



# CXCL8 and CXCR4 expression in synchronous bone metastasis in nasopharyngeal cancer

Rahmat Cahyanur<sup>1</sup>, Cosphiadi Irawan<sup>1</sup>, Lisnawati Rachmadi<sup>2</sup>, Marlinda Adham<sup>3</sup>, Achmad Fauzi Kamal<sup>4</sup>, Ahmad Rusdan Handoyo Utomo<sup>5</sup>, Mardiah Suci Hardianti<sup>6</sup>, Thariqah Salamah<sup>7</sup>, Muchtaruddin Mansyur<sup>8</sup>

1 Hematology Medical Oncology Division, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia—Cipto Mangunkusumo General Hospital, Jakarta, Indonesia

2 Department of Anatomical Pathology, Faculty of Medicine Universitas Indonesia—Cipto Mangunkusumo General Hospital, Jakarta, Indonesia

3 Department of Ear, Nose, and Throat, Head and Neck Surgery, Faculty of Medicine Universitas Indonesia—Cipto Mangunkusumo General Hospital, Jakarta, Indonesia

4 Department of Orthopedics and Traumatology, Faculty of Medicine Universitas Indonesia—Cipto Mangunkusumo General Hospital, Jakarta, Indonesia

5 Graduate School of Biomedical Sciences, Yarsi University, Jakarta, Indonesia

6 Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

7 Department of Radiology, Faculty of Medicine Universitas Indonesia—Cipto Mangunkusumo General Hospital, Jakarta, Indonesia

8 Department of Community Medicine, Faculty of Medicine Universitas Indonesia—Cipto Mangunkusumo General Hospital, Jakarta, Indonesia

**Corresponding author:** Rahmat Cahyanur, Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia—Cipto Mangunkusumo Hospital. Jl. Diponegoro No. 71, Jakarta, Indonesia.; Email: rahmat.cahyanur01@ui.ac.id

**Received:** 12 August 2025 ♦ **Accepted:** 12 January 2026 ♦ **Published:** 30 April 2026

**Citation:** Cahyanur R, Irawan C, Rachmadi L, Adham M, Kamal AF, Utomo ARH, Hardianti MS, Salamah T, Mansyur M. CXCL8 and CXCR4 expression in synchronous bone metastasis in nasopharyngeal cancer. *Folia Med (Plovdiv)* 2026;68(2):e168480. doi: 10.3897/foimed.68.e168480.

## Abstract

**Introduction:** Nasopharyngeal cancer is the most common head-and-neck cancer in Indonesia. It occurs in the mucosal epithelium of the nasopharyngeal region. This study aimed to evaluate the role of gene expression in the occurrence of synchronous bone metastasis in nasopharyngeal cancer.

**Materials and methods:** This was a cross-sectional study of patients with nasopharyngeal cancer conducted at Cipto Mangunkusumo Hospital, Jakarta, from 2018 to 2023. Gene expression differences were assessed using NanoString technology, with genetic material extracted from paraffin-embedded tissue samples. The analysis used a fold-change value of 1.5 to  $-1.5$  and an adjusted  $p$ -value ( $p$ -adj) of  $<0.05$ .

**Results:** Ninety-five patients with nasopharyngeal cancer were included in the study. Most were male and the most common sites of bone metastasis were the vertebrae (70.2%), ribs and sternum (57.4%), and pelvis (27.7%). Most bone metastases were characterized by 2–5 lesions and were predominantly osteoblastic (71.4%). The CXCL8 gene was downregulated to 0.29 (0.16–0.54,  $p$ -adj=0.009), while the CXCR4 gene was upregulated to 1.45 (1.16–1.82,  $p$ -adj=0.049), in the group with bone metastasis compared to the group without metastasis. Among patients with only bone metastasis, the CXCR4 gene showed a further increase in expression levels, up to 1.61 (1.25–2.07,  $p$ -adj=0.02).

**Conclusions:** The results of this study showed that out of the 80 genes, 2 genes play a role in primary bone metastasis. In the group with bone-involvement metastasis, CXCR4 was upregulated and CXCL8 was downregulated. In the bone-only metastasis group, only the CXCR4 gene was found to be upregulated.

## Keywords

CXCR4, CXCL8, nasopharyngeal cancer, synchronous bone metastasis

## Introduction

Nasopharyngeal cancer is a type of malignancy that occurs in the mucosal epithelium of the nasopharyngeal area, which is the region above the throat and behind the nose.<sup>[1]</sup> The incidence of nasopharyngeal cancer is 1.2 per 100,000 people globally. In Indonesia, nasopharyngeal carcinoma is the most common form of head-and-neck cancer, accounting for 28.4% of cases. The mortality rate from nasopharyngeal cancer in Indonesia is the second highest in Asia, following that of China.<sup>[2]</sup> The majority of nasopharyngeal cancer cases in Indonesia present at advanced or locally advanced stages (30.1% and 18.9%, respectively).<sup>[3]</sup>

Metastasis refers to the spread of cancer from its primary site to distant organs, and it is the leading cause of cancer-related morbidity and mortality.<sup>[4]</sup> The metastatic process involves multiple steps, including the detachment of cancer cells from the primary tumor, entry into the circulatory and lymphatic systems (intravasation), evasion of the immune system, extravasation through capillaries in organs, and proliferation within the target organ.<sup>[4,5]</sup>

Studies have shown that metastasis is not solely related to tumor size. A study by Sopik et al.<sup>[6]</sup> reported that, in breast cancer patients, the relationship between metastasis occurrence and tumor size is not linear. The occurrence of metastasis is also influenced by the biological characteristics of the tumor cell population, particularly the expression of specific genes that drive the metastatic process.<sup>[6,7]</sup> Several genes and proteins linked to bone metastasis, including *CXCR4*, *RANK*, *RANKL*, *BMP2*, osteopontin, *PTHrP*, *CXCL8*, and *SRC* have been reported to show increased expression levels in nasopharyngeal cancer. These proteins are encoded by the genes *CXCR4*, *RANK*, *TNFSF11*, *BMP2*, *OPN*, *PTHrP*, *IL-8*, and *SRC*, respectively. However, to date, the role of those genes and proteins with incident of synchronous bone metastasis (metastasis at initial diagnosis) in nasopharyngeal cancer have not been thoroughly investigated.<sup>[8-13]</sup>

Research on the pathomechanisms of bone metastasis in nasopharyngeal cancer is still limited. Gaining a deeper understanding of bone metastasis is crucial to inform potential future interventions and treatments. Identifying the key genes involved in bone metastasis could potentially prevent its occurrence by blocking the expression of these genes or the synthesis of related proteins in patients with nasopharyngeal cancer.

## Aim

This study investigates the differentially expressed gene (DEG) analysis in synchronous bone metastasis in nasopharyngeal cancer. We expect there is significant DEG involvement in bone metastasis.

## Materials and methods

This study was an analytical cross-sectional comparative study that evaluated the link between gene expression levels and synchronous bone metastasis in nasopharyngeal cancer. Conducted from January 2022 to March 2023, the study utilized paraffin blocks of nasopharynx at the Department of Anatomical Pathology and medical record data of nasopharyngeal cancer patients at Cipto Mangunkusumo General Hospital. Nucleic acid (mRNA) extraction from the paraffin blocks was performed in the laboratories of the Anatomical Pathology Department and NanoString technology was used to analyze gene expression in the Genetic Science. The inclusion criteria for this study were patients over the age of 18 with histopathological examination results showing nasopharyngeal cancer. The exclusion criteria were incomplete medical records, more than one primary cancer, or unavailable paraffin blocks. Bone metastasis was based on radiological results at initial diagnosis (CT scan, MRI, or PET CT). Bone metastasis categorized into two groups: bone-involvement metastasis along with other organs and bone-only metastasis.

The demographic and clinical characteristics were analyzed using univariate analysis in SPSS 28. Gene expression analysis was performed using the nSolver<sup>®</sup> 4.0 application, and quality control checks on imaging quality and binding density were done for each sample.<sup>[14]</sup> Differentially expressed gene analysis was conducted to compare gene expression between groups. The reference genes used in the study were *HPRT1*, *YARS*, *EIF3S7*, *PDCD1*, *PDCD1LG2*, *TIGIT*, and *TRATI*.<sup>[15]</sup> This study examined 80 target genes selected by the researchers using a customized panel, along with nine negative control genes and six positive control genes (**Table A1**). Results are presented in tables and volcano plots, with statistical significance defined as an adjusted *p* (*p*-adj) value of <0.05. The adjusted *p*-value was calculated using the Benjamini-Hochberg method to reduce the false discovery rate in the DEG analysis.

This study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia (approval number KEDN158/UN2.F1/ETIK/PPM.00.02/2022).

## Results

A total of 95 subjects were included in this study, consisting of 64 subjects in the metastasis group and 31 subjects in the non-metastasis group. **Table 1** provides an overview of the demographic and clinical characteristics of the subjects, divided into the metastasis and non-metastasis groups.

The majority of subjects, in both the non-metastasis and metastasis groups, had lymph node involvement, with rates of 90.3% and 98.4%, respectively. For subjects classified as N3, a larger proportion were in the non-T4 stage

**Table 1.** Clinical and demographic characteristics of the subjects

Subject characteristics	Metastasis (n=64)	Non-metastasis (n=31)
Age, average (years)	47.95 (13.35)	48.64 (9.77)
Sex		
Male, f (%)	49 (76.6)	23 (74.2)
Female, f (%)	15 (23.4)	8 (25.8)
Histopathology		
Keratinizing carcinoma, f (%)	0 (0)	2 (6.5)
Non-keratinizing squamous cell carcinoma, f (%)	64 (100)	29 (93.5)
Basaloid squamous cell carcinoma, f (%)	0 (0)	0 (0)
T stage		
T1, f (%)	1 (1.6)	1 (3.2)
T2, f (%)	25 (39.1)	5 (16.1)
T3, f (%)	11 (17.2)	10 (32.3)
T4, f (%)	27 (42.2)	15 (48.4)
N stage		
N0, f (%)	1 (1.6)	3 (9.7)
N1, f (%)	1 (1.6)	2 (6.5)
N2, f (%)	13 (20.3)	15 (48.4)
N3, f (%)	49 (76.6)	11 (35.5)
Lymph node status		
Bilateral, f (%)	54 (84.4)	26 (83.9)
N3 diameter $\geq$ 6 cm, f (%)	6 (9.4)	1 (3.2)
Radiology		
Parapharyngeal involvement, f (%)	54 (84.4)	26 (83.9)
Cranial base invasion, f (%)	17 (26.6)	10 (32.3)
Number of metastatic sites		
1 organ, f (%)	45 (70.3)	N/A
>1 organ, f (%)	19 (29.7)	N/A
Metastatic sites		
Liver, f (%)	18 (28.1)	N/A
Lung, f (%)	20 (31.3)	N/A
Bone, f (%)	47 (73.4)	N/A
Brain, f (%)	3 (4.7)	N/A

compared to the T4 stage (60.0% versus 40.0%). In the metastasis group, the proportion of subjects classified as N3 was greater, with 49 individuals (76.6%) compared to only 11 (35.5%) in the non-metastasis group. Most subjects with metastasis had involvement of only one organ, and the bone was the most common site of metastasis in 47 cases (73.4%).

## Subject characteristics based on bone involvement and bone-only metastasis

Among the 64 subjects with metastatic lesions, 47 had bone-involvement metastasis and 17 did not have bone metastasis. The demographic and clinical characteristics of the study participants based on bone involvement and bone only metastasis are outlined in **Table 2**.

**Table 2.** Distribution of subjects with bone-involvement and bone-only metastasis

Subject characteristics	Bone involvement (n=47)	Bone-only metastasis (n=28)
Age, average (years)	47.29 (13.23)	48.67 (13.66)
Sex		
Male, f (%)	35 (74.5)	22 (78.6)
Female, f (%)	12 (25.5)	6 (21.4)
T stage		
T1, f (%)	0 (0)	0 (0)
T2, f (%)	17 (36.2)	10 (35.7)
T3, f (%)	10 (21.3)	6 (21.4)
T4, f (%)	20 (42.6)	12 (42.9)
N stage		
N0, f (%)	1 (2.1)	1 (3.6)
N1, f (%)	1 (2.1)	1 (3.6)
N2, f (%)	9 (19.1)	5 (17.9)
N3, f (%)	36 (76.6)	21 (75.0)
Bone metastasis composition		
Bone, f (%)	28 (59.6)	
Bone with other organs, f (%)	19 (40.4)	
Bone metastatic sites (f=47)		
Axial		
Vertebra, f (%)	33 (70.2)	17 (60.7)
Costae dan sternum, f (%)	27 (57.4)	19 (67.9)
Cranium, f (%)	8 (17.0)	4 (14.3)
Appendicular		
Shoulder (scapula), f (%)	6 (12.8)	5 (17.9)
Clavicle, f (%)	3 (6.4)	3 (10.7)
Humerus, f (%)	3 (6.4)	2 (7.1)
Femur, f (%)	9 (19.1)	4 (14.3)
Cruris, f (%)	2 (4.3)	1 (3.6)
Pelvis, f (%)	13 (27.7)	5 (17.9)
Number of bone-metastatic sites		
1 site, f (%)		13 (46.4)
2–5 sites, f (%)		14 (50.0)
>5 sites, f (%)		1 (3.6)
Type of bone metastasis		
Osteoblastic, f (%)		20 (71.4)
Osteolytic, f (%)		6 (21.4)
Mixed, f (%)		2 (7.1)

Among subjects with bone-involvement metastasis, the most common locations were the vertebrae (70.2%), costae and/or sternum (57.4%), and pelvis (27.7%). Most of the subjects with bone metastases were in T4 and N3 stages, with 50.0% having 2–5 metastatic sites. Osteoblastic-type lesions were the most common, accounting for 71.4% of cases, as shown in Fig. 1.

### DEGs among the bone-involvement and non-metastasis groups

DEG analysis was performed by comparing gene expression levels in the bone-involvement metastasis group with the non-metastasis group (Table A2). A comparison of the expression levels of the top 40 genes that differed between the two groups can be seen in the volcano plot in Fig. 2a. Based on the DEG analysis, the *CXCL8* gene was downregulated and the *CXCR4* gene was upregulated, as detailed in Table 3.

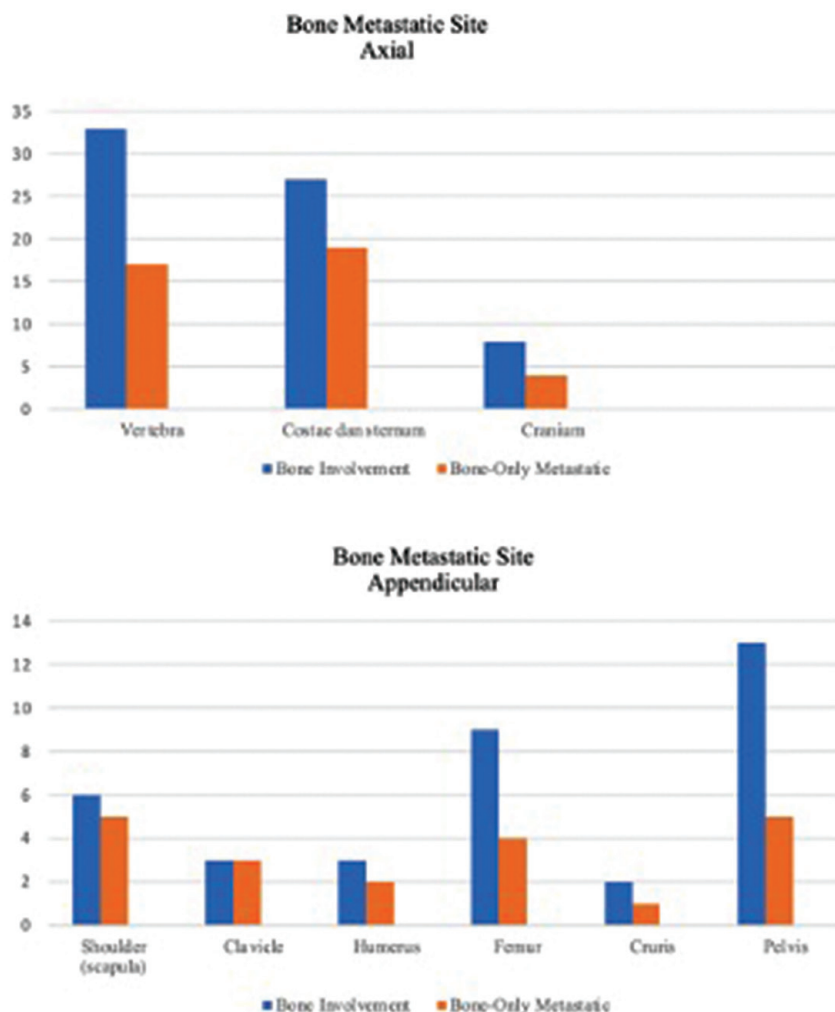
### DEGs between the bone-only and non-metastasis groups

DEG analysis was conducted by comparing subjects with only bone metastasis to those without metastasis (Table A3). The volcano plot in Fig. 2b illustrates the comparison of the expression levels of the top 40 genes that differed between the two groups. The *CXCR4* gene was found to be upregulated in subjects with bone metastasis only, as shown in Table 4.

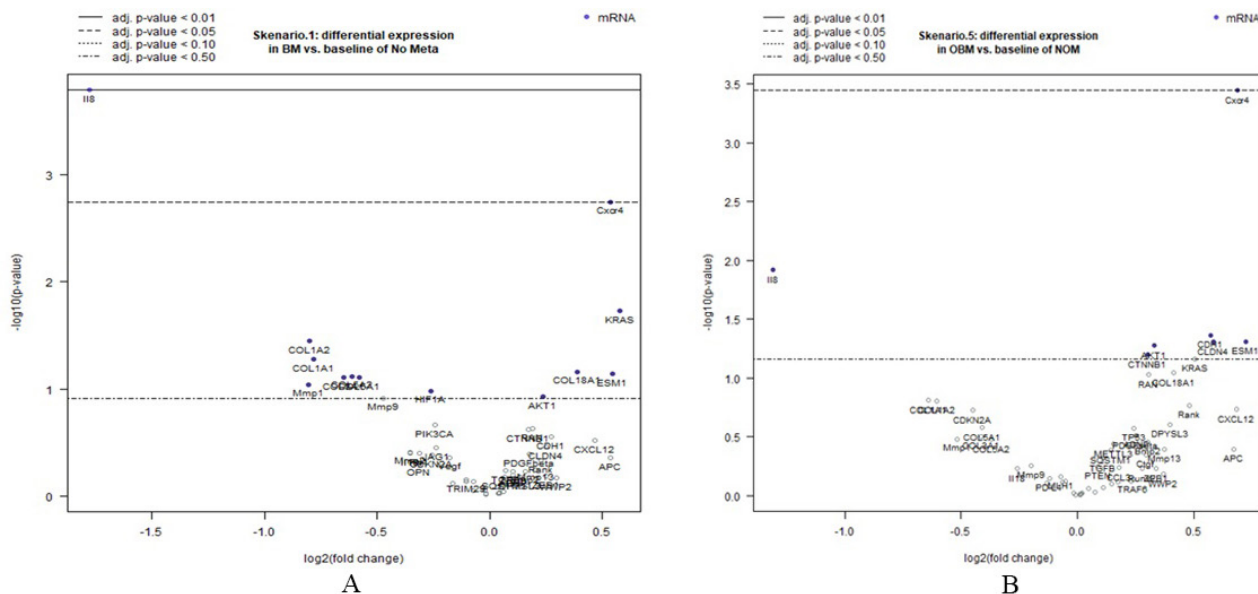
**Table 3.** Gene expression in the group with bone involvement metastasis against the subjects non-metastasis

Gene	Fold Change (CI 95%)	Adjusted <i>p</i> Value*
<i>CXCL8</i>	0.29 (0.16–0.54)	<b>0.009</b>
<i>CXCR4</i>	1.45 (1.16–1.82)	<b>0.049</b>

\**p* value <0.05, the Benjamini-Hochberg method



**Figure 1.** Distribution metastasis site among subjects with bone involvement and bone only metastasis.



**Figure 2.** Volcano plots of differentially expressed genes (DEGs) between (a) subjects with bone-involvement metastasis and those without metastasis and (b) subjects with bone metastasis and those without metastasis.

**Table 4.** Gene expression in the bone-only metastasis compared to the non-metastasis group

Gene	Fold change (CI 95%)	Adjusted <i>p</i> value*
<i>CXCR4</i>	1.61 (1.25–2.07)	<b>0.02</b>

\**p* value <0.05, the Benjamini-Hochberg method

## Discussion

### Subject characteristics

In this study, most subjects were male (75.8%), with an average age of 48 years at diagnosis. This finding is consistent with previous studies in Indonesia that have reported that nasopharyngeal carcinoma cases occur predominantly in males.<sup>[16]</sup> Adham et al.<sup>[17]</sup> reported that men were 2.4 times more likely to be diagnosed with nasopharyngeal cancer than women. A significant portion of the subjects in this study (73.4%) had bone metastasis, followed by lung and liver metastasis. This aligns with previous reports suggesting that bone is the most frequently involved organ in nasopharyngeal cancer.<sup>[18,19]</sup> Primary bone metastasis is the most common finding in patients who present with metastasis at the time of diagnosis, accounting for 64%–67% cases.<sup>[20]</sup> Most subjects with bone metastasis were male, with metastases primarily located in the vertebrae (70.2%), costae and sternum (57.4%), and pelvis (27.7%). These findings correspond with a previous report stating that the thoracic and lumbar vertebrae, sternum, costae, ilium, and femur are the most common metastatic sites.<sup>[20]</sup> This is associated with a greater presence of red bone marrow and more vascularization at those sites, which facilitate the

homing of cancer cells.<sup>[21]</sup> The majority of primary bone metastasis lesions in this study were identified at 2–5 sites (50.0%). This aligns with previous studies showing that primary metastasis in nasopharyngeal cancer typically involves only one organ but with multiple lesions.<sup>[22]</sup>

The most frequent type of bone metastasis lesion in this study was osteoblastic (71.4%). The radiological findings of this study, however, slightly differed from those of previous studies. In a study by Sham et al.<sup>[23]</sup> radiological images of bone metastasis in nasopharyngeal cancer showed lytic lesions (66%), mixed lesions (12.8%), and sclerotic lesions (21.2%). These differences likely stem from variations in criteria, as Sham et al.<sup>[23]</sup> defined bone metastasis as a radiological abnormality accompanied by pain resulting from the metastasis.

### Gene expression

Analysis of DEGs found that *CXCL8* expression levels decreased, while *CXCR4* expression levels significantly increased in the group with bone metastasis involvement. When comparing between the bone-only metastasis group and the non-metastasis group, only *CXCR4* showed a significant difference gene expression.

### *CXCR4*

The *CXCR4* gene is involved in adhesion, invasion, and the migration of cancer cells to bone<sup>[11]</sup>, as seen in breast, nasopharyngeal, kidney, lung, prostate, pancreatic, and ovarian cancers and melanoma. Higher levels of *CXCR4* expression in NPC linked to tumor growth, angiogenesis, metastasis, and treatment resistance. Additionally, increased *CXCR4* expression levels are linked to epithelial-mesenchymal transition (EMT) in various types of cancer. In ovarian

cancer, the upregulation of *CXCR4* is accompanied by increased expression levels of vimentin and snail, along with decreased expression levels of E-cadherin. EMT initiates metastasis, enabling cancer cells to migrate to other organs through the systemic circulation.<sup>[24,25]</sup>

The binding of *CXCR4* to its ligand, *CXCL12*, triggers a conformational change in the receptor molecule. This binding activates G proteins, leading to the conversion of GDP to GTP. The GTP molecule then binds to the  $\alpha$  and  $\beta\gamma$  subunits, causing them to dissociate. The  $\beta\gamma$  subunit activates two signaling pathways: phospholipase C- $\beta$  (PLC- $\beta$ ) and PI3K pathways. Meanwhile, the  $\alpha$  subunit affects gene transcription and expression through the PI3K-AKT-NF- $\kappa$ B, MEK1/2, and ERK1/2 pathways.<sup>[25]</sup> As a result, various cellular changes occur, including an increase in intracellular calcium levels, gene transcription, chemotaxis, cell survival, and cell proliferation.<sup>[25]</sup>

*CXCR4* expression has been linked to bone metastasis, especially in breast cancer.<sup>[26-29]</sup> The role of *CXCR4* in bone metastasis involves effects on the processes of cancer cell adhesion, invasion, and migration to the bone. Increased expression levels of *CXCR4* are linked to the development of bone metastases in breast cancer.<sup>[11]</sup> The findings of this study also confirmed the role of *CXCR4* in bone metastasis in nasopharyngeal cancer.

## CXCL8

*CXCL8* is typically challenging to detect under physiological conditions.<sup>[30]</sup> This chemokine plays a significant multifunctional role in modulating tumor proliferation, invasion, and migration through autocrine or paracrine signaling. The binding of *CXCL8* to *CXCR1/2* activates intracellular signaling pathways that regulate angiogenesis and cell proliferation, migration, survival, invasion, and motility.<sup>[31]</sup> Increased expression levels of *CXCL8* have been reported in nasopharyngeal cancer tissue.<sup>[32]</sup>

*CXCL8* has been shown to have different effects during the process of bone metastasis. Iguchi et al.<sup>[33]</sup> reported that in an in vivo study involving lung cancer cells, *CXCL8* has an inhibitory effect on the occurrence of bone metastasis. This effect arises from its inhibition of osteoclast resorption, which is a necessary initial step for bone metastasis to occur. Bone resorption allows cancer cells to penetrate the mineralized matrix within bone tissue. Other studies of breast cancer have shown that increased levels of IL-8 are associated with osteolytic bone metastasis due to heightened osteoclast formation and the stimulation of RANKL production through its binding to *CXCR1*.<sup>[34]</sup> A study of the role of IL-8 in multiple myeloma reported that increased *CXCL8* levels are linked to cell proliferation, osteoclast formation, and anti-apoptotic effects.<sup>[35-37]</sup> In this study, a decrease in *CXCL8* expression levels was observed in the group with bone-involvement metastasis. IL-8 promotes the maturation and differentiation of osteoclasts, thus enhancing bone resorption processes. The reduced expression

levels of *CXCL8* shifts the balance of bone remodeling toward a dominance of osteoblastic activity.<sup>[34,38]</sup>

This study is the first to simultaneously evaluate the role of the expression levels of multiple genes by measuring mRNA levels in patients with nasopharyngeal cancer, specifically focusing on bone metastases. The study's limitations included a retrospective design, small subgroup subjects, and a focus solely on the primary tumor by examining cancer cell gene expression levels without assessing the host microenvironment in metastases.

## Conclusions

Bone is the most common site of synchronous metastasis in advanced nasopharyngeal cancer. In this study, the predominant type of bone-metastatic lesion was osteoblastic. In the group with bone-involvement metastasis, *CXCR4* was upregulated and *CXCL8* was downregulated when compared to the non-metastasis group. In the bone-only metastasis group, only the *CXCR4* gene was found to be upregulated compared to the non-metastasis group.

## Ethical approval

The Health Research Ethics Committee of the Faculty of Medicine at Universitas Indonesia approved this study (Approval No. KEDN158/UN2.F1/ETIK/PPM.00.02/2022).

## Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalized human and animal cell lines were used in the present study.

## Conflict of interest

The authors have declared that no competing interests exist.

## Use of AI

No use of AI was reported.

## Funding

This work is supported by Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) Grant 2021–2022 No. NKB-130/UN2.RST/HKP.05.00/2021 and funded by the Ministry of Research and Technology of Republic of Indonesia, National Research, and Innovation Agency.

## Author contributions

All authors have contributed equally.

## Data availability

The authors declare that all data utilized in this study have been referenced or included in the article itself. The data may be shared upon reasonable request.

## Acknowledgements

The authors would like to extend their gratitude to Joshua Kurnia Tandi, MD; Idzhar Arrizal, MD; Claretta Vera Patricia Widya, MD; Alfonsus Pramudita, MD; Harits Adi Putra, MD; Endang Farihatul Izza, MD; Pinky Nur Alfaini, MD; and Tasyaa Fillahihasanah, MD, for their assistance in data collection. Special thanks are also given to Mrs. Supriyati and Mrs. Indriana Karmila from the Department of Anatomical Pathology for providing the paraffin-embedded samples.

## References

1. Akervall J, Kurnit DM, Adams M, et al. Overexpression of cyclin D1 correlates with sensitivity to cisplatin in squamous cell carcinoma cell lines of the head and neck. *Acta Otolaryngol* 2004; 124(7):851–7.
2. Salehiniya H, Mohammadian M, Mohammadian-Hafshejani A, et al. Nasopharyngeal cancer in the world: epidemiology, incidence, mortality and risk factors. *WCRJ* 2018; 5(1):e1046.
3. Faisal HH. [Analysis of survival and contributing factors in nasopharyngeal cancer patients in the ENT department of Dr. Cipto Mangunkusumo National Hospital [PhD thesis]. Jakarta: Universitas Indonesia; 2017 [Indonesian].
4. Seyfried TN, Huysentruyt LC. On the origin of cancer metastasis. *Crit Rev Oncog* 2013; 18(1-2):43–73.
5. Winberg RA. Moving out: invasion and metastasis. In: Winberg RA, editor. *The Biology of Cancer*. New York: Garland Science, Taylor & Francis Group; 2007. 587–654.
6. Sopik V, Narod SA. The relationship between tumour size, nodal status and distant metastases: on the origins of breast cancer. *Breast Cancer Res Treat* 2018; 170(3):647–56.
7. Yuan L, Guo F, Wang L, et al. Prediction of tumor metastasis from sequencing data in the era of genome sequencing. *Brief Funct Genomics* 2019; 18(6):412–8.
8. Ke L, Xiang Y, Guo X, et al. c-Src activation promotes nasopharyngeal carcinoma metastasis by inducing the epithelial-mesenchymal transition via PI3K/Akt signaling pathway: a new and promising target for NPC. *Oncotarget* 2016; 7(19):28340–55.
9. Qin H, Wang R, Wei G, et al. Overexpression of osteopontin promotes cell proliferation and migration in human nasopharyngeal carcinoma and is associated with poor prognosis. *Eur Arch Otorhinolaryngol* 2018; 275(2):525–34.
10. Resteghini C, Alfieri S, Quattrone P, et al. RANK expression in EBV positive nasopharyngeal carcinoma metastasis: a ready-to-treat target? *Oncotarget* 2017; 8(56):96184–9.
11. Wang M, Xia F, Wei Y, et al. Molecular mechanisms and clinical management of cancer bone metastasis. *Bone Res* 2020; 8(30):1–20.
12. Wang N, Wu Q-L, Fang Y, et al. Expression of chemokine receptor CXCR4 in nasopharyngeal carcinoma: pattern of expression and correlation with clinical outcome. *J Transl Med* 2005; 3:26–34.
13. Wang M-H, Zhou X-M, Zhang M-Y, et al. BMP2 promotes proliferation and invasion of nasopharyngeal carcinoma cells via mTORC1 pathway. *Aging* 2017; 9(4):1326–40.
14. Nanostring Technologies. Counter Advanced Analysis 2.0 User Manual 2018 [cited 2023 4 Agustus]. Available from: [https://nanostring.com/wp-content/uploads/MAN-10030-03\\_nCounter\\_Advanced\\_Analysis\\_2.0\\_User\\_Manual.pdf](https://nanostring.com/wp-content/uploads/MAN-10030-03_nCounter_Advanced_Analysis_2.0_User_Manual.pdf)
15. Guo Y, Chen JX, Yang S, et al. Selection of reliable reference genes for gene expression study in nasopharyngeal carcinoma. *Acta Pharmacol Sin* 2010; 31(11):1487–94.
16. Wei WI, Sham JS. Nasopharyngeal carcinoma. *Lancet* 2005; 365(9476):2041–54.
17. Adham M, Kurniawan AN, Muhtadi AI, et al. Nasopharyngeal carcinoma in Indonesia: epidemiology, incidence, signs, and symptoms at presentation. *Chin J Cancer* 2012; 31(4):185–96.
18. Shen L, Dong J, Li S, et al. M1 stage subdivision and treatment outcome of patients with bone-only metastasis of nasopharyngeal carcinoma. *The Oncologist* 2015; 20(3):291–8.
19. Chan J, Pilch B, Kuo T, et al. Tumours of the nasopharynx. In: Barnes L, Eveson JW, Reichart P, Sidransky D, editors. *World Health Organization pathology and genetics of head and neck tumours*. Lyon: IARC Press; 2005; 83–97.
20. Liao W, Tian M, Chen N. Characteristic and novel therapeutic strategies of nasopharyngeal carcinoma with synchronous metastasis. *Cancer Manag Res* 2019; 11:8431–42.
21. Ben-Ghedalia-Peled N, Vago R. Wnt signaling in the development of bone metastasis. *Cells* 2022; 11(23).
22. Qu W, Li S, Zhang M, et al. Pattern and prognosis of distant metastases in nasopharyngeal carcinoma: A large-population retrospective analysis. *Cancer Med* 2020; 9(17):6147–58.
23. Sham JS, Cheung YK, Chan FL, et al. Nasopharyngeal carcinoma: pattern of skeletal metastases. *Br J Radiol* 1990; 63(747):202–5.
24. Zi D, Tan J, Shu L, et al. CXCR4 mediated to epithelial-mesenchymal transition and stemness in epithelial ovarian carcinoma. *J Minim Invasive Gynecol* 2017; 24(7):S56–S7.
25. Chatterjee S, Behnam Azad B, Nimmagadda S. The intricate role of CXCR4 in cancer. *Advances Cancer Res* 2014; 124:31–82.
26. Kang Y, Siegel PM, Shu W, et al. A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell* 2003; 3(6):537–49.
27. Cospighi I, Atmakusumah TD, Siregar NC, et al. Bone metastasis in advanced breast cancer: analysis of gene expression microarray. *Clin Breast Cancer* 2018; 18(5):e1117–e22.
28. Sacanna E, Ibrahim T, Gaudio M, et al. The role of CXCR4 in the

- prediction of bone metastases from breast cancer: A pilot study. *Oncology* 2011; 80(3-4):225-31.
29. Zhang H-W, Sun X-F, He Y-N, et al. Bioinformatics analysis of breast cancer bone metastasis related gene-CXCR4. *Asian Pac J Trop Biomed* 2013; 6(9):732-8.
30. Liu Q, Li A, Tian Y, et al. The CXCL8-CXCR1/2 pathways in cancer. *Cytokine Growth Factor Rev* 2016; 31:61-71.
31. Liu B, Xu M, Guo Z, et al. Interleukin-8 promotes prostate cancer bone metastasis through upregulation of bone sialoprotein. *Oncol Lett* 2019; 17(5):4607-13.
32. Lo MC, Yip TC, Ngan KC, et al. Role of MIF/CXCL8/CXCR2 signaling in the growth of nasopharyngeal carcinoma tumor spheres. *Cancer Lett* 2013; 335(1):81-92.
33. Iguchi H, Ono M, Matsushima K, et al. Overproduction of IL-8 results in suppression of bone metastasis by lung cancer cells in vivo. *Int J Oncol* 2000; 17(2):329-33.
34. Bendre MS, Montague DC, Peery T, et al. Interleukin-8 stimulation of osteoclastogenesis and bone resorption is a mechanism for the increased osteolysis of metastatic bone disease. *Bone* 2003; 33(1):28-37.
35. Pellegrino A, Ria R, Pietro GD, et al. Bone marrow endothelial cells in multiple myeloma secrete CXC-chemokines that mediate interactions with plasma cells. *Br J Haematol* 2005; 129(2):248-56.
36. Kline M, Donovan K, Wellik L, et al. Cytokine and chemokine profiles in multiple myeloma; significance of stromal interaction and correlation of IL-8 production with disease progression. *Leuk Res* 2007; 31(5):591-8.
37. Herrero AB, García-Gómez A, Garayoa M, et al. Effects of IL-8 up-regulation on cell survival and osteoclastogenesis in multiple myeloma. *Am J Pathol Res* 2016; 186(8):2171-82.
38. Zhao M-N, Zhang L-F, Sun Z, et al. A novel microRNA-182/Interleukin-8 regulatory axis controls osteolytic bone metastasis of lung cancer. *Cell Death Dis* 2023; 14(5):298.

## Appendix

**Table A1.** Genes list

Genes	Class name		
<i>ADNP</i>	Endogenous	<i>NRAS</i>	Endogenous
<i>AKT1</i>	Endogenous	<i>OPG</i>	Endogenous
<i>APC</i>	Endogenous	<b>OPN</b>	<b>Endogenous</b>
<i>BRAF</i>	Endogenous	<i>PD-L1</i>	Endogenous
<b>Bmp2</b>	<b>Endogenous</b>	<i>PDGFbeta</i>	Endogenous
Bone sialoprotein ( <i>BSP</i> )	Endogenous	<i>PIK3CA</i>	Endogenous
<i>CCL3</i>	Endogenous	<i>PPAR-g</i>	Endogenous
<i>CDH1</i>	Endogenous	<i>PTEN</i>	Endogenous
<i>CDKN2A</i>	Endogenous	<b>PTHrP</b>	<b>Endogenous</b>
<i>CK7</i>	Endogenous	<i>RAN</i>	Endogenous
<i>CLDN4</i>	Endogenous	<i>RASSF1A</i>	Endogenous
<i>COL18A1</i>	Endogenous	<b>Rank</b>	<b>Endogenous</b>
<i>COL1A1</i>	Endogenous	<b>RankL</b>	<b>Endogenous</b>
<i>COL1A2</i>	Endogenous	<i>Runx2</i>	Endogenous
<i>COL3A1</i>	Endogenous	<i>S100A14</i>	Endogenous
<i>COL5A1</i>	Endogenous	<i>SPINK6</i>	Endogenous
<i>COL5A2</i>	Endogenous	<i>SQSTM1</i>	Endogenous
<i>CTNNB1</i>	Endogenous	<i>STK11</i>	Endogenous
<i>CXCL12</i>	Endogenous	<b>SRC</b>	<b>Endogenous</b>
<i>CXCL4</i>	Endogenous	<i>TGFB</i>	Endogenous
<i>CaSR</i>	Endogenous	<i>TP53</i>	Endogenous
<i>Ctgf</i>	Endogenous	<i>TRAF6</i>	Endogenous
<b>Cxcr4</b>	<b>Endogenous</b>	<i>TRIM29</i>	Endogenous
<i>DDR1</i>	Endogenous	<i>VPS35</i>	Endogenous
<i>DPYSL3</i>	Endogenous	<i>Vegf</i>	Endogenous
<i>ESM1</i>	Endogenous	<i>WWP2</i>	Endogenous
<i>FN1</i>	Endogenous	<i>ZEB1</i>	Endogenous
<i>HIF1A</i>	Endogenous	<i>ciRS-7</i>	Endogenous
<i>IL1alfa</i>	Endogenous	<i>EIF3S7</i>	Housekeeping
<i>IL6</i>	Endogenous	<i>HPRT1</i>	Housekeeping
<i>IL-11</i>	Endogenous	<i>PDCD1</i>	Housekeeping
<i>Il17</i>	Endogenous	<i>PDCD1LG2</i>	Housekeeping
<i>Il18</i>	Endogenous	<i>TIGIT</i>	Housekeeping
<b>IL-8</b>	<b>Endogenous</b>	<i>TRAT1</i>	Housekeeping
<i>JAG1</i>	Endogenous	<i>YARS</i>	Housekeeping
<i>KRAS</i>	Endogenous	<i>NEG_A</i>	Negative
<i>LGR6</i>	Endogenous	<i>NEG_B</i>	Negative
<i>MET</i>	Endogenous	<i>NEG_C</i>	Negative
<i>METTL3</i>	Endogenous	<i>NEG_D</i>	Negative
<i>MLH1</i>	Endogenous	<i>NEG_E</i>	Negative
<i>MUC16</i>	Endogenous	<i>NEG_F</i>	Negative
<i>Mmp1</i>	Endogenous	<i>NEG_G</i>	Negative
<i>Mmp13</i>	Endogenous	<i>NEG_H</i>	Negative
<i>Mmp2</i>	Endogenous	<i>POS_A</i>	Positive
<i>Mmp9</i>	Endogenous	<i>POS_B</i>	Positive
		<i>POS_C</i>	Positive
		<i>POS_D</i>	Positive
		<i>POS_E</i>	Positive
		<i>POS_F</i>	Positive

**Table A2.** DEGs bone involvement vs. non-metastasis

Genes	Log2 fold change	Linear fold change	P-value	BH. p-value
IL-8-mRNA	-1.78	0.292	0.000161	0.00901
CXCR4-mRNA	0.537	1.45	0.00178	0.0498
KRAS-mRNA	0.577	1.49	0.0186	0.347
COL1A2-mRNA	-0.8	0.575	0.0359	0.439
COL1A1-mRNA	-0.781	0.582	0.0525	0.439
COL18A1-mRNA	0.388	1.31	0.069	0.439
ESM1-mRNA	0.546	1.46	0.0723	0.439
COL5A2-mRNA	-0.612	0.654	0.0759	0.439
COL5A1-mRNA	-0.58	0.669	0.0783	0.439
COL3A1-mRNA	-0.65	0.637	0.0784	0.439
Mmp1-mRNA	-0.803	0.573	0.0921	0.469
HIF1A-mRNA	-0.261	0.835	0.104	0.484
AKT1-mRNA	0.238	1.18	0.118	0.498
Mmp9-mRNA	-0.474	0.72	0.124	0.498
PIK3CA-mRNA	-0.242	0.845	0.218	0.786
RAN-mRNA	0.193	1.14	0.235	0.786
CTNNB1-mRNA	0.171	1.13	0.239	0.786
CDH1-mRNA	0.275	1.21	0.282	0.877
CXCL12-mRNA	0.467	1.38	0.302	0.888
CLDN4-mRNA	0.25	1.19	0.35	0.888
JAG1-mRNA	-0.236	0.849	0.354	0.888
Mmp2-mRNA	-0.352	0.784	0.394	0.888
Il18-mRNA	-0.355	0.782	0.402	0.888
FN1-mRNA	-0.312	0.806	0.403	0.888
PDGFbeta-mRNA	0.177	1.13	0.409	0.888
CDKN2A-mRNA	-0.254	0.839	0.414	0.888
APC-mRNA	0.535	1.45	0.442	0.888
Vegf-mRNA	-0.176	0.885	0.444	0.888
Rank-mRNA	0.229	1.17	0.471	0.91
OPN-mRNA	-0.317	0.803	0.494	0.922
Mmp13-mRNA	0.203	1.15	0.565	0.958
TGFB-mRNA	0.0735	1.05	0.58	0.958
Bmp2-mRNA	0.161	1.12	0.589	0.958
TP53-mRNA	0.104	1.07	0.593	0.958
ADNP-mRNA	0.105	1.08	0.635	0.958
ZEB1-mRNA	0.25	1.19	0.651	0.958
DPYSL3-mRNA	0.135	1.1	0.662	0.958
SQSTM1-mRNA	0.0669	1.05	0.666	0.958
WWP2-mRNA	0.299	1.23	0.682	0.958
TRIM29-mRNA	-0.106	0.929	0.707	0.958
PD-L1-mRNA	-0.104	0.931	0.729	0.958
DDR1-mRNA	-0.0701	0.953	0.735	0.958
SRC-mRNA	-0.166	0.892	0.759	0.958
Ctgf-mRNA	0.0951	1.07	0.782	0.958
Runx2-mRNA	0.106	1.08	0.815	0.958

TRAF6-mRNA	0.139	1.1	0.822	0.958
METTL3-mRNA	0.0327	1.02	0.828	0.958
MLH1-mRNA	-0.0313	0.979	0.84	0.958
PTEN-mRNA	-0.0198	0.986	0.883	0.958
RASSF1A-mRNA	-0.0733	0.95	0.894	0.958
STK11-mRNA	0.0639	1.05	0.918	0.958
BRAF-mRNA	0.0419	1.03	0.938	0.958
VPS35-mRNA	0.0387	1.03	0.939	0.958
S100A14-mRNA	-0.0186	0.987	0.956	0.958
NRAS-mRNA	0.0419	1.03	0.956	0.958
CCL3-mRNA	-0.0143	0.99	0.958	0.958

**Table A3.** DEGs bone only vs. non-metastasis

Gen	Log2 fold change	Linear fold change	P-value	BH. p-value
<b>CXCR4-mRNA</b>	<b>0.685</b>	<b>1.61</b>	<b>0.000357</b>	<b>0.02</b>
IL-8-mRNA	-1.31	0.403	0.012	0.336
CDH1-mRNA	0.57	1.48	0.043	0.48
ESM1-mRNA	0.72	1.65	0.0487	0.48
CLDN4-mRNA	0.581	1.5	0.0491	0.48
AKT1-mRNA	0.33	1.26	0.053	0.48
CTNNB1-mRNA	0.301	1.23	0.0629	0.48
KRAS-mRNA	0.501	1.42	0.0685	0.48
COL18A1-mRNA	0.412	1.33	0.0902	0.522
RAN-mRNA	0.306	1.24	0.0933	0.522
COL1A1-mRNA	-0.642	0.641	0.155	0.703
COL1A2-mRNA	-0.604	0.658	0.156	0.703
Rank-mRNA	0.481	1.4	0.17	0.703
CXCL12-mRNA	0.682	1.6	0.185	0.703
CDKN2A-mRNA	-0.45	0.732	0.188	0.703
DPYSL3-mRNA	0.397	1.32	0.248	0.816
COL5A1-mRNA	-0.41	0.753	0.265	0.816
TP53-mRNA	0.241	1.18	0.267	0.816
ADNP-mRNA	0.253	1.19	0.308	0.816
PDGFbeta-mRNA	0.25	1.19	0.31	0.816
COL3A1-mRNA	-0.415	0.75	0.313	0.816
Mmp1-mRNA	-0.516	0.699	0.331	0.816
COL5A2-mRNA	-0.369	0.775	0.335	0.816
Bmp2-mRNA	0.302	1.23	0.351	0.818
METTL3-mRNA	0.152	1.11	0.366	0.82
APC-mRNA	0.669	1.59	0.402	0.822
Mmp13-mRNA	0.371	1.29	0.407	0.822
SQSTM1-mRNA	0.143	1.1	0.411	0.822
Ctgf-mRNA	0.291	1.22	0.45	0.869
TGFB-mRNA	0.105	1.08	0.486	0.907
Mmp9-mRNA	-0.2	0.871	0.557	0.921
PTEN-mRNA	0.0867	1.06	0.565	0.921
CCL3-mRNA	0.178	1.13	0.576	0.921

ZEB1-mRNA	0.338	1.26	0.587	0.921
Il18-mRNA	-0.258	0.836	0.59	0.921
Runx2-mRNA	0.277	1.21	0.592	0.921
WWP2-mRNA	0.366	1.29	0.657	0.966
MLH1-mRNA	-0.0723	0.951	0.685	0.966
PD-L1-mRNA	-0.121	0.92	0.712	0.966
TRAF6-mRNA	0.238	1.18	0.731	0.966
STK11-mRNA	0.227	1.17	0.75	0.966
HIF1A-mRNA	-0.055	0.963	0.751	0.966
PIK3CA-mRNA	-0.0632	0.957	0.772	0.966
BRAF-mRNA	0.175	1.13	0.775	0.966
Mmp2-mRNA	-0.131	0.913	0.776	0.966
VPS35-mRNA	0.144	1.11	0.802	0.976
SRC-mRNA	0.109	1.08	0.863	0.984
JAG1-mRNA	0.0467	1.03	0.866	0.984
NRAS-mRNA	0.0759	1.05	0.93	0.984
DDR1-mRNA	-0.0158	0.989	0.946	0.984
Vegf-mRNA	0.0167	1.01	0.948	0.984
TRIM29-mRNA	0.0128	1.01	0.968	0.984
FN1-mRNA	0.0113	1.01	0.978	0.984
S100A14-mRNA	-0.00808	0.994	0.983	0.984
OPN-mRNA	-0.0108	0.993	0.983	0.984
RASSF1A-mRNA	0.0122	1.01	0.984	0.984