Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Detection of circulating tumor cells in patients with pancreatic cancer by novel nano microfluidic chips and comprehensive nursing intervention

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ARTICLE INFO

Original paper

Article history:
Received: May 30, 2023
Accepted: September 17, 2023
Published: December 20, 2023

Keywords:

Comprehensive nursing, CTCs, Novel nano microfluidic chip, PC

ABSTRACT

It was to explore the detection efficiency of novel nano microfluidic chips on circulating tumor cells (CTCs) in pancreatic cancer (PC) patients and the effect of comprehensive nursing intervention on the life indicators of patients after PC surgery. PC, benign pancreas, and healthy volunteers were enrolled. The number and positive rate of CTCs in the subjects, the relationship between the number of CTCs in PC patients and clinical data, the recurrence rate, and the life indicators of patients after intervention were sorted out and analyzed. The positive rate of CTCs was 96.67% in PC group (29/30), 16.67% in benign pancreas group (5/30), and 0% in healthy controls. The positive CTCs in PC patients were correlated with vascular invasion (P<0.05). The recurrence rate after PC resection was 56.67% (17/30). The positive detection rate of nano microfluidic chip was 30/30 (100%), which was higher than against CellSeach 7/30 (23.33%), P<0.05. The physiological function score of intervention group (93.67±1.48) was higher as against controls (60.45±2.03), P<0.05. The social function score of intervention group (91.26±0.44) was higher as against controls (54.92±2.98), P<0.05. The novel nano microfluidic chip has high sensitivity and specificity for the detection of CTCs in PC patients. Postoperative comprehensive nursing intervention can improve the quality of life (QoL) and functional status of PC patients.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.14.19

Introduction

Pancreatic cancer (PC) is a malignant tumor with high incidence and mortality. Its early symptoms are not obvious, which often leads to difficulties in late diagnosis and treatment (1). Current clinical diagnosis and monitoring methods mainly rely on tissue biopsy and imaging examination, but these methods have certain limitations, such as trauma, invasion, low sensitivity, and insufficient specificity (2,3). Therefore, it is of great clinical significance to develop a non-invasive detection method with high sensitivity and specificity for early detection and monitoring of PC changes. In recent years, circulating tumor cells (CTCs) have attracted much attention as important biomarkers for early diagnosis and prognosis evaluation of PC (4). CTCs are cancer cells released from primary tumors, which survive and migrate in the blood circulation and have important metastatic potential (5). However, the traditional CTCs detection methods have some technical limitations, such as complex operation, low detection sensitivity, and poor specificity. To overcome these limitations, the combination of nanotechnology and microfluidic technology has shown great potential in the field of CTCs detection.

As a new type of detection platform, nano microfluidic chips have made significant progress in early cancer diagnosis and treatment monitoring (6,7). The emergence of nano-microfluidic chips provides a new solution for the detection of CTCs in PC patients. This technology combines nanotechnology with microfluidic technology to achieve the enrichment, separation, and analysis of CTCs in the blood through the design of miniature channels and nanostructures (8,9). The advantage of nano microfluidic chips lies in their high integration, high throughput, and high sensitivity, which enable them to effectively capture and separate rare CTCs while achieving accurate analysis of cell characteristics (10-12).

Based on the above advantages of nano microfluidic chips, this article further explored the application of novel nano microfluidic chips in the detection of CTCs in the peripheral blood of PC patients. It explored its relationship with postoperative recurrence and prognosis of PC patients, as well as the application effect of comprehensive nursing intervention in such patients.

Materials and Methods

Subjects

30 patients with PC treated in Chongqing Liangjiang New Area People's Hospital from 2020 to 2023 were selected as the PC group, 30 patients with chronic pancreatitis, IPMN, pancreatic serous cyst, pancreatic mucinous cyst, and mass type autoimmune pancreatitis as a benign group, and 30 healthy volunteers as healthy controls. PC group had 18 males and 12 females (63.59±7.82) years. The benign pancreas group had 20 men and 10 women (64.03±6.64) years. There were 17 men and 13 women in healthy controls (62.94±8.43) years. Approval by the Medical Ethics Committee of Chongqing Liangjiang New Area People's Hospital was obtained. The patients and

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their families agreed to sign the corresponding informed consent.

Inclusion criteria

(I) patients diagnosed as PC according to the relevant diagnostic criteria (13,14); (II) patients aged 18 years and above; (III) patients providing written informed consent and agreeing to participate in the trial; (IV) patients able to cooperate with the completion of relevant examinations and treatments.

Exclusion criteria

(I) patients with other serious systemic diseases or malignant tumors; (II) pregnant or lactating women; (III) patients received pancreatic surgery or other anti-cancer treatment; (IV) patients with intellectual or cognitive impairment, unable to provide valid research data; (V) patients with abnormal coagulation function that does not meet the safety standards.

Research methods

Venous blood samples were collected from three groups. The general clinical data (gender, age, clinical symptoms, tumor location, tumor size, pathological type, lymph node metastasis, nerve invasion, TNM staging) and CTCs number of each group were collected, and the correlation was analyzed. The detection efficiency of the novel nano microfluidic chip was evaluated by comparing the detection results of the novel nano microfluidic chip with those of the conventional detection method CellSearch. In addition, comprehensive nursing intervention was carried out for patients after PC surgery, and the life indicators of patients following the intervention were observed. The application outcomes of novel nano microfluidic chips and comprehensive nursing in the detection and treatment of PC patients were evaluated.

Novel nano microfluidic chip and CTCs in peripheral blood detection

Nanofluidic chips are an advanced technology platform for the detection and analysis of CTCs. It utilizes the properties of microfluidics and nanostructures to achieve efficient enrichment, separation, and characterization of CTCs (15). The following were the detailed steps of the nano microfluidic chip CTCs detection process (16,17): (I) Blood sample collection: Blood samples were collected from the venous blood of PC patients and pretreated with anticoagulants to ensure the integrity and reliability of the samples. (II) Sample pretreatment: The collected blood samples were subjected to pretreatment steps, such as erythrocyte lysis, and removal of cell debris and cell aggregates in plasma. These steps helped to reduce the presence of interferences and improve the detection efficiency of CTCs. (III) Microfluidic chip preparation: A nano-microfluidic chip suitable for CTCs detection was designed and prepared. Chips usually consisted of micrometer-level channels and structures, including microvalves, microcolumns, and microarrays for the enrichment, separation, and capture of CTCs. (IV) CTCs enrichment and separation: The pretreated blood samples were passed through the nano microfluidic chip, and the microchannel structure and surface modification characteristics in the chip were used to achieve the enrichment and separation of CTCs. The commonly used enrichment methods included immunomagnetic bead sorting and microfluidic capture technology. (V) CTCs fixation and staining: The enriched CTCs were fixed and stained on the chip. By using specific antibody labeling technology, antibodies that bind to specific antigens on the surface of CTCs were combined with fluorescent dyes to realize the visualization of CTCs. (VI) Image acquisition and analysis: Microscope or cell imaging system was used to collect images of fixed stained CTCs. The acquired images can be adopted for automatic or semi-automatic CTCs counting and qualitative analysis by image processing and analysis software. (VII) Data interpretation and result analysis: According to the number of CTCs, phenotypic characteristics and other relevant information, data interpretation, and result analysis were performed. The clinical significance of CTCs was evaluated by correlation analysis with clinicopathological features and prognostic information.

Statistical methods

SPSS 20.0 statistical software was adopted for data analysis. Measured data of continuous variables were expressed as means ± standard deviations. *t*-test was adopted for statistical analysis. Count data for categorical variables were expressed as percentages (%). χ^2 test was adopted. A *P*-value of less than 0.05 was considered statistically significant.

Results

Results of CTCs in peripheral blood

The positive rate of CTCs was 96.67% in the PC group (29/30), 16.67% in the benign pancreas group (5/30), and 0% in healthy controls. The number of CTCs was (41.58±30.75), (9.17±4.32), and 0, respectively (Figure 1).

The relationship between PC CTCs positivity and pathological characteristics and tumor stage

The positive rate of CTCs in the PC group was correlated with vascular invasion ($P<0.05$), but not with gender, age, clinical symptoms, tumor location, tumor size, pathological type, lymph node metastasis, nerve invasion, and TNM staging ($P>0.05$) (Table 1).

Recurrence of patients after PC resection

The recurrence rate following PC resection was 56.67% (17/30), including 8 cases of local recurrence, 6 cases of distant metastasis, and 3 cases of both (Figure 2).

Nano microfluidic chip for PC CTCs and CellSeach detection

The samples of PC patients were sent to the nano microfluidic chip and CellSeach for positive detection. The results suggested that the positive rate of the nano mi-

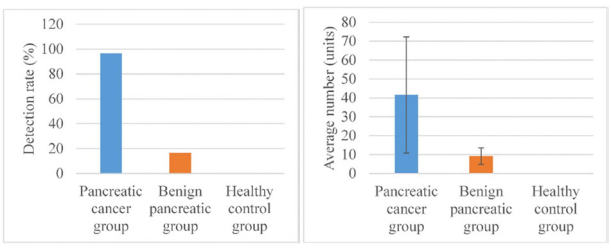

Figure 1. Positive detection rate and number of CTCs.

Table 1. Relationship between PC CTCs positivity and pathological characteristics and tumor stage.

Categories	Number (cases)	Number of CTCs	t / χ^2	*P*
Gender				
Male	18	40.26±33.84	2.235	0.162
Female	12	33.95±31.06		
Age (years)	63.59±7.82	39.13±30.77	1.056	0.413
Clinical symptoms				
Yes	19	38.91±37.35	2.025	0.079
No	11	26.92±27.54		
Tumor location				
Head of the pancreas	23	36.85±33.60	0.723	0.914
Body and tail of pancreas	7	39.67±35.77		
Tumor size (cm)	2.99±0.73	36.42±31.35	2.323	0.384
Type of pathology				
Adenocarcinoma	22	42.47±35.32	9.834	0.139
Squamous cell carcinoma	8	34.18±22.63		
Lymph node metastasis				
Yes	18	43.27±33.42	2.158	0.163
No	12	27.08±25.61		
Vascular invasion				
Yes	17	53.36±37.23	8.976	0.038
No	13	21.23±13.84		
Nerve invasion				
Yes	19	46.92±40.41	0.853	0.735
No	11	25.74±13.45		
TNM staging				
I/II	16	28.07±22.13	1.085	0.097
III/IV	14	48.34±36.93		

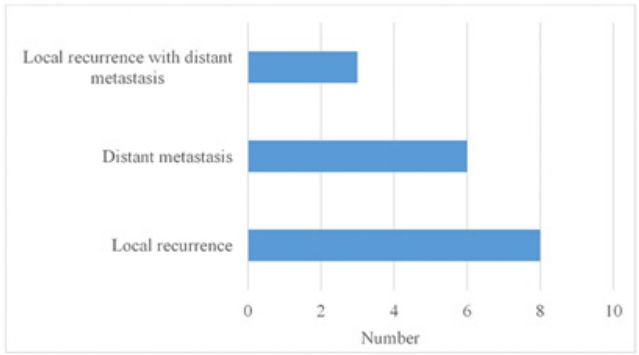

Figure 2. Recurrence of patients following PC resection.

crofluidic chip detection was 30/30 (100%), and that of CellSeach detection was 7/30 (23.33%). The positive rate of nano microfluidic chip detection was higher than CellSeach ($P<0.05$).

Effect of comprehensive nursing intervention

Patients after PC were given comprehensive nursing intervention and randomly divided into intervention groups and controls. The patients in the intervention group received comprehensive nursing intervention, while the patients in the controls only received routine nursing. The physiological function, social function, body pain, and mental health scores of the two groups following intervention were compared. The results revealed that: the physiological function score of the intervention group (93.67±1.48) was higher as against controls (60.45±2.03), $P<0.05$; The social function score of the intervention group (91.26±0.44) was superior as against controls (54.92±2.98), $P<0.05$. The body pain score of the intervention group (90.83±1.32) was superior as against controls (62.79±2.51), $P<0.05$; The mental health score of the intervention group (93.05±2.23) was superior as against controls (61.13±2.18), $P<0.05$ (Figure 3).

Discussion

PC is a highly aggressive malignancy with high morbidity and mortality (18,19). At present, the early diagnosis and treatment of PC still face many challenges, leading

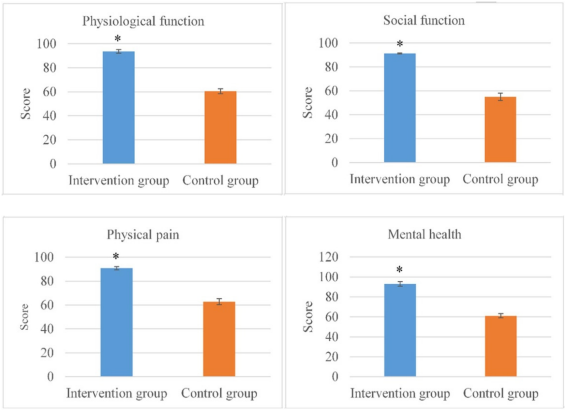

Figure 3. Comparison of life index scores of patients. Note: "*" indicates $P<0.05$ compared to controls.

to poor prognosis for patients. CTCs, as a biomarker of malignant tumor cells shedding in tumor tissues and entering the blood circulation, have important clinical significance (20). Detection and analysis of CTCs can provide important information for early diagnosis, treatment selection, and prognosis evaluation. However, traditional methods for CTCs detection have many limitations, such as low sensitivity, complex operation, and time consumption (21,22). This article aimed to explore the detection of CTCs by a novel nano microfluidic chip in PC patients and its application in comprehensive nursing intervention. The specific objectives include evaluating the feasibility and effectiveness of nano microfluidic chip in detecting CTCs in PC patients, analyzing the characteristics of CTCs in PC patients, and exploring their correlation with clinical characteristics. In addition, the nano microfluidic chip was compared with conventional methods such as CellSearch, and the effect of comprehensive care intervention on the quality of life (QoL) and functional status of PC patients was evaluated.

Firstly, CTCs were detected in PC patients by using nano-microfluidic chip. The results suggested that the positive detection rate of CTCs in the PC group was 96.67%, which was significantly higher than against the other two groups. The nanofluidic chip has high sensitivity and specificity in the detection of CTCs in PC patients. The number of CTCs in the PC group was clearly higher than in the other two groups, further confirming the potential of CTCs as a biomarker for PC. Further analysis showed that the presence of CTCs in the PC group was clearly correlated with vascular invasion, suggesting that CTCs may play an important role in the invasion and metastasis of PC. However, compared to clinical characteristics such as gender, age, clinical symptoms, tumor location, there was no significant correlation between CTCs positivity and these characteristics. This may imply that CTCs, as a systemic indicator, can reflect the clinical characteristics of PC more broadly. Studies related to breast cancer and colorectal cancer have confirmed that the number of CTCs in peripheral blood and clinical stage, lymph node metastasis, and distant metastasis have significant significance (23,24). This is somewhat different from the result of this article, considering that it is caused by the number of samples. In addition, the nano microfluidic chip was compared with the conventional CellSearch method. The positive rate of CTCs detected by nano microfluidic chip was clearly higher than that by CellSearch, and there was a significant statistical difference between the two methods. This indicates that nano microfluidic chips have higher sensitivity and accuracy in detecting CTCs in PC patients, which provides a more reliable basis for clinical diagnosis and treatment. It is possible that CellSearch relies on EpCAM-coated immunomagnetic beads to capture CTCs and then use CK, CD45, and DAPI to identify CTCs, while the invasion of solid tumor cells into the blood needs to undergo the EMT process, during which epithelial-derived markers may be lost (25). Pancreaticoduodenectomy is the most commonly used surgical procedure for PC. Many scholars say that in order to improve the surgical prognosis of patients, it is necessary to cooperate with effective nursing intervention methods during the perioperative period (26,27). In comprehensive nursing intervention for patients following PC, the patients were grouped: into intervention groups and controls, and the physiological function, social function, body pain, and mental health scores of the two groups were evaluated. The results revealed that the scores of physical function, social function, body pain, and mental health in the intervention group were clearly superior as against controls. This indicates that comprehensive nursing intervention can significantly improve the QoL and functional status of patients after PC.

Taken together, the positive rate of CTCs is related to vascular invasion, but the correlation with other clinical features is weak. Relative to traditional methods, nano microfluidic chips have better performance. In addition, comprehensive nursing intervention can markedly improve the QoL and functional status of patients following PC. These results provide new methods and strategies for early diagnosis, treatment selection, and care of PC.

Conclusion

Novel nano microfluidic chip has high performance in detecting CTCs in PC patients, and comprehensive nursing intervention following surgery can obviously improve the QoL and functional status of PC patients. Because the sample size of this article is relatively small, especially for some analysis subgroups, which may limit the accurate evaluation of the relationship between some factors and CTCs detection results, the research scope needs to be further expanded in future studies.

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