ORIGINAL RESEARCH

Surface-Enhanced Raman Spectroscopy of Pretreated Plasma Samples Predicts Disease Recurrence in Muscle-Invasive Bladder Cancer Patients Undergoing Neoadjuvant Chemotherapy and Radical Cystectomy

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Objective: To explore the value of surface-enhanced Raman spectroscopy analysis of pretreated plasma samples in prediction of bladder cancer (BCa) recurrence after neoadjuvant chemotherapy (NAC) and radical cystectomy (RC).

Patients and Methods: SERS was used to analyze plasma samples collected before biopsy and treatment in BCa patients undergoing NAC and RC. The value of clinicopathological parameters and distinctive SERS peaks in the prediction of disease recurrence were analyzed in Cox regression proportional hazard analysis and Log rank test. Principal component analysis and linear discriminant analysis (PCA-LDA) were employed to process spectral data and construct diagnostic algorithms.

Results: A total of 88 patients with 440 plasma SERS spectra were collected. The SRES spectra from recurrent patients were compared with patients who remained recurrence free. The SERS demonstrated higher levels of circulating free nucleic acid components in recurrent population, which is represented by significantly higher intensities at SERS peaks of 725 cm^{-1}, 1328 cm^{-1} and 1455 cm^{-1}. The SERS also detected significantly lower levels of tryptophan shown as lower significantly intensities at the 1558 cm^{-1}, which is proved to be an independent predictor of BCa recurrence. The addition of SERS peaks of 1558 cm^{-1} to classic clinicopathological predictors including pathological tumor stage, lymph node metastasis and pathological downstaging can significantly enhance the power of the predictive model from 0.66 to 0.76 in the area under curve (AUC) of receiver operating characteristic (ROC) curves. Meanwhile, the PCA-LDA diagnostic model based on SERS spectra reveals a high accuracy of 85.2% in prediction of disease recurrence and the AUC of 0.92 in the ROC curve. When validated in the leave-one-out cross-validation method, the accuracy of the model remained 84.1%.

Conclusion: We show that SERS analysis of plasma before NAC treatment can accurately classify patients with different risks of disease recurrence after surgery and improve the power of clinicopathological predictive models, thus refining clinical decision-making.

Keywords: Raman spectroscopy, bladder cancer, cancer recurrence, neoadjuvant chemotherapy, radical cystectomy

Introduction

Bladder cancer (BCa) is a common and heterogeneous disease with over 430,000 new cases and nearly 170,000 deaths annually worldwide.[1] Muscle-invasive bladder cancer (MIBC) is an advanced stage of disease that represents around

20% of newly diagnosed cases. Also, approximately 15% to 20% of non-muscle invasive bladder cancer progress to MIBC with a higher progression rate in high risk patients.[1]

Treatment paradigm for MIBC consists of cisplatin-based neoadjuvant chemotherapy (NAC) followed by radical cystectomy (RC) and pelvic lymph node dissection, which has been demonstrated to bring improved survival benefits than RC alone.[2] Nevertheless, around 40% of patients experience disease recurrence despite NAC and RC, which may require early disease monitoring and intensified treatment.[3] Studies have proposed various clinical features to improve risk stratification and prediction of disease recurrence and outcomes in BCa.[4] Tumor stage and lymph node involvement have been proved to be related to disease outcomes and survivals after surgery.[5] Also, pathological downstaging during NAC has an important prognostic role for survival outcomes.[6] Apart from clinical parameters, circulating tumor biomarkers have been found to predict oncologic outcomes of BCa and guide clinical management.[7,8] With the advent of liquid biopsy, circulating tumor DNA (ctDNA) also has growing significance in BCa diagnosis and monitoring. A previous study with a large cohort has shown that high levels of ctDNA in blood samples are associated with disease recurrence after radical cystectomy.[9] Since BCa has altered metabolism including lipid, amino acids and glycolysis, metabolic profiling of patients' samples including tissue, urine and blood have been successfully used for cancer diagnosis and recurrence monitoring.[10] Previous studies have employed liquid chromatography coupled with mass spectrometry (LC-MS) and proton nuclear magnetic resonance (^{1}H-NMR) to detect metabolites and identify bladder cancer patients.[11] However, these methods often involve complicated and time-consuming process. Novel noninvasive analytical methods are in urgent need to further expedite clinical translation.

Surface-enhanced Raman spectroscopy (SERS) is non-invasive optical analytical technology which can rapidly characterize biological samples with fingerprint chemical vibration and enable classification.[12] The convenient process and simple specimen requirement enable SERS to be widely applied in human malignancy diagnosis and monitoring with high accuracy.[13] A growing number of studies have shown that SERS can reflect biochemical alterations in biospecimens and achieve sensitive and non-invasive diagnosis of multiple malignancies including prostate cancer, breast cancer and lung cancer.[14–16] It has been reported that SERS analysis of blood serum can accurately detect prostate cancer in patients with "gray zone" prostate-specific antigen levels of 4–10 ng/mL with over 90% accuracy.[14] Additionally, a study including 427 prostate cancer patients has shown that SERS combined with deep learning algorithms can analyze metabolic differences in serum and therefore differentiate metastatic and non-metastatic prostate cancer.[17] In BCa, previous studies have demonstrated that Raman spectroscopy can be used to differentiate normal and cancerous bladder tissue with different tumor grade.[18,19] Furthermore, multiple studies have also shown that SERS analysis of serum and urine metabolites can accurately detect bladder cancer and classify tumor grade, promoting SERS based non-invasive diagnosis of BCa.[20,21] A multicenter study enrolling 340 patients utilized Raman spectroscopy to characterize epithelial cells in urine, which achieved rapid BCa diagnosis and tumor grading with high accuracies.[22] These promising results of Raman spectroscopy applied in BCa and other types of cancer reveal great potentials of its clinical translation. In the present study, we performed SERS analysis of plasma samples collected before biopsy and treatment in BCa patients undergoing NAC and subsequent RC and explored the predictive capacity of Raman spectra in disease recurrence.

Patients and Methods
Patients and Protocols

Patients diagnosed with BCa from March 2015 to August 2018 were included in the study. Inclusion criteria are as follows: 1) Patients had pathologically confirmed bladder urothelial cancer and underwent neoadjuvant cisplatin-based chemotherapy of gemcitabine and cisplatin regimen and subsequent radical cystectomy with pelvic lymph node dissection; 2) Patients had clinical stage of T2-4aN0M0; 3) Patients had no histories of antibiotics consumption or drug abuse within the last 4 weeks of biopsy; 4) None of patients had received any cancer treatment including radiotherapy, chemotherapy or immunotherapy before biopsy and blood sample collection; 5) Patients had no any other malignancy;

This study complies with the Declaration of Helsinki and was conducted following the approval from the Institutional Ethics Committee (IEC) of Shanghai RenJi Hospital (Approval No. Renji[2013]126). All patients included in the study had

written informed consent. For each patient, 5mL blood specimen was prospectively collected through peripheral vein after 12 hours of overnight fasting and before biopsy and any cancer treatment including neoadjuvant therapy and radical cystectomy. The blood samples were centrifuged at 3000 rpm for 10 min, and plasma was then collected and frozen at −80°C until SERS analysis.

Silver Nano-Particles Synthesis and Preparation

The Ag nanoparticles (NPs) were synthesized based on the sodium citrate thermal reduction method.[23] Accordingly, 1 mL of 0.1 M AgNO3 solution and 100 mL of deionized water were mixed and heated to boil. 1.9 mL of a 1% sodium citrate tribasic solution was then added to the mixed solution and kept boiling and stirred continuously until the color of mixed solution turned celadon. As shown in Figure 1, the transmission electron microscope was used to observe the Ag NPs.

SERS Analysis of Plasma Samples

To obtain higher concentration, Ag NP solution was then centrifuged at 7500 rpm for 10 minutes and the supernatant was discarded. During SERS measurement, 20 μL plasma were mixed with 20 μL of silver colloidal nanoparticles and incubated for 3 min at room temperature. Then 20 ul of the resulting mixture was dropped onto a silicon plate for SERS measurement.

The SERS spectra of samples was recorded using HORIBA Raman microscope (HORIBA Scientific) with a spectral resolution of approximately 1 cm^{-1}. 532-nm yttrium aluminum-garnet laser with 50mW was focused on sample surface and a Leica DM2500 microscope (Leica Microsystems, Wetzlar, Germany) with a L50× (N.A. 0.5) objective lens was used to obtain SERS spectra with acquisition time of 3 seconds. Five random spots on each sample surface were measured to acquire comprehensive SERE spectra.

Data Processing and Analysis

The raw SERS spectra were preprocessed by a Vancouver Raman algorithm based on the fifth order polynomial fitting method to remove fluorescence background.[24] And the processed SERS spectra underwent baseline correction and normalization. OriginPro 8.0 software (OriginLab, USA) was then utilized to generate mean and subtracted spectra of
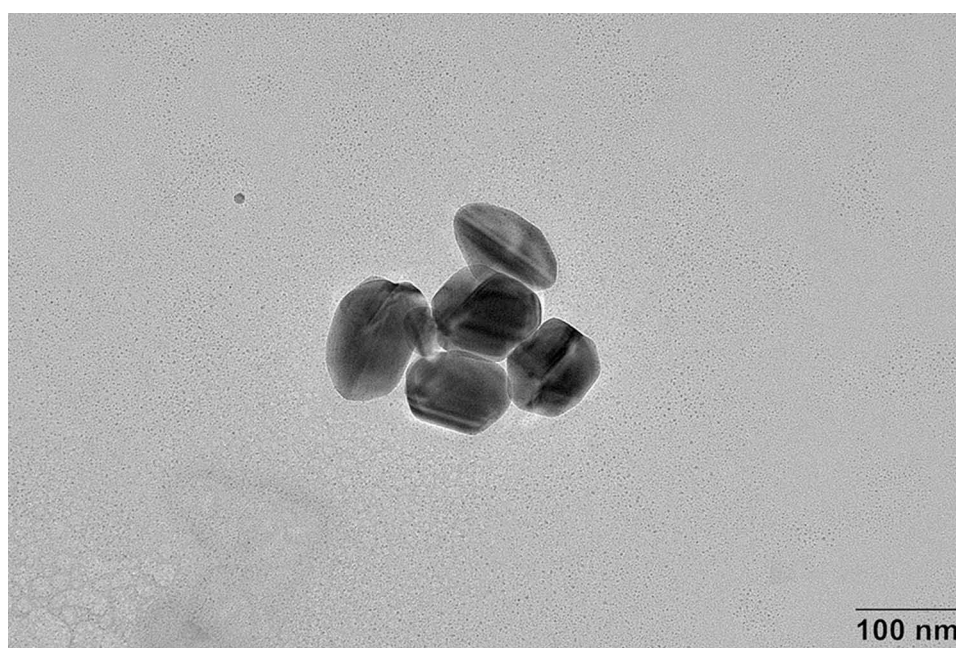

Figure 1 The transmission electron microscope (TEM) of Ag nanoparticles.

different patient groups. Clinical parameters and SERS data were compared between two groups of patients using Mann–Whitney *U*-test, independent-sample test, and chi-squared test appropriately. Association between all factors and disease recurrence was analyzed using Cox regression proportional hazard analysis. Principal component analysis and linear discriminant analysis (PCA-LDA) were used to process spectral data and develop discriminative algorithms. The PCA-LDA model was then validated by the leave-one-out cross-validation. The ROC curves and corresponding area under the curve (AUC) were generated for predictive models based on the PCA-LDA model and clinical parameters with or without Raman shifts. The SPSS Statistics software 22.0 (IBM) was used to perform the data analysis with p<0.05 as statistically significant.

Results

Overall, 88 BCa patients were included in the study. All patients underwent neoadjuvant cisplatin-based chemotherapy and subsequent cystectomy and lymph node dissection. With a median follow up time of 40 months, a total of 44 patients developed disease recurrence. As shown in Table 1, baseline characteristics including age, gender and clinical stages are comparable between the recurrent group and non-recurrent group. Compared with the non-recurrent group, patients in the recurrent group have significantly higher pathological tumor stages, lymph node metastasis rates and lower pathological downstaging rates, which indicates that recurrence patients harbor more adverse features.

SERS Spectra of the Plasma Samples

The mean SERS spectra of recurrent group and non-recurrent group were shown in Figure 2. There are several distinguishable intensity differences in SERS shifts between two groups including 638 cm^{-1}, 725 cm^{-1}, 1095 cm^{-1}, 1135 cm^{-1}, 1328 cm^{-1}, 1455 cm^{-1} and 1558 cm^{-1}. Table 2 shows that SERS shifts could be attributed to different biochemical assignment according to previous studies and Raman spectroscopy database.[24–27] When compared to non-recurrent group, SERS spectra of recurrent group have significantly higher signal intensities of SERS shift at 725 cm^{-1} (p<0.001), 1095 cm^{-1} (p<0.001), 1328 cm^{-1} (p<0.001), 1455 cm^{-1} (p<0.001) and significantly lower signal intensities at

Table 1 Demographics and Clinical Features of the Cohort

| Clinical Variables	Total	Recurrent Patients	Non-Recurrent Patients	p value
Case No. (%)	88	44 (50.0)	44 (50.0)	
Age (IQR), years	61.5 (58–66)	62 (57.5–66)	61 (58–66)	0.818
Gender				0.778
Female	15 (17.0)	8 (18.2)	7 (15.9)	
Male	73 (83.0)	36 (81.8)	37 (84.1)	
Clinical stage, No.(%)				0.263
T2N0M0	73 (83.0)	34 (77.3)	39 (88.6)	
T3N0M0	10 (11.4)	6 (13.6)	4 (9.1)	
T4aN0M0	5 (5.7)	4 (9.1)	1 (2.3)	
Pathological T stage, No.(%)				<0.001
T0	18 (20.5)	2 (4.5)	16 (36.4)	
Tis	4 (4.5)	2 (4.5)	2 (4.5)	
Ta-1	26 (29.5)	7 (15.9)	19 (43.2)	
T2	14 (14.9)	10 (22.7)	4 (7.8)	
T3	17 (19.3)	17 (38.6)	0 (0)	
T4	9 (10.2)	6 (13.6)	3 (6.8)	
Pathological N stage No.(%)				0.009
N0	79 (89.8)	36 (81.8)	43 (97.7)	
N1	9 (10.2)	8 (18.2)	1 (2.3)	
Pathological downstaging				<0.001
Yes	50 (58.4)	13 (29.5)	37 (84.1)	
No	38 (41.6)	31 (70.5)	7 (15.9)	

Abbreviation: IQR, interquartile range.
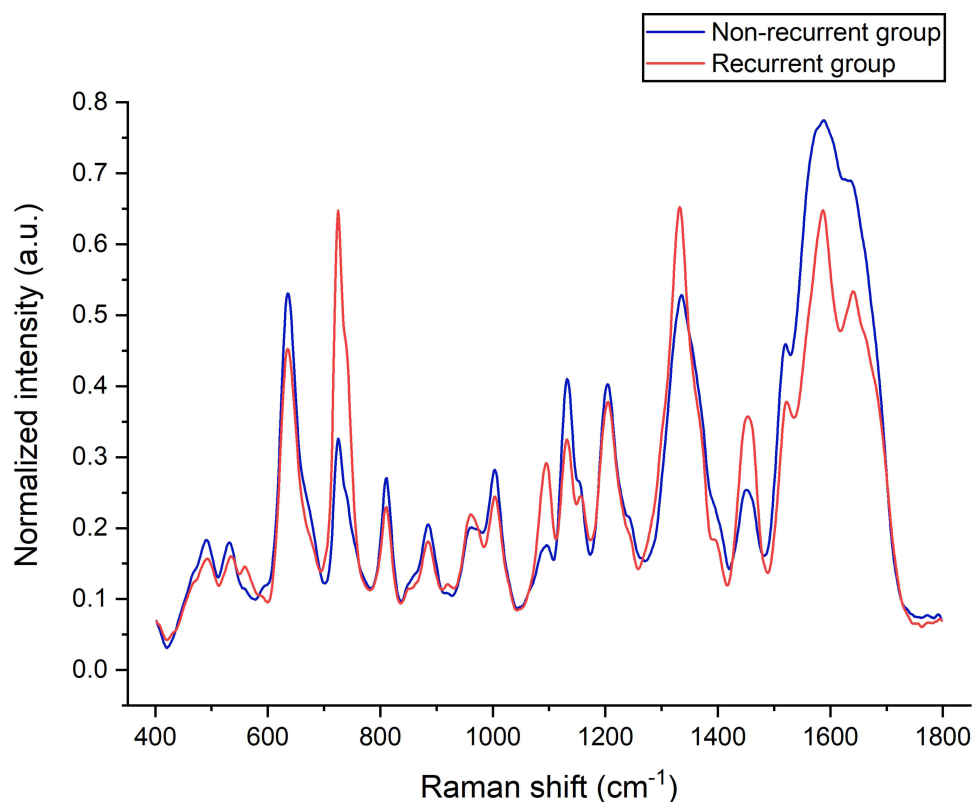
Figure 2 Mean SERS spectra of the recurrent group and non-recurrent group.

638 cm^{-1} (p=0.029), 1135 cm^{-1} (p=0.028) and 1558 cm^{-1} (p<0.001). Accordingly, this indicates that patients with cancer recurrence have higher concentrations of hypoxanthine, DNA, nucleic acids, deoxyribose and lower concentrations of tyrosine, d-mannose and tryptophan.

SERS Spectra in Prediction of Disease Recurrence

To evaluate the value of clinical variables and Raman peaks in predicting disease recurrence, we used Cox regression proportional hazard analysis. As shown in Table 3, pathological tumor stage, pathological lymph node metastasis, pathological downstaging and the intensities of Raman shifts of 725 cm^{-1}, 1095 cm^{-1}, 1135 cm^{-1}, 1328 cm^{-1}, 1455 cm^{-1} and 1558 cm^{-1}, were significantly associated with disease recurrence in univariate analysis. Notably, the pathological tumor

Table 2 Tentative Assignments of Significant SERS Shifts in Biological Samples[24–27]

Raman Shift (cm^{-1})	Major Assignment
638	C-C twisting mode/tyrosine
725	Hypoxanthine
890	δ(C−O−H)/Amino galactose
1095	Deoxyribonucleic acid, phosphodioxy group
1135	C-N stretch/D-mannose
1328	Nucleic acids and phosphates
1455	Deoxyribose
1558	Tryptophan
1583	C=C bending mode/phenylalanine
1655	Amide I/C=C lipid stretch

Abbreviation: SERS, Surface-enhanced Raman Spectroscopy.

Table 3 Cox Regression Proportional Hazard Analysis of Clinical Variables and SERS Shifts

Variables	Unadjusted			Adjusted		
	HR	(95% CI)	P	HR	(95% CI)	P
Pathological T stage			<0.001			<0.001
T0	Ref.	/	/	Ref.	/	/
Tis	7.15	1.00–51.37	0.051	11.69	1.59–85.93	0.016
Ta-1	2.87	0.59–13.91	0.191	2.06	0.41–10.22	0.378
T2	12.60	2.71–58.64	0.001	114.66	13.68–961.21	<0.001
T3	23.19	5.29–101.63	<0.001	370.25	35.85–3823.68	<0.001
T4	12.05	2.41–60.17	0.002	228.56	20.98–2489.71	<0.001
Pathological N stage				/	/	/
N0	Ref.	/	/			
N1	3.06	1.40–6.70	0.005			
Pathological downstaging	0.182	0.09–0.35	<0.001	0.055	0.01–0.32	0.001
Peak 638 cm^{-1}	0.23	0.04–1.28	0.093	/	/	/
Peak 725 cm^{-1}	8.55	3.20–22.90	<0.001	/	/	/
Peak 1095 cm^{-1}	715.45	36.35–14,082.31	<0.001	/	/	/
Peak 1135 cm^{-1}	0.06	0.01–0.41	0.005	/	/	/
Peak 1328 cm^{-1}	71.58	7.79–657.93	<0.001	/	/	/
Peak 1455 cm^{-1}	102.91	9.20–1151.23	<0.001	/	/	/
Peak 1558 cm^{-1}	0.035	0.009–0.14	<0.001	0.018	0.003–0.11	<0.001

Abbreviations: CI, Confidence Interval; HR, Hazard Ratio; Ref., Reference; SERS, Surface-enhanced Raman Spectroscopy.

stage, pathological downstaging and the intensity of Raman shift at 1558 cm^{-1} remained significantly in the multivariate model. The Raman shift intensities of 638 cm^{-1}, 725 cm^{-1}, 1004 cm^{-1}, 1095 cm^{-1}, 1135 cm^{-1}, 1328 cm^{-1}, 1455 cm^{-1} and 1558 cm^{-1} in two groups were shown in Figure 3. With the dividing cut-off intensity of 0.552 by Raman peak of 1558 cm^{-1}, patients were divided into low intensity group and high intensity group and compared by Log rank test. Figure 4 shows that the low intensity group has significantly higher risks of disease recurrence than the high intensity group (p<0.001), which suggests that the intensities of peak 1558 cm^{-1} can accurately distinguish patients with different prognosis.

Furthermore, we used PCA-LDA method to analyze SERS spectra and distinguish recurrent group and non-recurrent group. After extracting the first 9 principal components accounting for 84.3% of variances in the PCA process, we
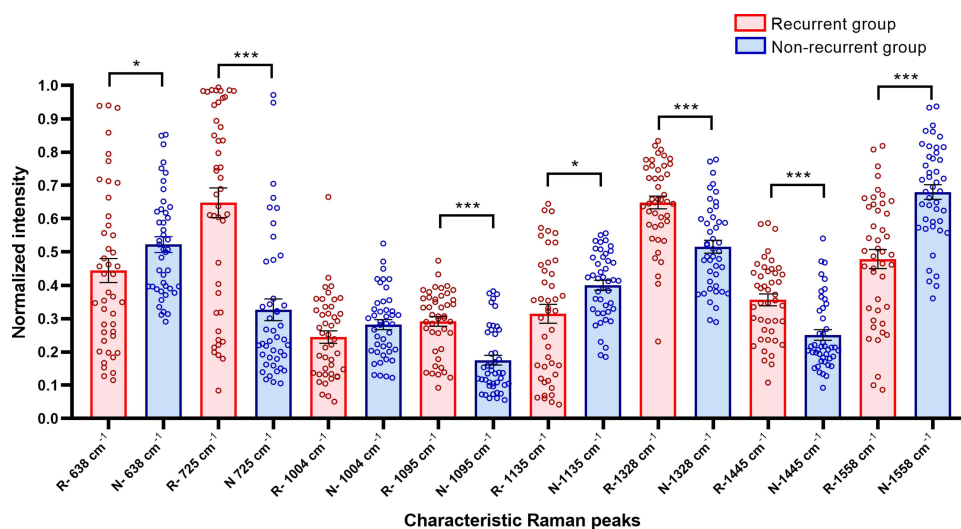

Figure 3 Distribution of significant SERS peaks' intensities for recurrent group and non-recurrent group. Bar graph shows the mean spectral intensities at each peak. Scatter diagram shows mean intensities of each sample in specific peaks, *p < 0.05, ***p < 0.001; Error bar indicates standard error of the mean (S.E.M.).
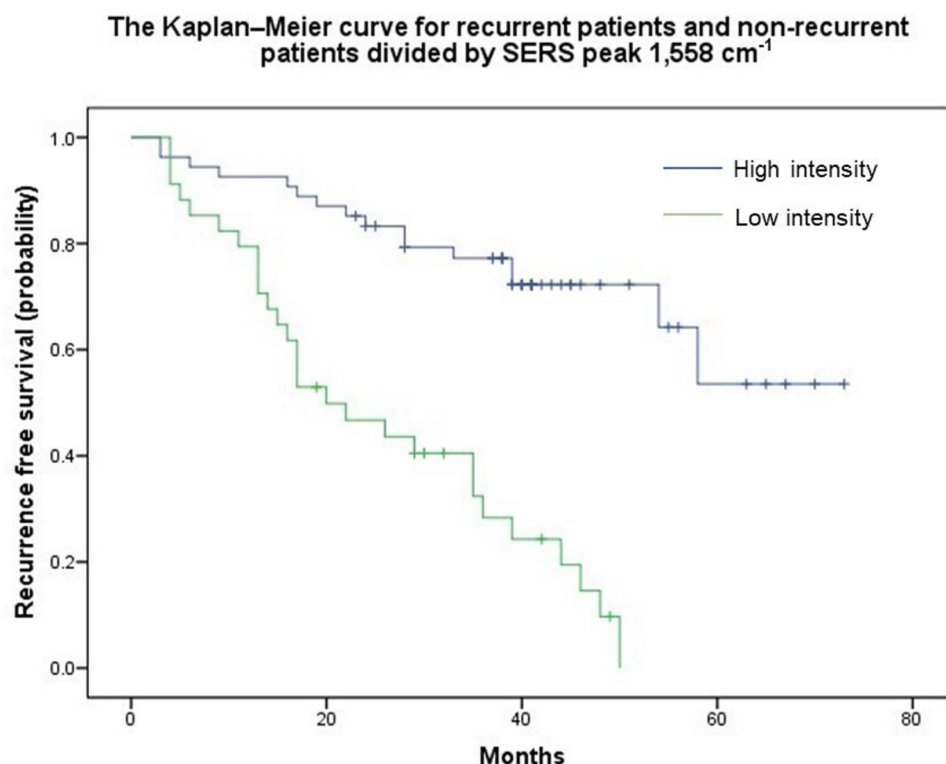
Figure 4 The Kaplan–Meier curve for recurrent patients and non-recurrent patients divided by SERS peak 1558 cm^{-1}.

performed LDA in the model and revealed a high accuracy of 85.2% in predicting disease recurrence, which is shown in Figure 5. As shown in Table 4, we also employed leave-one-spectrum-out cross-validation method to further test the discriminative model. We show that the validated diagnostic accuracy is 84.1%.

To compare the diagnostic values of clinical parameters and SERS spectra, we used ROC curve to assess the models of pathological tumor (pT) stage, pathological lymph node metastasis (pLNM) and pathological downstaging (pDS), and clinical variables combined with peak 1558 cm^{-1}, and the PCA-LDA model. As shown in Figure 6, the PCA-LDA model have the highest AUC of 0.92 (95% CI, 0.87–0.98) while the AUC of clinical variables was 0.66 (95% CI, 0.54–0.77). When combined with Raman peak 1558 cm^{-1}, the AUC of predictive model of clinical variables was improved to 0.76 (95% CI, 0.66–0.87).

Discussion

MIBC is an advanced and aggressive type of bladder cancer with a high rate of recurrence and unfavorable prognosis. Mostly, MIBC patients undergo recurrence within 2 years at the rate of approximately 40% even after definitive treatment.[3] Although it has been well-established that neoadjuvant chemotherapy (NAC) combined with RC can improve overall survival by 5–6% in MIBC, disease outcomes remain poor especially in patients with aggressive and adverse features.[28] Therefore, it is of great clinical significance to identify patients with high risk of recurrence and need for timely and intensified management while avoiding excessive treatment for patients with low risk of relapse. In this study, we utilized SERS technique to analyze plasma samples of BCa patients undergoing NAC and RC and investigate the value of Raman shifts in prediction of disease recurrence. We showed that SERS analysis can reflect differences of distinctive biochemical components between recurrent patients and non-recurrent patients including SERS peaks of 725 cm^{-1}, 1095 cm^{-1}, 1135 cm^{-1}, 1328 cm^{-1}, 1455 cm^{-1} and 1558 cm^{-1}, which are significantly associated with recurrence. The intensity of Raman shift 1558 cm^{-1} could further refine the predictive model based on clinicopathological parameters and enhance its predictive capability of BCa recurrence.
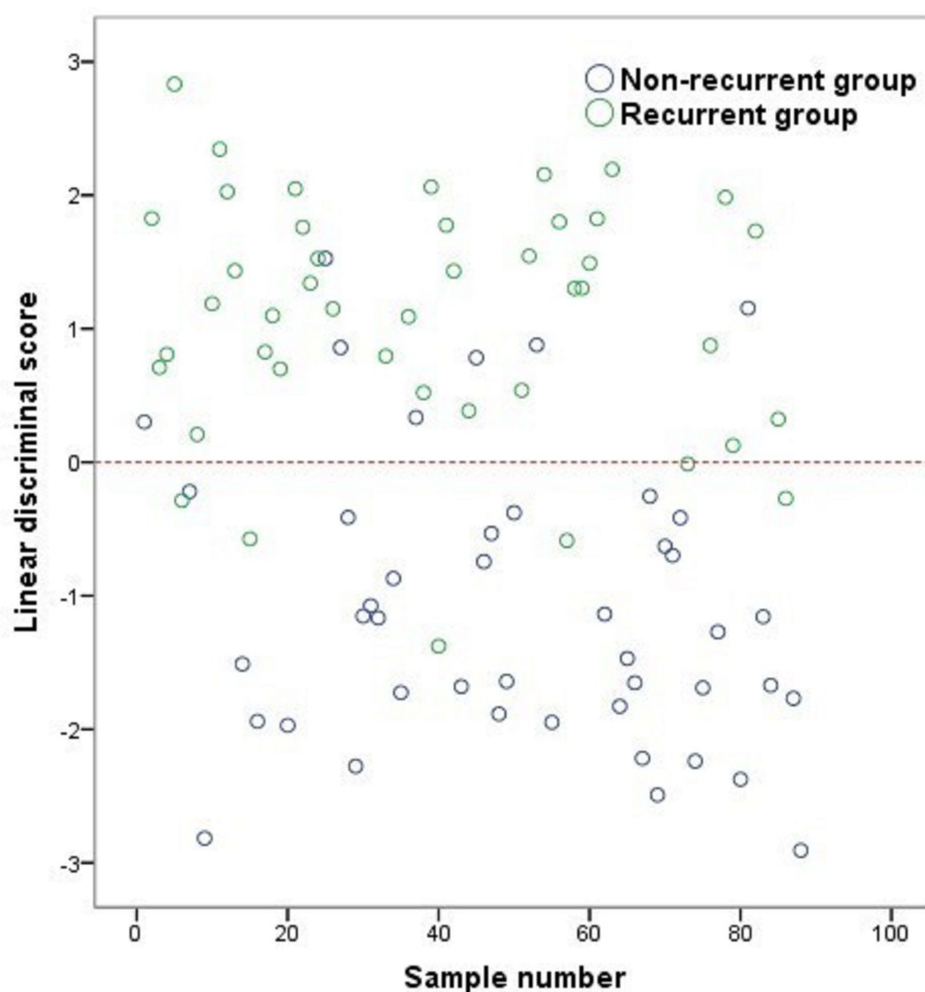
Figure 5 Scatter plot of linear discriminant (LDA) scores for recurrent group and non-recurrent group.

In recent years, SERS has been growingly studied and serves as a convenient and non-destructive analytic method in human disease.[13] Combined with metallic nano-substrate, signal intensity of SERS peaks can be enhanced 10^6 to 10^8 times and provide fingerprint information of analyzed samples including blood, urine and saliva, thus enabling biocomponent identification and disease assessment.[29] With its non-destructive nature and convenience, SERS analysis of human specimen has been extensively used in cancer diagnosis and assessment such as prostate cancer, gastric cancer and hepatocellular cancer, which yield high accuracies and promising potentials.[30–32]

In the present study, SERS spectra exhibited prominent differences between the recurrent group and non-recurrent group in plasma samples before treatment, implying that potential biocomponents could be related to disease recurrence. Specifically, significantly stronger intensities at Raman spectral peaks 725 cm^{-1}, 1328 cm^{-1}, and 1455 cm^{-1}, and

Table 4 The PCA-LDA Model of Recurrent and Non-Recurrent Groups Based on the Raman Spectra of Plasma with Leave-One-Spectrum-Out Cross-Validation

Group	Predicted Group		Total
	Non-recurrent (%)	Recurrent (%)	
Non-recurrent (%)	37 (84.1)	7 (15.9)	44 (100)
Recurrent (%)	7 (15.9)	37 (84.1)	44 (100)

Abbreviations: PCA, principal component analysis; LDA, linear discriminant analysis.
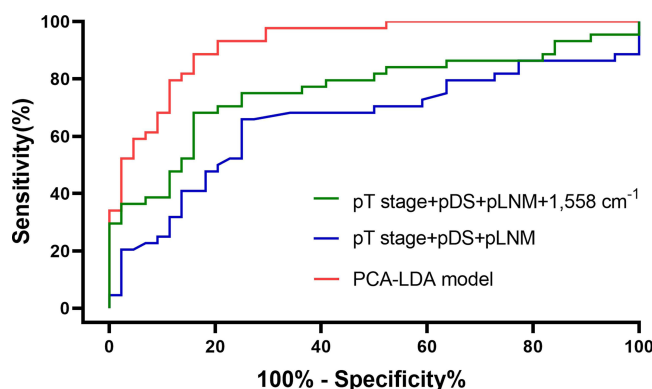
Figure 6 The receiver operating characteristic (ROC) curves of the PCA-LDA model, clinical variables of pathological tumor stage, lymph node metastasis and pathological downstaging, and clinical variables combined with Raman peaks of 1558 cm^{-1}.

lower intensities at 1135 cm^{-1} and 1558 cm^{-1} were identified in the recurrent group compared with non-recurrent group. According to tentative biochemical assignments of Raman shifts, the increased intensities in Raman peaks of 725 cm^{-1}, 1328 cm^{-1} and 1455 cm^{-1} indicate that higher concentrations of nucleic acid bases including, hypoxanthine, DNA/RNA bases and deoxyribose exist in plasma of recurrent patients. It has been shown that levels of circulating tumor DNA are significantly associated with disease recurrence in patients receiving RC.[9] Also, detectable plasma ctDNA in BCa patients before chemotherapy is highly prognostic and associated with disease recurrence.[33] Such evidence suggest that distinctive SERS shifts in present study correspond well to levels of circulating nucleic acids and can possibly predict recurrence in BCa patients. It is understandable that bladder cancer cells with more aggressive nature and abnormally accelerated cell proliferation and necrosis could cause increased release of nucleic components into circulation. In fact, most patients with metastatic BCa have significantly higher ctDNA fractions than localized BCa.[34] In concordance, a previous study using SERS to predict prostate cancer recurrence has reported that patients with early recurrence harbor elevated levels of nucleic acids in preoperative blood, which are represented in distinctive Raman peaks including 725 cm^{-1} and 1328 cm^{-1}.[24] Therefore, SERS could provide a rapid and accurate method for monitoring concentration of nucleic acids, which could be translated into prediction of disease recurrence in BCa patients.

Notably, lower intensities at the Raman shift of 1558 cm^{-1} suggest increased risk of BCa recurrence, which can serve as an independent predictor and greatly improve the predicting capabilities of clinical parameters. Accordingly, the Raman shift of 1558 cm^{-1} is assigned to tryptophan in circulation. Tryptophan is an essential amino acid that cannot be synthesized in human body. Considerable evidence has shown that tryptophan metabolites may have a role in human bladder carcinogenesis.[35] Abnormal tryptophan metabolism in patients with bladder carcinoma has been reported to strongly correlate with disease recurrence.[36] A recent study has shown that levels of plasma tryptophan are significantly decreased in BCa patients compared to healthy controls.[37] In fact, increased expression and activity of tryptophan-metabolizing enzymes in BCa patients can promote tryptophan metabolism, thus decreasing its circulating levels.[37,38] Also, enhanced consumption of tryptophan by tumor cells in synthesizing cellular protein and formatting cytoskeleton contribute to its decrease in circulation.[38] Similarly, it has been reported that circulating tryptophan levels also decrease in patients with lung, gastric, colorectal, breast, and prostate cancer, which suggests that abnormal tryptophan metabolism could be a symbolic hallmark of malignancy.[39] And SERS could rapidly reveal levels of tryptophan metabolites in plasma and assist in prediction of BCa progression.

In order to explore the predictive value of Raman peaks and clinical factors, we used Cox regression proportional hazard analysis to test these factors and found that the Raman shift of 1558 cm^{-1} was an independent predictor of disease recurrence in both univariate and multivariate analysis. With optimal cut-off value of signal intensity, SERS peak 1558 cm^{-1} could accurately differentiate recurrent patients and non-recurrent patients in Kaplan–Meier curve. Since pathological tumor stage and pathological downstaging are well-established predictors of disease outcomes and also significant in multivariate analysis in present study, we add SERS peaks to the prognostic model and found that SERS

peak 1558 cm^{-1} could significantly enhance the discriminatory power of models in ROC curve. Notably, SERS peak 1558 cm^{-1} is the only parameter before NAC treatment while pathological tumor stage, pathological lymph node metastasis and pathological downstaging represent tumor response after NAC.

Since SERS can reflect comprehensive biochemical information of blood, individual spectral peaks could omit other useful biochemical information. Integrated analysis of SERS spectra could further characterize differences between recurrent and non-recurrent patients. To this end, we employed PCA-LDA model for spectral data analysis and constructed a diagnostic algorithm for discrimination. The PCA-LDA model demonstrated a high accuracy of 85.2% for prediction and remained 84.1% when validated in leave-one-spectrum-out cross-validation method, which further supports the value of SERS spectra in prediction of BCa recurrence. Moreover, the ROC curve showed that the PCA-LDA model has the highest AUC outperforming clinical parameters combined with spectral peaks, which suggests that SERS revelation of biochemical alteration in plasma could be a more robust predictor of disease outcome than classic clinical factors.

Our study has several limitations. The present study is conducted in a monocentric and retrospective manner. A large cohort with prospective design is further needed for validation. Also, biochemical assignments of SERS peaks are based on previous studies and databases. Supportive methods such as mass spectrometry that could help identifies substances might be used to confirm the results. Despite these limitations, our study reported a successful application of SERS-based analysis of plasma in prediction of bladder cancer recurrence in a clinical cohort, which shows profound clinical significance in identifying patients suitable for personalized treatment strategies.

Conclusion

In this preliminary study, we show that SERS analysis of plasma before NAC treatment can accurately detect biochemical alterations in patients with or without disease recurrence. SERS spectra of pretreated plasma could help identifying patients with high risk of disease recurrence after surgery and improve the power of clinicopathological predictive models, thus refining clinical decision-making.

Acknowledgments

Disclosure

The authors have declared that no competing interest exists in this work.

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