DOI: https://doi.org/10.17816/uroved58165

非肌层浸润性膀胱癌的光动力荧光诊断研究

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本文就光动力学在非肌层浸润性膀胱癌诊断中的应用作一综述。介绍了光敏剂作用机理的现代数据，荧光膀胱镜检查的方法，在实际医学中使用光动力学诊断的临床研究结果。结果表明，与标准膀胱镜检查相比，光动力诊断显著提高了膀胱癌的检测效率。这种方法在原位癌（carcinoma *in situ*）和多局灶尿路上皮肿瘤的病例中尤其有价值。提高诊断水平可以提高手术治疗的根治性和延长无复发期。

关键词： 非肌层浸润性膀胱癌；荧光膀胱镜检查；光敏剂；光动力诊断。

引用本文：
Kasymov BG, Shanazarov NA, Muratov TM, Daniyarova GD, Zhumakayev AM, Kyzlasov PS, Mustafayev AT, Slesarevskaya MN, Kuzmin IV, Al-Shukri SKh. 非肌层浸润性膀胱癌的光动力荧光诊断研究. *Urology reports (St. Petersburg).* 2021;11(2):163-174. DOI: https://doi.org/10.17816/uroved58165

收稿日期： 2021年1月13日 **审稿日期：** 2021年6月4日 **出版时间：** 2021年6月23日

DOI: https://doi.org/10.17816/uroved58165

Photodynamic diagnostics of nonmuscle invasive bladder cancer

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The review is devoted to the application of photodynamic diagnosis of non-muscle invasive bladder cancer. The recent data on the mechanism of action of photosensitizers, the method of performing fluorescence cystoscopy are presented, and the results of clinical studies of the application of photodynamic diagnostics in practical medicine are presented. It has been shown that photodynamic diagnostics significantly increases the efficiency of detecting bladder cancer in comparison with standard cystoscopy. The application of this method is especially valuable in cases of carcinoma in situ and multifocal growth of urothelial tumors. Improvement in diagnostics makes it possible to increase the radicality of surgical treatment and to increase the duration of the relapse-free period.

Keywords: non-muscle invasive bladder cancer; fluorescence cystoscopy; photosensitizer; photodynamic diagnostics.

To cite this article:
Kasymov BG, Shanazarov NA, Muratov TM, Daniyarova GD, Zhumakayev AM, Kyzlasov PS, Mustafayev AT, Slesarevskaya MN, Kuzmin IV, Al-Shukri SKh. Photodynamic diagnostics of nonmuscle invasive bladder cancer. *Urology reports (St. Petersburg)*. 2021;11(2):163-174. DOI: https://doi.org/10.17816/uroved58165

Received: 13.01.2021 **Accepted:** 04.06.2021 **Published:** 23.06.2021

ECO•VECTOR

DOI: https://doi.org/10.17816/uroved58165

Фотодинамическая диагностика немышечно-инвазивного рака мочевого пузыря

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Обзорная статья посвящена применению фотодинамической диагностики немышечно-инвазивного рака мочевого пузыря. Приведены современные данные о механизме действия фотосенсибилизаторов, методике выполнения флуоресцентной цистоскопии, представлены результаты клинических исследований применения фотодинамической диагностики в практической медицине. Показано, что фотодинамическая диагностика существенно увеличивает эффективность выявления рака мочевого пузыря по сравнению со стандартной цистоскопией. Особенно ценным представляется применение данного метода в случаях carcinoma *in situ* и мультифокальном росте уротелиальных опухолей. Улучшение диагностики позволяет повысить радикальность хирургического лечения и увеличить длительность безрецидивного периода.

Ключевые слова: немышечно-инвазивный рак мочевого пузыря; флуоресцентная цистоскопия; фотосенсибилизатор; фотодинамическая диагностика.

Как цитировать:
Касымов Б.Г., Шаназаров Н.А., Муратов Т.М., Даниярова Г.Д., Жумакаев А.М., Кызласов П.С., Мустафаев А.Т., Слесаревская М.Н., Кузьмин И.В., Аль-Шукри С.Х. Фотодинамическая диагностика немышечно-инвазивного рака мочевого пузыря // Урологические ведомости. 2021. Т. 11. № 2. С. 163–174. DOI: https://doi.org/10.17816/uroved58165

Рукопись получена: 13.01.2021 **Рукопись одобрена:** 04.06.2021 **Опубликована:** 23.06.2021

ЭКО•ВЕКТОР

膀胱癌（BC）是泌尿系统最常见的肿瘤和最常见的恶性肿瘤之一，在男性肿瘤病理结构中占第7位，在女性肿瘤病理结构中占第17位[1]。在俄罗斯联邦，膀胱癌发病率为每年每10万人77.1例[2]。约75%的初诊患者为浅表性或非肌层浸润性膀胱癌（NMIBC），对应于Ta、T1、Tis分期，未见肌组织浸润[3]。在40岁以下的患者中，膀胱癌在无创期的检出率甚至更高[4]。

经尿道膀胱肿瘤切除术仍是治疗非肌层浸润性膀胱癌的主要方法[5]。同时，经尿道膀胱肿瘤切除术后肿瘤过程的复发率非常显著，12个月内复发率达到50%，5年内复发率达到78%，5年后膀胱癌进展的可能性为45%[6,7]。原发性经尿道膀胱肿瘤切除术后非肌层浸润性膀胱癌的高复发率与多种因素有关。主要是肿瘤的恶性程度，膀胱粘膜病变的多中心性，有无未被发现的*原位癌*灶，以及经尿道切除术中肿瘤细胞是否可能植入[8,9]。

肿瘤的早期诊断对膀胱癌患者的及时有效治疗起着决定性的作用。在常规的临床实践中，膀胱癌的初步检测，尿液细胞学，超声和膀胱镜检查与膀胱壁活检是[10]。

近150年来，膀胱镜检查一直在膀胱肿瘤的检测中发挥着主导作用。1878年，德国泌尿科医生Maximilian Nitze和奥地利医疗器械制造商Josef Leiter共同提出了第一台膀胱镜[11]。即使考虑到他们提出的膀胱镜在技术上的缺陷，Nitze和Leiter的发明的意义是很难被高估的。最大的问题是确保膀胱有足够的照明，在第一次膀胱镜中，钨丝被用于这个目的。未来，随着技术的发展，膀胱镜的光学性能得到了根本性的改善，提供了膀胱壁的高度可视化。当使用现代仪器，标准膀胱镜或白光膀胱镜时，可以发现大于3 mm的肿瘤。同时，粘膜扁平内皮病变（不典型增生、*原位癌*）和小肿瘤往往不被注意，30-75%的膀胱癌患者经尿道切除术后（2-6周）假复发[12-15]。此外，标准的白光膀胱镜检查往往不能区分炎症和肿瘤的变化，也不能准确判断肿瘤的边界和浸润深度[16-18]。因此，提高标准膀胱镜检查在膀胱癌检出方面的有效性，已被泌尿外科界公认为肿瘤学最重要的问题之一。在这个方向上的大量研究导致了膀胱癌光学成像新技术的出现。

实施的技术解决方案可以有条件地分为宏观、微观和分子三大类[18,19]。宏观方法包括光动力荧光诊断（PDD）、内镜窄带成像术（NBI）和内窥镜摄像系统（IMAGE 1 SPIES—Storz Professional Image Enhancement System）。这些诊断技术本质上与标准的白光膀胱镜检查相似，但具有显著更高的诊断效率[15]。显微成像方法，如共聚焦激光显微内窥镜和光学相干断层扫描，可以在细胞和亚细胞水平上以高分辨率实时检查膀胱黏膜，类似于组织学，这使我们可以把它们看作是一种光活检。分子方法是基于光学成像技术与肿瘤特异性分子试剂（如抗体）的荧光标记相结合的方法[20]。

光动力荧光诊断的原理是基于肿瘤细胞对光敏剂的选择性积累和当光敏剂被蓝光激发时的特征红光的检测[21]。关于光动力荧光诊断的可能性的研究是由J.F.Kelly和M.E.Snell（1976）的研究发起的。他们在切除膀胱的实验中证明了膀胱移行细胞肿瘤细胞中荧光的存在。静脉注射2 mg/kg血卟啉24小时后，*原位癌*灶（Cis）、粘膜异常增生和外生性肿瘤出现鲜红光晕，而膀胱正常粘膜及无血管肿瘤未见红光[22]。1982年，第一次进行了荧光膀胱镜检查，并使用了荧光支气管镜检查装置[23]。20世纪90年代，人们开始研究5-氨基乙酰丙酸（5-ALA）用于膀胱肿瘤光动力荧光诊断的可能性。该物质作为光敏剂的前体，是血红素合成的中间产物之一[24]。过量给药5-ALA可抑制血红素合成的最后阶段，抑制其前体—内源性原卟啉IX（Pp IX）的积累。在未改变的组织中，内源性原卟啉IX的铁螯合酶作用下，迅速被利用转化为血红素。肿瘤细胞中缺乏这种酶，导致内源性原卟啉IX水平暂时但显著升高。这导致了肿瘤和正常组织之间内源性原卟啉IX的高荧光对比，可达到10-15倍的值[25]。

对于膀胱肿瘤的光动力荧光诊断，在膀胱内注射光敏剂，然后它们被尿路上皮吸收并包含在细胞的血红素生物合成中，导致异常细胞内光活性卟啉的积累[13,15,26-32]。在蓝光（380-480 nm）的影响下，发育不良细胞会发出特有的红色荧光，这种红色荧光在未改变的尿路上皮的蓝色背景下很容易被观察到[28]。由于5-ALA活性形式的不稳定性，该物质以盐酸（Alasense药物）的形式产生。立即灌注前，将1.5 g Alasense用50毫升1.4%磷酸一氢钠稀释后注入已排空的膀胱。

在进行荧光膀胱镜检查之前，5-ALA暴露在膀胱中的时间尚未达成共识。因此，在S.H.Al-Shukri等人[33]的研究中，暴露时间为60分钟，然后持续120分钟，即使在膀胱排空后，由于5-ALA扩散到膀胱肿瘤细胞，内源性内源性原卟啉IX也会增加。N.A.Lopatkin等人[34]没有明确5-ALA溶液在膀胱内持续时间的时间参数：接触时间从1.5小时到3小时不等，平均为114.1分钟，而研究人员还允许接触时间减少到60-80分钟。

内源性原卟啉IX在蓝光下的荧光持续时间为10-20分钟[25,33]，然后荧光强度显著降低。这个过程叫光漂白。同时，蓝光下其速度明显高于白光，且与观测距离和光通量强度成正比。

DOI: https://doi.org/10.17816/uroved58165

大量的临床研究结果证实了5-ALA作为光敏剂的光动力荧光诊断膀胱癌的有效性[13,15,17,28-32,35,36]。I.Kausch等人[31]的一项系统综述表明，与标准膀胱镜检查的46-80%相比，光动力荧光诊断测膀胱癌的灵敏度在76-97%之间。两项荟萃分析的作者也证实了光动力荧光诊断的有效性，其中一项荟萃分析回顾了涉及900例患者的8项研究[15]，另一项荟萃分析回顾了涉及2807例患者的27项研究[32]。根据这些研究结果得出的一般结论是，光动力荧光诊断在检测膀胱癌方面的灵敏度比标准膀胱镜高20%。与此同时，其他两项研究并没有显示使用光动力荧光诊断比传统细胞镜检的膀胱癌检出率更高[37,38]。

2006年，欧洲泌尿外科医师协会推荐光动力荧光诊断方法用于Cis的诊断，同时注意到该技术的使用使Cis的检测提高了23%[31,32,39]。在A.M.Kamat等人的研究中[40]，41例患者中，有13例（32%）仅在光动力荧光诊断的帮助下检测到Cis。

光动力荧光诊断的另一个重要应用领域是发现复发风险高的膀胱癌。G.Mowatt等人[32]的系统综述和荟萃分析显示，在诊断高危肿瘤时，荧光膀胱镜的敏感性为89%，而白光膀胱镜的敏感性为56%。对于低复发风险的肿瘤，荧光膀胱镜的敏感性几乎与标准膀胱镜相似（92%比95%）[32]。光动力荧光诊断在多灶性膀胱肿瘤患者中被证明是非常有效的，这也可以被认为是其实施的指示[17]。

随着光动力荧光诊断应用经验的积累，我们发现5-ALA在生理pH下的亲水性和低脂肪溶解性等生化特性导致其生物利用度不足，在膀胱镜检查时组织荧光（光漂白）迅速消失，这限制了5-ALA在临床实践中的使用[3]。

通过使用另一种化合物—氨基乙酰丙酸己酯（HAL—hexylaminolevulinate）克服了这些限制，该化合物具有较高的脂肪溶解性，并能被尿路上皮细胞快速均匀地吸收。在尿路细胞内，氨基乙酰丙酸己酯转化为5-ALA，提供光动力荧光诊断[28,41]。

许多研究已经证实了氨基乙酰丙酸己酯的光动力荧光诊断的有效性，特别是在Cis检测方面。该技术检测膀胱肿瘤的灵敏度为49-100%[3,15,42]。与标准膀胱镜检查相比，氨基乙酰丙酸采用光动力荧光诊断的顺式检测频率约高25-30%[15,29,30]。

R.Chou等人[43]的系统综述分析了14项随机试验的结果，评价光动力荧光诊断的有效性，使用5-ALA（6项研究）和氨基乙酰丙酸己酯（9项研究）与白光膀胱镜在原发性或复发性经尿道膀胱肿瘤切除术后的诊断进行了比较。共有2906名患者参与了这些研究。研究结果分析表明，与白光膀胱镜相比，荧光膀胱镜在短期（3个月不到）和长期（12个月以上）可导致膀胱癌复发频率的降低。与此同时，膀胱癌的进展率和死亡率没有差异。然而，为了证实这些结果，似乎有必要进行额外的长期观察研究，以了解光敏剂在膀胱癌进展中的作用机制[43]。

在I.G.Rusakov等人[44]的研究中，对198例膀胱恶性病变患者进行了荧光膀胱镜检查—原发性（n=67）和复（n=131）膀胱移行细胞癌。以5-ALA为基础开发的药物Alasense作为光敏剂。制备3% Alacense无菌溶液进行膀胱给药，光敏剂灌注后2-3小时进行光动力荧光诊断。局部荧光光谱法测量的膀胱癌灶相对于正常粘膜的荧光对比度λ=408 nm最大，其值在10至35之间（平均约15）。在λ=532 nm时，荧光对比度从2到20（平均约6）。非特异性荧光病灶（炎症、中度异常增生、移行细胞乳头状瘤）荧光对比在λ=408 nm处不超过4，λ=532 nm处不超过2。作者认为，利用局部荧光光谱法测量肉眼荧光病灶的荧光光谱，定量评估尿路上皮荧光，提高了光动力学诊断膀胱肿瘤的信息价值和有效性。

在俄罗斯进行的一项多中心临床研究中，研究了使用Hexacene光敏剂（HAL）光动力荧光诊断膀胱肿瘤的有效性，并与标准膀胱镜进行了比较[45]。124例患者行膀胱内灌注0.2%Hexacene溶液50 ml，暴露1-2小时。从膀胱取出药物后，所有患者都接受标准膀胱镜检查，然后进行荧光诊断。局部荧光光谱显示在膀胱给Hexacene药后1小时内，Hexacene诱导的内源性原卟啉IX在肿瘤中的荧光水平比其在周围健康粘膜中的荧光水平平均高出5.8倍。与标准膀胱镜检查结果相比，光动力荧光诊断膀胱癌的有效性提高了24.4%（从75.6%到100%），诊断准确率提高了15.2%（从83.3%到98.5%），阴性预后值提高了33.5%（从66.5%到100%）。荧光诊断允许27.4%的患者额外识别白光无法检测到的肿瘤病灶。在4.0%的患者中，在荧光诊断时膀胱粘膜出现假阳性荧光，可能是由于炎症过程。接受活性剂量Hexacene药物的患者中没有出现不良反应、总体健康状况改变、血液和尿液检测[45]。

在The First Pavlov State Medical University of St. Petersburg泌尿系进行的一项研究中，比较了使用第二代光敏剂Fotoditazin的荧光膀胱镜和标准膀胱镜检测非肌层浸润性膀胱癌的诊断效果[8]。为了进行光动力荧光

DOI: https://doi.org/10.17816/uroved58165

诊断，将5mg Fotoditazin溶解在20ml盐水溶液中注射到膀胱。药物在膀胱内的暴露时间为60至90分钟。然后在白光和蓝光下依次行膀胱镜检查。根据本研究结果，标准膀胱镜在白光下的诊断灵敏度为62.3%，Fotoditazin的光动力学诊断为96.7%[8]。

S.I.Gorelov等人[46]对Fotoditazin和Alacense在膀胱癌光动力荧光诊断中的应用进行了比较评估。作者观察了144名膀胱癌患者，将他们分为三组。第一组20例患者膀胱内注射Alacense1.5 g光敏剂；第二组48例患者光敏剂为Fotoditazin，静脉给药剂量为0.7-1.4 mg/kg体重；第三组76例患者经膀胱内注射Fotoditazin，剂量为10.0 mg。所有给药方法的照射时间为1.5-2小时，照射后进行光动力荧光诊断。当发现肿瘤或膀胱粘膜荧光区域时，进行经尿道切除术。根据研究结果，作者得出结论，Fotoditazin和Alacense可以用于膀胱肿瘤的荧光诊断，因为它们在诊断膀胱乳头状病变和扁平病变时的敏感性和特异性相当，而且Fotoditazin荧光更强烈。此外，Fotoditazin可通过静脉和膀胱给药，但膀胱给药剂量比静脉给药少5倍。膀胱肿瘤的光动力荧光诊断与静脉注射Fotoditazin无显著差异。

在K.M.Gallaghera等人[47]的研究中，分析了345例原发性非肌层浸润性膀胱癌的治疗结果。将患者分为两组：第1组（*n*=153）行白光下经尿道膀胱肿瘤切除术，第2组（*n*=192）行荧光下经尿道膀胱肿瘤切除术。在无禁忌症的情况下，所有患者在经尿道膀胱肿瘤切除术后均行丝裂霉素C灌注24小时。当评估结果时，经尿道膀胱肿瘤切除术与光动力荧光诊断的优势被证明。同时，光动力荧光诊断组（*n*=192）的无复发生存率为52.9个月，而白光下光动力荧光诊断组（*n*=153）的无复发生存率为42.4个月。在随访的第1年和第3年，采用光动力荧光诊断的组复发率较低（21.5%和38.9%），而在采用常规膀胱镜的组中，这些指标分别为39.0%和53.3%。此外，作者还指出，光动力荧光诊断的优势并不取决于进行手术的外科医生的经验[47]。M.R.Lykke等人[48]也证实了通过光动力荧光诊断进行经尿道膀胱肿瘤切除术后膀胱癌复发频率降低了41%。国内研究证实了光动力荧光诊断经尿道膀胱肿瘤切除术在膀胱癌患者中的有效性[49]。

到目前为止，有出版物表明光动力荧光诊断具有积极的临床和经济效果。尽管引入光动力荧光诊断会增加手术成本，但从长期来看，通过减少膀胱癌的复发数量，可以显著节省成本[50]。P.U.Malmström等人[51]，分析了2032例初诊膀胱癌患者的治疗结果。他们发现，在初次经尿道膀胱肿瘤切除术期间使用HAL进行荧光膀胱镜检查，并在诊断后一年内对所有随后的经尿道膀胱肿瘤切除术患者拒绝进行23例膀胱切除术和180例经尿道膀胱肿瘤切除术。

与标准膀胱镜检查相比，光动力荧光诊断法在检测膀胱癌方面具有更高的灵敏度，这是毫无疑问的，并被大量研究的结果所证实[32]。同时，光动力荧光诊断的特异性略低于白光膀胱镜检查（63%比81%）[31]。这是由于粘膜在有限区域出现假阳性辉光，这可能是由以下几个原因引起的，特别是膀胱壁的炎症过程，包括最近的经尿道切除术后，以及卡介苗灌注（BCG—Bacillus Calmette-Guérin），特别是在实施这些手术后的前三个月[52,53]。D.Zlatev等人[54]估计膀胱癌光动力荧光诊断假阳性结果的频率在10-12%的病例中，并将其与内源性组织荧光团的自身荧光和免疫细胞积累光敏剂的高能力相关。因此，不建议对在前90天接受膀胱内免疫治疗或化疗的患者进行光动力荧光诊断[3]。

尿路上皮发育不良，特别是鳞状细胞化生，也是假阳性荧光最常见的原因之一。因此，在膀胱的颈部和尿道的前列腺部分，当粘膜出现这种变化时，几乎总是能观察到非特异性的红光。此外，当膀胱镜与膀胱壁呈锐角时，可能出现假阳性荧光。这种现象被称为切向辉光[55]。有一种简单的方法鉴别切线荧光和病理荧光。要做到这一点，使用活组织切片钳提起粘膜可疑区域，这将改变该区域的蓝光照明角度。如果荧光区域消失，则视此现象为切向荧光。如果红光持续，尽管操作，该区域应视为病理性，应进行经尿道膀胱肿瘤切除术[22]。

结论

与传统膀胱镜检查相比，光动力荧光诊断显著提高了非肌层浸润性膀胱癌的检测效率。准确的诊断可以增加手术治疗的根治性，最终增加无复发期的持续时间。目前，光动力荧光诊断被大多数国家泌尿外科团体推荐用于膀胱癌的诊断，特别是对于复发性膀胱癌、多灶性病变和Cis的高危患者。与此同时，与该技术的使用有关的一些问题还不清楚，需要进一步的研究来了解光敏剂的作用机制，开发新的药物和技术，以减少假阳性结果的数量和增加荧光膀胱镜的特异性。

附加信息

利益冲突作者声明，没有明显的和潜在的利益冲突相关的发表这篇文章。

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