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Optical biopsy of bladder cancer using confocal laser endomicroscopy

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Abstract

Objective Confocal laser endomicroscopy (CLE) is an emerging endoscopic technique that can provide in vivo histopathologic information. It may improve the diagnostic criteria for benign and neoplastic lesions of the bladder. In this study, we reported our experience with utilizing CLE imaging when treating bladder neoplasms, and investigated its diagnostic value with respect to histologic diagnosis.

Materials and methods Twenty-one patients scheduled for diagnostic cystoscopy or transurethral resection of the bladder tumor were enrolled prospectively. CLE was performed after intravesical fluorescein administration and confocal video sequences were reviewed and analyzed retrospectively. Histopathology served as reference standard for comparison.

Results Confocal laser endomicroscopy-based classification combined with white light cystoscopy (WLC) images was consistent with histopathology in 17 cases (81.0%). Consensus with histopathological results was found in six cases (85.7%) for low-grade urothelial carcinoma and eight cases (80.0%) for high-grade urothelial carcinoma.

Conclusion Confocal laser endomicroscopy was proved to be a useful technique that could complement white light cystoscopy by providing real-time histopathological information of bladder lesions.

Keywords Bladder cancer · Confocal laser endomicroscopy · Real-time histopathology · Urothelial carcinoma · Non-muscle-invasive bladder carcinoma

Introduction

Bladder cancer is currently the most common genitourinary malignancy worldwide, representing the 11th most commonly diagnosed malignancy [1]. Approximately, 70% of patients present with non-muscle-invasive bladder cancer, whereas the remaining are diagnosed as muscle-invasive bladder cancer [2]. Accurate individualized treatment depends on accurate initial diagnosis, which faces significant challenges. White light cystoscopy (WLC) is a crucial technique in identifying suspicious lesions, and detecting cancer and tumor recurrence in the bladder. However, it has several well-recognized shortcomings including inadequacy to detect flat lesions, especially discriminating between inflammation and malignancy lesions [3]. Although histopathology remains as the gold standard of bladder cancer diagnosis, it is not readily available to provide real-time histopathological information to guide endoscopic intervention.

Several optical imaging techniques have emerged to enhance local cancer management and to stratify treatment options more reasonable. Feasibility studies using fluorescence cystoscopy, narrow band imaging, and optical coherence tomography have been reported [4–6]. Confocal laser endomicroscopy (CLE) is a newly developed endoscopic imaging technique that complements WLC. It enables the endoscopic microscopy of mucosal lesions with real-time imaging of cellular architecture and morphology [7, 8]. In contrast to other imaging technologies, CLE has dynamic microscopic resolution, which constitutes the greatest strength of this technique [9]. The contrast is based on fluorescence excited by a specific laser. Administering a fluorescent label binding to the cells could enhance this fluorescence, thereby allowing visualization of cellular microarchitecture and morphology reminiscent of histology [10]. With current applications in the gastrointestinal tract [11,

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12], CLE use has also been attempted in the urinary tract for tract urothelial carcinoma diagnosis [13]. Although CLE diagnostic criteria for bladder lesion have been proposed, systemic criteria have not been established yet [14].

In this prospective study, we investigated the diagnostic yield of CLE in bladder cancer with the purpose to validate the CLE diagnostic criteria for benign and neoplastic bladder lesions.

Materials and methods

Instrumentation

Confocal laser endomicroscopy of bladder lesion was performed utilizing the Cellvizio clinical system (Cellvizio 100 series; Mauna Kea Technologies, Paris, France), consisting of a desktop computer with software for image processing, a 2.6-mm-diameter fiber-optic imaging probe for image acquisition and a laser scanning unit. The imaging probe was plugged into the working channel of the standard flexible and rigid endoscopes. Fluorescein, a contrast agent with an established safety profile, was used to stain the extracellular matrix and help visualize suspicious lesions. Confocal image was acquired via direct contact of the probe with urothelium and then recorded as video sequences at 12 frames per second (Fig. 1).

Protocol

The study was approved by the institutional ethics committees of Fudan University Shanghai Cancer Center (Shanghai, China).

Patient preparation and contrast agent

Consecutive patients with suspicious bladder mass scheduled for either diagnostic cystoscopy or transurethral resection of the bladder tumor (TURBT) were enrolled in the study and provided consent for CLE and using fluorescein as a contrast agent. After preliminary WLC evaluation, all patients received fluorescein intravesically. A total of 400 mL of 0.1% fluorescein was diluted in saline (0.9%

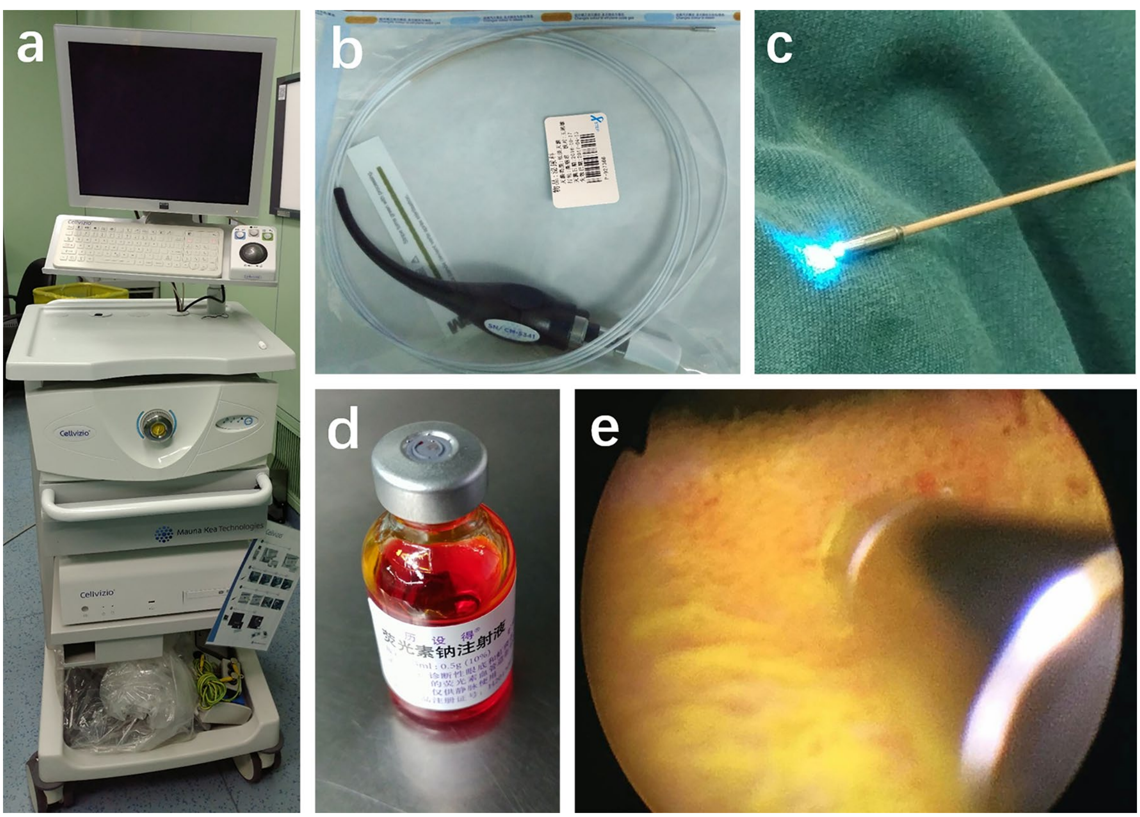

Fig. 1 System of confocal laser endomicroscopy. **a** Clinical-grade CLE system (Cellvizio) from Mauna Kea Technologies, equipped with software for image acquisition and processing. **b**, **c** The 2.6-mm laser probe within the working channel of the cystoscope. **d** Fluorescein, an FDA-approved contrast agent, which can be administered intravenously or topically for CLE imaging. **e** Direct probe contact of a papillary tumor for image acquisition

NaCl), and then instilled into the bladder through a sterile urinary catheter and left indwelling for five minutes.

CLE imaging and training program

Confocal laser endomicroscopy imaging was performed within 2–3 min after intravesical fluorescein administration. Regions of interest were identified when using WLC and consequently scanned by a manipulated CLE imaging probe. Different layers of the bladder epithelium were imaged as the operators varied probe pressure on surface of lesions. Normal- and abnormal-appearing tissue CLE images were reviewed combined with white light endoscopy in real time and recorded for further analysis offline. Finally, the operator biopsied or surgically resected imaged tissue specimens, which were then stained with hematoxylin and eosin (H&E) for corresponding histopathologic diagnosis.

At the start of this study, the observers participated in an online training provided by the company [8]. The course consisted of a teaching set as well as a validation set. Both sets comprise of intraoperative WLC and CLE videos from typical patients undergoing TURBT. Our impression was that a 1-day course is supposed to be enough to complete the learning process.

CLE image evaluation and data analysis

The real-time confocal video sequences were processed offline after preliminary assessment. Processed confocal images were then analyzed using the Cellvizio Viewer software (Mauna Kea Technologies) by two urologists (Yi-Ping Zhu and Jie Wu) independently, according to the proposed CLE features (architectural features: papillary configuration, tissue organization, and vascular features; cellular features: cohesiveness of cells, cellular morphology, definition of cell borders, and polarity of the cells) [8]. The two observers, who were blinded to all clinical and histopathological data, classified suspicious lesions as low-grade urothelial carcinoma, high-grade urothelial carcinoma, or benign lesion according to the World Health Organization 2004 classification based on the CLE features described above. Then, a joined consensus was reached after individual evaluation. Finally, CLE images were compared with the corresponding histopathology of the biopsied or resected tissue to validate the diagnostic accuracy of CLE.

Results

To compile the imaging atlas, we prospectively recruited patients scheduled to undergo TURBT at the Department of Urology, Fudan University Shanghai Cancer Center. 21 patients (mean age 61 years, range 32–81 years) were included in the study, whose clinicopathological characteristics are showed in Table 1. Histopathology of the patients revealed seven low-grade urothelial carcinomas, ten high-grade urothelial carcinomas, two cases of carcinoma in situ (CIS), an inflammation lesion, and one case of leiomyoma.

CLE-based classification combined with WLC images was consistent with histopathology in 17 cases (81.0%). Consensus with histopathological diagnosis was found in six cases (85.7%) for low-grade urothelial carcinoma and eight cases (80.0%) for high-grade urothelial carcinoma. The observers mistakenly diagnosed an inflammation lesion as CIS via CLE, while CLE-based diagnosis of CIS and leiomyoma cases was accurate. Figure 2 shows representative mosaic images of bladder lesions. No intraoperative complications or postoperative complications were recorded. For bladder neoplasm, architectural features and cellular features were utilized to identify benign lesions and low- and high-grade carcinomas [8]. With CLE, high-grade cancer showed cellular pleomorphism and markedly irregular architecture with indistinct cell borders, cohesiveness of cells was lost and sometimes tortuous vessels in fibrovascular stalk could be observed. Yet, low-grade tumor exhibited characteristically organized papillary structures, organized cell pattern and distinct cell borders in contrast to high-grade cancer (Table 2). Furthermore, low-grade urothelial carcinoma exhibited cell polarity,

Table 1 Patient characteristics and diagnoses

Case	Gender	Age	CLE + WLC	Histopathology
1	Male	73	High grade	High grade
2	Female	66	Low grade	Low grade
3	Male	32	Leiomyoma	Leiomyoma
4	Male	46	Low grade	Low grade
5	Male	55	CIS	Inflammation
6	Male	57	Low grade	High grade
7	Male	71	High grade	High grade
8	Female	68	High grade	High grade
9	Male	63	Low grade	Low grade
10	Female	62	Low grade	High grade
11	Male	56	High grade	Low grade
12	Male	72	Low grade	Low grade
13	Male	71	High grade	High grade
14	Male	49	Low grade	Low grade
15	Male	58	Low grade	Low grade
16	Male	81	High grade	High grade
17	Female	72	CIS	CIS
18	Male	49	High grade	High grade
19	Male	65	High grade	High grade
20	Male	63	High grade	High grade
21	Male	60	CIS	CIS

CIS carcinoma in situ

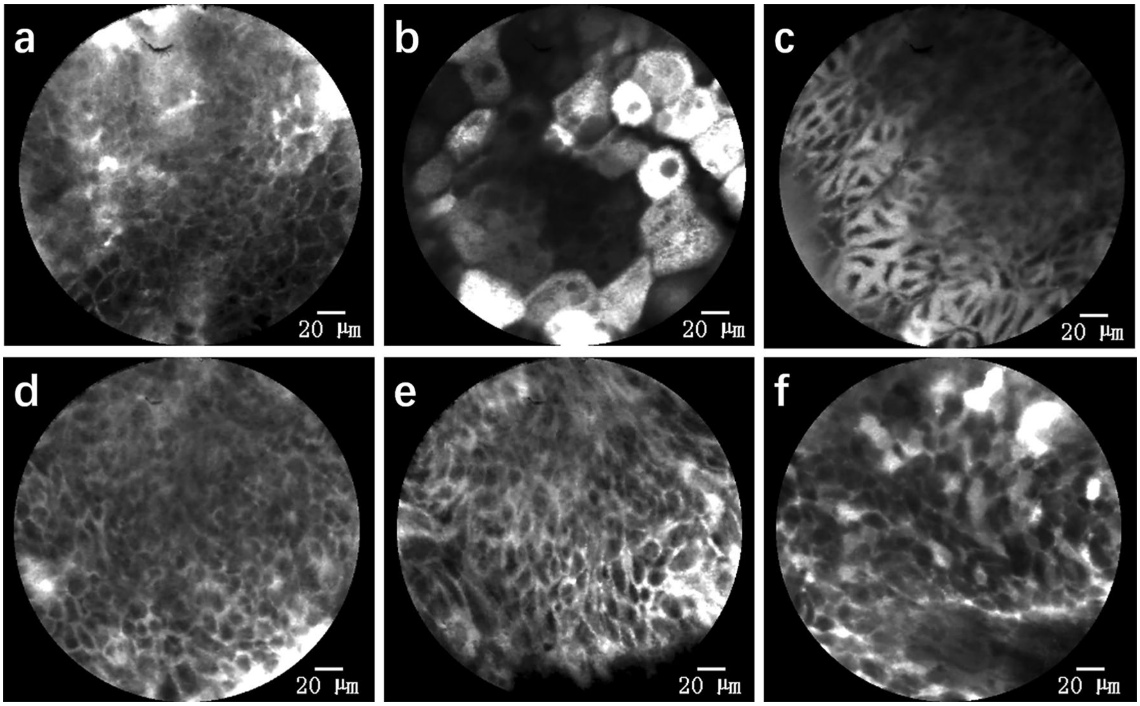

Fig. 2 CLE feature of **a** normal bladder urothelium. **b** Perivesical fat. **c** Leiomyoma of bladder: typical spindle cells. **d** Low-grade urothelial carcinoma: crowding of uniform-appearing cells. **e** High-grade urothelial carcinoma: pleomorphic and distorted sheet of cells. **f** Carcinoma in situ (CIS): large pleomorphic cells, loss of cellular cohesiveness, and indistinct cellular borders

Table 2 CLE image characteristic of bladder cancer

CLE feature	Inflammation	Low-grade UC	High-grade UC	CIS	Leiomyoma
Architectural	Flat	Papillary	Papillary	Flat	Papillary
Vascular features	n/a	Fibrovascular stalk	Tortuous vessels in fibrovascular stalk	n/a	n/a
Organization of cells	n/a	Organized	Disorganized	Disorganized	Disorganized
Cohesiveness of cells	Small, clustered cells	Cohesive	Not cohesive	Not cohesive	Spindle cells
Cellular morphology	Monomorphic	Monomorphic	Pleomorphic	Pleomorphic	Monomorphic
Definition of cell borders	Distinct	Distinct	Indistinct	Indistinct	Distinct
Polarity of cells	n/a	Present	Not present	Not present	n/a

UC urothelial carcinoma, *CIS* carcinoma in situ

which was defined by Leim et al. as a relative orientation of cells and nuclei in the same direction [10].

Bladder leiomyoma is a rare and benign mesenchymal tumor. Characteristic histopathological feature of this neoplasm is spindle cell proliferation with focal areas of chronic inflammation [15]. In this study, we explored CLE image characteristics of a bladder leiomyoma, which was compared to corresponding WLC images and H&E staining from a subsequent biopsy (Fig. 3). With CLE, bladder leiomyoma showed homogeneous, dark gray spindle cells, with distinct cell borders.

Discussion

Confocal laser endomicroscopy is a newly developed endoscopic imaging technology which can provide in vivo tissue histopathology during endoscopy. It can generate a 500–1000 magnification of dynamic histological images of tissue with high accuracy, a so-called “optical biopsy”. CLE was first applied for screening and surveillance of colorectal and gastric cancers, Barrett’s esophagus, and *H. pylori*-associated gastritis [16]. Soon afterwards, the

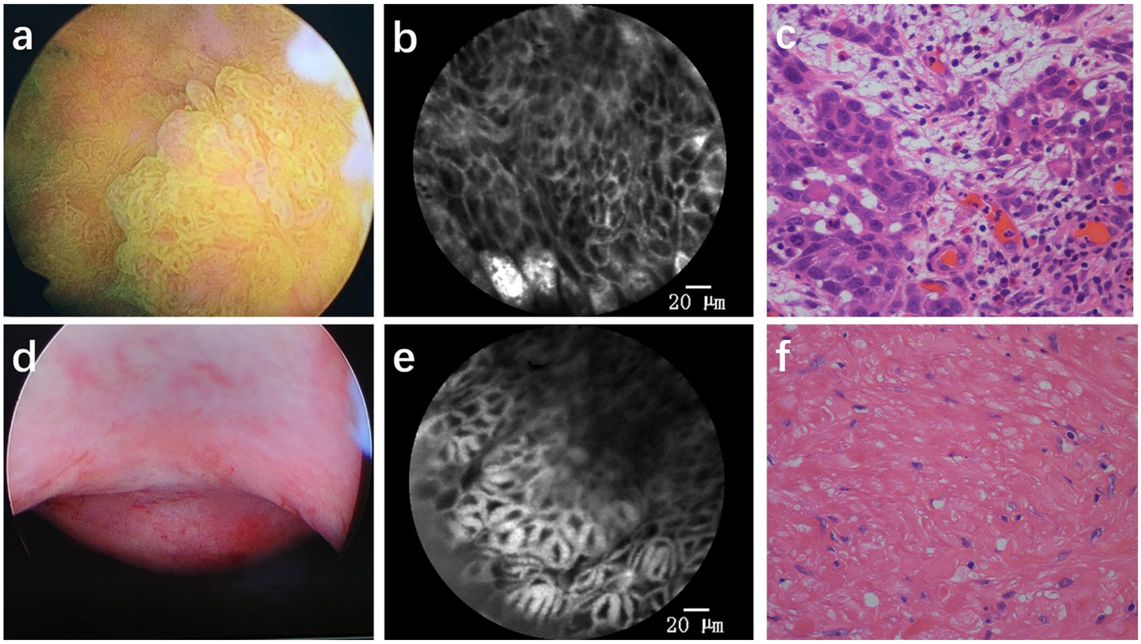

Fig. 3 Characteristic in vivo microscopy features of urothelial neoplasms is shown with corresponding WLC images and hematoxylin and eosin (H&E) staining from subsequent biopsy. **a**–**c** High-grade urothelial carcinoma, CLE showed disorganized cell pattern, with pleomorphic cells and indistinct cell borders. **d**–**f** Leiomyoma, CLE showed crowding of monomorphic spindle cells, with distinct cell borders

diagnostic scope of CLE expanded to pulmonology, neurosurgery, as well as urology [17–19].

The relatively high recurrence rate and long-term survival make bladder cancer one of the most expensive malignancies to manage. Recurrence and progression are more common in high-grade tumors, representing a large public health burden [2]. In addition, some apparent early tumor recurrences may not represent true recurrences of completely resected tumors. In fact, inadequate visualization of WLC could lead to missed diagnosis or inadequate initial resection. Hence, visual detection of bladder cancer and identification of high-grade tumors are crucial for its management. Thus, accurate CLE criteria for bladder cancer grading are of significant beneficial during the decision-making process. And the relevant patients could reap huge fruits from a conservative management of bladder cancer.

Sonn et al. [20] described the CLE features of normal urothelium, and low- and high-grade urothelial carcinomas for the first time in 2009. In 2013, Chang et al. [8] proposed diagnostic criteria for bladder lesions based on CLE and in 2018, Liem et al. [10] validated and renovated the criteria.

In the current study, we described the previously proposed CLE diagnostic criteria for bladder lesions with histopathological correlation. Due to unreliability of the purely WLC-based diagnosis, we did not make a WLC-based tumor evaluation. The CLE-based classification combined with WLC evaluation was in accordance with histopathological diagnosis in 81.0% of the cases, authenticating the significant value of CLE in real-time bladder cancer stratification. Notably, we first explored CLE imaging characterization of a bladder leiomyoma, which exhibited crowding of monomorphic spindle cells with distinct cell borders. In addition, the failure to diagnose an inflammation lesion suggested that the CLE features of flat lesions may be much more complicated than expected, making accurate differential diagnosis between CIS and inflammatory tissue challenging. However, the low number of cases limits the veracity of our results, as only two cases of CIS and one case of inflammatory tissue were analyzed.

There are several limitations to our study. First, the number of patients enrolled in the study was limited. A larger and multicenter trial is needed to ascertain the diagnostic value of CLE. Second, the sensitivity and specificity for CLE-based tumor evaluation need to be improved and a diagnostic nomogram needs to be developed, since an almost 20% variation from final histopathology diagnosis is far from satisfactory. In addition, manipulation for steady direct contact, which is crucial for the full estimation of a region, could be difficult especially for the novices. Therefore, the analysis of recorded image sequence based on the inadequate contact may come to a biased diagnosis regarding to the whole tumor. Despite the limitations mentioned above, CLE is a promising technology that may play a central role in the conservative management of bladder carcinoma in the future. The ability to visualize real-time histopathologic features makes histologic information during TURBT as well as cystoscopy possible. The former could confirm radicality of TURBT and better stratified patients who could benefit from a conservative treatment. The latter could not only optimize the expenditure of conservative bladder cancer management but also enhance the follow-up quality of urothelial carcinoma patients. Another advantage of CLE is the ability to explore and estimate the entire surface of the suspected lesion, while the biopsy could only provide pathological information of one or several well-defined areas which may cause misdiagnosis. Moreover, CLE may also be used for minute lesion that is difficult for biopsy like upper tract urothelial carcinoma. Finally, although we only achieved the overall diagnostic accuracy of 81.0% for bladder cancers, the false-negative rate is literally zero. We believe that with improvement of diagnostic criteria of CLE and accumulation of experience, seasoned urologists may be able to make histopathologic diagnoses independently and with higher accuracy.

Including CLE, several significant advances in endoscopic technology have contributed in the conservative management of bladder cancer in the last decade. Narrow band imaging (NBI) is a high-resolution endoscopic technique that can differentially penetrate mucosa to enhance visualization of mucosal vasculature, which can enhance the contrast between normal urothelium and hypervascular cancer tissue [21]. Optical coherence tomography (OCT) is another optical biopsy technology that can provide real-time and cross-sectional images of tissue morphology at a resolution of 10–20 μm [22]. Fluorescence cystoscopy, also known as photodynamic diagnosis (PDD), is capable to provide wide-field fluorescence imaging, which is based on fluorescence such as 5-ALA. The specific photosensitizing agent accumulates significantly more in tumor cells than in normal cells, and then enables a surgeon to visualize cancer tissues under specific excitation wavelength [4]. In the future, PDD and NBI could be combined with CLE to achieve multimodal imaging of the bladder cancer, which is supposed to be a solution of evaluation of flat hyperemic areas and may dramatically alter the current management of bladder cancer.

This study performed a preliminary investigation of the efficacy and feasibility of CLE-based diagnosis of bladder cancer. CLE proved to be a promising complement to the white light endoscopy techniques for improvement of endoscopic management of bladder lesions and may enable real-time tumor grading of urothelial carcinoma. However, accurate differentiation of flat hyperemic areas remains challenging and further validation from prospective multicenter studies, as well as cost–benefit analyses, is expected.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration with its later amendments.

Informed consent Informed consent was obtained from all individual participants included in the study.

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