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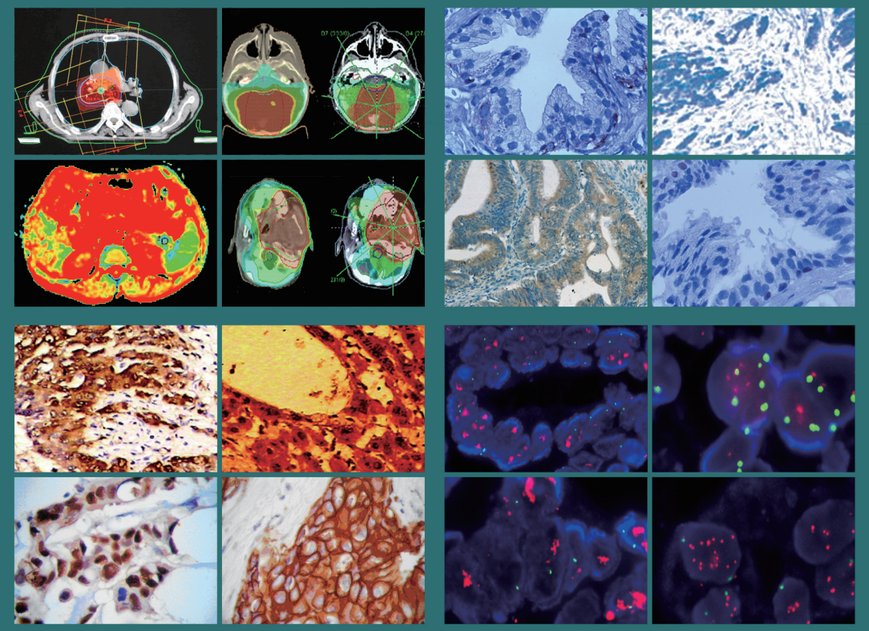

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Aims & Scope

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ORIGINAL ARTICLE

Efficacy and adverse effects of olanzapine in the treatment of moderate to severe refractory neuropathic pain*

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Abstract

Objective The aim of the study was to investigate the efficacy and adverse effects of olanzapine in the treatment of moderate to severe refractory neuropathic pain.

Methods Forty patients with digestive system cancer were enrolled, who had moderate to severe refractory neuropathic pain; the patients were treated with olanzapine for 2 weeks at a daily dosage of 5 mg to 10 mg per night according to patients' response and tolerability, combined with conventional analgesic therapy. Pain intensity was evaluated by using a Numeral Rating Scale (NRS) at baseline, 3 days, and 2 weeks after therapy. The Pittsburg Sleep Quality Index (PSQI) was evaluated at baseline and 2 weeks after therapy. Data on adverse events were recorded. The dosage of conventional analgesics was adjusted over time based on the severity of pain.

Results The mean pain score decreased by 2.575 ± 1.318 ($P < 0.000$) at 3 days and by 3.400 ± 1.614 ($P < 0.000$) at 2 weeks; 30% of the patients experienced significant pain relief at 3 days and 50% at 2 weeks. The PSQI decreased by 4.725 ± 2.828 ($P < 0.000$) at 2 weeks. The adverse events induced by olanzapine included sleepiness, weight gain, dizziness, fatigue, dry mouth, and constipation; all the side effects were mild.

Conclusion When combined with conventional analgesic therapy, olanzapine was effective in relieving pain and sleep disturbance, and was well-tolerated among patients with refractory neuropathic pain.

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Key words: Olanzapine; refractory cancer pain; neuropathic pain; efficacy; adverse effect

According to a review of the published literature [1], the incidence of pain ranges from 33% to 59% in the patients with cancer who are undergoing radical treatment, and reaches up to 64% in patients with metastatic, advanced, or terminal phase cancer. Under the guidance of the WHO cancer pain relief program, the control of cancer pain has become increasingly more pro-active and standardized; however, cancer pain does not appear to be effectively controlled in almost half of patients.

For patients with neuropathic pain, the control of symptoms is much more difficult. Neuropathic pain results from injury to the peripheral or central nervous system; this type of pain may be described as burning, sharp, or shooting. Studies have suggested that 69% of neuropathic pain is tumor-related, whereas up to 43% may be treatment-related [2]. Opioid-based analgesic therapy is recommended for both tumor- and treatment-related neuropathic pain. The use of a combination of tricyclic antidepressants (e.g., amitriptyline and nortriptyline) and anticonvulsants (e.g., gabapentin and pregabalin) in addition to the basic use of opioids can improve pain control [3–6]. Despite of, the treatment of moderate and severe cancer pain, especially when combined with neuropathic pain, remains very difficult.

Olanzapine is a thiophene benzodiazepine derivative; as a newer atypical antipsychotics, it has been identified as useful for the management of several symptoms commonly encountered in palliative care and it is used

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widely for chemotherapy-induced nausea and vomiting [7]. This paper retrospectively analyzed the efficacy and adverse reaction of 40 patients with refractory neuropathic pain admitted to the Department of Digestive Oncology, Cancer Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan, China, and these patients were all treated with olanzapine on the basis of conventional analgesia.

Materials and methods

Clinical materials

In total, 40 patients with cancer were admitted to the Department of Digestive Oncology, Cancer Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan, China, were enrolled. The median age of patients was 57 years of age (range: 26–69 years). There were 26 males and 14 females. The ECOG score of 40 patients was 1–2 (that of 10 was 2 and the rest was 1). There were 19 patients with rectal cancer, 3 patients with esophageal cancer, 7 patients with pancreatic cancer, 2 patients with anal cancer, and 9 patients with liver cancer; 26 had advanced-stage disease. All 40 patients experienced refractory pain, defined as pain symptoms that could not successfully be relieved after analgesic drug treatment for 1–2 weeks, all of which met the diagnostic criteria for neuropathic pain, that is, at least met the first and second criteria of the grading system of IASP 2008 [8]. All patients scored ≥ 2 on the ID Pain scale [9]. The clinical characteristics of the 40 patients are shown in Table 1.

Methods

For enrolled patients, the self-control method was used, and all Numeral Rating Scales (NRS) scores was recorded for all patients. The initial dose of olanzapine was 5 mg orally, once per night. Changes in the NRS score and adverse events were recorded. The Pittsburg Sleep Quality Index (PSQI) was used to assess the patients' sleep status before and 2 weeks after treatment.

In accordance with the principle of the WHO analgesia ladder, opioids should be adjusted at any time in parallel with changes in pain.

Observation indices

Assessment of pain

Pain levels were measured by self-reporting on a 0–10 NRS, where 0 is no pain and 10 is the worst pain the patient can imagine. The three levels of pain intensity referred to in the algorithm are mild pain (1–3), moderate pain (4–6); and severe pain (7–10). The clinical grading of pain was as follows. (1) Mild pain: the pain was tolerable, and the patient could live normally without the disturbance of sleep; (2) Moderate pain: the pain was obvious and unbearable, requiring the use of analgesics, and sleep was disturbed; (3) Severe pain: the pain was intolerable and sleep was seriously disturbed, which may have been accompanied by the disturbance of vegetative nervous system or passive body posture.

According to the changes in NRS score before and after medication, the degree of pain relief was evaluated as mild (< 25%), moderate (25%–49%), and obvious (≥ 50%).

Patients chose a number that represented their pain level, based on their individual experience of pain, and the pain score was registered by the medical staff. Pain score was recorded three times per day from the start of medication. If breakthrough pain occurred, it was evaluated and recorded at any time.

Assessment of sleep quality

The PSQI was used to evaluate the patients' sleep status [10]. PSQI is a widely used sleep quality scale. Patients filled in 18 self-rating items before and after 2 weeks treatment. There were seven evaluation items for PSQI: sleep quality, time to fall asleep, sleep time, sleep efficacy, sleep disorders, hypnotic drugs, and daytime function. The sum of the seven items was the total score of PSQI, and a total score of greater than seven could be diagnosed as insomnia.

Adverse reactions

The adverse reactions of patients treated with

Table 1 Clinical characteristics of 40 patients with refractory cancer pain

	n	%
Gender		
Male	26	65.0
Female	14	35.0
Age (years)		
> 60 y	14	35.0
≤ 60 y	26	65.0
Pain causes		
Tumor	26	65.0
Surgery	6	15.0
Radiotherapy	4	10.0
Surgery + radiotherapy	4	10.0
Stage		
II	1	
III	13	
IV	26	
ECOG score		
0	3	7.5
1	27	67.5
2	10	25.0
Cancer type		
Rectal cancer	19	47.5
Esophageal cancer	3	7.5
Pancreatic cancer	7	17.5
Anal cancer	2	5.0
Liver cancer	9	22.5

olanzapine were recorded, and the degree of adverse reactions was closely observed. Vital signs were measured at least once per day.

Statistical methods

SPSS 22.0 statistical software was used for data processing. A paired *t*-test was used to analyze changes in the NRS score and PSQI score before and after treatment for each patient, and $P < 0.05$ was considered to represent a statistically significant difference.

Results

Analysis of analgesic effect

The enrolled patients all experienced moderate or severe pain: 22 patients (55.0%) had moderate pain (NRS 4–6) and 18 patients (45.0%) with severe pain (NRS 7–10).

After 3 days treatment with added olanzapine, 90% (36/40) of patients experienced pain relief, and the pain relief rate reached 92.5% (37/40) after 2 weeks of treatment. The PSQI score of all 40 patients after 2 weeks of treatment was lower than before treatment.

The mean NRS of the 40 patients was 6.675 ± 1.328 at baseline, 4.100 ± 1.008 after 3 days with the addition of olanzapine treatment, and 3.275 ± 0.988 after 2 weeks.

The proportion of pain relief after 3 days and 2 weeks are shown in Table 2.

Assessment of sleep status

The PSQI score was 13.700 ± 3.566 before olanzapine treatment and 8.975 ± 2.486 after 2 weeks of treatment. The changes in NRS and PSQI score after the addition of olanzapine treatment are shown in Table 3.

Summary statistics were compared by using a paired *t*-test. Compared with the baseline values, the NRS score after 3 days of medication decreased by 2.575 ± 1.31 (*t*-value, 12.354; $P < 0.000$), and the NRS score decreased by 0.825 ± 0.747 (*t*-value, 6.983; $P < 0.000$) after 2 weeks of administration compared to that after 3 days. Compared with the baseline values, the PSQI score decreased by 4.725 ± 2.283 (*t*-value, 10.566; $P < 0.000$) after 2 weeks of treatment. All differences were statistically significant.

Adverse reaction

The patients were continuously monitored for adverse reactions during treatment. Fifteen patients (37.5%) experienced somnolence after olanzapine medication, 3 patients (7.5%) reported weight gain after 2 weeks of drug administration, 6 patients (15.0%) experienced dizziness, 7 patients (17.5%) experienced fatigue, 1 patient (2.5%) experienced a mild extrapyramidal reaction, which manifested as dysphoria and fidgeting for unknown reasons, 2 patients (5.0%) experienced grade I transaminase elevation, 2 (5.0%) patients experienced nausea but no vomiting, and 14 (32.5%) patients complained of mild xerostomia after treatment. All the above adverse reactions were mild and tolerable, and no drug withdrawal or reduction treatment was given.

Table 2 Pain relief after introduction of olanzapine [*n* (%)]

	No relief (%)	Mild relief (%)	Moderate relief (%)	Obvious relief (%)
After 3 days	4 (10.0)	5 (12.5)	19 (47.5)	12 (30.0)
After 2 weeks	3 (7.5)	7 (17.5)	10 (25.0)	20 (50.0)

Table 3 NRS and PSQI changes before and after introduction of Olanzapine

	Before medication	Three days after medication	Two weeks after medication
NRS score	6.675 ± 1.328	4.100 ± 1.008	3.275 ± 0.988
PSQI score	13.700 ± 3.566		8.975 ± 2.486

Discussion

Psychiatric drugs have been used for the treatment of pain for more than 30 years [11]. Phenothiazine drugs such as chlorpromazine and propofol are used for the treatment of headache, and psychoactive drugs, such as haloperidol and fluphenazine, are reported to be used in the treatment of neuropathic pain, especially for patients with mood disorders, anxiety, depression, and other adverse emotions [11–12].

It has been reported that the incidence of insomnia in patients with cancer is as high as 20%–90% [13–16], especially in patients with pain. Apart from the psychological inducement of fear and anxiety caused by disease and treatment, pain caused by disease and treatment, and adverse reactions caused by drugs, are important factors affecting sleep [17]. Sleep dysfunction can induce different levels of anxiety and depression, which will significantly affect the quality of life of patients with cancer. Studies have shown that patients with sleep disorders often also experience a decrease in central 5-hydroxytryptamine (5-HT) content [18], and that changes in central 5-HT level can significantly affect the emotional state of patients, resulting in anxiety, depression, and other symptoms.

Olanzapine, as a new atypical psychotropic drug, has been used widely in various fields, such as antiemetic therapy for cancer patients and treatment for depression [7, 19] owing to its affinity for a variety of neurotransmitters [19–20], including the dopamine (D) D1, D2, D3, D4, 5-hydroxytryptamine (5-HT) 5-HT2A, 5-HT2C; histamine H1; adrenal α1 and M1 receptors. Olanzapine acts on the 5-hydroxytryptamine/dopamine receptor, downregulates

the 5-HT2 receptor and its antagonism to the dopamine receptor is relatively weak, which may be the key effect through which olanzapine improves sleep and alleviates depressive symptoms in patients [21]. Many studies have suggested that olanzapine can improve sleep in healthy people [22–23]. A small study showed that when opioid drugs were used to treat moderate and severe cancer pain, the addition of 2.5–7.5 mg olanzapine daily, which can effectively control pain, reduce the use of opioids, relieve pain-related anxiety, depression, delirium, and cognitive impairment, improve the patients' sleep, and improve the quality of life of patients with pain.

In this study, all 40 enrolled subjects were patients with refractory cancer pain that could not be satisfactorily controlled by conventional analgesic therapy for 1–2 weeks. There were 26 patients with advanced cancer, and 13 patients with stage IIb and IIIb rectal cancer were enrolled because of the poor control of anal pain caused by surgery and radiotherapy. One patient with stage III esophageal cancer had poor control of symptoms of chest wall pain after surgery. For the patients with refractory pain, we added olanzapine on the premise of basic analgesic treatment; after 3 days, only 4 patients (10.0%) showed no improvement in pain symptoms, after 2 weeks, 37 patients (92.5%) showed varying levels of relief in pain symptoms. After 3 days administration of olanzapine, pain symptoms were relieved slightly in 9 cases (22.5%), moderately in 19 cases (47.5%), and significantly in 12 cases (30%). However, after 2 weeks of medication, 50% of the patients' pain has been significantly improved. The overall pain improvement is obvious.

The total average score of PSQI before medication was 13.700 ± 3.566; the lowest score was 7 and the highest score was 20. All 40 patients in the group had sleep disorders of different levels and all met the diagnostic criteria for insomnia. After the administration of olanzapine for 2 weeks, the overall mean score of the PSQI re-evaluation was 8.975 ± 2.486; the lowest score was 4, and the highest score was 12. This was a statistically significant decreased. The PSQI total score of in 8 (20%) patients was lower than 7. This suggests that the sleep condition of patients with olanzapine was significantly improved. After severe pain was controlled, the use of opioids also decreased.

Conclusion

This study showed that the incidence of adverse reactions to low-dose olanzapine for the treatment of refractory pain in patients with cancer was low, and that most of the adverse reactions were mild and well-tolerated. Olanzapine is cheap, only 8 yuan per day for 5 mg, and administered once per day orally. In summary, in patients with cancer with refractory neuropathic pain, a low dose of olanzapine added to the conventional analgesic therapy, which has clear analgesic effect, results in mild adverse reactions, can be well tolerated, and a low price, can be considered for selected cases.

Conflicts of interest

The authors indicate no potential conflicts of interest.

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ORIGINAL ARTICLE

Experimental study on the relationship between traumatic stress and tumor growth, proliferation, and metastasis*

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Abstract

Objective This study aimed to investigate the relationship between traumatic stress and tumor growth, proliferation, and metastasis.

Methods A scalding method was used as an injurious factor to induce traumatic stress in Wistar rats. The rats were randomly divided into three groups—the control group, mild-scald group, and severe-scald group, with 14 rats in each group. Wistar rats were used to subculture the Walker-256 cell line for the generation of tumor ascites. Tumor cells from the ascites were cultured and used to establish a rat subcutaneous xenograft model. After 7 days, the mild-burn group and the severe-burn group were subjected burns to 10% and 15% of their backs, respectively. Blood was taken from the tail vein of rats at different times to detect changes in blood cortisol, IL-1β, and TNF-α levels. Pathological specimens were collected 14 days later, and immunohistochemistry was performed to examine vascular endothelial growth factor (VEGF), proliferating cell nuclear antigen (PCNA), E-cadherin, and vimentin.

Results Cortisol, IL-1β and TNF-α levels were significantly higher in the scalding groups than in the control group. Tumor examination was performed after 14 days. The changes in tumor size showed that the tumor volume in the control group (0.593 ± 0.195 cm^3) and the mild-scald group (0.782 ± 0.344 cm^3) were not significantly different. However, the tumor volume was significantly larger in the severe-burn group (1.806 ± 0.838 cm^3) than in the control and the mild-burn groups($P < 0.05$). Tumor tissue immunohistochemistry showed that the percentage of cells expressing PCNA in the control group, mild-scald group, and severe-scald group was 57.1%, 71.4% and 85.7%, respectively, and the differences among the groups were statistically significant. The number of VEGF-positive cells in the mild- and severe-scald groups was significantly higher than those of the control group ($P < 0.05$). The number of E-cadherin-positive cells in the tumor tissues was significantly lower in the severe-scald group than that in the control and mild-scald groups. Vimentin showed the opposite trend in the tumor tissue, and the differences were statistically significant ($P < 0.05$).

Conclusion Different degrees of a traumatic response in tissues caused by scalding can cause a corresponding stress response in the body. The release of inflammatory mediators; increase in VEGF, PCNA and vimentin in the tumor tissue; and decrease in E-cadherin lead to a change in tumor tissue growth and metastasis. Traumatic stress is associated with tumor growth, proliferation, and metastasis.

Key words: stress response; Wistar rats; tumor; scalding

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Surgery is the treatment of choice for cancer. However, the recurrence and metastasis of postoperative tumors is the main factor that affects the efficacy of treatment. Surgical traumatic stress interferes with the function of the immune system through the bidirectional regulation of the neuroendocrine system and the immune system,

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ultimately inducing and accelerating the development of the tumor and the surveillance of the tumor cells by the immune system [1]. However, the effect of the stress caused by different degrees of trauma on tumor growth and metastasis are unclear. In this study, a stress response induced by scalding rats was used as a model to study this problem.

Materials and methods

Materials

Forty-two male Wistar rats and 1 young Wistar rat were provided by the Experimental Animal Center of Beijing Academy of Military Medical Sciences. The average body weight of the rats was 120 g (120 ± 20) g. The young rats were weaned for 2 weeks and were used to generate ascites tumors to provide tumor cells. The Walker-256 cell line was provided by the PLA General Hospital.

Preparation and collection of tumor cells

The frozen tube containing the Walker-256 cell line was removed from the -80 °C liquid nitrogen freezer and thawed in a 37 °C water bath before washing with PBS. The supernatant was removed, and 2 mL of PBS was added. The cells were resuspended. One milliliter of cell suspension was injected into the abdominal cavity of the young rat. After 5 days, the ascites of the young rat was collected, washed with approximately 30 mL of PBS three times, and centrifuged to prepare concentrated tumor cells, which were used for further study.

Generation of a rat tumor model

The model was made by subcutaneously transplanting the tumor cells. Tumor cells were counted, and a suspension with a cell concentration of approximately 2×10^8/ mL was made with PBS. Injections with 0.3 mL of cell suspension, were targeted to the left groin of Wistar rats. After 5 days, tumor nodules with a diameter of approximately 0.3 cm were deemed to be successful.

Grouping and injury of the experimental animals

Days after inoculation, scalding was used as an injury method to induce systemic stress. Rats were divided into three groups—the control group, mild-scald group, and moderate-scald group, with 12 rats in each group. According to the Rubner formula, the body surface area of the rat S (m^2) = 0.0913 × body weight (kg) and the scald area = S × percentage of burned area (%). A rectangular template for the different areas was generated. The scalding model was executed as follows. In the mild-burn group, the rats were fixed on a wooden board, the skin of the back was immersed for 5 s in 100 ° C water; the scalded area was approximately 10% of the skin on the back. In the severe-scald group, the rats were fixed on wooden boards, and the skin protruding from the back was immersed in 100° C water; the scalded area was approximately 15% of the skin on the back. Immediately after the scalding, the animals were dried with dry gauze, and the back was coated with iodophor to prevent infection. According to the degree of the scalding, the rats were intraperitoneally injected with physiological saline solution. In the experimental control group, no injury or fluid replacement was performed, however, the rest of the treatments were the same as those in the experimental group.

Indicator detection

Serum cortisol, IL-1β, and TNFα levels were determined by ELISA at different times. Fourteen days after the systemic injury, the rats were sacrificed, and the tumor tissues were observed with light microscopy. Immunohistochemistry was used to detect the expression of proliferating cell nuclear antigen (PCNA), vascular endothelial growth factor (VEGF), E-cadherin, and vimentin in the tumor tissues.

Statistical analysis

SPSS 19.0 statistical software was used to analyze the measurement data, which are represented as mean ± standard, and each group was analyzed by one-way ANOVA. Multiple comparisons were performed with LSD or Dunnett T3 tests. The count data and rate were analyzed with a chi-square test.

Results

Tumor characteristics

Before the injury, the volume of the subcutaneous tumors in each group of rats increased without any spontaneous regression. There was no significant difference in the size of the tumors between the groups. After mild and moderate burns were generated, the tumor growth of the rats in the experimental groups was significantly different from that in the control group. Fourteen days after the injury, the rats were sacrificed, the tumor tissues of the rats were recovered, and the tumor sizes were measured. The results showed that the tumor volume of the control group was small and the morphology was regular. The tumor volume of the mild- and moderate-scald groups was significantly higher than that of the control group. The difference in tumor volume among the three groups was statistically significant ($P < 0.05$; Table 1).

Table 1 Change in the transplanted tumor volume in the rats of each group (cm^3) (n = 14)

Group	1 (d)	3 (d)	14 (d)
Control	0.044 ± 0.017	0.212 ± 0.098[a]	0.593 ± 0.195[a]
Mild-scald	0.058 ± 0.020	0.348 ± 0.186[ab]	0.782 ± 0.344[ab]
Severe-scald	0.059 ± 0.028	0.543 ± 0.639[ab]	1.806 ± 0.838[ab]

[a] Compared with the group on the first day; [b] compared with the control group (P < 0.05).

Changes in the various test indicators

There was a distinct inflammatory response caused by different types of trauma.

(1) Serum cortisol concentration: After the animal was injured, data were compared regarding changes over time and changes resulting from the degree of the injury. At different observation times, there was a significant difference in the serum cortisol levels between the same group of rats after injury. The rat serum cortisol began to rise 1 day after scalding, reached a peak after 3 days, and gradually decreased after 14 days; these differences were statistically significant. When comparing differences that resulted from the degree of the injury, the serum cortisol concentration in the control group (59.01 ± 8.51), mild-scald group (89.01 ± 12.26 μg/L), and moderate-scald group (129.01 ± 23.98 μg/mL) were significantly different one day after scalding (P < 0.05). The serum cortisol concentration in the severe-scald group (P < 0.05) was significantly higher than that in the control and mild-scald groups. This change persisted, with the lowest levels seen in the moderate-burn group on day 14 postinjury (Table 2).

(2) Serum IL-1β and TNF-α concentrations: The serum IL-1β and TNF-α levels based on the time and extent of the trauma also showed changes similar to those observed for cortisol. The differences among the cytokine levels in the groups at 1 day after scalding was statistically significant. The rat serum IL-1β and TNF-α levels began to rise 1 day after scalding and reached a peak after 3 days.

After 14 days, the degree of scalding was positively correlated with the increase in serum IL-1β and TNF-α levels, and the differences were statistically significant. (Tables 3 and 4).

Table 2 Changes in the plasma cortisol levels in the rats (μg/mL)(n = 14)

Group	1 (d)	3 (d)	14 (d)
Control	59.01 ± 8.51	61.08 ± 12.43[a]	60.07 ± 12.39[a]
Mild-scald	89.01 ± 12.26	100.12 ± 17.31[ab]	73.26 ± 15.62[ab]
Severe-scald	129.01 ± 23.98	141.01 ± 28.51[ab]	95.78 ± 15.44[ab]

[a] Compared with the group on the first day; [b] compared with the control group (P < 0.05).

Table 3 Changes in the plasma IL-1 β levels in the rats (n = 14)

Group	1 (d)	3 (d)	14 (d)
Control	5.8 ± 1.5	6.1 ± 1.9[a]	5.9 ± 1.7[a]
Mild-scald	91.8 ± 10.3	145.8 ± 17.5[ab]	40.2 ± 9.3[ab]
Severe-scald	156.2 ± 21.9	205.3 ± 26.4[ab]	55.78 ± 10.1[ab]

[a] Compared with the group on the first day; [b] compared with the control group (P < 0.05).

Table 4 Changes in the plasma TNF-α levels in the scalded rats (n = 14)

Group	1 (d)	3 (d)	14 (d)
Control	6.3 ± 1.7	6.5 ± 1.9[a]	6.5 ± 1.7[a]
Mild-scald	43.5 ± 7.5	65.0 ± 14.2[ab]	31.5 ± 9.7[ab]
Severe-scald	86.2 ± 14.9	124.1 ± 19.8[ab]	53.8 ± 12.3[ab]

[a] Compared with the group on the first day; [b] compared with the control group (P < 0.05).

Immunohistochemical expression of PCNA, VEGF, E-cadherin, and vimentin in rat tumor tissues

The expression of PCNA in the mild- and moderate-scald groups was higher than that in the control group. PCNA expression in the control group was noted in 50.0% of the tissue. However, PCNA expression in the mild-burn group was observed in 71.4% of the tissue, and its expression in the severe-burn group was noted in 85.7% of the tissue. These differences were statistically significant. The VEGF immunohistochemical analysis of the rat tumor tissue showed that the VEGF expression patterns were similar to those of PCNA. The positive proportion of E-cadherin in the tissue gradually decreased with the severity of scalding. In contrast, vimentin positivity gradually increased with the severity of the scalding (Table 5).

Discussion

While the biological behavior of tumors has received increasing attention, research on the relationship between the tumor and the whole organism has also continued to progress. Several studies have shown that tissue microenvironmental changes are closely related to tumor growth. Surgery has a significant effect on the

Table 5 Immunohistochemical expression of PCNA ,VEGF,E-cadherin and Vimentin in tumor tissue (n = 14)

Group	PCNA	VEGF	E-cadherin	Vimentin
Control	57.1%	35.7%	75.7%	14.3%
Mild-scald	71.4%[a]	64.3%[a]	68.3%[a]	21.4%[a]
Severe-scald	85.7%[a]	85.7%[a]	28.7%[a]	51.4%[a]

[a] Compared with the control group (P < 0.05).

recurrence and metastasis of some malignant tumors. The surgical removal of tumor lesions causes local changes at the surgical site and systemic changes in the body [1–2]. When the surgical trauma is greater, the traumatic stress response increases, including neuroendocrine reactions, cytokine changes, metabolic changes, and other possible biological responses [3-6]. The damage caused by different degrees of burns can lead to different degrees of systemic reactions. In this case, the hypothalamus, pituitary, and adrenal axes are excited, and corticosteroids are released in large quantities to maintain the stability of the body environment [7–8]. The results of this experiment also suggest that the amount of cortisol that is released differs based on the degrees of the burn; the level of corticosteroids released increases with the increasing burn severity.

The traumatic response of the body can further stimulate the systemic inflammatory response because of the stress experienced. Studies have shown that changes in inflammatory mediators, especially the release of large amounts of inflammatory mediators, can promote tumor progression by altering the tumor microenvironment [9]. An example of this is a massive release of TNF-α, which can eventually lead to the disruption of the body's homeostasis, changes in the tumor microenvironment, and the promotion of tumor growth. Studies have found that surgical trauma can cause sympathetic nervous system excitability throughout the neuroendocrine system, releasing catecholamines, which directly inhibit the function of immune cells (NK cells) and promote cytokine secretion by Th cells that indirectly inhibit cellular immune function, thereby promoting tumor cell growth and metastasis [10].

Another study has found that TNF can promote the expression of vascular endothelial cell surface adhesion molecules, thereby promoting the formation of tumor neovascularization [11]. Systemic blood redistribution during traumatic stress, including small blood vessel contraction, and paralysis can cause local tissue ischemia and hypoxia. The blood supply of tumor tissues can also be affected. Insufficient blood supply or ischemia-reperfusion injury occurs in tumor tissues, while the hypoxic environment can upregulate HIF-1 and regulate the expression of VEGF, increasing VEGF levels *in vivo*. VEGF can also accelerate the growth of solid tumors while promoting tissue regeneration [11–12]. As an important protein in cell proliferation, proliferating cell nuclear antigen (PCNA), also known as cyclin, also undergoes significant changes during the systemic inflammatory response. The expression of PCNA is closely related to the proliferation of cells. The detection of the number of PCNA-positive cells in tumor tissues can reflect the proliferation activity of tumor tissues and is a good marker for evaluating the state of cell proliferation. PCNA is currently widely used in the diagnosis and treatment of tumors and during prognostic evaluation [13]. In this study, the expression of PCNA in the experimental groups was higher than that in the control group ($P < 0.05$). The expression of PCNA in the severe-burn group was higher than that in the mild-burn group ($P < 0.05$), indicating that traumatic stress can enhance the expression of PCNA in tumor cells and promote tumor proliferation. E-cadherin maintains cell morphology, cell movement, and adhesion. Vimentin maintains cell shape, cytoplasmic integrity, and cytoskeletal stability. Decreased expression of E-cadherin changes the cytoskeleton, degrades the basement membrane, enhances activity, and causes high tumor invasiveness [12]. High expression of vimentin causes metastasis of tumor cells and can be used to evaluate the risk of metastasis. This study found that the expression of E-cadherin in tumor tissues gradually decreased with the increase in degree of scalding, but the expression of vimentin gradually increased as the degree of scalding increased. As the degree of scalding worsens, the stress response in rats increases, resulting in enhanced tumor invasion.

The study results indicate that different degrees of trauma lead to different degrees of the traumatic stress response and the resulting systemic inflammatory response accelerates tumor growth, through factors such as cell proliferation and vascular endothelial cytokines, and promotes changes to tumor cell morphology, movement, and activity. We speculate that with the increased stress response and the release of inflammatory factors, changes can occur in the tumor microenvironment and then activate the epithelial-mesenchymal transition of tumor cells, enhancing the movement capacity of tumor cells, which it turn may cause tumor cells to invade and metastasize. Tumor recurrence and metastasis are critical factors affecting survival after surgery. Thus, these issues deserve further study.

Conflicts of interest

The authors indicate no potential conflicts of interest.

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ORIGINAL ARTICLE

Prognostic factors for pN2 non-small cell lung cancer: a comprehensive evidence from 73 studies involving 23,772 patients*

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Abstract

Obojective Non-small-cell lung cancer (NSCLC) is a common malignancy. pN2 NSCLC, with pathologically confirmed ipsilateral mediastinal/subcarinal nodes metastasis, has been known as a very heterogeneous subgroup in terms of its anatomical, biological and patient characteristics. Prognostic factors based on patient characteristics were not well determined yet in this subgroup, and there is currently no standard treatment recommendation for these heterogeneous pN2 subjects. Apparent disagreements and inconsistency exist in study reports concerning the prognostic significance of certain factors in pN2 NSCLC, especially regarding to the issue about whether skip N2 metastasis benefit from surgery.

Methods We therefore performed this comprehensive summary of the published literatures to draw a more precise and less uncertain conclusion. After a comprehensive literature search, a total of 73 studies involving 23,773 subjects were included according to eligibility criteria.

Results As expected, most of the investigated factors, such as old age, male, advanced pathological T stage, advanced clinical N stage, multiple N2 stations, extended surgical resection (pneumonectomy), and incomplete resection, but not post-operation treatment (eg. chemotherapy and radiotherapy) were significantly associated with poor survival. However, skip N2 metastasis was favourable prognostic factors in operable pN2 NSCLC subjects. Other factors (histological type and primary tumour side) were neutral in terms of association with overall survival. We highlighted a number of important prognostic factors for pN2 NSCLC patients. Particularly, patients with skip N2 disease benefit from surgery.

Conclusion Our findings could be used as reference information for decision-making in clinical practice and future study design.

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Key words: non-small cell lung cancer; meta-analysis; prognostic factors; overall survival

Annually, more than one million deaths are attributed to lung cancer, the most common malignancy worldwide [1]. Non-small cell lung cancer (NSCLC) accounts for 80% of lung cancer cases. pN2 NSCLC, with pathologically confirmed ipsilateral mediastinal/subcarinal node metastases, is a heterogeneous subgroup in terms of its anatomical, biological, and patient characteristics [2]. Prognostic factors based on patient characteristics are not yet well-characterized in this subgroup. Furthermore, there was no major improvement regarding N descriptors in the lung cancer tumor node metastasis (TNM) stage classification system until 2017 in the 8th edition by AJCC/UICC [3], where not only metastatic lymph node location but also the numbers of involved nodes were considered. Treatment options have varied from surgery alone to surgery in combination with adjuvant and/or neo-adjuvant therapies [4–6]; however, there is currently no standard treatment recommendation for these heterogeneous pN2 subjects. Consequently, pN2 patient survival outcomes vary to a large extent and the reported 5-year overall survival rate ranges from 10 to 40% [3, 7].

To improve the prognosis of patients with pN2 disease, several clinical trials have evaluated the effectiveness of different treatment modalities, such

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surgery and with or without pre-/post-operative adjuvant therapies. However, the results achieved limited success [8–10]. Although recently developed techniques, including video-assisted thoracoscopic surgery for lobectomy and lymphadenectomy, have been used and assessed in clinical practice, their actual impacts on prognosis, especially long-term outcomes, remain controversial [11–12]. Therefore, determining prognostic factors continues to be of utmost importance for the clinical management of pN2 NSCLC.

To address this point, numerous retrospective or prospective studies have repeatedly reported certain clinicopathological features as independent prognostic factors, such as age, gender, histology type, primary tumor side/location/size, N2 stations, and skip N2 metastasis. In addition, several studies revealed that the surgery and degree of resection, as well as post-operative adjuvant therapy, were associated with long-term outcomes (see our included studies). However, apparent disagreements and inconsistencies concerning the prognostic significance of certain factors in pN2 NSCLC exist in these reports. For example, although many studies indicated that multiple N2 station involvement independently predicted worse prognosis compared with a single N2 station, others reported that no significant difference was found between them.

Considering the aforementioned discrepancies among prior studies, a comprehensive summary of the published literature is essential to reach a more precise and certain conclusion. A meta-analysis based on pooled data from single studies is one of the best methods to provide high level evidence to be integrated into clinical guidelines [13]. Herein, with a quantitative synopsis of studies published in the last few decades, we performed a meta-analysis to examine the prognostic significance of reported factors in pN2 NSCLC patients. Our report may provide clues and references for optimal clinical management of this specific subgroup, as well as guidance for future research designs.

Materials and methods

Literature search and study selection

A systematic literature search of the PubMed, EMBASE, and Cochrane Library databases up to March 2019 was conducted. For each database, all possible combinations of the following search terms were used: “non-small cell lung cancer”, “NSCLC”, “N2 disease”, “lymph node”, and “survival”. The publication language was limited to English. Reference lists of the included studies, as well as relevant systematic reviews, were checked manually to identify additional related studies. We collected published studies assessing the prognostic value of clinicopathological features and treatment elements in patients with pN2 NSCLC. All of the included subjects were pathologically proven to have N2 metastases by means of preoperative mediastinoscopy, lymph node biopsy, or mediastinal lymph node dissection at the time of resection. Overall survival (OS) was the only endpoint considered. Prognostic factors of interest could be any of those reported in prior studies; however, we only included those factors with reported or calculable hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) obtained by multivariate analysis. Definitions of these factors were in accordance with those described in the original studies. Studies containing relevant data with the following exclusion criteria were considered eligible: (1) Letters, reviews, case-reports, conference abstracts; (2) Studies that discussed the relationship between clinicopathological features or treatment strategies and OS in patients with NSCLC not proven to be pN2 disease; and (3) Articles in which multivariate HR values for OS were not reported and could not be calculated using other information. A systematic literature search of the PubMed, EMBASE, and Cochrane Library databases up to March 2019 was conducted. For each database, all possible combinations of the following search terms were used: “non-small cell lung cancer”, “NSCLC”, “N2 disease”, “lymph node”, and “survival”. The publication language was limited to English. Reference lists of the included studies, as well as relevant systematic reviews, were checked manually to identify additional related studies. We collected published studies assessing the prognostic value of clinicopathological features and treatment elements in patients with pN2 NSCLC. All of the included subjects were pathologically proven to have N2 metastases by means of preoperative mediastinoscopy, lymph node biopsy, or mediastinal lymph node dissection at the time of resection. Overall survival (OS) was the only endpoint considered. Prognostic factors of interest could be any of those reported in prior studies; however, we only included those factors with reported or calculable hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) obtained by multivariate analysis. Definitions of these factors were in accordance with those described in the original studies. Studies containing relevant data with the following exclusion criteria were considered eligible: (1) Letters, reviews, case-reports, conference abstracts; (2) Studies that discussed the relationship between clinicopathological features or treatment strategies and OS in patients with NSCLC not proven to be pN2 disease; and (3) Articles in which multivariate HR values for OS were not reported and could not be calculated using other information.

Statistical analyses

For prognostic factors, HR point estimates and 95% CIs were extracted. The Cochran Q test and I2 statistic were used to evaluate between-study heterogeneity

and as guidance for model selection for meta-analytic pooling. Statistical heterogeneity was defined as a Cochran Q test $P < 0.05$ or I2 statistic > 50% [14]. In the presence of statistical heterogeneity, a random-effects model was used; otherwise, we used a fixed-effects model for meta-analysis [15]. For pooled analysis with statistical heterogeneity, we either performed a sensitivity analysis by removal of individual studies to test stability of the results with newly recalculated pooled HRs, or conducted a meta-regression with publication year, sample size, and country (non-Asian or Asian, represented in the model by 0 and 1, respectively) as covariates to investigate possible explanations for heterogeneity. Egger's linear regression test and funnel plots were used for evaluating publication bias[16]. When publication bias was suspected (an asymmetry funnel plot or Egger's test $P < 0.05$), a trim-and-fill analysis was performed to further investigate publication bias and result stability [17]. Pooled estimates with 95% CIs not covering "1" were considered statistically significant. All meta-analyses were performed using R version 3.4.3, which was also used for result visualization. Reporting was according to the PRISMA guidelines.

Results

Characteristics of included studies

The scheme used for the literature search and study selection is shown in Fig. 1. Two authors selected full-text articles independently after a comprehensive review of potentially relevant citations. Finally, 73 studies with 23,772 patients were considered eligible for subsequent analyses (Table 1). Except for three very large studies (two based on the National Cancer database [18–19] and one based on the SEER database [20]) that included thousands of

Table 1 Basic information of included studies

Authors	Year	Country	pN2 patients	Male	Mean or Median Age (y)
Nakanishi	1997	Japan	53	33	66
Tanaka	1997	Japan	155	111	60.8
Suzuki	1999	Japan	242	140	63
Andre	2000	France	686	606	61
Bueno	2000	USA	103	59	59
Fukuse	2000	Japan	76	52	62.3
Ichinose	2001	Japan	406	291	62.4
Tomita	2003	Japan	60	42	63.9
Ueda	2003	Japan	147	102	NA
Inoue	2004	Japan	154	99	62
Tanaka	2004	Japan	99	64	62.2
Casali	2005	Italy	183	153	64
Martin	2005	USA	353	208	63
Port	2005	USA	78	39	64
Takenaka	2005	Japan	118	80	62
Benoit	2006	France	142	NA	NA
Iwasaki	2006	Japan	142	94	NA
Ohta	2006	Japan	94	52	65.5
Sakao	2006	Japan	53	38	63
Cerfolio	2008	USA	148	89	66
Lee	2008	Korea	358	283	61
Matsuguma	2008	Japan	91	50	NA
Misthos	2008	Greece	302	240	62
Decaluwe	2009	Belgium	92	68	64
Mohamed	2009	Egypt	78	58	NA
Ratto	2009	Italy	277	229	NA
Zou	2009	China	183	129	NA
Kim	2010	Korea	217	170	60.5
Ma	2010	China	173	130	NA
Sakao	2010	Japan	106	57	61
Scotti	2010	Italy	175	145	NA
Dai	2011	China	221	160	60
Fontaine	2011	UK	146	69	66
Meacci	2011	Italy	40	36	58.7
Nakagiri	2011	Japan	121	79	65
Sakao	2011	Japan	45	22	61
Zheng	2011	China	720	515	57
Amini	2012	USA	61	27	61
Baba	2012	Japan	46	35	68
Funakoshi	2012	Japan	103	55	62.7
Ito	2012	Japan	40	12	65
Hishida	2013	Japan	97	74	66
Shah	2013	USA	55	35	62
Sonobe	2013	Japan	496	325	NA
Yan	2013	China	115	81	62
Zheng	2013	China	180	124	57.7
Askoxylakis	2014	Germany	71	48	59
Ichinose	2014	Japan	67	43	65
Kim	2014	Korea	129	88	62.1
Lee	2014	Korea	355	275	60
Legras	2014	France	871	712	61.2
Lim	2014	Korea	104	86	61
Tsitsias	2014	UK	68	33	66
Wang	2014	China	263	168	NA
Cao	2015	China	208	129	59.4
Fu	2015	China	204	143	58
Hsieh	2015	Taiwan	108	56	60.2
Kawasaki	2015	Japan	121	81	66.6
Lee	2015	Korea	105	79	62
Mikell	2015	USA	2115	991	64
Robinson	2015	USA	4483	2094	NA
Uehara	2015	Japan	287	169	62
Feng	2015	China	357	209	NA
Yang	2015	USA	111	70	62
Yoo	2015	Korea	250	145	59
Garelli	2016	France	982	706	61
Kim	2016	Korea	574	444	58.8
Spaggiari	2016	Italy	141	104	63
Tamura	2016	Japan	182	127	64.6
Guerrera	2017	Italy	279	168	63
Wang	2017	China	112	77	NA
Kou	2018	China	2949	1457	NA
Xu	2018	China	246	175	59

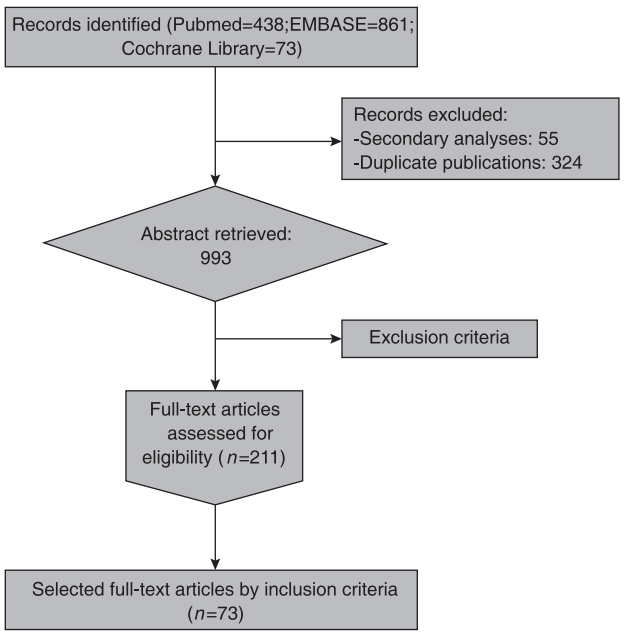

Fig. 1 Flow diagram of the meta-analysis

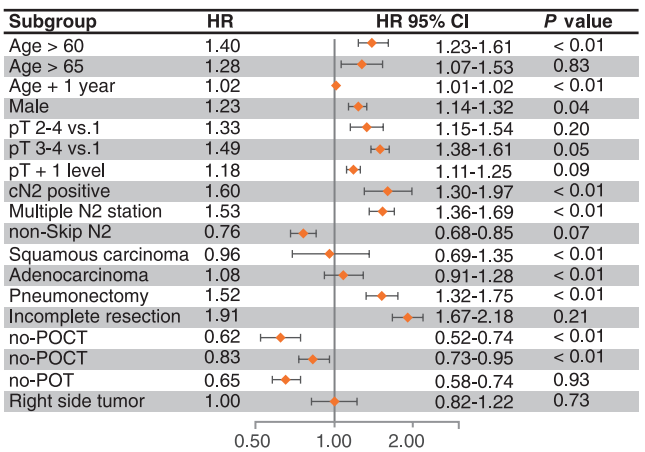

Fig. 2 Meta-analytical pooled results of investigated factors and their prognostic value. The vertical line with a value of "1" represents the risk boundary. For each factor, a black dot equals the HR value and the length of the colored line equals the 95% CI. A poor prognosis factor was defined as its HR value together with the corresponding 95% CI located outside of the risk boundary. pT, pathological T stage; cN, clinical N stage; POCT, postoperative chemotherapy; PORT, postoperative radiotherapy; POT, postoperative treatment; HR, hazard ratio; CI, confidence interval

patients, all other eligible studies were retrospective cohort studies with sample sizes less than 1000. Basic information of these included studies is provided in Table 1.

Pooled analysis

Results of the meta-analytical pooled analysis are summarized in Fig. 2.

Among demographic factors, age ≥ 60 (HR = 1.40, 95% CI = 1.23–1.61), age ≥ 65 (HR = 1.28, 95% CI = 1.07–1.53), 1 year increment in age (HR = 1.02, 95% CI = 1.01–1.02), and male (HR = 1.23, 95% CI = 1.14–1.32) were all negative prognostic factors for OS.

Among clinicopathologic factors, advanced pathologic T stage (pT2-4 *vs.* 1: HR = 1.33, 95% CI = 1.15–1.54; pT3–4 *vs.* 1–2: HR = 1.49, 95% CI = 1.38–1.61; and 1 level increment: HR = 1.18, 95% CI = 1.11–1.25), positive clinical N2 disease (cN2 vs. cN0–1: HR = 1.60, 95% CI = 1.30–1.97), and multiple N2 station involvement (HR = 1.53, 95% CI = 1.36–1.69) were prognostic factors significantly associated with poor survival. Interestingly, the presence of skip N2 metastasis was a significant protective prognostic factor in the operable pN2 NSCLC group (HR = 0.76, 95% CI = 0.68–0.85). In contrast, there was no significance regarding histological type of tumor (squamous vs. non-squamous carcinoma: HR = 0.96, 95% CI = 0.69–1.35; and adenocarcinoma vs. non-adenocarcinoma: HR = 1.08, 95% CI = 0.91–1.28).

For surgical treatment, both, extended operation type and incomplete resection, were negatively associated with OS (pneumonectomy vs. lobectomy: HR = 1.52, 95% CI = 1.32–1.75; R1 + R2 vs. R0: HR = 1.91, 95% CI = 1.67–2.18, respectively).

Post-operative treatment was associated with improved OS (post-operative radiotherapy (PORT) vs. no-PORT: HR = 0.83, 95% CI = 0.73–0.95; post-operative chemotherapy (POCT) vs. no-POCT: HR = 0.62, 95% CI = 0.52–0.74; and post-operative adjuvant treatment (POT) vs. no-POT: HR = 0.65, 95% CI = 0.58–0.74).

Heterogeneity

Statistical heterogeneity was detected in the meta-analyses for comparisons concerning age (cut-off line 60 and 1 year increment), gender, clinical N2 disease, operation type, single/multiple N2 stations, histological type, and post-operative treatment (PORT and POCT). As shown in Fig. 3, none of the pooled effects changed significantly after adjustment for influential studies. According to results of the meta-regression with country (Asian or non-Asian), sample size, and year of publication as covariates, country was a possible source of heterogeneity for analysis on age ≥ 60 vs. age < 60 (P = 0.01). Varying sample sizes might have contributed to the heterogeneity found for the analysis concerning clinical N2 disease (P = 0.042), and country and sample size could be sources of heterogeneity for analyses on single vs. multiple N2 stations (P = 0.007 and 0.0002, respectively). Furthermore, year of publication was a possible explanation for the heterogeneity found in meta-analyses on POCT vs. non-POCT (P = 0.005) and PORT vs. non-PORT (P = 0.02). The meta-regression results for other analyses were all non-significant.

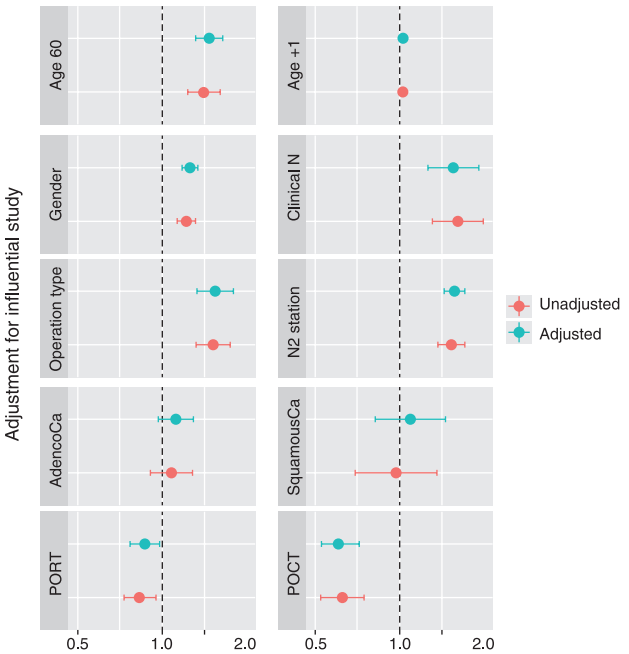

Fig. 3 Pooled effects after adjusting for influential individual studies. POCT, postoperative chemotherapy; PORT, postoperative radiotherapy; POT, postoperative treatment

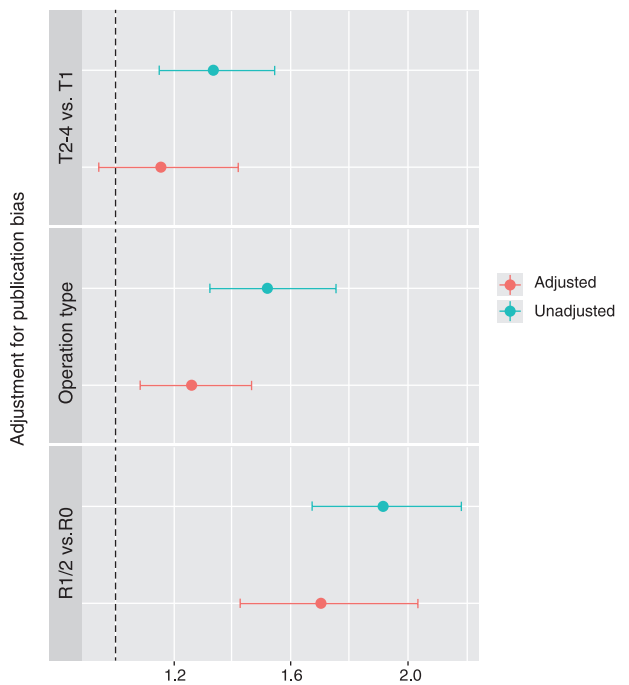

Fig. 4 Adjusted publication bias with the trim-and-fill method regarding several prognosis factors. T, pathological T stage; R, resection completeness

Publication bias

Publication bias was suspected for comparisons regarding pathological T stage (T2-4 *vs.* T1, $P = 0.037$), operation type ($P = 0.025$), and surgical completeness ($P = 0.018$). Results before and after adjustment by the trim-and-fill method are shown in Fig. 4. Funnel plots for each comparison are provided in Supplementary Data 4.

Discussion

Abundant literature focusing on the prognosis factors of pN2 NSCLC have been published; however, there has been a lack of definitive consensus among these researchers. pN2 NSCLC remains a contested issue due to apparent heterogeneity regarding its anatomical, biological, and patient characteristics. This has resulted in problematic prognosis and difficult treatment decisions. In addition to studies indicating a possible correlation between demographic characteristics (such as age, gender, and smoking history) and outcomes, certain studies showed that the outcomes of patients with pN2 NSCLC could be further influenced by tumor parameters (*e.g.*, size, side, location, and histology type) and N2 patterns (single/multiple N2 station involvement, skip N2 metastasis, and lymphadenopathy). However, in terms of the prognostic significance of these clinicopathological factors, varying opinions are held by different investigators, based on their own experience and study results. Thus, to end this chaos and find new clues for further investigation, a comprehensive synopsis of currently available data is one of the best methods to reach a relatively decisive conclusion. To the best of our knowledge, this meta-analysis, which comprehensively summarized all potential prognosis factors reported previously by applying standardized statistical methods, is the first contribution focusing on the pN2 NSCLC subgroup.

In the present study, dozens of important clinical, pathological, and treatment factors were included in the meta-analysis to assess the pN2 NSCLC patients. Notably, we applied a stringent filter for including eligible studies; only those factors with reported HRs and corresponding 95% CIs obtained from multivariate analyses were considered. According to our results, old age, male gender, advanced pathological T stage, positive clinical N2 disease, multiple positive N2 stations, extended surgery, and incomplete resection were negative prognostic factors, while post-operative treatments, including radiotherapy, chemotherapy, or bimodality therapy, improved the overall survival.

Skip N2 metastasis accounts for one third of overall cases of pN2 NSCLC [21], and is the most debated factor regarding whether skip N2 metastasis benefit from surgery. Our pooled evidence indicated that skip N2 metastasis was a favourable prognostic factor in operable pN2 NSCLC subjects, though the reasons for that remain unknown. Li *et al* reported that there was no difference

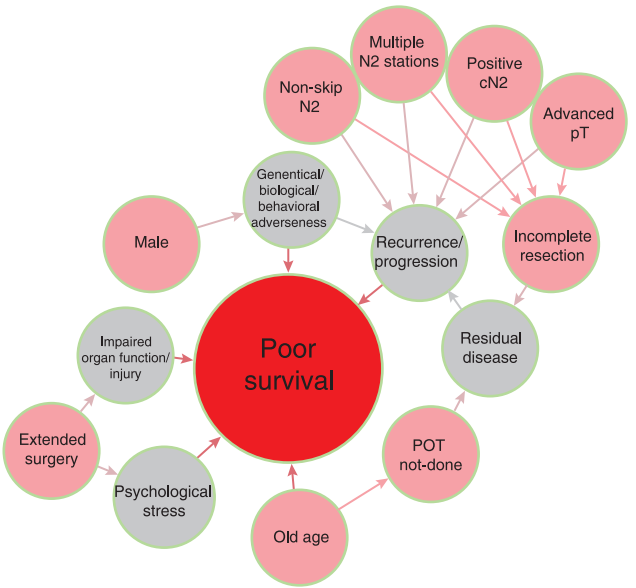

Fig. 5 Hypothetical prognostic model of investigated and latent factors, and their plausible associations with survival outcome

in terms of well-identified genetic alterations, but a significantly lower incidence of lymphovascular invasion was observed in pN2 NSCLC tumors with skip N2 compared to non-skip N2 cases [21].

Towards better interpretation of our results, we propose a hypothetical prognosis model (Fig. 5). Several latent factors are included in this model. Each directed edge between two factor nodes indicates a hypothetical causal relationship, which can be translated as "the source factor cause, or increase the possibility of the target factor". For example, according to this model, older people naturally have a shorter remaining life-span, and old age also precludes post-operative treatments, increasing the possibility of residual disease, that is a causal factor of disease recurrence or progression and results in poor survival. This model provides an overview of the potential causal relationships among the factors and the outcome. However, because data from observational studies form the basis of our study, our findings and hypotheses embedded in the prognostic model should be treated with caution and be further verified in well-designed clinical trials. Nevertheless, we suggest that prognostic factors determined as significant in the present study should be taken into consideration in further relevant studies, especially for research design and data analysis. In addition, the hypothetical relations proposed in our prognostic model incorporating latent factors may be a good starting points for subsequent confirmatory studies.

There are several limitations of the present study. Firstly, except for the prognostic factors investigated above, the biological and genetic backgrounds of individuals with pN2 NSCLC will largely affect outcomes and should be taken into account. Consistent with this, several studies found that lower PD-L1 expression and increased tumor-infiltrating lymphocytes predicted good prognosis in pN2 NSCLC[22–24], while mutation of the epidermal growth factor receptor gene and p16 gene deletion shortened OS in this group[25–26].

Secondly, statistically significant heterogeneity was detected in the meta-analysis of certain comparisons. Adjustment for influential individual studies, which were identified by a sensitivity analysis, did not change many of the pooled effects, indicating these studies were not important sources of heterogeneity. In contrast, meta-regression analyses indicated that country, year of publication, and sample size were possible sources of heterogeneity. In addition, we should be aware that, in each study, different combinations of factors were adopted for the Cox model (i.e., the statistical model used to calculate multivariate HRs and 95% CIs) and this difference may be an important source of heterogeneity. For example, whether including a certain factor into the Cox model for a given dataset will influence, to some degree, the final results. Nevertheless, the use of heterogeneous Cox models in different studies is quite common, and may inevitably introduce heterogeneity into meta-analyses.

Lastly, another important limitation of our study is publication bias. Although we cannot exclude the possibility that the detected publication bias is a consequence of selective reporting, we think it might be better to treat this publication bias as a reflection of the true effect. Unlike a meta-analysis of interventions, the present study focused on the association between certain factors and OS, and these factors were not involved with any artificial interests (i.e., the main cause of intended selective reporting). Therefore, the risk that our results were influenced by artificial selective reporting is relatively low. On the other hand, if true associations do exist, it is possible that a large proportion of studies reported results consistent enough to cause asymmetry of the funnel plot. Whether the suspected publication bias in our study is a genuine or false finding needs to be confirmed in further studies.

To summarize, our results support that the following factors are important prognostic factors for pN2 NSCLC: age, gender, pathological T stage, clinical N2 disease, number of involved N2 stations, skip N2 disease, operation type, completeness of surgery, and postoperative treatments. Our findings could be used as reference information for clinical decision-making and as guidance for the design of future studies.

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Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Analysis of the relationship between deep venous catheter-related infection and post-operative complications in patients receiving minimally invasive esophagectomy

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Abstract

Objective The aim of the study was to evaluate catheter-related infection rate (CRIR) for patients receiving minimally invasive esophagectomy (MIE), to identify the optimal catheterization approach and relationship between CRIR and post-operative complications.

Methods In total, 168 patients with esophageal carcinoma and undergoing MIE combined with pre-operative deep venous catheterization (DVC) were analyzed in our institution (Qingdao Municipal Hospital, China), from 2014 to 2018. After completing DVC, catheter-tips together with intraductal venous blood samples were sent to the microbiology lab for bacterial strain culture. CRIR was statistically evaluated for the following clinical variables: gender, age, smoking status, drinking status, past history, tumor location, histologic grade, pathological T, N, and M category, anastomotic location, anastomotic leakage, anastomotic stricture, chylothorax, pneumonia, recurrent laryngeal nerve (RLN) injury, reflux esophagitis, catheterization site, and catheter-locking days.

Results Among the 144 patients recruited in our study, 105 catheters were inserted into the jugular vein and 39 catheters into the subclavian vein. The median age of these patients was 63 years (range: 42–79 years), and the median catheter-locking period was seven days (range: 4–21 days). Four catheters were identified with three types of strain colonizations, including *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Blastomyces albicans*. Statistical data showed that patients diagnosed with catheter-related infection were likely to incur anastomotic leakage (66.67%, $P < 0.001$) and pneumonia (27.27%, $P < 0.001$); features such as tumors located in the upper esophagus (13.6%, $P = 0.003$), and over seven catheter-locking days (10.00%, $P < 0.001$) were attributed to a high CRIR.

Conclusion Although both jugular and subclavian veins can be catheterized for patients with MIE, DVC is associated with more than seven catheter-locking days and upper esophagectomy, due to high CRIR. Furthermore, catheter-related infection is related to anastomotic leakage and pneumonia.

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Key words: deep venous catheterization (DVC); catheter-related infection (CRI); minimally invasive esophagectomy (MIE); complications

Deep venous catheterization (DVC) is a dependable and convenient approach for medical staff to perform safety infusion, anti-shock, and surveillance procedures. The common sites of insertion include the femoral, jugular and subclavian veins [1–2]. Despite remarkable advantages, the drawbacks of this technique cannot be ignored. Catheter-related bloodstream infection (CRBSI) is one of the most common complications of DVC, and often results in prolonged hospitalization, increasing costs, and even a high risk of mortality [3]. With the popularization of Enhanced Recovery After Surgery (ERAS), many scholars recommended that reducing catheter-days and selecting a suitable insertion site can alleviate the complications of DVC [4–5]. However, there

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is limited research attention regarding catheter-related infection rates (CRIR) in patients undergoing minimally invasive esophagectomy (MIE).

Therefore, we conducted the present study to evaluate CRIR in different insertion sites, in order to identify the relationship between CRIR, post-operative complications and eligible local insertion sites for patients with MIE.

Materials and methods

Initially, 168 cases from 2014 to 2018 treated in the Department of Cardiothoracic Surgery of our institution (Qingdao Municipal Hospital, China) were screened, and all underwent MIS and pre-operative DVC. To minimize confusion, we designed a brief methodology to confirm the type of catheter-related infection, by identifying strain colonization of the catheter-tip and intra-catheter blood. Furthermore, some patients not meeting the criteria were excluded.

Exclusion criteria

Some necessary exclusion criteria were applied in order to streamline and enforce the research. The details were as follows: (1) Patients owing to multiple catheters during hospital stay; (2) Occurrence of unexpected exfoliation of catheter; (3) Catheter-based mechanical complications (e.g. artery injury, local hematoma and pneumothorax), and deep-vein thrombosis.

Process of catheterization and identification of strains

Under the full shield of sterilization, including surgical hand antisepsis, disposable surgical long-sleeved gowns, sterile gloves, caps, and masks for manipulators, as well as sterile drapes, antiseptics and dressings for patients, catheterization was completed using the Seldinger technique [6]. In addition, catheters were removed based on the nutritional status and emergence of post-operative complications. Given that early enteral nutrition support was beneficial to improve the patient's condition and conserve medical expenses, catheters were removed when normal oral feeding had resumed.

Catheter-tips, together with intraductal venous blood, were sent to the microbiology lab for bacterial strain culture. Any type of bacterium or fungus confirmed by the clinical laboratory was recorded.

Statistical analysis

After data collection, the relevant variables were categorized and statistical differences among categorical variables were analyzed using Pearson's chi-square test. A two-sided P value less than 0.01 was considered statistically significant. All statistical analyses were performed using SPSS version 19.0.

Results

A total of 144 catheter samples were obtained for our study. In total, 105 catheters were inserted into the jugular vein and 39 catheters into the subclavian vein. For all patients (115 males and 29 females) receiving catheterizations, the median age was 63 years (range: 42–79 years), and the median catheter-locking days was seven (range: 4–21 days). Four catheters were confirmed to possess three types of strain colonization, including *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Blastomyces Albicans*, which were identified in the tip of the catheters or intra-catheters. The detailed relationship between catheter-tip infections and post-operative complications for all patients is listed in Table 1. The statistical analysis revealed that patients diagnosed with catheter-related infection were likely to experience anastomotic leakage (66.67%, $P < 0.001$), pneumonia (27.27%, $P < 0.001$), features such as tumors located in the upper esophagus (13.6%, $P = 0.003$), and over seven catheter-locking days (10.00%, $P < 0.001$), which were attributed to a high CRIR.

Discussion

With the popularization of the DVC technique, perioperative fluid infusion and central venous pressure monitoring has become simple and reliable [2, 7]. Most surgeons and anesthetists consider the subclavian vein as a primary insertion site, because of its low CRIR. However the risk of mechanical complications such as arterial injury, obvious hematoma, and pneumothorax are greater than the jugular vein insertion site [1, 8]. The Centers for Disease Control and Prevention guidelines for preventing intravascular catheter-related infections recommend to "use a subclavian site, rather than a jugular or a femoral site, in adult patients" [9]. However, research has revealed that subclavian vein catheterization is associated with a high incidence rate of mechanical complications [2, 4, 8]. A large-scale multicentric randomized trial, which analyzed a total of 3471 catheters in 3027 patients using three procedures, demonstrated that subclavian-vein catheterization was associated with a lower risk of bloodstream infection and deep vein thrombosis, and a higher risk of pneumothorax, compared to jugular-vein or femoral-vein catheterization [8]. In our study, there was no significant difference between subclavian vein and jugular vein catheterization in CRIR, but prolonged catheter-locking days and upper esophagectomy resulted in high CRIR. Therefore, we consider it beneficial for patients with MIE to receive short-term catheterization, especially for those with upper thoracic esophagectomy.

Under the guidance of ERAS, the early removal of catheters and performing enteral nutrition support

Table 1 The relationship between the catheter-tips infection and post operative complications in patients receiving MIE

Variable	*n*	Catheter-related infection rates (%)	*P*
	144	2.78	
Gender			0.806
Male	115	2.61	
Female	29	3.45	
Age (years)			0.620
≤ 65	91	3.30	
> 65	53	1.89	
Smoking status			0.473
Never	48	4.17	
Ever	96	2.08	
Drinking status			0.208
Never	104	3.85	
Ever	40	0	
Past history			0.284
No	70	4.29	
Yes	74	1.35	
Tumor location			0.003
Upper	22	13.64	
Middle	105	0.95	
Lower	17	0	
Histologic grade			0.184
G1	32	0	
G2	79	5.06	
G3	33	0	
Pathological T category			0.362
PT1	21	0	
PT2	20	0	
PT3	81	4.94	
PT4	22	0	
Pathological N category			0.428
PN0	86	4.65	
PN1	26	0	
PN2	20	0	
PN3	12	0	
AJCC stage			0.052
PI stage	27	0	
PII stage	59	6.78	
PIII stage	58	0	
Anastomotic location			0.330
Neck	27	0	
Chest	117	3.42	
Anastomotic leakage			< 0.001
No	141	1.42	
Yes	3	66.67	
Anastomotic stricture			0.767
No	141	2.84	
Yes	3	0	
Chylothorax			0.700
No	139	2.88	
Yes	5	0	
Pneumonia			< 0.001
No	133	0.75	
Yes	11	27.27	
RLN injury			0.623
No	138	2.90	
Yes	8	0	
Reflux esophagitis			0.767
No	141	2.84	
Yes	3	0	
Catheterization site			0.924
Jugular vein	105	2.86	
Subclavian vein	39	2.56	
Catheter-locking days			0.001
≤ 7 days	104	0	
> 7 days	40	10.00	

Note: MIE: minimally invasive esophagectomy; RLN: recurrent laryngeal nerve

could minimize the risk of CRBSI. A meta-analysis conducted by Chinese scholars revealed that early oral-feeding or nasogastric feeding is superior to DVC infusion when alleviating CRIR in patients with acute pancreatitis [10]. After analyzing the variables of MIE patients, we found that high CRIR results in a high risk of anastomotic leakage and pneumonia, attributed to blood stream infection by systemic veins, arising from strain colonization of catheter-tips. Thus, it is essential to strictly manage catheter-locking days, not only to lower the risk of catheter-tip infection, but also strengthen enteral nutrition.

Some limitations of this research cannot be ignored. Firstly, the homogeneousness of retrospective data was difficult to guarantee accurately and therefore some deviation exists in the statistical results. Furthermore, the absence of a peripheral blood test for recruited samples may reduce the authority of our study. However, our research still has importance: (a) both jugular and subclavian veins can be catheterized for patients with MIE, (b) DVC for more than seven days and upper esophagectomy leads to high CRIR, and (c) MIE patients with catheter-related infection are at risk of serious post-operative complications. We hope more large-scale, randomized controlled trials are carried out in the future to further verify these issues.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

The study of selective primary culture and determination of a breast cancer cell line in vitro*

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Abstract

Objective The successful establishment of a tumor cell bank is based on the premise that the target cells can be cultured by a legitimate approach. In this experiment, we used primary culture to select and detect breast cancer cells *in vitro*, which can provide experimental ideas and methods for the establishment of a living tumor tissue cell bank.

Methods Fifty-two specimens were collected over a two-year period from people with breast cancer who needed surgical treatment in our hospital. Cells were isolated and used to establish successful cell culture. Cell activity and cell purity were measured before liquid nitrogen cryopreservation.

Results (1) At the initial culture stage, cells grew with adherence. Cell multiplication could be seen after the cell medium was exchanged three times. Cell viability was above 86%, while the viability of the target cells was above 75%, as detected by hematoxylin and eosin (HE) staining. (2) The number of breast cancer cells decreased, while the number of fibroblasts increased after five rounds of passage. (3) The success rate was 73.08%, which did not include polluted cells and those that were not successfully cryopreserved.

Conclusion (1) breast cancer cells could be selected from primary culture *in vitro* through an appropriate method. (2) Exchange of the cell medium and further cell passage improved cell multiplication. (3) The experimental results could be monitored using trypan blue and HE staining. (4) The success of breast cancer cell culture *in vitro* could be used as a reference for other cell culture, so as to establish a tumor tissue cell bank.

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Key words: breast cancer; primary culture; Trypan blue staining; hematoxylin and eosin (HE) staining

The incidence and mortality of malignant tumors have been on the rise for the past few years, resulting in a research hotspot [1]. Tumor cell lines with rapid propagation, a convenient source, and propagate have received more attention. However only 30%–40% of the original genetics has been retained [2]. Therefore, a way to keep the genome stabile and retain rapid propagation will provide better help for clinical research.

Breast cancer has reduced prevalence, but poor prognosis. As a malignant tumor, it seriously threatens the physical and mental health of humans [3–4]. There are many methods for the culture of breast cancer [5]. Our laboratory used enzyme digestion to culture breast cancer cells and summarized the experimental problems to lay the foundation for our future work, to establish a human breast cancer cell line and bank.

Materials and methods

General materials

Patients with breast cancer at the Inner Mongolia People's Hospital from February 2017 to January 2019 were recruited. All patients provided signed informed consent before treatment. Patients ranged in age from

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28 to 74 years, with an average age of 47.67 ± 11.78 years. All patient samples were digitally coded to ensure anonymization.

Inclusion criteria

(1) First diagnosis of breast cancer;

(2) First surgical treatment of breast cancer;

(3) Never received radiation therapy;

(4) Never received chemotherapy.

Patients had to satisfy all the above requirements to be included in the study.

Experimental reagents

Experimental reagents for cancer cell culture

(1) Tissue separation system (CHI SCIENTIFIC, USA) for breast cancer cell culture;

(2) Digestive buffer for breast cancer tissues;

(3) Tissue washing liquid for breast cancer tissues;

(4) Basic culture medium for breast cancer cell culture;

(5) Culture medium supplements;

(6) Culture serum breast cancer cell.

Experimental reagents for detection of activity

Trypan blue reagent and Dulbecco's phosphate-buffered saline (DPBS) buffer solution.

Experimental reagents for hematoxylin and eosin (HE) staining

Xylene, anhydrous alcohol, sterile water for injection, hematoxylin, eosin, 1% hydrochloric acid alcohol, and neutral gum.

Experimental apparatus

VS-840K-U super-clean worktable (Suzhou Antai Air Tech Co., LTD., China), MCO-5AC carbon dioxide incubator (SANYO, Japan), OPTEC-BDS200 inverted biological microscope (Chongqing Optec Instrument Co., LTD., China), MD192 low temperature refrigerator (SANYO, Japan), MVE XC47/11-6 liquid nitrogen jar (MVE, USA), HW.SY21-K Electro-Thermostatic Water Bath (Beijing Changfeng Instrument Co., LTD., China), AL104-IC electronic balance (Shanghai Mettler Toledo Instrument Co., LTD., China), incubator shakers (Shanghai Kuangbei Industrial Co., LTD., China), HC-2062 high speed centrifuge (Anhui Zhongke Zhongjia Scientific Instrument Co., LTD., China), a liquid handler from Dragon (100–1000 μL), and a liquid handler from Eppendorf (10/20–200 μL).

Experimental methods

Cell culture

(1) Breast cancer tissue was collected during the operation and the specimens were sent to the laboratory under sterile conditions.

(2) The muscle and adipose tissue around the breast cancer tissue were removed on the super-clean worktable. Tissue was rinsed with sterile liquid for at least ten minutes, twice.

(3) Under sterile conditions, the tissue was cut into fragments with 0.2–0.5 mm^2 diameters.

(4) Tissue was placed in a 37 °C incubator shaker with digestion solution until the tissue mass could not be observed by the naked eye.

(5) After digestion, an aliquot of cells was used for cell counting while another aliquot was used to start the cell culture.

(6) Cells were examined by regular microscopic examination during cell passage.

(7) Once cells were determined to be viable and the cell purity coefficient was determined, they were cryopreserved in liquid nitrogen.

Detection of cancer cell viability

Trypan blue (0.4%) was added to the cell suspension, and the living and dead cells were counted after 3 minutes. The living cell rate (%) was calculated as the total number of living cells/(total number of living cells + total number of dead cells) ×100%.

Purity test of cancer cells

Cells were smeared onto a microscope slide and HE staining was performed. The HE stained cells were used for pathological diagnosis and to test the purity of the cancer cells by microscopic examination.

Results

Number of cancer cells obtained

The size range of tissue samples was 0.25–1.2 cm^3, with an average of 0.68 ± 0.25 cm^3. The number of cancer cells obtained by digestion was 4.03–14.00 × 10^5 cells/mL, with an average of 8.76 ± 2.35 × 10^5 cells/mL. The digestion time was 10–24 h, with an average of 14.62 ± 1.81 h.

Cell culture results

(1) Most of the cells grew with adherence after 24 h (Fig. 1a). After 48 h, all of the cell lines grew with adherence (Fig. 1b).

(2) Cell started to rapidly propagate with the appropriate shape of cells after 9–10 days and three rounds of passage (Fig. 1c). The cells were tested for viability before cryopreservation; all cells were above 86% viability, with HE stained cells above 75% (Fig. 1d–1e).

(3) After five rounds of passage, a large number of dead tumor cells were observed and they stopped multiplying (Fig. 1f).

(4) Of the 52 patients, 38 cell lines were successfully cryopreserved with the number off cells at least 3 × 10^6 cells/mL. Of the remaining samples, eight had too low a cell population, seven cases were contaminated with

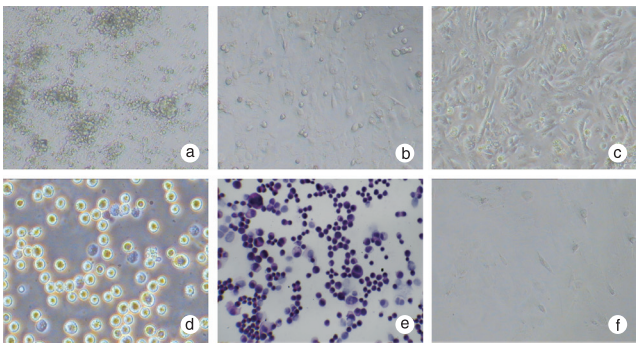

Fig. 1 Primary culture and determination. (a) Cell culture after 24 hours (×100); (b) Cell culture after 48 h (× 400); (c) Cell culture after 9 days (× 400); (d) Cell viability, (× 400, 90.30%); (e) HE consequence, (× 400,81.90%); (f) After 5 times of passages(× 400)

bacteria, one case had an fungal infection, and six were not successfully cryopreserved. The success rate was 73.08%.

Discussion

A tumor tissue bank refers to an institution that systematically collects and stores tumor tissues after surgical resection [6]. Traditionally, tumor tissues were stored under low temperature conditions with a temperature from -195°C to -80°C. However, research shows that after a long time at low temperature, the biological activity of macromolecules in tumor tissues decreases with the passage of time [7–8]. The muscle and adipose tissue which surrounds the tumor tissue also influences the obtained cells. In this experiment, tumor cells were cultured and frozen *in vitro*, which ensured a high number of tumor cells and reduced the difference between the cultured and the original cells.

Cell culture refers to a culture technique in which cells are extracted from tissues in the body, which mimics the environment in which cells grow in the body, and where cells are cultured and passaged in the proper conditions to maintain the original structure and function of the cells [9]. As a rapidly developing experimental technology in modern biological science, cell culture has been applied in various experimental studies.

The basis of successful primary cell culture is to acquire specific target cells. Understanding the growth characteristics of cancer cells guides the time to change the culture medium and when to passage cells [10–12]. Cell activity can be influenced if the cells contact experimental reagents for too long a time [13–15]. Thus, determining the correct time to combine the cells with experimental reagents seems particularly important. How to prepare frozen stock solution is as important as when to cryopreserve the cell culture [16–17].

Deficiencies in the experiment

It is necessary to explore the possible causes of contamination and then strengthen the awareness of aseptic technique after cells have been contaminated. Additionally, it is necessary to determine better culture methods to improve the growth and proliferation ability of cells to avoid the cessation of growth after the fifth cell passage.

In summary, the establishment of a tissue bank can not only provide necessary materials for follow-up experiments, but also provides certain guiding significance for the progress of clinical work. Our laboratory used enzyme digestion to culture breast cancer cells *in vitro*. We not only obtained a high enough number of tumor cells for cryopreservation, but also reduced the genetic difference between the cultured and the original cells. The success of primary culture of breast cancer cells *in vitro* provides an idea for subsequent primary culture of other cells, and at the same time, provides the basis for establishment of a living tumor tissue cell bank.

Conflicts of interest

The authors indicate no potential conflicts of interest.

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ORIGINAL ARTICLE

Benefit of adjuvant chemoradiotherapy in patients with pathologically lymph node-positive and locally advanced gastric cancer

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Abstract

Objective This study aimed to compare the effectiveness of adjuvant chemoradiotherapy (CRT) and adjuvant chemotherapy (ChT) for T3–4/N+ gastric cancer (GC) following D2/R0 dissection, and identify the specific subgroups that could benefit from adjuvant CRT.

Methods All eligible patients were divided into the CRT group and ChT group. We assessed the survival outcomes and patterns of recurrence for each group, and determined the prognostic factors for survival by performing Cox proportional risk regression analyses.

Results A total of 192 gastric cancer patients were included in the study. The estimated 3-year and 5-year disease-free survival (DFS) probabilities in the CRT and ChT groups were 52.9% *vs.* 36.7% (P = 0.024) and 41.2% *vs.* 31.1% (P = 0.148), respectively, and the estimated 3-year and 5-year overall survival (OS) probabilities were 82.4% *vs.* 70.0% (P = 0.044) and 52.0% *vs.* 35.6% (P = 0.022). Patients in the CRT group had a lower risk of locoregional recurrence than those in the ChT group (20.6% *vs.* 34.4%; P = 0.031). The subset analyses revealed that patients with stage N1–2 disease were more likely to benefit from adjuvant CRT than from adjuvant ChT (DFS: 53.1% *vs.* 36.4%; P = 0.039; OS: 53.1% *vs.* 38.6%; P = 0.036).

Conclusion For locally advanced gastric cancer patients with LN+, adjuvant CRT showed superior survival benefits compared with adjuvant ChT alone. Patients with N1–2 achieved better survival from adjuvant CRT.

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Key words: locally advanced gastric cancer; adjuvant chemoradiotherapy; adjuvant radiotherapy; lymph node-positive; survival and prognosis

Gastric cancer (GC) is the fifth most common malignant tumor in the world and one of the major causes of cancer-related deaths. Surgical resection is the main treatment paradigm of non-metastatic GC. Currently, radical D2 resection has become the globally recognized standard surgery, especially in Asian countries. However, a high rate of locoregional recurrence and distant metastasis occurred after surgery [1–2]. Therefore, adjuvant treatment is the key to improving survival outcomes after gastrectomy.

Two phase III clinical studies, the CLASSIC trial from Korea and the Japanese ACTS-GC trial, established the value of postoperative adjuvant chemotherapy (ChT) and recommended the routine use of adjuvant ChT in GC patients who underwent D2-dissection in East Asia. However, the overall survival (OS) of patients with node-positive (N2–3) disease has not improved in the ACTS-GC trial, and fewer T3 and T4 lesions had been reported in patients in the CLASSIC trial (comprising 44% of patients) [3–4]. Therefore, adjuvant ChT alone appeared to have a limited clinical value.

The ARTIST trial was a large phase III randomized clinical study that evaluated the issues described above [5]. This study did not determine the superiority of adjuvant ChT over adjuvant chemoradiotherapy (CRT). However, an unplanned subgroup analysis of node-positive patients reported significant disease-free survival (DFS). Approximately 60% of the enrolled patients had an early-

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stage disease (stages Ib/IIa), of which over 20% had T1 or T2 lesions [6].

Although the results of previous studies were not satisfactory, radiotherapy (RT) is still considered a promising therapeutic modality. Numerous studies have demonstrated that a considerable percentage of relapses following radical surgery occurred in the tumor bed or the anastomotic or regional lymph node, which provided further rationale for the utilization of adjuvant RT [7–9]. In China, most patients with gastric cancer are usually diagnosed in the later stages, with a resulting poor prognosis. Adjuvant CRT has been considered as the best option for patients with a higher risk of locoregional recurrence. In recent years, some randomized controlled trials have confirmed the clinical efficacy of postoperative CRT for certain gastric cancer patients [10–11]. The National Comprehensive Cancer Network guidelines indicated that GC patients with pathological T3–4 Nx or TxN+ stage should receive postoperative adjuvant RT. This study therefore aimed to compare the effectiveness of adjuvant CRT and adjuvant ChT for T3-4/N+ GC following D2/R0 dissection, and identify the specific subgroups that could benefit more from adjuvant CRT.

Materials and methods

This was a retrospective cohort study conducted in patients with locally advanced GC, who were surgically treated at the Affiliated Hospital of Qingdao University between June 2010 and June 2014. Patients (1) aged between 18 and 75 years, who underwent R0 resection and D2 lymphadenectomy; (2) with pathologically confirmed stage T3-4N+M0 adenocarcinoma of the stomach or gastroesophageal junction according to the 7th edition of the American Joint Committee on Cancer staging system; and (3) who received systemic postoperative adjuvant CRT or ChT were included in the study. Patients (1) who had undergone palliative surgery; (2) who underwent R1 dissection confirmed as positive surgical resection margins; (3) with incomplete function of important organs such as the heart, liver, kidney, and bone marrow; (4) who had other malignant tumors; and (5) who lacked detailed medical records including surgical records, pathology data, and adjuvant therapy regimens were excluded. Informed consent was signed by each patient included in the study. The research was approved by the ethics committee of the Affiliated Hospital of Qingdao University.

All eligible patients agreed to undergo total/subtotal gastrectomy with D2 lymph node dissection without a routine splenectomy or caudal pancreatectomy. Total gastrectomy was defined as the total resection of the stomach, while subtotal gastrectomy was defined as resection of the proximal or distal two-thirds of the stomach.

RT was administered using intensity-modulated radiation therapy using a 6-MV linear accelerator. The clinical target volume (CTV) included the tumor bed, areas of anastomosis, and regional lymph nodes. The tumor bed was delineated based on preoperative imaging and the intraoperative description provided by the surgeon. For cancer in the lower one-third of the stomach, RT was administered in the duodenal stump; for tumors in the esophagogastric junction + upper one-third of the stomach, RT was performed after conducting esophageal and intestinal anastomosis. Regional lymph nodes included in the CTV such as the perigastric, hepatoduodenal, or hepatic portal; pancreaticoduodenal; splenic hilum or splenic artery and para-aortic LNs; and the specific CTV-node (CTV-n) varied according to the location of the tumors. The planning target volume was determined by expanding a margin of 0.5–1.0 cm based on the CTV, after considering breathing movements or positioning errors.

In the CRT group, the patients were treated with one to two cycles of ChT followed by CRT (45 Gy of radiation at 1.8 Gy per day, 5 days per week, for 5 weeks with concurrent ChT) and four or five subsequent cycles of ChT after surgery. Concurrent ChT regimens included oral capecitabine (625 mg/m^2 twice daily for 14 consecutive days followed by a 7-day rest period, for 21 days) or oral S-1 (40 mg/m^2 twice daily for 14 consecutive days followed by a 7-day rest period, for 21 days). In the ChT alone group, the patients were given six to eight cycles of ChT. The ChT regimens primarily included a combination of treatments using 5-fluorouracil or oral fluorouracil derivatives along with platinum, epirubicin, or taxanes.

After systemic treatments, all patients were followed up every 3 months for the first 2 years, at 6-month intervals until 5 years, and then annually thereafter. The follow-up consisted of history and physical examination, laboratory tests (serum tumor biomarkers, blood count, hepatic function, and renal function), computed tomography scans of the chest, gastrointestinal tract ultrasonography, and a yearly gastroscopy.

Recurrence was diagnosed by histological biopsy, cytological examination, or radiographic evidence based on the patient's reviewed or follow-up receipt in the medical record. Only sites of first recurrence and metastasis were recorded and analyzed. Recurrences that occurred at the remnant stomach, anastomosis, duodenal stump, tumor bed, and regional lymph nodes were considered as locoregional recurrences. Recurrences that occurred inside the abdominal cavity were defined as abdominal metastases. Distant metastasis referred to the spread of cancer to some sites outside the abdominal cavity such as the liver and supraclavicular lymph nodes.

The study aimed to compare the 3-year and 5-year DFS as well as the OS of patients who received adjuvant CRT and ChT after D2 resection. Finally, the subgroups who benefited more from adjuvant CRT were identified.

All statistical analyses were carried out using SPSS statistical software for Windows, version 24.0 (SPSS, Chicago, IL, USA), and the χ^2 test or t-test/Wilcoxon rank sum test were performed to detect the differences in the baseline characteristics and the significance differences in the 3-year and 5-year DFS and OS between the two arms. All survival outcomes were compared using the log-rank test and were plotted using Kaplan-Meier survival curves. The Cox proportional risk regression model was used for assessing the prognostic factors for survival by univariate and multivariate analyses. A P value of < 0.05 was regarded as significant, and all P-values were two sided.

Results

Study population and clinicopathological characteristics

A total of 192 GC patients were recruited in the study between June 2010 and June 2014. The baseline characteristics are summarized in Table 1. The investigated baseline characteristics of the two arms were balanced and comparable.

Treatment delivery and safety

Patients who underwent adjuvant CRT were provided a median radiotherapy dose of 45 Gy (range: 45.0–50.0 Gy). In the CRT and ChT groups, 81.4% (83/102) and 70.0% (63/90) of the patients finished the planned ChT, respectively (P = 0.065); primary CRT regimens delivered in both groups consisted of XELOX, SOX, and FOLFOX. The most common adverse events were gastrointestinal reaction (asthenia/anorexia, nausea/vomiting, and abdominal pain/diarrhea) and hematological toxicity (leukocyte/neutropenia and hepatic insufficiency). Neither treatment-related death nor grade 3/4 adverse events occurred in either group.

Survival prognostic factors

On the evaluation date, the median follow-up time of the entire group was 54 months (range: 6–109 months). A total of 126 patients died (63 deaths in both groups), and 127 had recurrence (63 patients in the CRT arm and 64 patients in the ChT arm). The estimated 3-year and 5-year DFS probabilities of the CRT and ChT arms were 52.9% *vs.* 36.7% (P = 0.024) and 41.2% *vs.* 31.1% (P = 0.148), respectively (Fig. 1). The estimated OS probability was 82.4% *vs.* 70.0% (P = 0.044) and 52.0% *vs.* 35.6% (P = 0.022) in the same consecutive order (Fig. 2). Tables 2 and 3 list the results of the univariate and multivariate analyses for DFS and OS, respectively. Adjuvant CRT, nodal status, and tumor diameter were regarded as independent prognostic factors for DFS and OS in the univariate and multivariate analyses. Additionally, the Cox proportional hazard regression model showed that tumor location, histopathology, and differentiation were also important survival prognostic factors.

Table 1 The baseline patient characteristics in the adjuvant CRT and adjuvant ChT groups

Characteristics	CRT group		ChT group		P value
	n = 102	%	n = 90	%	
Gender					
Male	78	76.5	66	73.3	0.616
Female	24	23.5	24	26.7	
Age (years)					
≤ 60	79	77.5	56	62.2	0.021
> 60	23	22.5	34	37.8	
Tumor location					
GEJ + upper 1/3	13	12.7	7	7.8	0.461
Middle 1/3	25	24.5	29	32.2	
Lower 1/3	60	58.8	49	54.4	
Multiple/diffuse	4	3.9	5	5.6	
Histopathology					
Adenocarcinoma	92	90.2	78	86.7	0.518
Mucinous adenocarcinoma	6	5.9	5	5.6	
SRC adenocarcinoma	4	3.9	7	7.8	
Differatiantion					
Mid-low	24	23.5	16	17.8	0.327
Low	78	76.5	74	82.2	
Diameter (cm)					
Mean (SD)	5.71	(2.59)	5.51	(2.41)	0.581
Median (range)	5.25	(1.5-15.0)	5.00	(1.5-14.0)	
Tumor depth					
T3	65	63.7	49	54.4	0.191
T4	37	36.3	41	45.6	
No. resected nodes					
< 16	28	27.5	19	21.1	0.308
≥ 16	74	72.5	71	78.9	
N stage					
N1	17	16.7	17	18.9	0.913
N2	32	31.4	27	30.0	
N3a	44	43.1	36	40.0	
N3b	9	8.8	10	11.1	
Operation type					
Proximal	8	7.8	6	6.7	0.556
Distal	63	61.8	50	55.6	
Total	31	30.4	34	37.8	
Chemotherapy regimen					
Single	4	3.9	5	5.6	0.133
Doublet	89	87.3	83	92.2	
Triplet	9	8.8	2	2.2	

CRT, chemoradiotherapy; ChT, chemotherapy; GEJ, gastroesophageal junction; SRC, signet ring cell; SD, standard deviation

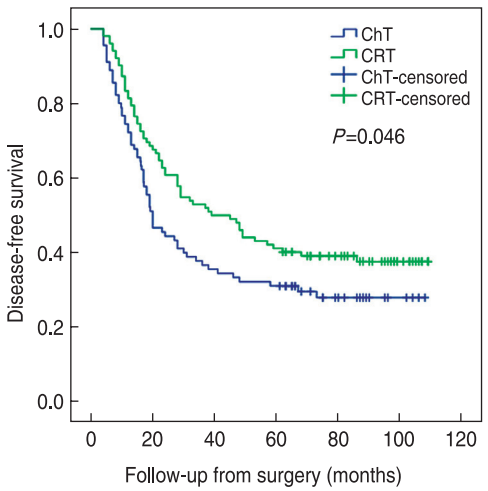

Fig. 1 DFS curves of patients in the two groups. ChT, chemotherapy; CRT, chemoradiotherapy; DFS, Disease-free Survival

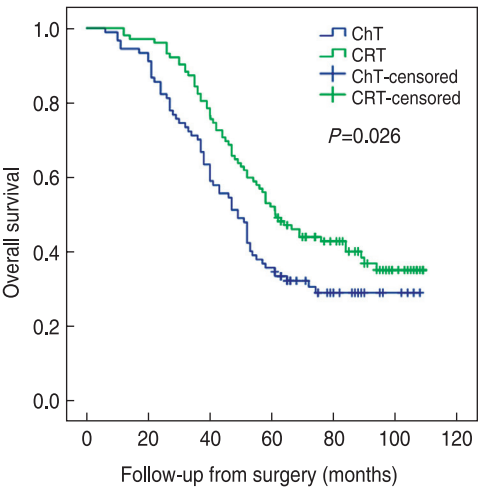

Fig. 2 OS curves of patients in the two groups. ChT, chemotherapy; CRT, chemoradiotherapy; OS, Overall Survival

Table 2 Univariate and multivariate analysis of clinical characteristics in relation to DFS

Prognostic factors	*n*	Univariate analysis			multivariate analysis		
		HR	95%CI	*P*	HR	95%CI	*P*
Gender							
Male	144	Ref.			Ref.		
Female	48	0.865	0.574–1.303	0.488	0.835	0.549–1.270	0.399
Age (years)							
≤ 60	135	Ref.			Ref.		
> 60	57	1.128	0.773–1.645	0.533	0.906	0.610–1.346	0.626
Tumor location							
GEJ + upper 1/3	20	Ref.			Ref.		
Middle 1/3	54	0.460	0.254–0.831	0.010	0.365	0.198–0.675	0.001
Lower 1/3	109	0.628	0.370–1.064	0.084	0.546	0.316–0.942	0.030
Multiple/diffuse	9	0.582	0.229–1.477	0.254	0.477	0.184–1.234	0.127
Histopathology							
Adenocarcinoma	170	Ref.			Ref.		
Mucinous adenocarcinoma	11	2.162	1.126–4.151	0.020	3.048	1.529–6.077	0.002
SRC adenocarcinoma	11	0.883	0.388–2.008	0.766	1.400	0.578–3.389	0.456
Differatiantion							
Mid-low	40	Ref.			Ref.		
Low	152	1.534	0.968–2.430	0.069	1.712	1.042–2.815	0.034
Diameter (cm)							
≤ 6	137	Ref.			Ref.		
> 6	55	1.739	1.204–2.512	0.003	1.750	1.182–2.592	0.005
N stage							
N1–2	93	Ref.			Ref.		
N3a–3b	99	1.889	1.322–2.698	< 0.001	1.890	1.305–2.736	0.001
Group							
ChT	90	Ref.			Ref.		
CRT	102	0.705	0.497–0.999	0.049	0.610	0.422–0.882	0.009

DFS, disease free survival; CRT, chemoradiotherapy; ChT, chemotherapy; GEJ, gastroesophageal junction; SRC, signet ring cell; HR, hazard ratio; CI, confidence interval; Ref, reference

Recurrence pattern

We collected the patients' imaging data and endoscopy results during the follow-up period, and then compared the patterns of recurrence between the two treatment groups (Table 4). There was no significant difference in the total recurrence rate (61.8% in the CRT group *vs.*

Table 3 Univariate and multivariate analysis of clinical characteristics in relation to OS

Prognostic factors	*n*	Univariate analysis			Multivariate analysis		
		HR	95%CI	*P*	HR	95%CI	*P*
Gender							
Male	144	Ref.			Ref.		
Female	48	0.865	0.574–1.304	0.490	0.837	0.551–1.273	0.406
Age							
≤60	135	Ref.			Ref.		
>60	57	1.159	0.794–1.691	0.445	0.924	0.624–1.370	0.695
Tumor location							
GEJ+upper 1/3	20	Ref.			Ref.		
Middle 1/3	54	0.460	0.253–0.834	0.011	0.372	0.201–0.686	0.002
Lower 1/3	109	0.596	0.351–1.010	0.055	0.529	0.307–0.914	0.022
Multiple/diffuse	9	0.606	0.239–1.539	0.292	0.503	0.194–1.302	0.157
Histopathology							
Adenocarcinoma	170	Ref.			Ref.		
Mucinous adenocarcinoma	11	2.170	1.130–4.169	0.020	2.444	1.242–4.810	0.010
SRC adenocarcinoma	11	0.864	0.380–1.966	0.728	1.368	0.572–3.271	0.481
Differatiantion							
Mid-low	40	Ref.			Ref.		
Low	152	1.548	0.968–2.474	0.068	1.589	0.971–2.600	0.066
Diameter (cm)							
≤ 6	137	Ref.			Ref.		
> 6	55	1.887	1.304–2.732	0.001	1.881	1.266–2.795	0.002
N stage							
N1–2	93	Ref.			Ref.		
N3a–3b	99	1.910	1.335–2.735	< 0.001	1.836	1.270–2.654	0.001
Group							
ChT	90	Ref.			Ref.		
CRT	102	0.674	0.475–0.958	0.028	0.587	0.407–0.848	0.004

OS, overall survival; CRT, chemoradiotherapy; ChT, chemotherapy; GEJ, gastroesophageal junction; SRC, signet ring cell; HR, hazard ratio; CI, confidence interval; Ref, reference

71.1% in the ChT group; $P = 0.172$), peritoneal seeding (24.5% in the CRT group *vs.* 27.8% in the ChT group; $P = 0.607$), and distant metastasis (21.6% in the CRT group *vs.* 24.4% in the ChT group; $P = 0.636$) between the two groups. However, patients in the CRT group had a lower risk of locoregional recurrence than those in the ChT group (20.6% *vs.* 34.4%; $P = 0.031$). In addition, patients with N3 stage had higher rates of total recurrence than those with N1–2 stage (76.8% *vs.* 54.8%; $P = 0.001$).

Subgroup analysis

The results of the subgroup analysis for DFS and OS are shown as a forest plot in Fig. 3 and Fig. 4. Patients in most subsets showed improvements in DFS and OS after undergoing adjuvant CRT. In patients with N1–2 stage disease, a tumor diameter of >6 cm, mucinous adenocarcinomas and SRC, and low differentiation, the adjuvant CRT proved to be a significant beneficial factor (all $P < 0.05$). We further mapped the survival curves of DFS and OS based on the different lymph node states; the patients with pathologically N3a–3b (pN3a–3b) had a higher risk of recurrence and mortality than those with pN1–2 (DFS, $P < 0.001$; OS, $P < 0.001$) (Fig. 5a; Fig. 6a). Patients with pN1-2 were more likely to benefit from adjuvant CRT compared with adjuvant ChT (DFS: 53.1% vs. 36.4%; P=0.039; OS: 53.1% *vs.* 38.6%; $P = 0.036$) (Fig. 5b; Fig. 6b). However, no significant survival advantage from adjuvant CRT was observed in patients with N3a–3b stage disease (DFS: 24.5% *vs.* 21.7%; $P = 0.383$; OS: 24.5% *vs.* 21.7%; $P = 0.254$) (Fig. 5c; Fig. 6c). Due to the small

Table 4 Recurrence patterns

Relapse status	CRT group, *n* (%)		ChT group, *n* (%)	
	N1–2	N3	N1–2	N3
	n = 49	*n* = 53	*n* = 44	*n* = 46
No relapse	26 (53.1)	13 (24.5)	16 (36.4)	10 (21.7)
Relapse	23 (46.9)	40 (75.5)	28 (63.6)	36 (78.3)
Local/regional	7 (14.3)	14 (26.4)	12 (27.3)	19 (41.3)
Peritoneal	10 (20.4)	15 (28.3)	11 (25.0)	14 (30.4)
Distant	6 (12.2)	16 (30.2)	10 (22.7)	12 (26.1)
CRT	0.674	0.475–0.958	0.587	0.407–0.848

ChT, chemotherapy; CRT, chemoradiotherapy; Some people have multiple sites of relapse at the same time

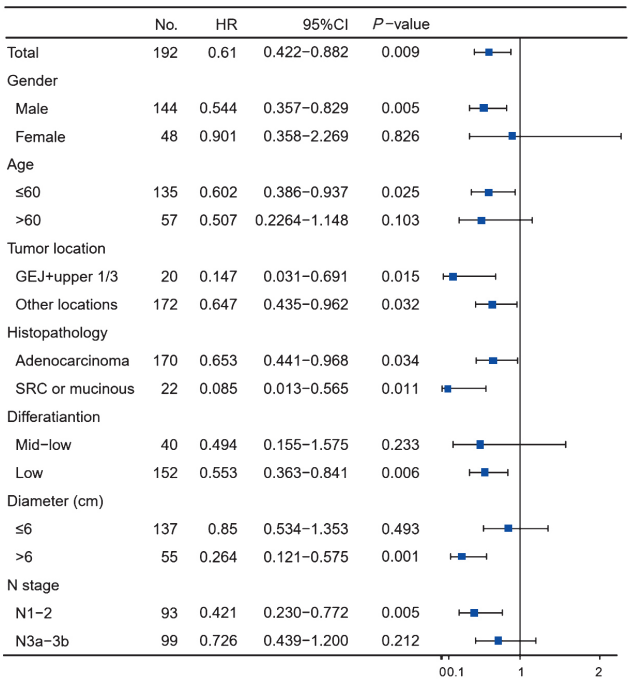

Fig. 3 Forest plot of subgroup analysis for DFS. HR < 1 favors adjuvant CRT and HR > 1 favors adjuvant ChT. ChT, chemotherapy; CRT, chemoradiotherapy; GEJ, gastroesophageal junction; SRC, signet ring cell; CI, confidence interval; HR, hazard ratio; DFS, disease-free survival

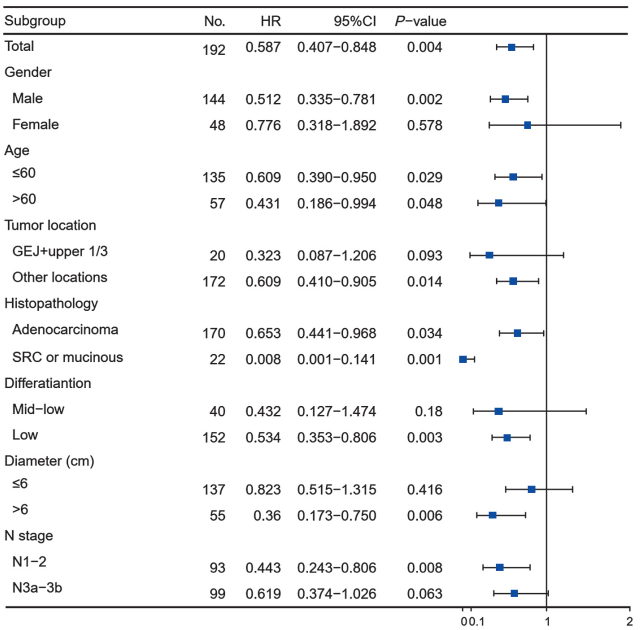

Fig. 4 Forest plot of subgroup analysis for OS. HR < 1 favors adjuvant CRT and HR > 1 favors adjuvant ChT. ChT, chemotherapy; CRT, chemoradiotherapy; GEJ, gastroesophageal junction; SRC, signet ring cell; CI, confidence interval; HR, hazard ratio; OS, overall survival

number of patients and uneven distribution in the other subgroups (55 patients with tumor diameters of >6 cm, 22 patients with mucinous adenocarcinomas and SRC, and 152 patients with low differentiation), the survival results of those subgroups should be interpreted dialectically, but the survival curves were not shown. Further clinical studies are warranted to characterize the effectiveness of combined CRT for these subgroups of patients.

Discussion

There remains a lack of consensus regarding the choice of adjuvant therapy for GC patients who underwent radical resection, especially in countries where D2 dissection is the standard surgery. Extended D2 resection provides more accurate staging and is associated with reduced locoregional recurrence and GC-related mortality risk, compared with D1 resection [12]. The research results from the ACTS-GC and the CLASSIC trials established the importance of postoperative adjuvant ChT in GC patients who underwent D2 resection. However, it remains

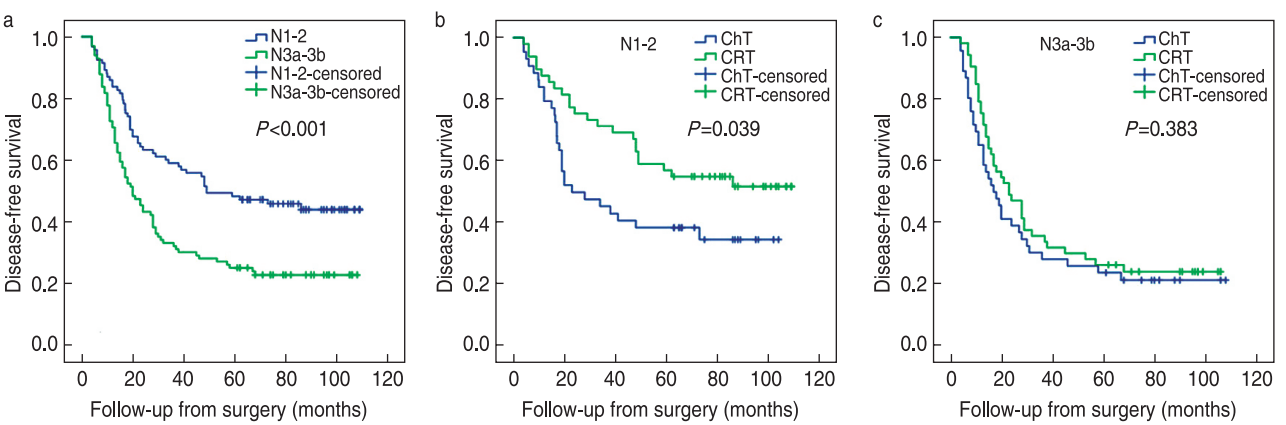

Fig. 5 Survival curves showed significant survival difference (DFS) in different LN stage (a).ChT, chemotherapy; CRT, chemoradiotherapy; DFS, disease-free survival. The patients with N1-2 stage disease (b) benifited from CRT, but the patients with N3a-3b stage disease (c) not

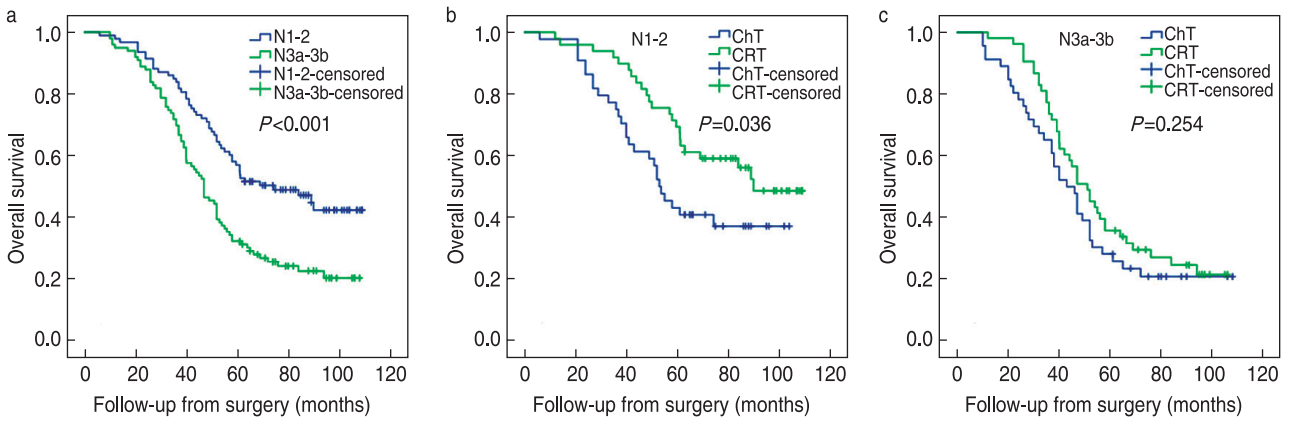

Fig. 6 Survival curves showed significant survival difference (OS) in different LN stage (a). ChT, chemotherapy; CRT, chemoradiotherapy; OS, overall survival. The patients with N1-2 stage disease (b) benifited from CRT, but the patients with N3a-3b stage disease (c) not

controversial whether the addition of RT provides further survival benefits. The ARTIST study was the first clinical study to assess the effectiveness of postoperative CRT in GC patients who underwent D2 resection. The results showed that the addition of RT to XP CRT did not significantly reduce the percentage of relapses. However, the subgroups with positive lymph nodes and intestinal type were more likely to benefit from the addition of RT. This finding indicated that it is important to explore the predominant population of adjuvant CRT cases. Therefore, we carried out a retrospective study to compare the role of adjuvant CRT and adjuvant ChT in locally advanced GC patients with positive lymph nodes who underwent D2 gastrectomy.

In this study, we only included GC patients with pathological T3–4 and pN+ stages, which was consistent with China's actual situation, because most patients in China are usually diagnosed in the later stages of the disease. Our patients mainly received doublet or triplet ChT regimens using 5-fluorouracil or oral fluorouracil derivatives, which was similar to those used in previous studies and has been shown to be well-tolerated. However, the consistency of the ChT regimens still needs to be determined using a series of clinical trials in the future. Our study demonstrated that patients with locally advanced cancer who received adjuvant CRT had significant improvements in their 3-year and 5-year DFS and OS, respectively. Although no significant difference was observed in the 5-year DFS, our results should be interpreted with caution due to the small number of relapses occurring after 3 years and the possible lower statistical efficiency. Our results did not show an improvement in the 5-year OS of patients who underwent adjuvant CRT or adjuvant ChT (5 year OS: 52.0% *vs.* 35.6%) compared with those reported in the ARTIST study (5 year OS: 75.0% *vs.* 73.0%). This could be due to the fact that 60% of the patients from each group in the ARTIST study had stage IB and II disease; therefore, they had better survival prognosis than those with late-stage disease. By contrast, our patients were usually in the later stages of the disease and, therefore, had a worse prognosis owing to the more prominent negative survival effect of advanced stage.

Subgroup analysis identified that patients with the N1–2 stage disease and poorly differentiated, larger tumor sizes (> 6 cm) showed a significantly prolonged survival after adjuvant CRT. Lymph node staging was an independent prognostic factor, which could predict distant metastasis and survival [13–14]. Kim *et al* [15] and a subgroup analysis of the ARTIST trial revealed an improvement in the DFS in pN+ patients who received CRT. In our cohort, all patients were pathologically lymph node positive and showed a significant improvement in the 3-year DFS (52.9% in the CRT arm *vs.* 36.7% in the ChT arm; P = 0.024), which was consistent with the reports of previous studies. In addition, those patients who received adjuvant CRT showed a significant improvement in their 3-year and 5-year OS. RT was reported to be the most effective locoregional therapeutic modality in patients with a high risk of relapse after surgery [16]. In our study, the CRT arm (20.6%) had lower rates of locoregional relapse than the ChT arm (34.4%; P = 0.031). This result indicated that RT might improve patient's survival through the process of locoregional control. However, patients with stage pN3 did not benefit from RT because of the high incidence of distant metastasis and peritoneal dissemination [17-19]. After further stratifying the lymph node staging into separate subgroups, we found that adjuvant CRT prolonged the survival of patients with stage pN1-pN2 disease, while no significant survival difference was shown between the two treatment strategies for patients with the pN3 classification.

In our study, tumor size showed a unique predictive value; patients with tumor size > 6 cm had superior

survival rates after receiving CRT. Maruyama Index (MI) was a quantitative standard for assessing the adequacy of lymph node dissection in gastric cancer; an MI of < 5 was considered a strong independent predictor of better disease-free survival and OS in gastric cancer patients, and tumor size was one of the seven variables [20–21]. Tumor size was consequentially considered as an essential prognostic factor in some solid tumors such as breast, lung, and liver cancer; however, for patients with lymph node metastasis, a tumor size stage system showed a significant improvement in predictive accuracy in the subgroup survival analysis [22]. Tumor size was associated with the degree of invasion and lymph node metastases in GC. We hypothesized that larger tumors were more likely to undergo micrometastases after surgery and that RT improved locoregional control by facilitating the clearance of the subclinical lesions.

The patients with the major forms of carcinoma had superior survival outcomes from adjuvant CRT based on our subset analysis, including adenocarcinoma, mucinous adenocarcinoma, and signet ring cell (SRC) carcinoma. However, there might be some bias due to the limited number of patients with mucinous adenocarcinomas and SRC types in our study ($n = 22$). In addition, Charalampakis et al. reported that tumors with a higher percentage of SRC were more likely to be resistant to RT [23]. Hence, further studies are warranted to solve this contradiction.

There were several limitations in the study. First, the sample size of the study was small. Second, it was difficult to avoid bias due to the retrospective nature of the study. Third, it was difficult to select a standard adjuvant ChT regimen, although the final distribution of ChT treatments was balanced in the two groups. Finally, due to the uneven distribution of patients in different subgroups, after combining these cases to perform a series of subgroup analyses, the results of these analyses should be cautiously interpreted. To confirm our findings, multi-center, prospective, large sample clinical trials should be conducted to obtain more rigorous results.

Conclusion

Our study showed the value of adjuvant CRT in locally advanced GC patients treated with D2 resection. For locally advanced GC patients with positive lymph nodes, the addition of adjuvant CRT showed superior clinical benefits in both OS and DFS, compared with adjuvant ChT alone. Using a subgroup analysis, we identified that high-risk patients are suitable for CRT; however, the results and significance of such subgroup analysis need to be confirmed because of the uneven distribution of patients among some subgroups, as well as the lower test efficiency. Further prospective clinical trials are needed to verify the efficacy and to characterize the predominant population of patients treated with adjuvant CRT.

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Ethics approval and consent to participate

The research was approved by the ethics committee of Affiliated Hospital of Qingdao University. Informed consent was signed by each patient included in the study.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Evaluation of the safety and efficacy of glucocorticoid therapy for hyperbilirubinemia in patients with hepatocellular carcinoma who have undergone transcatheter arterial chemoembolization

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Abstract

Objective The aim of this study was to analyze the safety and efficacy of glucocorticoid treatment for hyperbilirubinemia in patients with hepatocellular carcinoma (HCC) who have undergone transcatheter arterial chemoembolization (TACE).

Methods We conducted a retrospective analysis of the clinical data of 198 patients with HCC who were admitted to The Fifth Medical Center of PLA General Hospital from June 2014 to August 2019 and underwent TACE therapy. The patients were divided into glucocorticoid (GCC) treatment group and control group. Standard liver-protecting procedures were used in both groups. The treatment group also received intravenous injections of methylprednisolone sodium succinate for 3–5 days. Reduction in bilirubin concentration, mean duration of hospitalization, and complications were compared between the two groups to investigate the safety and efficacy of GCCs for treatment of hyperbilirubinemia after TACE treatment.

Results Bilirubin concentrations were significantly lower in the treatment group than in control group on days 3 and 5 after GCC/conventional liver-protecting treatment ($P < 0.05$). The treatment group had significantly shorter durations of total post-surgery hospitalization, and recovery time than the control group (14.5 ± 4.6 days vs. 17.5 ± 6.6 days, $P < 0.001$; 9.2 ± 3.3 days *vs*. 11.8 ± 5.4 days, $P = 0.001$; 7.0 ± 3.3 days vs. 9.3 ± 4.6 days, $P < 0.001$). No GCC-associated complications were detected in the treatment group.

Conclusion Short-term use of GCCs to treat hyperbilirubinemia in patients with HCC who have undergone TACE is safe and associated with rapid decline in bilirubin concentration and shorter hospital stay compared with patients who did not receive GCCs.

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Key words: glucocorticoid; primary liver cancer; hyperbilirubinemia;transcatheter arterial chemoembolization (TACE)

Primary liver cancer (PLC) is the fifth most common cancer worldwide and the third leading cause of cancer deaths [1]. About 90% of PLC are hepatocellular carcinoma (HCC) [2–3]. Transcatheter arterial chemoembolization (TACE) is currently one of the most used methods of treating intermediate- or advanced-stage HCC [1–4]. TACE has been shown to slow tumor growth and vascular invasion and increase the life expectancy of individuals with HCC. Because most of these patients also have hepatic cirrhosis, their liver function is often severely compromised after a TACE procedure. The main manifestations include increased serum aminotransferase and bilirubin, decreased albumin and cholinesterase, and impaired blood coagulation, all of which can slow recovery and prolong hospital stay [5–6].

Glucocorticoids (GCCs) are widely used to treat chronic hepatitis and liver failure [7–9]. However, such treatment may induce adverse reactions such as infection, gastrointestinal tract (GIT) bleeding, and viral replication. Therefore, administration of GCCs to patients with liver disease remains controversial [10–11]. Recent findings show that GCCs could protect against chemotherapy-induced

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apoptosis and improve adverse reactions to tumor necrosis such as edema, inflammation, pain, and electrolyte disturbances [12–13]. Additionally, GCCs can inhibit adverse reactions to chemotherapy-induced cytotoxicity such as nausea and vomiting. We hypothesized that GCCs would rapidly alleviate the inflammatory response resulting from tumor necrosis after TACE, facilitating improved liver function and reduced bilirubin concentrations. We also hypothesized that early, low dose, and short-term administration of GCCs would alleviate its adverse effects. In the present study, we retrospectively analyzed clinical data of 198 patients with HCC who were admitted to The Fifth Medical Center of PLA General Hospital from June 2014 to August 2019 and developed hyperbilirubinemia (HBR) after TACE operation, to investigate the safety and efficacy of administering GCCs to treat post-TACE HBR.

Materials and methods

Patients

Data of 5124 patients with HCC who were admitted to The Fifth Medical Center of PLA General Hospital from June 2014 to August 2019 and treated with TACE were retrospectively analyzed. All patients had liver function tests on days 2 to 5 after TACE procedure. Bilirubin concentrations were mildly increased (17.1–51.3 μmol/L) in 937 patients, and 198 patients had HBR (≥ 51.3 μmol/L). Only the latter were included in our study. All patients had been diagnosed with HCC based on imaging examinations or pathology findings in accordance with the Barcelona clinic liver cancer (BCLC) staging criteria [14]. Among them, 13, 93, and 92 patients were classified as stage A, stage B, and stage C HCC, respectively. All underwent TACE. These patients' baseline characteristics are as shown in Table 1. There was no statistical difference between the two groups ($P > 0.05$).

Table 1 The baseline characteristics of 198 patients with HCC

Items	Treatment (n = 102)	Control (n = 96)	P
Age (year)	54.5 ± 8.7 (Range: 35–72)	56.8±8.9 (Range: 34–83)	0.074
Gender			0.291
Male	93	83	
Female	9	13	
Tumor Size (cm)	7.0 ± 3.3 (Range: 1.0–18.5)	6.4 ± 3.3 (Range: 0.9–18.2)	0.209
< 5	24	33	
≥ 5, < 8	41	36	
≥ 8	37	27	
Etiology			0.607
HBsAg Positive	90	84	
Anti-HCV Positive	8	10	
Alcohol	3	2	
Unknown	1	0	
Child-Pugh			0.343
Class A	61	51	
Class B	41	45	
PVTT			0.309
Absence	72	58	
Branch PVTT	23	28	
Main PVTT	7	10	
EHS			0.120
Presence	25	15	
Absence	77	81	
BCLC			0.982
Stage A	7	6	
Stage B	48	45	
Stage C	47	45	

Note: HBsAg:hepatitis B surface antigen; HCV:hepatitis C virus; PVTT:portal vein tumor thrombus; BCLC:barcelona clinic liver cancer

Treatment protocols

TACE treatment: Standard preoperative preparation was performed. The Seldinger technique was used for femoral arterial catheterization, followed by angiography of the common hepatic and superior mesenteric arteries to investigate tumor staining and portal vein filling. Fluorouracil (0.5–1.0 g), epirubicin (20–40 mg), and super-lipiodol (5–25 mL) were slowly injected after super-selective catheterization of the tumor-feeding arteries, with the volume of lipiodol depending on the tumor size. Gelatin sponge particles were then administered to embolize these arteries.

TACE can be performed in the following [3]: (i) Patients with intermediate- or advanced-stage PLC who cannot be managed surgically, but do not have severe liver or kidney dysfunction. Those patients may have large tumor masses (occupying < 70% of the liver), multiple nodular lesions, partial obstruction of the main portal vein, or complete obstruction of the main portal vein with a compensatory collateral vascular network connecting to the hepatic arteries. Their liver function must be Grade A or B according to the Child–Pugh system and ECOG scores 0–2. (ii) Patients with small lesions, who do not qualify for or are unwilling to undergo surgery or ablation procedures.

Contraindications to TACE include [3]: (i) Severe irreversible coagulation dysfunction; (ii) Complete obstruction of the main portal vein by tumor thrombus with little collateral vascular network development; (iii) Active infection that cannot be treated simultaneously; (iv) Extensive metastases with expected survival time less than 3 months; (v) Severe complications resulting from decompensated cirrhosis, such as GIT bleeding, hepatic encephalopathy, massive ascites, or hepatorenal syndrome; (vi) Cachexia or multi-organ failure; (vii) Tumor occupying ≥ 70% of the

liver; and (viii) Severely decreased numbers of peripheral leukocytes and platelets, with leukocytes < 2.0×10^9/L and platelets < 50×10^9/L.

Liver-protecting procedures: The 198 patients were retrospectively allocated to GCC treatment (n = 102) and control groups (n = 96) according to whether they had received GCCs. Both groups received conventional liver-protecting treatment (*i.e.*, glycyrrhizic acid, glutathione, polyene phosphatidylcholine, and ademetionine) to treat their post-surgery liver injury. The treatment group also received methylprednisolone sodium succinate (MSS, 40–80 mg i.v., q.d., for the first three days, followed by gradual reduction of dosages until discontinuation, which occurred within 7 days).

Follow-up and outcomes

Both groups were followed up with liver function tests on days 0, 3, 5, and 30 after GCC/conventional liver-protecting treatment. Changes in total bilirubin (TBIL), direct bilirubin (DBIL), alanine transaminase (ALT), and aspartate aminotransferase (AST) concentrations, and duration of hospitalization and healing were recorded. Adverse effects of the procedure were classified on a scale of 0–4 in accordance with the CTCAE v3.0 guidelines [15].

Statistical analysis

SPSS 19.0 was used for statistical analysis. Quantitative data are expressed as mean ± standard deviation . Discrete variables are expressed as the number of cases and percentages, and between-group comparisons were performed using the χ^2 test. P < 0.05 was considered to denote statistical significance.

Results

Comparison of major biochemical indexes

Concentrations of TBIL, DBIL, ALT, and AST on admission did not differ significantly between the treatment and control groups (P < 0.05, Table 2). Concentrations of all four of these biochemical variables gradually increased after TACE, but still did not differ significantly between the two groups (P > 0.05, Table 2). These liver function indexes improved in both groups after introduction of GCC/conventional liver-protecting treatment. Three days after commencing these treatments, the treatment group had significantly lower concentrations of TBIL, DBIL, ALT, and AST than the control group (P < 0.05, Fig. 1). Five days later, the treatment group had significantly lower concentrations of TBIL and DBIL than the control group (Fig. 1). On day 30, there were no differences in the

Table 2 Comparison of major liver function indicators before and after TACE treatment between the two groups (mean ± standard)

Group	Cases	Pre-TACE				Post-TACE			
		TBII (μmol/L)	DBII (μmol/L)	ALT (U/L)	AST (U/L)	TBII (μmol/L)	DBII (μmol/L)	ALT (U/L)	AST (U/L)
Treatment	102	24.7 ± 9.9	12.8 ± 7.1	41.3 ± 27.2	55.5 ± 35.5	68.9 ± 17.3	40.8 ± 19.6	335.9 ± 254.3	344.5 ± 267.60
Control	96	27.4 ± 10.5	15.0 ± 8.6	41.9 ± 21.8	57.1 ± 34.7	66.6 ± 18.8	37.6 ± 17.1	314.1 ± 243.4	331.7 ± 317.5
P value		0.069	0.111	0.826	0.390	0.145	0.376	0.489	0.652

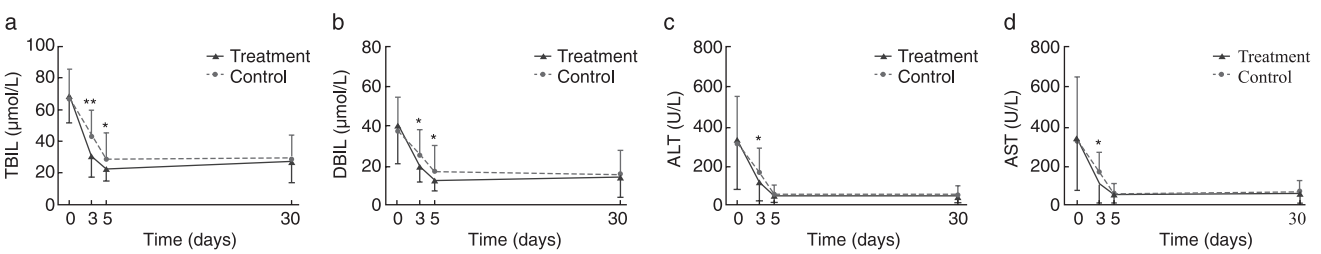

Fig. 1 Comparisons of major liver function indexes between the two groups after administration of GCC/conventional liver protecting procedures on day 0, 3, 5, and 30.

(a, b) Patients in both groups had significantly decreased TBIL and DBIL concentrations compared with pretreatment concentrations. On days 3 and 5, the treatment group had significantly lower TBIL and DBIL concentrations than the control group. (c, d) Both groups had significantly decreased ALT and AST concentrations compared with pretreatment concentrations. On days 3 and 5, the treatment group had significantly lower ALT and AST concentrations than the control group.

**P < 0.01; *P < 0.05

Table 3 Comparison of total duration of hospitalization and post-surgery recovery time between the two groups (mean ± standard)

Group	Cases	Time		
		Total hospitalization duration (day)	Post-surgery hospitalization duration (day)	Symptom improvement time (day)
Treatment	102	14.5 ± 4.6	9.2 ± 3.3	7.0 ± 3.3
Control	96	17.5 ± 6.6	11.8 ± 5.4	9.3 ± 4.6
P value		0.000	0.001	0.000

concentrations of TBIL, DBIL, ALT, and AST between the two groups ($P > 0.05$, Fig. 1).

Comparison of symptom improvement and mean duration of hospitalization between groups

All 198 patients had varying levels of fever, pain, nausea, and vomiting after undergoing TACE. Symptom improvement was defined as follows: body temperature lower than 37.5°C, pain score reduced to less than three points on the visual analog scale [16], and nausea/vomiting lower than Grade 2. Improvement time was defined as the time needed to resolve fever, pain, nausea, or vomiting. The improvement time in the treatment and control groups was 7.0 ± 3.3 days and 9.3 ± 4.6 days, respectively; this difference was significant ($P = 0.000$, Table 3). The duration of hospitalization was 14.5 ± 4.6 days and 17.5 ± 6.6 days, respectively ($P = 0.000$), whereas the duration of post-surgery hospitalization was 9.2 ± 3.3 days and 11.8 ± 5.4 days, respectively ($P = 0.001$). That is, all these variables differed significantly between the two groups (Table 3).

GCC-related complications and adverse effects

Of the 102 patients in the treatment group, 4 developed hyponatremia/hypochloremia and 1 developed temporary hyperglycemia during a fast. These adverse effects resolved completely on taking appropriate steps to correct the electrolyte disturbance and discontinuing GCCs. No hypertension, infection, bleeding, osteoporosis, endocrine disorder, or other corticosteroid-related symptoms occurred.

Discussion

TACE is currently accepted as the most commonly used treatment for patients with unresectable HCC [2–4]. TACE can provide high concentrations of chemotherapy drugs localized at tumor areas, simultaneously reducing the tumor blood supply by artery embolization. The increased duration of drug exposure increases the anti-tumor effects, resulting in ischemia, hypoxia, and apoptosis in tumor cells [17]. However, more than 90% of the patients with HCC have pre-existing cirrhosis, and chemotherapeutic drugs and lipiodol-based embolization agents will damage the remaining normal liver tissue, and further reduce the already impaired hepatic functional reserve. These patients have raised transaminase and bilirubin concentrations and may present evidence of decompensation of liver function such as ascites and GI hemorrhage [17–18]. The main mechanism underlying these beneficial effects is the stabilization of lysosome membranes which inhibits the release of mediators of liver cell necrosis or inflammation, reduces damage to vascular endothelial cells, and promotes bile excretion from biliary capillaries [19–21]. Grieco *et al* [22] followed up patients who underwent TACE for 12 months and reported that the mortality rates were 16/17 and 47/81, respectively, in the patients who did and did not develop liver failure post-procedure. It has been proposed that post-TACE increases in bilirubin and transaminase are mainly attributable to embolization agents causing cellular ischemia, hypoxia, and death, chemotherapy drug toxicities, release of inflammatory factors, and stress responses. GCCs, a broad-spectrum steroid immunosuppressant, is synthesized in and released from the adrenocortical fascicular zone and has anti-inflammatory, anti-shock, anti-allergic, and immunosuppressive effects. GCC is widely used to treat severe liver diseases and liver failure [7–9]. Studies have found that GCCs can accelerate recovery of liver function after TACE, shorten hospitalization, and reduce fatal complications [22–24]. However, in all of these studies preventive GCCs were administered before TACE, which potentially increases GCC-associated adverse effects and risk of infection.

In the present study we retrospectively analyzed the safety and efficacy of administration of GCCs to treat HBR in 198 patients with HCC treated with TACE. The treatment and control groups did not differ significantly in pre-TACE tumor size, tumor number, portal vein tumor thrombus status, extrahepatic metastasis status, Child–Pugh classification of liver function, bilirubin concentration, or transaminase concentration. After TACE, the treatment group had higher bilirubin concentrations than the control group. In both groups, bilirubin and transaminase concentrations decreased after GCC/conventional liver-protecting treatment; however, the treatment group had significantly lower bilirubin concentrations on days 3 and 5, and significantly shorter time to symptom improvement and shorter mean duration of hospitalization than the control group. Four patients in the treatment group developed hyponatremia/hypochloremia, one patient developed hyperglycemia, and none developed hypertension, infection, bleeding, osteoporosis, endocrine disorder, or other corticosteroid-

related symptoms, indicating that GCC treatment of post-TACE HBR in patients with HCC is safe and effective.

Ogasawara *et al* [23] administered dexamethasone (DMS) to prevent postembolization syndrome after TACE in patients with HCC. The treatment group received 20 mg DMS and 3 mg granisetron (GT) intravenously immediately prior to undergoing TACE and continued to receive 8 mg DMS daily for two days thereafter. The control group received saline and 3 mg GT immediately prior to undergoing TACE and continued with saline for two days thereafter. These researchers found that DMS was significantly effective in preventing fever, anorexia, nausea, and vomiting. However, DMS did not significantly prevent increased ALT, AST, or BIL levels. In a prospective, random, double-blind, controlled study of 88 patients, Yang *et al* [24] found that preventive administration of DMS was independently associated with the alleviation of postembolization syndrome. Feng *et al* [19] administered DMS plus ginsenoside to prevent postembolization syndrome. The treatment group received 2.25 mg DMS orally plus 200 mg ginsenosides orally, b.i.d., from 3 days before TACE and continued these medications for an additional 4 days after undergoing TACE. These researchers reported that the treatment group not only had significantly less nausea, vomiting, and fever after TACE surgery than the control group, but also had satisfactory reduction in ALT, AST, and BIL concentrations. However, whether GCCs should be administered preventively to all patients about to undergo TACE is still unclear, particularly for those who do not have severely impaired liver function. In our large study, only 3.86% (198/5124) of patients had bilirubin concentrations greater than 50.1 μmol/L after TACE. Most patients recovered normal liver function with conservative liver-protecting treatment; thus only a few patients required GCC treatment. We therefore did not administer GCCs preventively but prescribed it only after bilirubin concentrations had increased, to minimize blindness and other adverse effects.

The GCCs used in the present study was MSS. Compared with DMS, MSS has a rapid effect, is an active form of corticosteroid and can play a pharmacological role without being transformed by the liver. MSS has twice the binding affinity for GCC receptor and a five times stronger anti-inflammatory effect than DMS. Therefore, it relieves fever and liver function impairment more rapidly, and stresses the liver less in patients with postembolization syndrome after TACE. Additionally, MSS has lesser mineralocorticoid activity, such as water and sodium retention, than DMS. Therefore, we used MSS for short-term, pulse treatment and achieved favorable outcomes. No water or sodium retention, osteoporosis, peptic ulcer, or abnormal glucose tolerance occurred.

In the present study, we administered early, small-dose, short-term MSS treatment to patients with HCC who had significantly increased bilirubin concentrations after TACE. The treatment group attained decreased bilirubin levels in a significantly shorter time and had a significantly higher rate of effectiveness than the control group. No secondary infection, GIT bleeding, or increase in glucose concentration occurred. Compared with conventional liver-protecting treatment, treatment with GCC was associated with quicker recovery from postembolization syndrome and shorter total duration of hospitalization, which reduced the psychological and economic pressure on patients. Compared with the existing prophylactic application of glucocorticoid [19, 23–24] in the treatment of TACE postoperative embolism syndrome, it reduced blindness and side effects. However, the sample size of the present study was relatively small, and GCC efficacy, safety, and long-term effects on prognosis in patients with HCC need to be further confirmed by a large, prospective, randomized, controlled study to provide stronger evidence for its future promotion in clinical practice.

In conclusion, short-term administration of GCCs to treat HBR in patients with HCC who have undergone TACE is safe and is associated with rapid decline in bilirubin concentration and shorter hospital stay compared with patients who did not receive GCCs.

Ethics approval and consent to participate

Patient data were used after obtaining approval from the ethics committee of The Fifth Medical Center of PLA General Hospital, and the study was performed with the written consent of the patients included, in compliance with the Helsinki Declaration.

Conflicts of interest

The authors declare no potential conflicts of interest.

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ORIGINAL ARTICLE

Association between diabetes mellitus, hypertension, hyperlipidemia, chronic viral hepatitis, and the risk of multiple myeloma: a case-control study

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Abstract

Objective This case-control study aimed to investigate whether diabetes mellitus (DM), hypertension, hyperlipidemia, and chronic viral hepatitis are risk factors for multiple myeloma (MM). Moreover, the clinical characteristics of MM patients with or without the abovementioned exposure factors were analyzed.

Methods In total, 340 MM patients and 680 patients with benign diseases who were hospitalized from January 2012 to December 2017 were classified under the case group and control group, respectively. Data about medical history of DM, hypertension, hyperlipidemia and chronic viral hepatitis were collected by reviewing medical records. Univariate and multivariate analyses were conducted to compare the history of DM, hypertension, hyperlipidemia, and viral hepatitis between the two groups. Considering DM, hypertension, hyperlipidemia, and chronic viral hepatitis as exposure factors, clinical characteristics, such as renal function and presence of fungal and other types of infections, between the exposed and non-exposed groups were analyzed.

Results No significant difference was observed in the prevalence of DM, hypertension, and hyperlipidemia between the case and control groups. MM patients had a higher prevalence of chronic viral hepatitis than those with benign diseases. No significant difference was observed in the prevalence of renal dysfunction, fungal infection, and non-fungal infections in MM patients with or without DM, hypertension, and hyperlipidemia. MM patients with chronic viral hepatitis had a significantly higher prevalence of non-fungal infections during hospitalization than those without.

Conclusion No significant association was noted between MM and DM, hypertension, and hyperlipidemia. Chronic viral hepatitis is correlated to a significantly higher risk of MM, and MM patients with chronic viral hepatitis were more susceptible to non-fungal infections during hospitalization. Although a non-significant trend was observed in this study, we believe that DM and hypertension might be associated with a higher risk of MM. Thus, large-scale studies must be conducted to validate the results of the current study.

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Key words: multiple myeloma (MM); diabetes mellitus (DM); hypertension; hyperlipidemia; chronic viral hepatitis; case-control study

Multiple myeloma (MM) is a malignant disease characterized by clonal plasma cell proliferation and is the second most prevalent blood cancer. Moreover, this condition is incurable among elderly individuals [1]. The common clinical manifestations of MM include bone pain, anemia, renal insufficiency, infection, and hypercalcemia. The incidence of MM in China is about 1 per 100 000 population, which is lower than that in western countries. However, with the aging population, its incidence increases annually. The etiology of MM is not fully understood, and specific cytogenetic abnormalities, such as t(4;14), t(14;16), t(14;20), 1q+, 1p– and 17p–, are considered the molecular risk factors of this condition. Studies have shown that microRNA-32 may play an important role in the development of MM [2]. Moreover, clinical epidemiological studies have shown that the risk factors of MM may include pesticide exposure, radiation, obesity, viral infections [3–6], frequent consumption of

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pickled foods, including sauerkraut, and experiencing negative life events [7].

Hyperglycemia, hypertension, and hyperlipidemia are often referred to as the "three high". In recent years, researchers have shown increasing attention to the relationship between these conditions and malignant tumors. Some studies conducted in other countries have shown that diabetes mellitus (DM) may be a risk factor of MM. In addition, DM may be associated with a higher risk of monoclonal gammopathy of unknown significance (MGUS) converting to MM and may lead to poor prognosis in MM patients [8]. Chronic hepatitis is extremely common in China. Some researchers believe that individuals with chronic viral hepatitis may have a higher risk of hematological malignancies. Through a systematic search of literature in the database, we found that no study has assessed the association between the three high and the risk of MM in China. In addition, the correlation between chronic viral hepatitis and the incidence of MM has not been clearly reported. Therefore, this case-control study aimed to explore whether DM, hypertension, hyperlipidemia, and chronic viral hepatitis are risk factors for the onset of MM based on interviews and the case data of 340 MM patients and 680 patients with benign diseases who were matched according to age, gender, and admission year. In addition, in MM patients with or without the above-mentioned exposure factors, clinical characteristics, such as renal function, fungal infections, and non-fungal infections, were analyzed and compared to explore whether they are relevant and can be used as reference for the clinical prevention and treatment of MM.

Patients and methods

Patients

In this study, 340 MM patients admitted to Department of Hematology, Zhongnan Hospital of Wuhan University (China) from January 2012 to December 2017 were included in the case group. Meanwhile, patients of the same age, gender, and admission year who were admitted to the hospital due to benign diseases were classified under the control group with 1:2 pairs. Clinical data, including gender; age; history of hypertension, DM, hyperlipidemia, and viral hepatitis; presence of co-infection with fungal and non-fungal infections; and renal insufficiency, were collected.

As judgement might be affected, patients who did not meet the abovementioned criteria but presented with malignant tumors, other endocrine diseases, or mental diseases at a young age and those with incomplete data were excluded.

Diagnostic criteria

1. Multiple myeloma: in accordance with the Chinese MM Guidelines for Diagnosis and Treatment (revised in 2017) [9].

2. Hypertension: mean systolic blood pressure ≥ 140 mmHg or mean diastolic blood pressure ≥ 90 mmHg or intake of antihypertensive drugs (according to the 2010 Chinese Guidelines for the Prevention and Treatment of Hypertension) [10].

3. Hyperlipidemia, defined as meeting at least one of the following criteria: total cholesterol level ≥ 6.22 mmol/L, low-density lipoprotein cholesterol level ≥ 4.14 mmol/L, and triglyceride level ≥ 2.25 mmol/L (according to the 2016 Guidelines for the Prevention and Treatment of Dyslipidemia in Chinese adults) [11].

4. Diabetes mellitus: (1) Symptoms of DM and random blood glucose level ≥ 11.1 mmol/L (random blood glucose defined as blood glucose assessed at any given time). The common symptoms of DM include polyuria, thirst, and weight loss without other causes. (2) Fasting blood glucose level ≥ 7.0 mmol/L (fasting status defined as no caloric intake for at least 8 h). (3) Blood glucose level ≥ 11.1 mmol/L based on a 2-h oral glucose tolerance test. The test was still performed as required by the World Health Organization. (4) Without the symptoms of DM but meeting one of the abovementioned criteria. A diagnosis of DM was made if one of the three criteria was still met during consultation on the next day (diagnostic criteria for DM proposed by the American Diabetes Association Standards of Medical Care in Diabetes-2019) [12].

5. Chronic viral hepatitis: Hepatitis B, refer to the Guidelines of Prevention and Treatment for Chronic Hepatitis B (2019 version) [13]. Hepatitis C, meet the Guidelines for the Prevention and Treatment of Hepatitis C (2019 version) [14].

Statistical analysis

The R3.5.2 software (Mathsoft, USA) was used for analysis. Using Fisher's exact test and the Cochran-Mantel-Haenszel test, a univariate analysis was performed to assess differences in the history of exposure to DM, hypertension, hyperlipidemia, and viral hepatitis between the case group and control group after the stratification of variables, such as age, gender, and admission year. A multivariate logistic regression analysis was performed to analyze the abovementioned factors. The Fisher's exact test was used to compare the prevalence of renal dysfunction, fungal infections, and non-fungal infections among MM patients with or without the abovementioned exposure factors. A P value < 0.05 was considered statistically significant.

Results

Comparison of the exposure state of DM, hypertension, hyperlipidemia, and chronic viral hepatitis in MM patients and those with benign diseases

Among the 340 MM patients, 197 were men and 143 women, with a median age of 62.5 (24–89) years. Moreover, 45 (13.2%), 77 (22.6%), 5 (1.5%), and 18 (5.3%) MM patients presented with DM, hypertension, hyperlipidemia, and chronic viral hepatitis, respectively. Meanwhile, there were 680 patients with benign diseases. Among them, 394 were men and 286 women, with a median age of 62.5 (24–89) years. Moreover, 80 (11.8%), 128 (18.8%), 14 (2.1%), and 5 (0.7%) patients presented with DM, hypertension, hyperlipidemia, and chronic viral hepatitis. The exposure rate of chronic viral hepatitis in the case group was significantly higher than that in the control group, and other exposure factors were not significantly different between the two groups (Table 1).

Comparison of renal dysfunction and presence of fungal or non-fungal infections during hospitalization among MM patients with or without the abovementioned exposure factors

MM patients with chronic viral hepatitis were found to have a significantly higher incidence of non-fungal infections during hospitalization than those without [odds ratio (OR) = 4.2, $P = 0.01$]. The trend of renal dysfunction and fungal infections increased during hospitalization in MM patients with diabetes compared with those without. However, the difference was not statistically significant

Table 1 Association between myeloma and diabetes, hypertension, hyperlipidemia, and viral hepatitis

Exposures		Subjects (n = 1020)		Unstratified analysis			Stratified by age		
		Myeloma	Control	OR	95% CI	P*	OR	95% CI	P**
Diabetes	Yes	45	80	1.14	[0.76, 1.72]	0.54	1.15	[0.77, 1.70]	0.56
	No	295	600						
Hypertension	Yes	77	128	1.26	[0.90, 1.76]	0.16	1.28	[0.92, 1.78]	0.16
	No	263	552						
Hyperlipidemia	Yes	5	14	0.71	[0.20, 2.11]	0.63	0.71	[0.25, 1.99]	0.68
	No	335	666						
Chronic viral hepatitis	Yes	18	5	7.53	[2.66, 26.2]	< 0.01	7.55	[2.78, 20.5]	< 0.01
	No	322	675						

Exposures		Stratified by gender			Stratified by year			Multivariate analysis		
		OR	95% CI	P**	OR	95% CI	P**	OR	95% CI	P***
Diabetes	Yes	1.14	[0.77, 1.69]	0.57	1.14	[0.77, 1.69]	0.57	1.12	[0.75, 1.67]	0.59
	No									
Hypertension	Yes	1.26	[0.92, 1.74]	0.17	1.26	[0.92, 1.74]	0.18	1.30	[0.94, 1.81]	0.15
	No									
Hyperlipidemia	Yes	0.71	[0.25, 1.99]	0.68	0.71	[0.25, 1.98]	0.68	0.67	[0.24, 1.89]	0.45
	No									
Chronic viral hepatitis	Yes	7.53	[2.77, 20.5]	< 0.01	7.42	[2.75, 20.0]	< 0.01	7.83	[2.88, 21.3]	< 0.01
	No									

Note: OR: odds ratio; 95% CI: 95% confidence interval; P*: P-value by Fisher's exact test; P**: P-value by Cochran-Mantel-Haenszel test; P***: P-value by multivariate logistic regression analysis

Table 2 The relationship between exposure factors and complications during hospitalization in MM patients

Complications during hospitalization		Exposure factors											
		Hypertension			Diabetes			Hyperlipidaemia			Chronic viral hepatitis		
		Yes	No	P	Yes	No	P	Yes	No	P	Yes	No	P
Renal dysfunction	Yes	14	33	0.26	10	37	0.10	0	47	1.00	1	46	0.49
	No	63	230		35	258		5	288		17	276	
Fungal infection	Yes	6	25	0.82	6	25	0.27	0	31	1.00	3	28	0.22
	No	71	238		39	270		5	304		15	294	
Non-fungal infection*	Yes	30	130	0.12	22	138	0.87	2	158	1.00	14	146	0.01
	No	47	133		23	157		3	177		4	176	

Note: *: Fungal and chronic hepatitis virus infections are not included

($P > 0.05$). MM patients with chronic viral hepatitis are at increased risk of fungal infections. No statistically significant association was observed between other exposure factors and comorbidities (Table 2).

Discussion

In recent years, the incidence of hyperglycemia, hypertension, and hyperlipidemia is increasing. According to the International Diabetes Federation (IDF) statistics, the number of DM patients worldwide reached 415 million in 2015. China has the highest number of DM patients globally, with 151 million recorded cases [15]. According to the data published on October 25, 2017, after adjusting for age and gender, the prevalence rate of hypertension in China is 37.2%. That is, 517 million people present with hypertension. The incidence rate of hyperlipidemia is extremely high, reaching up to 95%, among individuals aged > 65 years. The conditions associated with the three high are not only limited to vascular, nerve, and skin lesions, and several studies in China and other countries have confirmed that the three high is also closely correlated to malignant tumors. The American Diabetes Association and the American Cancer Association jointly issued a statement that DM is a risk factor for multiple types of malignant tumors, such as lung, colorectal, and breast tumors. Meanwhile, a history of DM is associated with a higher mortality from cancer. Individuals with malignant tumors had a higher risk of DM than those without [16]. Moreover, the incidence of DM varies among patients with different types of tumors. In a study of 205 type 2 DM patients with malignant tumors, gastrointestinal tumors accounted for 46.9% of all tumors, followed by hematologic tumors (26%) [17]. In a study by Tan LL *et al*, gastrointestinal tumors are the most common malignant tumors in patients with DM, followed by hematological and lung tumors [18]. The abovementioned studies have indicated that DM is closely correlated to hematologic malignancies. DM can increase the incidence of diffuse large B-cell lymphoma by 1.41 times [19]. MM is a malignant blood disorder. What is the correlation between DM and MM? Currently, no study in China has assessed this association.

Several scholars believe that hypertension and malignant tumors are both proliferative lesions with a similar pathogenesis. Therefore, hypertension may promote the development of malignant tumors [20]. Its mechanism may be correlated to the overexpression of cellular oncogenes in hypertension, low-level inflammation, insulin resistance, insulin-like growth factor, and the renin–angiotensin system. Hyperlipidemia is considered a susceptible factor and is associated with the pathology of cancer. Fat catabolism in the body produces free radical compounds with extra electrons, which can increase the activity of carcinogens and cause cancer [21]. However, there is no study in China showing that hypertension and hyperlipidemia are associated with hematological malignancies. In this study, the incidence of DM (13.2%) and hypertension (22.6%) in the case group was slightly higher than that in the control group, and the incidence of hyperlipidemia (1.5%) was lower in the case group than in the control group. Although the difference was not statistically significant, individuals with diabetes and hypertension are more likely to have an increased risk of MM. Studies conducted in different regions have shown that DM is associated with the development of hematological tumors. Moreover, studies in other countries have revealed that IgA MM can occur secondary to hyperlipidemia [22]. Thus, to obtain more accurate epidemiological data of the three high in MM patients, multicenter and cross-regional prospective studies must be conducted in the future.

China has a high incidence of hepatitis. That is, the incidence of chronic viral hepatitis is always leading in class A and B infectious diseases in China [23]. From 2004 to 2013, the average annual incidence of hepatitis B infection was 72.61 per 100 000 population, and that of hepatitis C was about 15.51 per 100 000 population. An insufficient understanding of the immune mechanism of hepatitis infections has restricted the development of clinically effective interventions [24]. After the occurrence of the disease, it may gradually develop into hepatic cell carcinoma, which has an extremely high mortality rate. Moreover, studies in the literature have reported that hepatitis C is correlated to the development of hematological tumors. Nieters *et al* have conducted large, multi-center controlled studies that included 1807 patients with newly diagnosed malignant lymphoid diseases, and results showed that the risk of B-cell non-Hodgkin lymphoma and large B-cell lymphoma in hepatitis C virus RNA(+) patients was two and three times higher than that in normal individuals, respectively [25]. A Swedish cohort study has shown that the risk of MM is significantly higher in individuals with hepatitis C [26]. In the study by Wang LR *et al*, the incidence rate of hepatitis B was higher in MM patients than in healthy individuals [27]. The results of these studies are consistent with those of our study. That is, the incidence of chronic viral hepatitis in MM patients is significantly higher than that in the general population. Recent studies have shown that viral infection plays multiple roles in the process of carcinogenesis by causing an increase in genomic instability, cancer-promoting genetic mutations, signal pathway interruption, and tumor suppressor gene inhibition [28]. Meanwhile, hepatitis virus is a chronic antigenic stimulus to the body, which can cause immune system disorder and, subsequently, lymphocyte malignant proliferative diseases. Thus, this mechanism may be

responsible for the association between viral hepatitis and MM. Hepatitis is often in the state of inapparent infection in MM patients, and this process may also directly lead to MM [29]. No study has assessed the correlation between MM and hepatitis D and E in China and other countries. However, Pavlova and other scholars have reported that patients with blood diseases, particularly those with hematological malignancies, are more likely to develop hepatitis G than the general population [30].

What factors affect the clinical characteristics of MM patients? Studies have shown that MM patients are at risk of bacterial infections during hospitalization. At this period, combined DM, hospitalization time of 20 days, and high tumor stage based on the International Staging System are the independent risk factors of infections in MM patients [31]. A clinical study of 164 patients has shown that elevated creatinine and decreased serum albumin levels are independent risk factors for poor prognosis among MM patients [32]. Our study compared the clinical characteristics of MM patients with or without DM, hypertension, hyperlipidemia, and chronic viral hepatitis, and results showed that there is no significant difference in the prevalence of renal dysfunction, fungal infections, and other infections in MM patients with or without DM, hypertension, and hyperlipidemia. However, MM patients with DM are more likely to develop renal dysfunction and fungal infections during hospitalization. MM patients with chronic viral hepatitis have a significantly higher incidence of non-fungal infections during hospitalization than those without (OR = 4.2, $P = 0.01$), and the trend in the incidence of fungal infections has also increased. This result may be correlated to viral hepatitis, particularly in individuals with severe hepatitis who have impaired liver function and low immune function, which results in weak resistance and increased susceptibility to pathogens. By contrast, MM patients with viral hepatitis will have a longer hospital stay and more contact with pathogenic bacteria and medical staff, which lead to a higher risk of nosocomial infections. To date, no study has assessed the incidence of other infections in MM patients with chronic viral hepatitis in China and other countries.

In conclusion, individuals with DM and hypertension have an increased risk of MM. Thus, large-scale prospective studies must be conducted to validate the results of our study. Moreover, chronic viral hepatitis significantly increased the risk of MM. MM patients with chronic hepatitis are a special group with a significantly increased incidence of other infections. Hence, the replication of hepatitis virus must be closely monitored, and the prognosis of MM should be improved by preventing viral infection and providing antiviral treatment.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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