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·文献综述·

乳腺癌前哨淋巴结转移术中检测进展：一步法核酸扩增技术

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摘　要　乳腺癌已成为女性最常见的恶性肿瘤之一，腋窝淋巴结（ALN）转移是乳腺癌最主要的转移方式，其是判断预后和指导术后辅助治疗方案决策最重要的指标。腋窝淋巴结清扫（ALND）是评估 ALN 状态最准确的方法，亦是造成上肢淋巴水肿、疼痛、感觉障碍等并发症的主要原因。随着早期乳腺癌的增多，ALN 阴性乳腺癌已占据一半以上，如对所有乳腺癌患者行 ALND，将只有少部分患者受益，大部分患者接受了过度治疗。乳腺癌前哨淋巴结（SLN）是乳腺癌患者肿瘤细胞经淋巴结转移的首道屏障。历经数十年发展，前哨淋巴结活检（SLNB）已成为乳腺癌患者 ALN 状态分期的标准程序，常用于确定乳腺癌治疗方式的选择。准确、快速的 SLN 术中诊断可以使 SLN 阴性的乳腺癌患者避免 ALND，SLN 阳性患者通过一次手术完成 ALND，避免二次手术的费用负担和手术风险。术中检测 SLN 是否转移常用的病理学方法是冷冻切片和印片细胞学检查，这两种常规病理检测方法仅检测 SLN 的代表性切片，均存在敏感度较低、主观性、非标准化、检测的组织量少（远远低于 5%）、没有统一诊断标准等缺点，因此临床上迫切需要一种结果准确，操作简便的新型检测方法。近年来分子诊断技术迅速发展，一步法核酸扩增（OSNA）是通过逆转录环介导的靶向细胞角蛋白 19mRNA 等温扩增法精确检测术中乳腺癌淋巴结转移的分子诊断方法。OSNA 检测运作时间约 30~40 min，在 SLN 检测分析中，OSNA 的准确率、敏感度均优于常规病理学检测方法。术中快速定量可区分宏转移和微转移，指导手术方案，并且在检测 SLN 微转移时，OSNA 也更胜一筹。笔者对 OSNA 检测在乳腺癌 SLN 转移中的进展及应用前景做一综述。

关键词　乳腺肿瘤；前哨淋巴结；一步法核酸扩增；综述
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Progress in intraoperative detection of sentinel lymph node metastasis in breast cancer: one-step nucleic acid amplification technique

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Abstract　Breast cancer has become one of the most common malignant tumors among women, and axillary lymph node (ALN) metastasis is the most important mode of breast cancer metastasis, which is the most important indicator to estimate the prognosis and guide the decision of postoperative adjuvant treatment.

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Axillary lymph node dissection (ALND) is the most accurate way to evaluate ALN status, and meanwhile is the main cause of complications such as upper extremity lymphedema, pain, and sensory impairment. With the increased detection of early-stage breast cancer, ALN negative breast cancer has accounted for more than half of all breast cancer patients, if ALND is performed in all breast cancer patients, only a small number of patients will benefit from it, and the majority of patients are overtreated. Sentinel lymph nodes (SLNs) are the first barrier of lymph node metastasis in patients with breast cancer. After decades of development, sentinel lymph node biopsy (SLNB) has become a standard procedure for ALN staging in breast cancer patients and is often used to determine the choice of treatment. Accurate and rapid intraoperative diagnosis of SLN enables SLN-negative breast cancer patients to avoid ALND, and SLN-positive patients to complete ALND through a single operation to avoid the cost burden and surgical risk of a second operation. Frozen section and touch imprint cytology examinations are commonly used to detect intraoperative metastatic SLN. These two routine pathological tests only detect representative sections of SLN. Both of them have shortcomings such as low sensitivity, subjectivity, non-standardization, small amount of detected tissue (far less than 5%), and no unified diagnostic criteria. Therefore, a new detection method with accurate results and simple operation is urgently needed in clinical practice. In recent years, molecular diagnostic techniques have developed rapidly. One-step nucleic acid amplification (OSNA) is a molecular diagnostic method for accurate detection of intraoperative lymph node metastasis of breast cancer through reverse transcriptional loop mediated isothermal amplification of targeted cytokeratin 19 mRNA. The operation time of OSNA detection is about 30 to 40 min. In SLN detection and analysis, the accuracy and sensitivity of OSNA are better than those of the conventional pathological detection methods. Rapid intraoperative quantification can distinguish the macrometastases from micrometastases and guide surgical protocols. In addition, OSNA is superior in detecting SLN micrometastases. Here, the authors address the progress and application prospect of OSNA detection in SLN metastasis of breast cancer.

Key words Breast Neoplasms; Sentinel Lymph Node; One-Step Nucleic Acid Amplification; Review

CLC number: R737.9

乳腺癌是女性最常见的恶性肿瘤之一，2020年全球最新癌症数据表明，乳腺癌取代肺癌，成为全球第一大癌，随着乳腺癌发病率及病死率的增加，乳腺癌的治疗越趋重要[1-2]。腋窝淋巴结（axillary lymph node，ALN）转移情况已成为乳腺癌患者整体生存最重要的预后指标，因此腋窝淋巴结清扫术（axillary lymph node dissection，ALND）是早期乳腺癌手术治疗的一部分，亦是造成上肢淋巴水肿等乳腺癌术后并发症的主要原因[3]。20世纪90年代发现的一种微创、能高度准确检测ALN转移的方法，即前哨淋巴结活检（sentinel lymph node biopsy，SLNB），历经数十年发展，SLNB已成为早期乳腺癌患者的标准治疗方法[4-5]。一系列大样本前瞻性临床试验证实了SLNB的安全性，SLNB可以提供准确的ALN分期，前哨淋巴结（sentinel lymph node，SLN）阴性及低负荷SLN阳性患者SLNB替代ALND的腋窝复发率和并发症发生率很低，为其提供了循证医学的证据[6-13]。准确、快速的SLN术中诊断变得尤为重要，冷冻切片（frozen section，FS）和印片细胞学（touch imprint cytology，TIC）是术中病理检查的常用方法，FS特异度几乎100%，敏感度也较细胞学高，但假阴性率约为10%~20%；TIC特异度高但敏感度低，所以两者的准确性都受到限制，敏感度在57%到74%之间[14-15]。FS和TIC没有统一的诊断标准，因此，临床上迫切需要一种结果准确、操作简便的新型检测方法。一步法核酸扩增（one-step nucleic acid amplification，OSNA）是一种通过细胞角蛋白（cytokeratin 19，CK19）检测SLN转移的方法，既简便又快速，适合于SLN转移的术中检测[16]。

1　乳腺癌SLN检测的临床意义及现状

乳腺癌目前治疗方式仍以手术治疗为主。随着乳腺外科保守理念的流行，不仅乳房能保留，腋窝也可以有条件的保留[17]。SLNB无疑是乳腺外科领域的一个跳跃式的进展。将SLN定义为从原发肿瘤向区域淋巴结引流的第一站淋巴结，它可以为1个或1组，是乳腺肿瘤淋巴结转移首先经过的解剖位置。以SLN的状态评价整个区域淋巴结的状态，即以SLNB病理结果评价区域淋巴结有无受侵，其重要性及有效性毋庸置疑，目前腋窝SLNB已成为乳腺癌患者手术治疗的标准处理模式[18-22]。当检出SLN阴性可避免ALND，同时也可避免术后上肢淋巴水肿等乳腺癌术后并发症；检出SLN阳性患者可一次完成ALND，可以避免患者二次手术费用的负担和手术风险，减少住院时间。SLNB与示踪剂的关系密不可分，临床中常用的是亚甲蓝注射液联合核素示踪剂（^{99m}Tc标记的硫胶体），可使检出率增加的同时降低假阴性率[23]。在临床工作中，SLN是否转移通过FS的组织病理学或TIC检测，并通过术后常规的病理学检测来确认SLN是否有转移。

1.1　SLN转移灶类型判定标准

SLN的准确诊断对于腋窝的准确分期、确定术后治疗方案及降低术后腋窝并发症等至关重要。根据AJCC乳腺癌TNM分期第8版，SLN转移分为宏转移、微转移和孤立肿瘤细胞（isolated tumor cell，ITC）。宏转移：病理学检测的阳性淋巴结存在1个以上>2 mm的肿瘤病灶，其他阳性淋巴结至少存在微转移。微转移：肿瘤病灶最大径>0.2~2 mm，或单张组织切片不连续，抑或接近连续的细胞簇<200个细胞。ITC：单个细胞或最大径≤0.2 mm的小细胞簇，单张组织切片不连续或接近连续的细胞簇≤200个细胞簇。

1.2　FS快速病理检查

目前FS在SLN是否转移中的诊断应用非常广泛，Godazande等[24]报道的FS的准确率为78.4%，假阴性率为20.9%，并认为在评估低肿瘤负荷（<2 mm的微转移灶）的淋巴结时敏感度较低。2011年一项包括13 062例患者的Meta分析[25]显示，FS对任何SLN转移灶的总体敏感度为73.0%，其中宏转移的敏感度为94.0%，微转移和ITC的敏感度为40%。Lanitis等[26]研究表明，FS的准确率为96.1%，假阴性率为11.7%（ITC：6.8%，微转移：7.7%，宏转移：4.0%）这也说明FS在SLN宏转移检出率更高。Shojaee等[27]结果认为，FS的敏感度，特异度和诊断准确性分别为24%、90%和43%，在FS中，SLN阳性和阴性分别为15.7%和84.3%，而在最终病理中，SLN阳性和阴性分别为41.0%和58.8%。这一差异源于FS的假阴性率，为31.3%。总而言之，FS有较高的准确率和较低的假阴性率，但其缺点是切片较厚，染色欠佳，与常规石蜡病理不能完全符合，而且易耗损组织。

1.3　TIC病理检查

TIC是检测SLN转移的一种简单且经济高效的技术。Uno等[28]对367例患者507枚淋巴结进行分析，结果表明，TIC诊断SLN转移的敏感度为84.1%，特异度为100.0%，准确率为97.4%；TIC可检测出全部的宏转移，而只检出约50.0%的微转移，未检测出ITC。一项回顾性研究[29]对1 227例乳腺癌患者评估TIC检测SLN的敏感度，结果表明，TIC的敏感度为68.6%，特异度为99.8%。Hashmi等[30]比较114例患者TIC和FS检测SLN转移的准确性，结果表明，TIC的敏感度，特异度和诊断准确率分别为83.7%、98.5%和92.1%，而FS的敏感度，特异度和诊断准确性分别为93.9%、100%和97.4%；检测微转移时TIC和FS的敏感度分别为14.3%和57.1%，诊断准确率分别为90.3%和95.8%；检测宏转移时TIC的敏感度和特异度分别为95.2%和98.5%，而FS的敏感度和特异度为100%。尽管FS的总体敏感度高于TIC，但TIC检测宏转移的敏感度与FS相当。综上，TIC具有不耗损标本、操作简便、廉价等优点，而且通过增加取样面积和多层面印片提高诊断的准确率。

1.4　术后常规病理检查

中国抗癌协会乳腺癌诊治指南与规范（2019年版）[31]推荐SLN术后常规病理检查的金标准是逐层切片行病理检测。推荐将SLN沿长轴切成2 mm厚的组织块，对每个组织块进行逐层或连续切片病理检测，3层切片间距为0.2~0.5 mm。而常规病理检测技术中也存在着一些缺点，其不能对大量的SLN进行分析、细胞学的敏感度在33.0%~73.0%，在微转移和浸润性小叶癌中常常出现假阴性结果，而且病理组织学分析是一种昂贵的分析SLN方法，还需要病理学家分析标本以得出最终的结论[32]。

2 OSNA检测乳腺癌前哨淋巴结转移

在当今的精准医疗时代，应准确评估SLN是否转移。常规的FS和TIC对SLN诊断具有较高的敏感度和特异度，但其存在主观性、非标准化、检测的组织量少等缺点，需要寻求更为准确的术中快速分子诊断技术。OSNA是一种仅需一步操作即可以匀浆标本做为模板，对靶向核酸基因扩增并检测的技术。采用OSNA技术，可以对淋巴结中的目标基因进行高精度、快速、简便的检测。

2.1 OSNA的原理

OSNA在选择目的基因的标记物时，先从人类遗传基因数据库中选择了45个在乳腺/乳腺癌组织中高表达，而在正常淋巴结组织中低表达的遗传基因，然后使用定量逆转录多聚酶链技术的方法，筛选出在转移阳性的淋巴结组织中高表达，而在转移阴性的淋巴结组织中低表达的7个候选遗传基因。进一步研究发现，这7个候选遗传基因在转移阳性和转移阴性的淋巴结组织中表达量存在很大的差异。最终将在转移阳性淋巴结组织中表达量最高的CK19mRNA选为最适合于乳腺癌淋巴结转移检测的遗传基因标志物，CK19标记物在95%以上的乳腺癌中均有表达，避免了基因组DNA扩增（或CK19基因假扩增）[33-34]。CK19在乳腺上皮细胞表达，而在淋巴结中不表达，当检测到SLN中CK19 mRNA扩增时，高度怀疑该淋巴结有乳腺癌转移[35]。近年来发展的定量逆转录多聚酶链技术检测一般需要数小时，包括mRNA的提取、纯化和cDNA合成等多个步骤，不适于术中检测SLN[35]。为了减少术中检查所需的时间，OSNA依据反转录-环状介导等温DNA扩增方法，在mRNA不纯化的情况下进行基因扩增，通过在恒定温度下用引物和DNA聚合酶II的混合物对样本完成检测[36]。OSNA可同时检测4个完整的SLN，仅需30~40 min[16]。

2.2 OSNA的结果分析

从每个SLN的中心取出1个非常薄的切片（1 mm）进行病理检查后，剩余的淋巴结切片在4 mL的Lynorhag裂解缓冲液（Sysmex，Kobe，Japan）pH为3.5中均化，并在室温下进行短暂离心，然后使用RT-LAMP对RD-100i系统中的2 μL上清液进行分析。结果分为阴性（$<2.5\times10^2$拷贝/μL）、（+）即微转移（$>2.5\times10^2$拷贝/μL，$<5.0\times10^3$拷贝/μL）、（++）即宏转移（$>5.0\times10^3$拷贝/μL）和+i（常规样品抑制，稀释样品$\geq2.5\times10^2$拷贝/μL）。分类为+i的患者也被认为是阳性[37]。

2.3 OSNA在临床中的应用

OSNA从2007年开始应用于临床，无论在术中还是术后都是一种准确的检测SLN转移的方法[38]。这种新兴技术具有半定量、可重复性、准确性和分析整个SLN的优点，被越来越多的中心所采用[39]。Szychta等[40]分析了105个SLN，中心1 mm的SLN切片用于FS检查，用OSNA分析SLN的2个层面，结果表明，与FS相比，OSNA更容易检出SLN转移（$P<0.000\ 1$）；不一致的结果有8个SLN，OSNA检测为阳性（2个检测结果为ITC，6个检测结果为微转移）而FS检测为阴性；OSNA的敏感度为100%，特异度为90.47%。Hintzen等[41]分析了271例患者459个SLN，对比常规组织学检查和OSNA检查结果，表明两组之间的宏转移相似，差异无统计学意义（组织学检出率为17.9%，OSNA检出率为16.2%，$P=0.715$）；在微转移方面，OSNA优于常规组织学检测（组织学检出率为11.4%，OSNA为25.0%，$P=0.004$）；在分析乳腺癌中SLN是否转移，OSNA是一项高度敏感的技术，在微转移方面更胜一筹。Santaballa等[37]研究也表明，OSNA较病理学更易检出SLN微转移（$P=0.000\ 7$）。在常规病理学检测为ITC时，据报道，OSNA对小体积转移灶的检测更敏感，OSNA检测有时会受到抑制，这会导致假阴性结果（<250拷贝/μL），如在稀释样品中（>250拷贝/μL）就为阳性[42]。有研究者[43]认为当结果为（100~250拷贝/μL）相当于ITC，还有待进一步研究确定。国内王永胜等[9]入组了全国5个乳腺病中心552例原发性乳腺癌患者，共探及SLN 1 188枚，OSNA的准确度、敏感度、特异度分别为91.4%、83.7%和92.9%，对于SLN微转移，OSNA的敏感度优于FS（$P=0.289$），显著优于TIC（$P=0.007$）。

OSNA技术还具有预测非前哨淋巴结（non-sentinel lymph node，nSLN）转移的风险。Cuffolo等[44-45]报道总肿瘤负荷（total tumor load，TTL）似乎对nSLN转移有预测价值。TTL定义为所有阳性SLN中CK19 mRNA的总数。利用TTL可以确定一个阈值，在该阈值下可以避免不必要的ALND。Peg等[46]试图利用TTL作为早期乳腺癌的预后指标，对950例患者进行研究发现，TTL与无病生存（$P=0.000\ 004$）、局部无复发生存（$P=0.001\ 4$）和总生存（$P=0.003\ 2$）相关；表明TTL是早期乳腺癌患者评估ALN转移的一个潜在预后分期工具；对于SLN

阳性手术后未进行 ALND 的患者，这一分组方法（低危复发风险 TTL<2.5×10^4 拷贝/μL，高危复发风险 TTL>2.5×10^4 拷贝/μL）可能会对后续治疗策略有所帮助；这项新的数据证实了 TTL 在腋窝转移低负荷患者中的临床价值，可以部分地取代没有进行 ALND 患者的腋窝分期信息。TTL 作为一种预测工具，在应用于临床之前，显然还需要进一步的验证研究。为避免 ALND 的并发症，OSNA 预测 nSLN 转移的潜力将继续受到关注。同时，OSNA 在新辅助治疗方面也具有一定的意义。新辅助治疗（neoadjuvant treatment，NAC）后淋巴结常常发生纤维化或萎缩，导致取材时难以识别。Peña 等[47–48]研究表明在 NAC 后患者的 SLN 转移检测能力方面，OSNA 是一种敏感度高、特异度高和可重复的诊断技术。Vieites 等[49]对 314 例患者行 NAC 后进行术中 SLN 活检，结果表明，TTL 可以预测 nSLN 的转移（曲线下面积=0.87），截止值为 1.5×10^4 拷贝/μL 时，阴性预测值为 90.5%；经过 5 年的随访，≤2.5×10^4 拷贝/μL 患者的 DFS>2.5×10^4 拷贝/μL 患者（P=0.001 7）。Espinosa-Bravo 等[50]研究表明，针对在 NAC 治疗后的 SLN 阳性患者，OSNA 对 SLN 的精确评估可降低 18.5% 二次 ALND 手术。

3　结　论

随着早期乳腺癌检出的增多，ALN 阴性患者已占据一半以上，如对所有患者进行 ALND，将只有一小部分患者受益，大部分患者接受了过度治疗。SLN 为腋窝分期的金标准，精准检出 SLN 是否有转移是外科医生所追求的。OSNA 是第一个对转移淋巴结中靶基因 mRNA 进行半定量检测的方法，初步试验取得令人满意的效果。在操作时更标准化，检测敏感度更高，术中快速定量可区分宏转移和微转移，指导手术方案，运用术中 SLN 定量结果制定预后模型进行深入的临床分析，操作简单，可重复性更高，可更精准的明确 SLN 的转移数目，同时可为乳腺癌的腋窝分期提供准确而及时的信息，避免患者进行二次手术，以便临床医生制订后续的手术方案及治疗措施。该技术有望成为一种新的手段，服务于乳腺癌患者，在中国推广使用。

参考文献

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020[J]. CA Cancer J Clin, 2020, 70(1):7–30. doi: 10.3322/caac.21590.

[2] Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries[J]. CA Cancer J Clin, 2021, 71(3): 209–249. doi: 10.3322/caac.21660.

[3] Rupp J, Hadamitzky C, Henkenberens C, et al. Frequency and risk factors for arm lymphedema after multimodal breast-conserving treatment of nodal positive breast Cancer - a long-term observation[J]. Radiat Oncol, 2019, 14(1): 39. doi: 10.1186/s13014–019–1243–y.

[4] 梁培德, 曾焕城, 贺丽芳, 等. 乳腺癌腋窝前哨淋巴结探查术的研究进展[J]. 肿瘤学杂志, 2020, 26(11):995–1001. doi:10.11735/j.issn.1671–170X.2020.11.B014.
Liang PD, Zeng HC, He LF, et al. Progress on Axillary Sentinel Lymph Node Biopsy for Breast Cancer[J]. Journal of Chinese Oncology, 2020, 26(11): 995–1001. doi: 10.11735/j. issn. 1671–170X.2020.11.B014.

[5] 宋文静, 贺鑫, 龚鹏举, 等. 基于临床病理大数据的早发乳腺癌腋窝淋巴结转移风险预测模型构建[J]. 中国普通外科杂志, 2020, 29(11):1293–1302. doi:10.7659/j.issn.1005–6947.2020.11.002.
Song WJ, He X, Gong PJ, et al. Establishment of risk prediction model for axillary lymph node metastasis in breast cancer at early age based on clinicopathologic big data[J]. Chinese Journal of General Surgery, 2020, 29(11): 1293–1302. doi: 10.7659/j. issn.1005–6947.2020.11.002.

[6] Veronesi U, Paganelli G, Viale G, et al. Sentinel-lymph-node biopsy as a staging procedure in breast cancer: update of a randomised controlled study[J]. Lancet Oncol, 2006, 7(12): 983–990. doi: 10.1016/S1470–2045(06)70947–0.

[7] Krag DN, Anderson SJ, Julian TB, et al. Sentinel-lymph-node resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer: overall survival findings from the NSABP B-32 randomised phase 3 trial[J]. Lancet Oncol, 2010, 11(10): 927–933. doi: 10.1016/S1470–2045(10)70207–2.

[8] Fleissig A, Fallowfield LJ, Langridge CI, et al. Post-operative arm morbidity and quality of life. Results of the ALMANAC randomised trial comparing sentinel node biopsy with standard axillary treatment in the management of patients with early breast cancer[J]. Breast Cancer Res Treat, 2006, 95(3): 279–293. doi: 10.1007/s10549–005–9025–7.

[9] 王永胜, 欧阳涛, 王启堂, 等. 中国前哨淋巴结活检多中心协作研究 CBCSG-001 最新资料报告[J]. 中华乳腺病杂志: 电子版, 2009, 3(3):265–272. doi:10.3969/j.issn.1674–0807.2009.03.004.
Wang YS, Ouyang T, Wang QT, et al. Chinese joint multi-center study of sentinel lymph node biopsy(CBCSG-001): latest news[J]. Chinese Journal of Breast Disease: Electronic Version, 2009, 3(3): 265–272. doi:10.3969/j.issn.1674–0807.2009.03.004.

[10] Hunt KK, Ballman KV, McCall LM, et al. Factors associated with

　

local-regional recurrence after a negative sentinel node dissection: results of the ACOSOG Z0010 trial[J]. Ann Surg, 2012, 256(3): 428−436. doi: 10.1097/SLA.0b013e3182654494.

[11] Galimberti V, Cole BF, Viale G, et al. Axillary dissection versus no axillary dissection in patients with breast cancer and sentinel-node micrometastases (IBCSG 23−01): 10-year follow-up of a randomised, controlled phase 3 trial[J]. Lancet Oncol, 2018, 19(10): 1385−1393. doi: 10.1016/S1470−2045(18)30380−2.

[12] Giuliano AE, Ballman K, McCall L, et al. Locoregional Recurrence After Sentinel Lymph Node Dissection With or Without Axillary Dissection in Patients With Sentinel Lymph Node Metastases: Long-term Follow-up From the American College of Surgeons Oncology Group (Alliance) ACOSOG Z0011 Randomized Trial[J]. Ann Surg, 2016, 264(3): 413−420. doi: 10.1097/SLA.0000000000001863.

[13] Donker M, van Tienhoven G, Straver ME, et al. Radiotherapy or surgery of the axilla after a positive sentinel node in breast cancer (EORTC 10981−22023 AMAROS): a randomised, multicentre, open-label, phase 3 non-inferiority trial[J]. Lancet Oncol, 2014, 15 (12):1303−1310. doi: 10.1016/S1470−2045(14)70460−7.

[14] Bernet L, Cano R, Martinez M, et al. Diagnosis of the sentinel lymph node in breast cancer: a reproducible molecular method: a multicentric Spanish study[J]. Histopathology, 2011, 58(6): 863−869. doi: 10.1111/j.1365−2559.2011.03836.x.

[15] Khaddage A, Berremila SA, Forest F, et al. Implementation of molecular intra-operative assessment of sentinel lymph node in breast cancer[J]. Anticancer Res, 2011, 31(2):585−590.

[16] Shimazu K, Miyake T, Okuno J, et al. One-step Nucleic Acid Amplification Can Identify Sentinel Node-negative Breast Cancer Patients With Excellent Prognosis[J]. Anticancer Res, 2019, 39(3): 1447−1454. doi: 10.21873/anticanres.13261.

[17] Watkins EJ. Overview of breast cancer[J]. JAAPA, 2019, 32(10): 13−17. doi: 10.1097/01.JAA.0000580524.95733.3d.

[18] Zahoor S, Haji A, Battoo A, et al. Sentinel Lymph Node Biopsy in Breast Cancer: A Clinical Review and Update[J]. J Breast Cancer, 2017, 20(3):217−227. doi: 10.4048/jbc.2017.20.3.217.

[19] Chen JJ, Chen JY, Yang BL, et al. Comparison of molecular analysis and touch imprint cytology for the intraoperative evaluation of sentinel lymph nodes in primary breast cancer: results of the China Breast Cancer Clinical Study Group (CBCSG) 001c trial[J]. Eur J Surg Oncol, 2013, 39(5): 442−449. doi: 10.1016/j.ejso.2013.02.005.

[20] Gherghe M, Bordea C, Blidaru A. Sentinel lymph node biopsy (SLNB) vs. axillary lymph node dissection (ALND) in the current surgical treatment of early stage breast cancer[J]. J Med Life, 2015, 8(2):176−180.

[21] Imoto S, Yamauchi C, Komoike Y, et al. Trends in axillary treatment for breast cancer patients undergoing sentinel lymph node biopsy as determined by a questionnaire from the Japanese Breast Cancer Society[J]. Breast Cancer, 2017, 24(3): 427−432. doi: 10.1007/s12282−016-0721−4.

[22] Garcia-Tejedor A, Falo C, Quetglas C, et al. Feasibility, accuracy and prognosis of sentinel lymph node biopsy before neoadjuvant therapy in breast cancer. A prospective study[J]. Int J Surg, 2017, 39:141−147. doi: 10.1016/j.ijsu.2017.01.106.

[23] 王骥, 吴瑞, 刘晓, 等. 吲哚菁绿联合亚甲蓝双示踪法在国内乳腺癌前哨淋巴结活检中应用价值的Meta分析[J]. 中国普通外科杂志2020,29(5):532−542.doi:10.7659/j.issn.1005−6947.2020.05.003.
Wang J, Wu R, Liu X, et al. Application value of dual-tracer method of indocyanine green plus methylene blue in sentinel lymph node biopsy of breast cancer in China: a Metaanalysis[J]. Chinese Journal of General Surgery, 2020, 29(5): 532−542. doi: 10.7659/j.issn.1005−6947.2020.05.003.

[24] Godazande G, Moradi S, Naghshvar F, et al. Is Necessary Intraoprative Frozen Section In Sentinel Lymph Node Biopsy For Breast Cancer Patients?[J]. Asian Pac J Cancer Prev, 2020, 21(3): 647−651. doi: 10.31557/APJCP.2020.21.3.647

[25] Liu LC, Lang JE, Lu Y, et al. Intraoperative frozen section analysis of sentinel lymph nodes in breast cancer patients: a meta-analysis and single-institution experience[J]. Cancer, 2011, 117(2):250−258. doi: 10.1002/cncr.25606.

[26] Lanitis S, Peristeraki S, Chortis P, et al. The value of the intraoperative assessment of the SLN via frozen section in the post Z0011 era[J]. J Gynecol Obstet Hum Reprod, 2020, 50(8):101991. doi: 10.1016/j.jogoh.2020.101991.

[27] Shojaee L, Abedinnegad S, Nafisi N, et al. Sentinel Node Biopsy in Early Breast Cancer Patients with Palpable Axillary Node[J]. Asian Pac J Cancer Prev, 2020, 21(6): 1631−1636. doi: 10.31557/APJCP.2020.21.6.1631.

[28] Uno Y, Akiyama N, Yuzawa S, et al. The value and practical utility of intraoperative touch imprint cytology of sentinel lymph node(s) in patients with breast cancer: A retrospective cytology-histology correlation study[J]. Cytojournal, 2020, 17: 11. doi: 10.25259/Cytojournal_80_2019.

[29] Pétursson HI, Kovács A, Mattsson J, et al. Evaluation of intraoperative touch imprint cytology on axillary sentinel lymph nodes in invasive breast carcinomas, a retrospective study of 1227 patients comparing sensitivity in the different tumor subtypes[J]. PLoS One, 2018, 13(4): e0195560. doi: 10.1371/journal.pone.0195560.

[30] Hashmi AA, Naz S, Ahmed O, et al. Diagnostic Accuracy of Intraoperative Touch Imprint Cytology for the Diagnosis of Axillary Sentinel Lymph Node Metastasis of Breast Cancer: Comparison With Intraoperative Frozen Section Evaluation[J]. Cureus, 2021, 13(1):e12960. doi: 10.7759/cureus.12960.

[31] 中国抗癌协会乳腺癌专业委员会. 中国抗癌协会乳腺癌诊治指南与规范(2019年版)[J]. 中国癌症杂志, 2019, 29(8):609−679. doi:10.19401/j.cnki.1007−3639.2019.08.009.
Breast Cancer Society of Chinese Anti-Cancer Association. Guidelines and standardization of diagnosis and treatment of breast

cancer of Chinese Anti-Cancer Association (2019 edition)[J]. China Oncology, 2019, 29(8): 609−679. doi: 10.19401/j. cnki. 1007−3639.2019.08.009.

[32] Costin AI, Păun I, Vârcuş F, et al. Intraoperative assessment of sentinel lymph nodes in early-stage breast cancer[J]. Rom J Morphol Embryol, 2018, 59(4):1033−1039.

[33] Schmitt AO, Specht T, Beckmann G, et al. Exhaustive mining of EST libraries for genes differentially expressed in normal and tumour tissues[J]. Nucleic Acids Res, 1999, 27(21): 4251−4260. doi: 10.1093/nar/27.21.4251.

[34] Tsujimoto M, Nakabayashi K, Yoshidome K, et al. One-step nucleic acid amplification for intraoperative detection of lymph node metastasis in breast cancer patients[J]. Clin Cancer Res, 2007, 13 (16):4807−4816. doi: 10.1158/1078−0432.CCR−06−2512.

[35] Horimoto Y, Tanabe M, Kazuno S, et al. Elucidation of inhibitory effects on metastatic sentinel lymph nodes of breast cancer during One-Step Nucleic Acid Amplification[J]. Sci Rep, 2018, 8(1):7563. doi: 10.1038/s41598−018-25911-w.

[36] Shigematsu H, Ozaki S, Yasui D, et al. Comparison of CK-IHC assay on serial frozen sections, the OSNA assay, and in combination for intraoperative evaluation of SLN metastases in breast cancer[J]. Breast Cancer, 2018, 25(2):191−197. doi: 10.1007/s12282−017-0811-y.

[37] Santaballa A, De La Cueva H, Salvador C, et al. Advantages of one step nucleic acid amplification (OSNA) whole node assay in sentinel lymph node (SLN) analysis in breast cancer[J]. Springerplus, 2013, 2:542. doi: 10.1186/2193−1801−2−542.

[38] Shi F, Liang Z, Zhang Q, et al. The performance of one-step nucleic acid amplification assay for intraoperative detection of sentinel lymph node macrometastasis in breast cancer: An updated meta-analysis[J]. Breast, 2018, 39: 39−45. doi: 10.1016/j.breast.2018.03.005.

[39] Fougo JL, Amendoeira I, Brito MJ, et al. Sentinel node total tumour load as a predictive factor for non-sentinel node status in early breast cancer patients - The porttle study[J]. Surg Oncol, 2020, 32: 108−114. doi: 10.1016/j.suronc.2019.11.008.

[40] Szychta P, Westfal B, Maciejczyk R, et al. Intraoperative diagnosis of sentinel lymph node metastases in breast cancer treatment with one-step nucleic acid amplification assay (OSNA) [J]. Arch Med Sci, 2016, 12(6):1239−1246. doi: 10.5114/aoms.2016.62902.

[41] Hintzen KFH, de Rooij L, Schouten N, et al. Molecular analysis of sentinel lymph nodes in patients with breast cancer using one-step nucleic acid amplification (OSNA): Does not lead to overtreatment in the current era of de-escalating axillary management[J]. Surg Oncol, 2020, 35:224−228. doi: 10.1016/j.suronc.2020.09.010.

[42] Cserni G. Intraoperative analysis of sentinel lymph nodes in breast cancer by one-step nucleic acid amplification[J]. J Clin Pathol, 2012, 65(3):193−199. doi: 10.1136/jclinpath-2011−200301.

[43] Laia BV, Marcos MB, Refael CM, et al. Molecular diagnosis of sentinel lymph nodes for breast cancer: one step ahead for standardization[J]. Diagn Mol Pathol, 2011, 20(1): 18−21. doi: 10.1097/PDM.0b013e3181eb9b30.

[44] Cuffolo G, Gahir-Atwal HK, Smith B. One-step nucleic acid amplification CK19 copy number for sentinel node biopsy in breast cancer: Identification of new cutoffs to predict nonsentinel axillary node involvement[J]. Breast J, 2020, 26(10): 2002−2005. doi: 10.1111/tbj.13977.

[45] Abe M, Kagara N, Miyake T, et al. Highly sensitive detection of sentinel lymph node metastasis of breast cancer by digital PCR for RASSF1A methylation[J]. Oncol Rep, 2019, 42(6):2382−2389. doi: 10.3892/or.2019.7363.

[46] Peg V, Sansano I, Vieites B, et al. Role of total tumour load of sentinel lymph node on survival in early breast cancer patients[J]. Breast, 2017, 33:8−13. doi: 10.1016/j.breast.2017.02.011.

[47] Peña KB, Kepa A, Cochs A, et al. Total Tumor Load of mRNA Cytokeratin 19 in the Sentinel Lymph Node as a Predictive Value of Axillary Lymphadenectomy in Patients with Neoadjuvant Breast Cancer[J]. Genes (Basel), 2021, 12(1): 77. doi: 10.3390/genes12010077.

[48] Batt J, Schrire T, Rayter Z. Can one stop nucleic acid sampling (OSNA) predict nodal positivity following neoadjuvant chemotherapy? A prospective cohort study of 293 patients[J]. Breast J, 2021, doi: 10.1111/tbj.14233. [Online ahead of print]

[49] Vieites B, López-García MÁ, Martín-Salvago MD, et al. Predictive and prognostic value of total tumor load in sentinel lymph nodes in breast cancer patients after neoadjuvant treatment using one-step nucleic acid amplification: the NEOVATTL study[J]. Clin Transl Oncol, 2021.doi:10.1007/s12094−020−02530−4.[Onlineaheadofprint]

[50] Espinosa-Bravo M, Navarro-Cecilia J, Ramos Boyero M, et al. Intraoperative assessment of sentinel lymph node by one-step nucleic acid amplification in breast cancer patients after neoadjuvant treatment reduces the need for a second surgery for axillary lymph node dissection[J]. Breast, 2017, s31: 40−45. doi: 10.1016/j.breast.2016.10.002.

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