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Serum CCL2 and serum TNF-α – Two new biomarkers predict bone invasion, post-treatment distant metastasis and poor overall survival in nasopharyngeal carcinoma

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ABSTRACT

Purpose: To evaluate the prognostic potential of serum CCL2 (sCCL2) and serum TNF-α (sTNF-α) in nasopharyngeal carcinoma (NPC) before treatment by analysing the expression of these two markers.

Experimental design: Both sCCL2 and sTNF-α were prospectively detected in 297 NPC patients with enzyme-linked immunosorbent assay (ELISA) before treatment. The correlations between sCCL2 level or sTNF-α level and patient's survival were evaluated.

Results: For sCCL2, the 5-year overall survival (OS) and 5-year distant metastasis-free survival (DMFS) of high expression group and low expression group were 64% versus 81% and 67% versus 84% ($P < 0.05$), respectively. For sTNF-α, the 5-year OS and 5-year DMFS of high expression group and low expression group were 62% versus 79% and 66% versus 82% ($P < 0.05$), respectively. The 5-year OS and 5-year DMFS for both positive patients, one marker positive patient and both negative patients were 53% versus 77% versus 85% and 58% versus 80% versus 86% ($P < 0.05$), respectively. Concentrations of sCCL2 and sTNF-α in patients with large skull base invasion were higher than those without or with small skull invasion ($P < 0.05$). Patients who developed bone metastasis alone after radical treatment had higher pre-treatment concentrations of sCCL2 and sTNF-α than those without metastasis ($P < 0.001$). Multifactorial Cox regression analyses demonstrated that T/N/M classification, chemotherapy, sCCL2 level and sTNF-α level were independent predictors of OS and DMFS of NPC patients.

Conclusion: High expression levels of sCCL2 and sTNF-α predict bone invasion, post-treatment distant metastasis and poor overall survival in NPC patients.

1. Introduction

Nasopharyngeal carcinoma (NPC) is the most common head and neck cancer in South of China, though it is rarely seen in the Western country. With the characteristics of early cervical lymph node metastasis, special anatomic site, sensitivity to radiotherapy and chemotherapy, NPC is treated with radiation as the radical treatment method. Radiotherapy combined

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with chemotherapy has been proven to be superior to radiotherapy alone in advanced stage NPC patients with benefit in overall survival and disease specific survival. However, the 3-year metastasis rates for advanced stage patients were reported to be as high as 31% even when treated with chemoradiotherapy.[1] This suggests that distant metastasis remains the major reason for the treatment failure of NPC.

The mechanism of metastasis is very complicated. As early as in 1889, Stephen Paget first reported that metastasis depends on crosstalk between selected cancer cells (the 'seeds') and specific organ microenvironments (the 'soil'), and only when the seeds and soil were compatible, the metastasis could be formed.[2] Many studies focused on the nature of the 'seeds', such as the oncogenes, tumour suppress genes, stem cells and so on, but the properties of the 'soil' also play a very important role in the development of metastasis.

Since there are numerous inflammatory cells infiltrating in nasopharyngeal carcinoma and tumour-associated macrophages (TAMs) is one of the major component of the infiltrated inflammatory cells,[3] we supposed that the 'soil' of NPC would have some specific characteristics. It has been confirmed that TAMs can promote tumour progression and increase metastatic potential in breast cancer and prostate cancer.[4,5] Chemokine CCL2 (chemokine C–C motif ligand 2 or monocyte chemotactic protein-1, MCP-1) is one of the major factors, which act as a chemotactic factor for monocyte and macrophage.[6] Several reports have indicated that serum CCL2 (sCCL2) expression is associated with tumour progression, lymph node metastasis, stroma formation and dissolution and clinical aggressiveness.[7–10] On the other hand, tumour necrosis factor-alpha (TNF-α) is one of the important cytokines secreted by the macrophage. The expression change of serum TNF-α (sTNF-α) level is closely related to tumour progression and prognosis in many cancers including NPC.[11–13] But with the limitation of short following-up time in the study about NPC, the observation about the relationship between TNF-α and NPC metastasis was not very satisfactory.

We hypothesised that CCL2 and TNF-α might be over-expressed in the serum of NPC patients and might be new metastatic biomarkers in NPC patients. In this prospective study, we provided the first evidence that sCCL2 was over-expressed before treatment in certain NPC patients. NPC patients with high sCCL2 level or high sTNF-α level before treatment would develop more distant metastasis and subsequent poorer overall survival after radical therapy. Furthermore, patients with large skull base invasion or developed bone metastasis after treatment had especially high concentrations of sCCL2 or sTNF-α. This suggested that sCCL2 and sTNF-α might get the 'soil' in the bone ready to favour the formation of bone metastasis in NPC patients. The findings here will provide evidence to develop anti-CCL2 and anti-TNF-α drugs to treat and prevent bone metastasis in certain patients in the future.

2. Experimental design

2.1. Patients and sera

With $\alpha = 0.05$, $\beta = 0.10$, $P_1 = 0.8$ and $P_2 = 0.6$, we estimated that we had to enrol at least 109 patients in each group. Consecutively and newly identified NPC patients who enroled in Nasopharyngeal Carcinoma Department of Sun Yat-Sen University Cancer Centre were studied and the serum samples from these patients were collected prospectively before treatment. The patient eligibility criteria are as follows: aged between 18 and 60 years; no contraindications for chemotherapy and radiotherapy; nasopharyngeal biopsy confirmed WHO type II or type III; stage I to IV_{a+b} (by UICC staging system 2002); no chemotherapy, surgery or radiotherapy before; Karnofsky (KPS) score $\geqslant 80$. All NPC patients had undergone routine checkups including head and neck magnetic resonance imaging (MRI), chest X-ray, abdominal ultrasonography and bone scan before the treatment and every 3 months after completion of radical treatment with the exception of bone scan which was repeated every 6 months or when the patients complained of bone pain. The primary end-point of the study was overall survival (OS). The secondary end-points were distant metastasis-free survival (DMFS) and local–regional relapse-free survival (LRRFS). The survival time was recorded since the completion of radical treatment. Based on the MRI before treatment, we defined patients with more than two sites of skull base invasion as large skull base invasion and no more than two sites of skull base invasion as small skull base invasion. This study was approved by the Ethics Review Board of Sun Yat-Sen University Cancer Centre and all the patients signed the informed consent to attend this study.

All the patients were treated with continuously definitive radiotherapy with a daily fraction of 2.0 Gy and five fractions per week by linear accelerator (6–8 MV). The radiation dose to the nasopharynx, lymph node-positive area and lymph node-negative area ranged from 60–78 Gy, 60–70 Gy and 50–60 Gy, respectively. Radiotherapy was completed within 6–8 weeks. The NPC patients with stage III, IV_a or IV_b disease received platinum-based chemotherapy. Two cycles of 5-fluorouracil (5-FU) (4.0 g/m^2) and cisplatin (80 mg/m^2) every 3 weeks were employed in inductive chemotherapy. Two to three cycles of high-dose cisplatin (80 mg/m^2) every 3 weeks were employed in concurrent chemoradiotherapy.

2.2. Detection of sCCL2 and sTNF-α levels with enzyme-linked immunosorbent assay (ELISA)

The concentrations of sCCL2 and sTNF-α were measured with commercially available human CCL2 or TNF-α quantitative ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the instructions provided by the manufacturer. After the reaction, a value at wavelength of 450 nm was measured with enzyme-linked spectrophotometer, and the concentrations of sCCL2 and sTNF-α were calculated from the standard curves, separately. All analyses were made in duplicate and the mean value was used for statistical analysis.

2.3. Statistical analysis

All statistical analyses were performed using SPSS 16.0 software. The Chi-square test was used for comparison of data among groups. The Spearman nonparametric rank correlation test was used for bivariate correlation analysis. Relationships between sCCL2 level or sTNF-α level and the clinical characteristics were evaluated with the pearson χ^2 test.

Means comparison was performed using a one-way ANOVA test. Receiver operator characteristic (ROC) curves were constructed by plotting sensitivity versus (1-specificity), the areas under the curves (AUC) were analysed with the Hanley and McNeil method. Survival rates were assessed by life tables. Kaplan–Meier analysis and Log-rank test were used to compare the difference of survival rate. Multivariate analysis was performed with the Cox proportional hazards model to analyse factors related to prognosis. A two-tailed *P*-value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Clinical characteristic and following-up

From February 2002 to October 2005, 297 eligible patients were enroled in this study. Pathologic diagnosis was WHO type II in 47 cases and WHO type III in 250 cases. According to the UICC (2002) staging system of NPC, 4 patients were stage I, 44 patients were stage II, 143 patients were stage III and 106 patients were stage IV_{a+b} (Table 1). Totally 201 patients were diagnosed with skull base invasion by MRI, including 138 with clivus invasion, 137 with base of sphenoid bone invasion, 80 with petrous apex invasion and 29 with pterygoid process invasion. Among these patients, 81 patients with 1 site of skull base invasion, 69 patients with 2 sites of skull base invasion, 39 patients with 3 sites of skull base invasion and 12 patients with 4 sites of skull base invasion. With 2 sites of skull base invasion as the median, we defined large skull base invasion as more than two sites of skull base invasion and small skull base invasion as no more than two sites of skull base invasion.

All patients completed radical radiotherapy. Totally 244 patients received platinum-based chemotherapy, including 89 patients received inductive chemotherapy and radiotherapy, 61 patients received concurrent chemoradiotherapy and 94

Table 1 – Clinical characteristics of 297 NPC patients and their relationships with sCCL2 level or sTNF-α level.

Clinical factor	Cases (*n* = 297)	sCCL2		*P*-value	sTNF-α		P- value
		High level *n* = 161(54%)	Low level *n* = 136(46%)		High level *n* = 130(44%)	Low level *n* = 167(56%)	
Sex							
Male	227(76%)	121(40%)	106(36%)	0.573	106(36%)	121(40%)	0.067
Female	70(24%)	40(14%)	30(10%)		24(8%)	46(16%)	
Ages (years)							
<50	200(67%)	111(37%)	89(30%)	0.521	84(28%)	116(39%)	0.377
⩾50	97(33%)	50(17%)	47(16%)		46(16%)	51(17%)	
T staging							
T1 + T2	92(31%)	51(17%)	41(14%)	0.776	36(12%)	56(19%)	0.900
T3 + T4	205(69%)	110(37%)	95(32%)		94(32%)	111(37%)	
N staging							
N0 + N1	179(60%)	95(32%)	84(28%)	0.628	79(27%)	100(33%)	0.877
N2 + N3	118(40%)	66(22%)	52(18%)		51(17%)	67(23%)	
Clinical staging							
I + II	48(16%)	26(8%)	22(8%)	0.995	17(6%)	31(10%)	0.203
III + IV_{a+b}	249(84%)	135(46%)	114(38%)		113(38%)	136(46%)	
Chemotherapy							
No	53(18%)	33(11%)	20(7%)	0.107	22(8%)	31(10%)	0.663
Inductive	89(30%)	49(17%)	40(13%)		38(13%)	51(17%)	
Concurrent	61(20%)	25(8%)	36(12%)		31(10%)	30(10%)	
Inductive plus concurrent	94(32%)	54(18%)	40(14%)		39(13%)	55(19%)	
Local–regional relapse							
No	259(87%)	141(47%)	118(40%)	0.834	114(38%)	145(49%)	0.825
Yes	38(13%)	20(7%)	18(6%)		16(6%)	22(7%)	
Distant metastasis							
No	226(76%)	112(38%)	114(38%)	0.004	88(30%)	138(46%)	0.003
Yes	71(24%)	49(16%)	22(8%)		42(14%)	29(10%)	
Progression							
No	195(66%)	96(32%)	99(34%)	0.017	75(25%)	120(41%)	0.011
Yes	102(34%)	65(22%)	37(12%)		55(19%)	47(15%)	
Death							
No	217(73%)	105(35%)	112(38%)	0.001	83(28%)	134(45%)	0.002
Yes	80(27%)	56(19%)	24(8%)		47(16%)	33(11%)	

Abbreviations: NPC, nasopharyngeal carcinoma; sCCL2, serum chemokine (C–C motif) ligand 2 or monocyte chemotactic protein-1; and sTNF-α, serum tumour necrosis factor-alpha.

patients received inductive chemotherapy and concurrent chemoradiotherapy. Five patients with stage III disease refused chemotherapy and received radiotherapy alone.

All of the living patients were followed up at least 50 months (range from 50 months to 78 months with a median of 65 months). During the following period, totally 80 patients died, 77 patients died of disease recurrence and 3 patients died of non-neoplastic diseases. One hundred and two patients developed disease recurrence, including 64 with distant metastasis, 31 with local–regional relapse and 7 with both distant metastasis and local–regional relapse. The metastasis first occurred in bone alone in 17 patients, lung alone in 13 patients, liver alone in 20 patients, multiple organs in 19 patients (7 of them included bone metastasis) and other organs including 1 case of mediastinal lymph node metastasis and 1 case of pleural metastasis.

3.2. *Expression of sCCL2 and sTNF-α and their relationships with each other and clinical characteristics*

The median concentrations of sCCL2 and sTNF-α in the 297 NPC patients were 340 ng/l (25–2038 ng/l) and 427 ng/l (21–1920 ng/l), respectively. When the concentrations of sCCL2 and sTNF-α were analysed as continuous variables, there were no significant differences found within the same T/N/M classification patients regarding either marker. Also, there was no correlation found between sCCL2 concentration and sTNF-α concentration according to Spearman's nonparametric rank correlation test ($P = 0.608$). To further understand their roles in bone invasion of NPC, we analysed the differences of sCCL2 and sTNF-α concentrations among patients with different degrees of skull base invasion. The results showed that NPC patients with large skull base invasion before treatment had higher concentrations of sCCL2 and sTNF-α than those without skull base invasion or with small skull base invasion (Fig. 1A, Table 2).

To find out the impact of expression levels of sCCL2 or sTNF-α to the survival of NPC patients, we constructed ROC curves between death events and censors and selected 311 ng/l for sCCL2 and 454 ng/l for sTNF-α as the cut-off points for the binary variables analysis. The patients were divided into high level group and low level groups accordingly. In the case of genders, ages, WHO pathological classification, T staging, N staging, clinical staging, with or without chemotherapy and local–regional recurrence, there were no significant differences found between high level group and low level group. However, high sCCL2 and high sTNF-α level were positively associated with post-treatment distant metastasis, progression and poor survival (Table 1).

3.3. *Prognostic value of sCCL2 level and sTNF-α level*

To understand the impact of high or low sCCL2/sTNF-α level to the survival status of NPC patients, we analysed the 5-year OS and 5-year DMFS of these patients with Kaplan–Meier analysis. The results showed that both the 5-year OS and the 5-year DMFS of high sCCL2 level group were significantly lower than low sCCL2 level group (OS: 64% versus 81%, $P = 0.001$; DMFS: 67% versus 84%, $P = 0.003$). Also, both the 5-year OS and the 5-year DMFS of high sTNF-α level group were found significantly lower than low sTNF-α level group (OS: 62% versus 79%, $P = 0.001$; DMFS: 66% versus 82%, $P = 0.001$) (Fig. 2).

However, there were no significant differences found in the 5-year LRRFS between the two groups regarding either sCCL2 level or sTNF-α level.

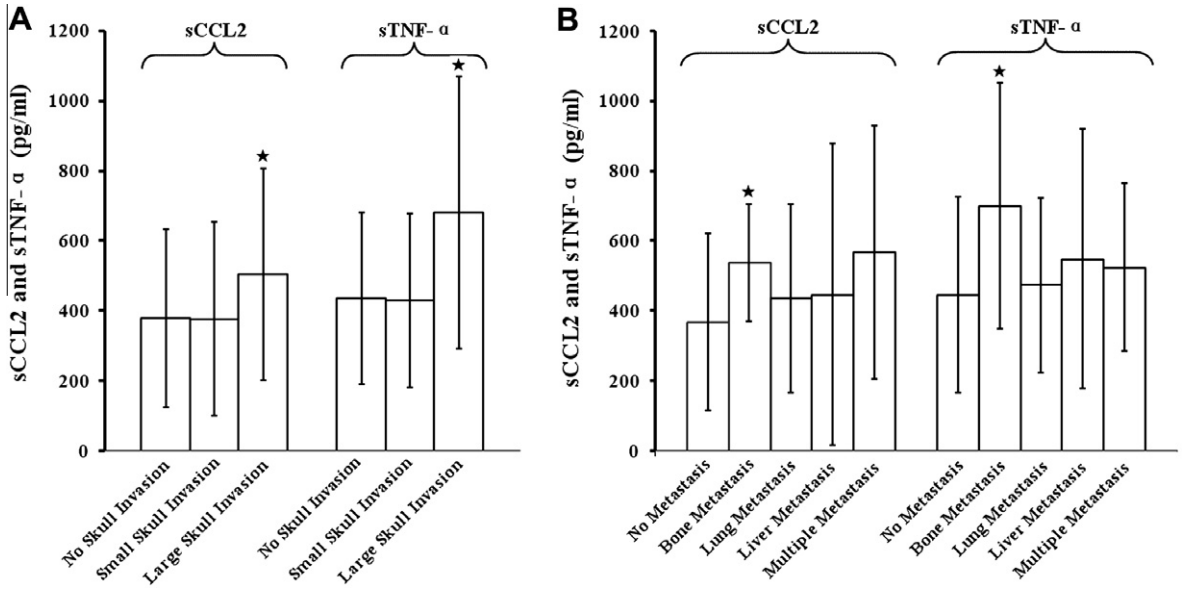

Fig. 1 – Concentrations of serum CCL2 and serum TNF-α in patients with nasopharyngeal carcinoma regarding different skull base invasion and distant metastatic status. (A) Pre-treatment concentrations of serum CCL2 and serum TNF-α in patient with large skull base invasion were significantly higher than those without skull base invasion and small skull base invasion. (B) Pre-treatment concentrations of serum CCL2 and serum TNF-α in patients who developed bone metastasis alone after the treatment were significantly higher than those without metastasis.

Table 2 – The mean concentrations of sCCL2 and sTNF-α within the same T/N/M classification and different degrees of skull base invasion in NPC patients.

	sCCL2 (pg/ml)	P-value	sTNF-α (pg/ml)	P-value
T classification				
T1 (n = 22)	331 ± 187	0.357	468 ± 255	0.385
T2 (n = 70)	414 ± 264		436 ± 239	
T3 (n = 119)	382 ± 283		471 ± 307	
T4 (n = 86)	433 ± 296		517 ± 315	
N classification				
N0 (n = 62)	353 ± 220	0.445	509 ± 311	0.768
N1 (n = 117)	425 ± 303		463 ± 295	
N2 (n = 94)	402 ± 286		475 ± 269	
N3 (n = 24)	400 ± 233		455 ± 315	
Clinical staging				
Stage I (n = 4)	399 ± 209	0.597	513 ± 276	0.303
Stage II (n = 44)	398 ± 295		406 ± 197	
Stage III (n = 143)	380 ± 268		475 ± 294	
Stage IV_{a+b} (n = 106)	429 ± 284		505 ± 319	
Skull base invasion				
No (n = 96)	380 ± 254	0.012	436 ± 245	0.000
Small (n = 150)	378 ± 276		431 ± 249	
Large (n = 51)	505 ± 303		681 ± 389	

Abbreviations: NPC, nasopharyngeal carcinoma; sCCL2, serum chemokine (C–C motif) ligand 2 or monocyte chemotactic protein-1; and sTNF-α, serum tumour necrosis factor-alpha.

Since the similar prognostic roles of sCCL2 level and sTNF-α level in NPC patients as we had found above, we expected that combination of sCCL2 level and sTNF-α level to be a better prognostic indicator. Further analysis showed that 5-year OS and 5-year DMFS for both high patients, one marker high patients and both low patients were 53% versus 77% versus 85% ($P < 0.001$) and 58% versus 80% versus 86% ($P < 0.001$), respectively, which could stratify the NPC patients into three layers with different prognosis (Fig. 3).

3.4. *Mean concentration of sCCL2 or sTNF-α expression and bone metastasis*

To determine if sCCL2 or sTNF-α was related to the specific organ metastasis after treatment, we analysed the differences of concentration of sCCL2 or sTNF-α as continuous variables regarding to the different metastatic status. We found that (a) both the mean concentration of sCCL2 and the mean concentration of sTNF-α in patients without metastasis were significantly lower than those who developed bone metastasis alone after treatment (sCCL2: 369 ± 253 versus 537 ± 168, $P = 0.013$; sTNF-α: 447 ± 279 versus 701 ± 351, $P < 0.001$); (b) no significant difference of either sCCL2 mean concentration or sTNF-α mean concentration was observed between patients without metastasis and those who developed lung metastasis alone, liver metastasis alone or multiple organ metastasis (sCCL2: 369 ± 253 versus 437 ± 269 or versus 447 ± 430.956 or versus 568 ± 361, $P > 0.05$; sTNF-α: 447 ± 279 versus 474 ± 250 or versus 549 ± 370 or versus 525 ± 239, $P > 0.05$) (Fig. 1B).

3.5. *Cox proportional hazards model analyses*

The associations of age, gender, WHO pathological classification, T staging, N staging, clinical staging, chemotherapy, sCCL2 level and sTNF-α level with 5-year OS and 5-year DMFS were performed with Cox proportional hazards model analyses. The analyses demonstrated that T staging, N staging, clinical staging, chemotherapy, sCCL2 level and sTNF-α level were independent predictors of OS and DMFS of NPC patients (Table 3).

4. Discussion

CCL2 is a member of the chemokine superfamily with a potent chemoattractant for monocytes, natural killer cells and memory T lymphocytes.[6] Its influence on tumourgenesis and metastasis may occur through two distinct mechanisms: (1) a direct effect on tumour cell growth and function and (2) an indirect effect on the tumour microenvironment by the regulation of macrophage mobilisation and infiltration into the tumour stroma.[14,15] Growing evidence suggests that CCL2 may act as paracrine and autocrine factors on prostate cancer and regulate the migration and invasive properties of cancer cells, resulting in enhanced proliferation and invasion of prostate cancer.[16]

In this study, we first reported that sCCL2 concentration before the treatment was a good indicator of post-treatment survival and distant metastasis in NPC patients. The 5-year OS and 5-year DMFS for high sCCL2 level group were much lower than the low level group. But there was no significant difference found in the 5-year LRRFS between the two groups. Furthermore, we demonstrated that sCCL2 level was an independent predictor of OS and DMFS of NPC patients by Cox proportional hazards model analyses. Tang KF and colleagues reported that expression of CCL2 was observed in 14 of 17 biopsies containing NPC tumour cells with variable intensities but not in the four biopsies from NPC patients not containing tumour cells.[17] These suggested that the sCCL2 might be

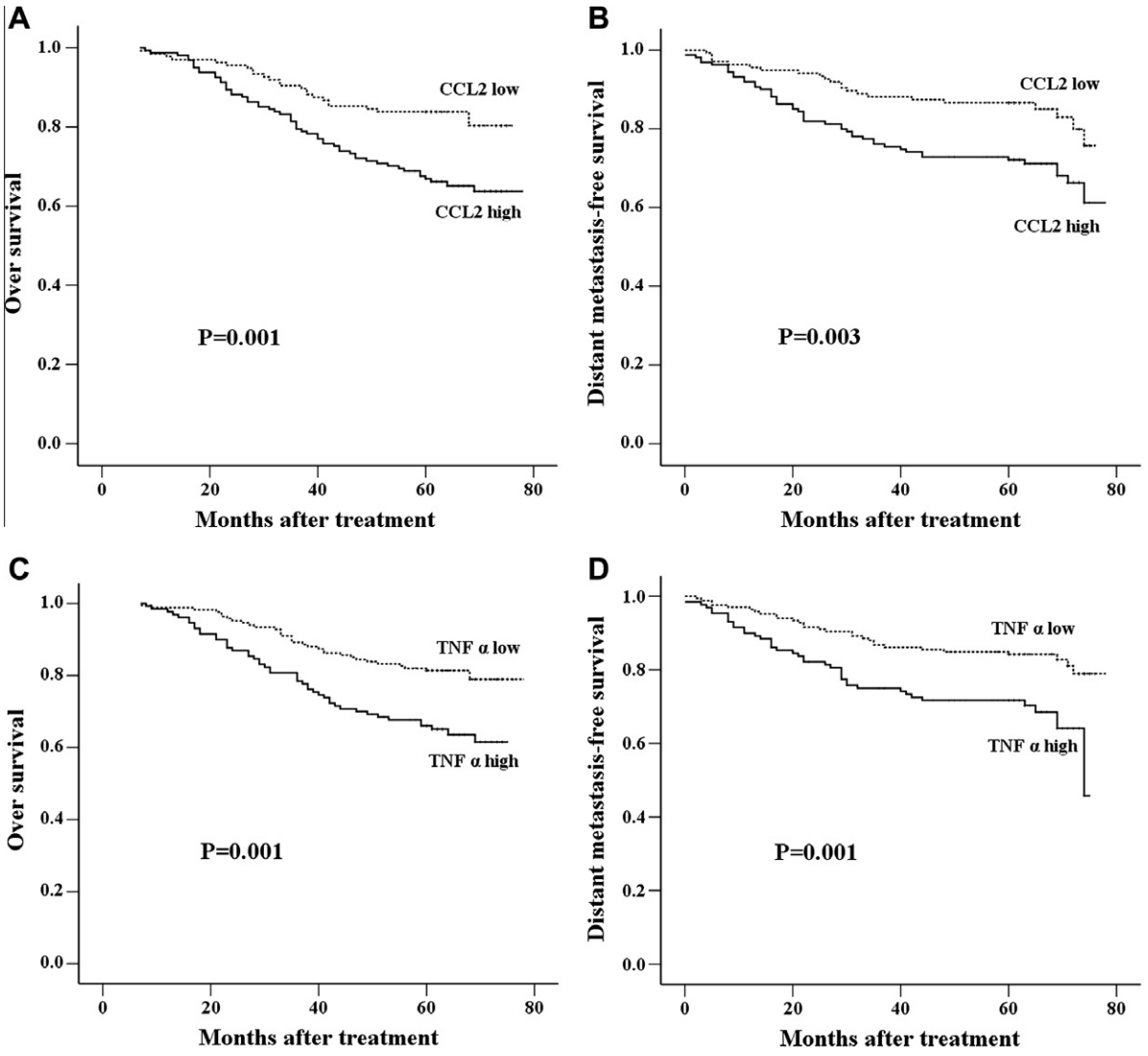

Fig. 2 – The 5-year overall survival and 5-year distant metastasis-free survival curves regarding to serum CCL2 level and serum TNF-α level, respectively. (A,B) The 5-year overall survival and 5-year distant metastasis-free survival curves of high sCCL2 level group and low sCCL2 level group. (C,D) The 5-year overall survival and 5-year distant metastasis-free survival curves of high sTNF-α level group and low sTNF-α level group.

derived from the secretion of NPC cells and those cells might be very important in the development of metastasis of NPC.

Experimental and clinical studies have confirmed that the level of tumour-derived CCL2 is significantly correlated with TAM density in ovarian cancer, breast cancer, oesophagus cancer and so on.[18–20] It is well established that TAM can secret a series of cytokines including TNF-α and vascular epithelial growth factor (VEGF) in the tumour stroma and stimulate the growth of tumour cells and/or promote tumour cell migration and metastasis.[7,21,22] Regarding the close relationship of CCL2 and TAMs, we detected the serum expression level of sTNF-α at the same time, which is one of the most important cytokines secreted by the macrophage. We found sTNF-α was also high expressed in some NPC patients and a further analysis showed that the 5-year OS and 5-year DMFS in the sTNF-α high level group were significantly lower than the ones in the low level group. In addition, sTNF-α level also was an independent predictor of OS and DMFS in NPC patients. When we combined the two biomarkers as a prognostic indicator, we found patients with both high levels of sCCL2 and sTNF-α had a worse prognosis than those with either high or low level. There were almost half the patients with both high levels of sCCL2 and sTNF-α who developed metastasis after the treatment. These suggested sCCL2 and sTNF-α might play very important roles in the development of metastasis.

LU and colleagues showed that monocytes can develop to become osteoclasts and enhance bone metastasis of prostate cancer under the stimulation of CCL2.[23] Bone is the most common organ that gets involved when distant metastasis developed in NPC. At the primary site, skull base easily got involved in NPC patients. We suppose that high level of sCCL2 or sTNF-α might be closely related with the bone invasion ability and the bone metastatic ability of NPC. In this research we are among the first to show that NPC patients with large

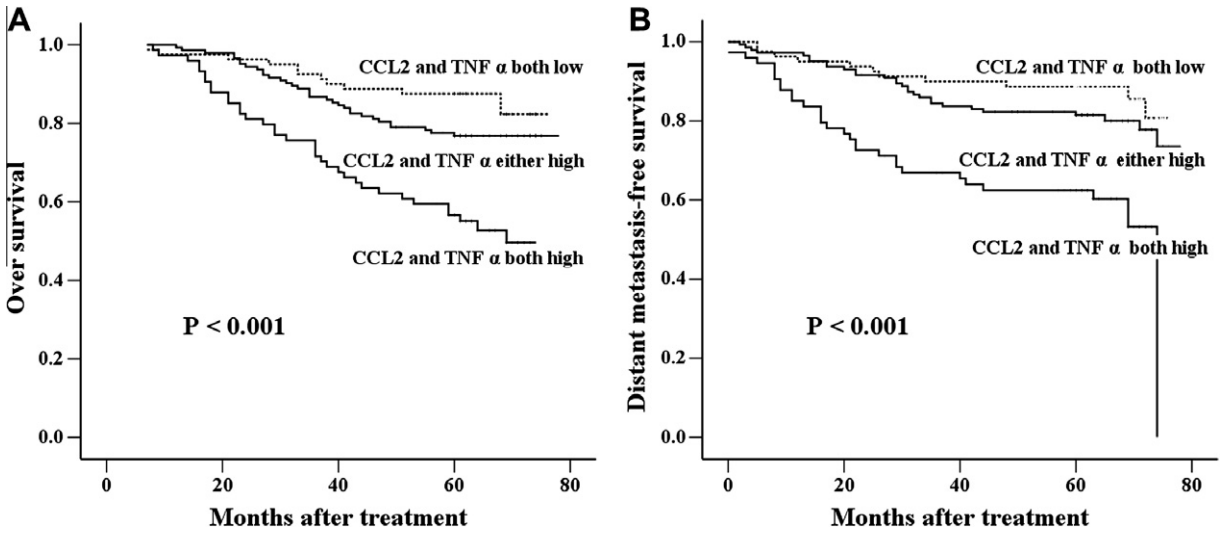

Fig. 3 – The 5-year overall survival and 5-year distant metastasis-free survival curves when combined serum CCL2 level and serum TNF-α level as a prognostic indicator.

Table 3 – Multivariate analysis of Cox proportional hazards model for 5-year OS and 5-year DMFS (n = 297).

Prognosis factor	Wald	Sig	Exp(B)	95% Confidence interval (CI) for Exp(B)	
				Lower	Upper
OS					
Age	3.653	0.056	1.565	0.989	2.476
Gender	2.715	0.099	0.604	0.331	1.100
WHO	0.494	0.482	0.818	0.467	1.433
T staging	9.629	0.002	2.174	1.331	3.550
N staging	8.941	0.003	1.640	1.186	2.268
Clinical staging	6.364	0.012	3.247	1.300	8.105
Chemotherapy	18.606	0.000	0.288	0.163	0.507
sCCL2	8.806	0.003	2.083	1.283	3.383
sTNF-α	5.458	0.019	1.728	1.092	2.735
DMFS					
Age	2.639	0.104	1.514	0.918	2.496
Gender	2.837	0.092	0.571	0.298	1.096
WHO	0.107	0.743	0.901	0.482	1.683
T staging	13.895	0.000	2.860	1.646	4.969
N staging	7.915	0.005	1.625	1.159	2.280
Clinical staging	20.038	0.000	9.695	3.586	26.211
Chemotherapy	44.725	0.000	0.132	0.073	0.239
sCCL2	6.157	0.013	1.916	1.146	3.201
sTNF-α	5.000	0.025	1.762	1.072	2.894

Abbreviations: OS, overall survival; DMFS, distant metastasis-free survival; sCCL2, serum chemokine (C–C motif) ligand 2 or monocyte chemotactic protein-1; and sTNF-α, serum tumour necrosis factor-alpha.

skull base invasion have higher concentrations of sCCL2 and sTNF-α than those without or with small skull base invasion. At the same time, the concentrations of sCCL2 and sTNF-α were not different within the same T/N/M classification. This means that the concentration differences of sCCL2 and sTNF-α among patients with different degrees of skull base invasion are not due to the different tumour burden. Additionally, the pre-treatment concentrations of sCCL2 and sTNF-α in NPC patients who would develop bone metastasis alone were significantly higher than those who would not develop metastasis, though with the limited 17 bone metastasis alone cases in our study. Further research to confirm their roles in bone metastasis are on the way. However, all these suggests that sCCL2 and sTNF-α might be specific biomarkers of bone invasion ability and bone metastatic ability in NPC patients. We hypothesised that high level of sCCL2 or sTNF-α might prepare the 'soil' in the bone to facilitate the formation of bone metastasis in NPC patients.

However, we cannot find the correlation between sCCL2 and sTNF-α in NPC patients. sCCL2 can be secreted by the normal cells such as bone marrow endothelial cells under stimulation and also can be secreted by the tumour cells.[24,25] At the same time, though macrophages are a major source of TNF-α, a variety of other cells, including fibroblasts, astrocytes,

Kupffer cells, smooth muscle cells, keratinocytes and tumour cells can also secret TNF-α.[26] These suggest that the sources of sCCL2 and sTNF-α in NPC patients are very complicated and may not be simply due to their relationship with TAMs. With the fact that high levels of sCCL2 and sTNF-α predict more distant metastasis, especially bone metastasis, we expect further researches about the mechanism of the two cytokines in the distant metastasis of NPC.

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Conflict of interest statement

None declared.

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