.8) au,后者的荧光强度高于前者($t = 20.718, P < 0.001$)(表2)。

2.3 随访结果

8例下颌骨ORNJ患者,下颌坏死骨刮除术1例,下颌骨节段性切除联合腓骨肌皮瓣5例,下颌骨边缘性切除联合股前外侧瓣1例,下颌骨边缘性切除联合胸大肌皮瓣1例。术后12个月,疗效评估2例治愈,5例有效,1例无效,术后好转率达87.5%。所选示范病例手术前后的曲面断层片对比情况见图2。

3 讨论

手术边界应有新鲜血液从骨面渗出是颌骨坏死骨质彻底性手术治疗成功的关键。目前,临床外科医生主要依靠术前临床检查、术前影像学检查和术中通过视觉观察骨断端缺血情况,结合经验判断坏死骨切除范围。因在影像学上颌骨坏死患者死骨与周围骨组织边界不清,要在术前准确界定死骨范围有一定困难,而术中往往发现实际坏死范围大于影像学显示范围,若未彻底去除死骨,术后也极易复发^[7-8]。尽管大多数颌骨坏死治疗需要手术干预,但目前还没有制定出确定手术范围的指南。因此,临床上需要一种能在术中辅助进行坏死骨质界定评估的方法。

表1 8例下颌骨放射性骨坏死患者临床资料

Table 1 Clinical data of all eight patients with osteoradionecrosis of the mandible

Patients	Genoler	Age/year	Primary tumor	BS clinical stages	Surgical treatment	Preoperative soft tissue injury	Preoperative trismus grading	Postoperative effect
1	Male	50	Right oropharyngeal carcinoma	II	Segmental jaw resection combined with fibular osteomyocutaneous flap	Swellings and ulcerations	II	Effective
2	Male	69	Carcinoma of the right neck lymph node	II	Segmental jaw resection combined with fibular osteomyocutaneous flap	Suppuration and intraoral sinus tracts	II	Recovery
3	Male	45	Nasopharyngeal carcinoma	II	Simple curettage	Abscess	I	Ineffective
4	Male	67	Right buccal carcinoma	II	Marginal jaw resection combined with anterolateral femoral free flap	Ulcerations and abscess	II	Effective
5	Male	50	Nasopharyngeal carcinoma	III	Segmental jaw resection combined with fibular osteomyocutaneous flap	Swellings and extraoral sinus tracts	III	Effective
6	Female	50	Nasopharyngeal carcinoma	II	Segmental jaw resection combined with fibular osteomyocutaneous flap	Ulcerations	III	Recovery
7	Male	67	Carcinoma of the left tonsil	II	Marginal jaw resection combined with pectoralis major myocutaneous flap	Abscess and swellings	II	Effective
8	Female	67	Nasopharyngeal carcinoma	III	Segmental jaw resection combined with fibular osteomyocutaneous flap	Intraoral and extraoral sinus tracts	III	Effective

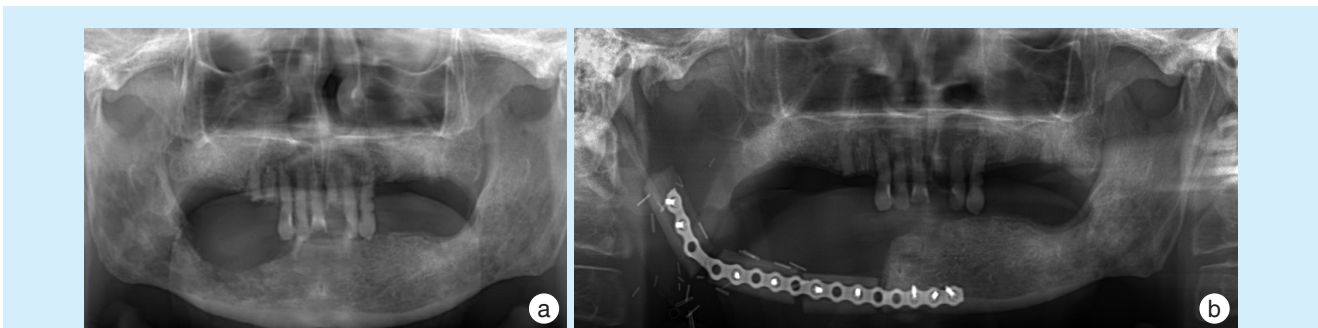
BS: bone destruction; soft tissue injury



表2 8例下颌骨放射性骨坏死患者术中坏死骨质边界扩大切除前后骨截面荧光强度

Table 2 Comparison of fluorescence intensity in the bone cross-section before and after resection of mandible osteoradionecrosis in 8 patients with an enlarged border of necrotic bone

Patients	1	2	3	4	5	6	7	8	Average fluorescence intensity/length
Before extended resection/au	107	108	103	114	107	108	112	106	108.8 ± 3.4
After extended resection/au	237	246	229	205	245	208	235	215	226.2 ± 15.8
Enlarge the length of the excision/cm	0.8	0.9	1.1	1.0	0.9	0.8	1.2	0.9	0.95 ± 0.14
<i>t</i>									20.718
<i>P</i>									< 0.001



a: panoramic radiograph showed bone destruction and necrosis before resection of the mandibular osteonecrosis; b: panoramic radiograph showed that the remaining bone tissues healed well and that the necrotic bone was successfully removed 12 months after segmental jaw resection combined with reconstruction using a fibular osteomyocutaneous flap

Figure 2 Panoramic radiographs of the mandibular osteoradionecrosis in patient #2 showed that bone tissues healed well 12 months after surgery

图2 曲面断层片示下颌骨放射性骨坏死患者#2手术治疗后12个月骨质愈合良好

随着吲哚菁绿在腹腔镜肝切除及胃癌根治术、乳腺癌荧光示踪哨位淋巴结活组织检查和子宫内膜癌哨位淋巴结切除等领域的普及并具有较好的诊疗效果,吲哚菁绿荧光成像技术在口腔鳞状细胞癌(oral squamous cell carcinoma, OSCC)手术治疗中的应用也愈来愈受到关注,即使仍处于起步阶段,但在术中辅助评估 OSCC 原发灶切除范围、辅助评估 OSCC 切缘状态、示踪 OSCC 颈部哨位淋巴结、辅助监测皮瓣血运等临床诊疗方面都具有良好的应用价值,但其在颌骨坏死的临床诊疗中还未有研究报道。基于吲哚菁绿增强的渗透性和滞留效应,其经静脉注射后与血浆蛋白迅速结合,形成 7 nm 左右的纳米粒子^[9-11]。除此之外,有研究报道,在双膦酸盐相关颌骨坏死大鼠模型中静脉注射吲哚菁绿后解剖坏死骨骼和正常骨骼并进行 NIF 成像和荧光强度定量检测,最终通过病理检查验证了:坏死骨的受累程度可通过吲哚菁绿 NIF 成像进行术中估计^[12]。

本课题组尝试利用吲哚菁绿与人体液结合的特性来界定 ORNJ 患者的颌骨切除范围,以期获得有更加彻底的新鲜血液流出的骨面。在本研究中,通过两步法切除下颌骨死骨和扩大切除边界后得到的新鲜骨创面,经 NIF 成像测量验证后远比仅凭肉眼和影像学结果行死骨切除具有更强更稳定的荧光信号,根据荧光信号针对性扩大切除后的手术边界更有可能具有丰富的血运以促进后期的伤口愈合以及抗感染,对于降低残留坏死骨质复发的几率具有一定优势,而尽可能少切正常骨质和切净潜在坏死病灶正是颌骨坏死手术中亟需完善的关键。本组 8 例 ORNJ 患者术后 12 个月疗效评估好转率达 87.5%,初步显示吲哚菁绿 NIF 技术应用于 ORNJ 患者术中坏死骨质界定具有一定的辅助价值。

为了获得较好的手术疗效,除了运用吲哚菁绿辅助明确死骨切除范围,对于骨坏死进展到晚期的患者,下颌骨死骨扩大切除同期联合血管化组织瓣修复对预后和减少骨坏死再次发生起到的作用也是不可忽视的。本研究中有 7 例患者行下颌骨死骨扩大切除联合腓骨肌皮瓣、股前外侧皮瓣和胸大肌皮瓣修复重建,不仅恢复了下颌骨的外形结构,还有助于颌骨的血供重建,特别是在下颌管内下牙槽动脉损伤、颌骨表面骨膜广泛剥离的情况下可保护剩余暴露骨组织,减少感染和颌骨再次坏死的风险,术后并发症也较单纯颌骨切

除的患者更少^[13-14]。本研究有 1 例 ORNJ 患者经死骨刮治术后随访仍出现扩大区域面积的坏死骨质,不排除吲哚菁绿荧光成像技术可能仍存在穿透深度有限、检测设备特异性和灵敏性有限等不足,导致一些远离肉眼可见的或者影像学上明显的潜在坏死骨质被忽视。基于本次研究结果,笔者课题组提出将 (226.2 ± 15.8) au 作为吲哚菁绿引导下 ORNJ 坏死骨质界定荧光阈值以供临床参考及进一步验证。本研究不足在于仅运用吲哚菁绿荧光成像技术比较扩大切除前后新鲜截骨创面的荧光强度差异,单纯死骨截面的荧光强度未明确以及扩大切除的范围距离有待进一步研究。有研究提出了一项定量光诱导荧光(quantitative light induced fluorescence, QLF)技术,非红色荧光显示硬化骨组织和板层状骨组织,亮红色荧光显示细菌入侵和骨溶解所致的感染状态,暗红色荧光显示以颗粒状组织为主,伴有炎症和骨基质缺失^[15]。在 QLF 引导下的颌骨坏死手术,保留非红色荧光区,去除骨的强、弱红色荧光区可能是有用的,吲哚菁绿荧光成像技术联合该技术有望解决上述不足。

综上,对 ORNJ 患者术中应用吲哚菁绿荧光成像技术辅助坏死骨质界定,有助于进一步明确手术的切除范围。

【Author contributions】 Kang ZQ took part in the surgery, collected data, and wrote the main manuscript. Wang YP took part in the surgery and collected clinical data. He YL analyzed the data and prepared the table. Cai YK prepared figures 1 and 2. Huang ZQ designed the study, took part in the surgery and approved the final manuscript. All authors read and approved the final manuscript.

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(编辑 张琳, 栾修文)



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《口腔疾病防治》入选 2022 年度中国高校科技期刊建设示范案例库优秀科技期刊

2022年12月1日,在中国高校科技期刊研究会第26次年会上发布了2022年度中国高校科技期刊建设示范案例库·杰出/百佳/优秀科技期刊入库案例名单。由南方医科大学口腔医院主办的科技期刊《口腔疾病防治》入选2022年度中国高校科技期刊建设示范案例库优秀科技期刊。

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