Received: 10 February 2026 | Revised: 19 March 2026 | Accepted: 1 April 2026

DOI: 10.1111/jne.70184

ORIGINAL ARTICLE

Journal of Neuroendocrinology WILEY

The emerging role of chemokines and chemokine receptors in the biological and clinical behaviour of pituitary neuroendocrine tumours: An exploratory transcriptomic study

Ana Luísa Silva[1,2] | Sayka Barry[3] | Ana Hipólito[1,2] | Mariana de Griné Severino[4] | Rita Joaquim[5] | Charlotte Hall[3] | Tiago Oliveira[6] | Dolores López-Presa[6] | Gonçalo Borrecho[7] | Francisco Tortosa[8] | Ema Nobre[4] | Claudia C. Faria[9,10,11] | Márta Korbonits[3] | Pedro Marques[1,12]

[1]Faculdade de Medicina, Universidade Católica Portuguesa, Lisbon, Portugal

[2]Instituto de Saúde Ambiental da Faculdade de Medicina da Universidade de Lisboa (ISAMB-FMUL), Lisbon, Portugal

[3]Centre for Endocrinology, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK

[4]Endocrinology Department, Hospital de Santa Maria, Unidade Local de Saúde de Santa Maria (ULSSM), Lisbon, Portugal

[5]Faculty of Medicine, Lisbon University, Lisbon, Portugal

[6]Pathology Department, Hospital de Santa Maria, Unidade Local de Saúde de Santa Maria (ULSSM), Lisbon, Portugal

[7]Pathology Department, Unidade Local de Saúde de Coimbra, Coimbra, Portugal

[8]Pituitary Tumor Unit, Pathology Department, Hospital CUF Descobertas, Lisbon, Portugal

[9]Neurosurgery Department, Hospital de Santa Maria, Unidade Local de Saúde de Santa Maria (ULSSM), Lisbon, Portugal

[10]Faculdade de Medicina da Universidade de Lisboa, Clínica Universitária de Neurocirurgia, Lisbon, Portugal

[11]GIMM—Gulbenkian Institute for Molecular Medicine, Lisbon, Portugal

[12]Pituitary Tumor Unit, Endocrinology Department, Hospital CUF Descobertas, Lisbon, Portugal

Correspondence
Pedro Marques, Pituitary Tumor Unit, Endocrinology Department, Hospital CUF Descobertas, Lisbon, Portugal & Faculdade de Medicina, Universidade Católica Portuguesa, Lisbon, Portugal.
Email: pedro.miguel.sousa.marques@gmail.com

Funding information
Neuroendocrine Tumor Research Foundation; Fundação Bial; Sociedade Portuguesa de Endocrinologia, Diabetes e Metabolismo

Abstract

The chemokine network in the microenvironment of pituitary neuroendocrine tumours (PitNETs) may modulate tumour biology, aggressiveness, and treatment responses. We aimed to study the role of various chemokines and chemokine receptors in defining PitNET phenotype and clinical outcomes. We included 96 patients (51 females) with available snap-frozen PitNET tissue from surgery between 2014 and 2020. Chemokine and chemokine receptors were studied by RT-qPCR. Fold difference in mRNA expression was calculated using the ΔΔCt method; chemokine and receptor expression levels were normalised to the expression of the control gene *TBP*, and expressed relative to a reference sample. Ten chemokines and receptors were studied (*CCL2*, *CCL3*, *CCL4*, *CXCL8*, *CX3CL1*, *CCR2*, *CCR4*, *CCR5*, *CXCR1*, *CXCR2*), and their expression correlated with clinico-pathological and outcome data, as well as other available microenvironment-related data. We found strong positive

Ana Luísa Silva and Sayka Barry have contributed equally to this manuscript.

correlations between all chemokines and chemokine receptors. Higher chemokine and receptor expression levels were seen in patients who had pituitary apoplexy (*CCR2*, *CXCR1*), hypopituitarism at diagnosis (*CCL2*, *CCR4*), Ki-67 >3% (*CCL4*, *CXCR2*), as well as in patients who required re-operation (*CCL3*, *CXCL8*, *CXCR2*), multimodal therapy (*CCL2*), and had active disease at last-follow-up (*CCL2*). There was a positive correlation between the number of pituitary surgeries and expression levels of *CCL3*, *CXCL8*, *CX3CL1*, *CXCR1*, and *CXCR2*. Compared to nonfunctioning-PitNETs, somatotropinomas had higher expression of *CCL2*, *CCL4*, and *CCR2*, and lower expression of *CX3CL1* and *CCR4*. Expression of *CDH1* (encoding E-cadherin) correlated negatively with *CCL2*, *CCL4*, *CCR2*, *CCR4*, and *CXCR2*, while the expression of *ZEB1* (mesenchymal marker) positively correlated with *CCL3*, *CCL4*, and *CX3CL1*. PitNETs expressing higher levels of *CCL4*, *CX3CL1*, *CCR4*, *CCR5*, and *CXCR1* had more and bigger vessels. Somatotropinomas treated pre-operatively with somatostatin analogues were associated with higher expression of *CCL2*, *CCR4*, *CXCR1*, and *CXCR2*, while nonfunctioning-PitNETs pre-surgically treated with dopamine agonists were associated with lower expression of *CCL3*, *CCL4*, *CX3CL1*, *CCR5*, *CXCR1*, and *CXCR2*. Our data suggests that chemokines and chemokine receptors may be involved in the modulation of different tumorigenic mechanisms in PitNETs, including tumour proliferation, epithelial-to-mesenchymal transition, and angiogenesis, and may be associated with more aggressive and difficult-to-treat disease.

KEYWORDS
chemokine receptors, chemokines, epithelial-to-mesenchymal transition, microenvironment, pituitary neuroendocrine tumour

1 | INTRODUCTION

Chemokines are a group of chemoattractant cytokines that act in their cell surface chemokine receptors controlling autocrine or paracrine communications within and between individual cell types, playing key roles in immunity, inflammation, repair and cell growth, as well as in cancer.[1,2] The chemokine system encompasses about 50 human genes encoding chemokine ligands and more than 20 corresponding transmembrane G-protein coupled chemokine receptors.[1,3] Chemokines are subdivided into 4 groups depending on the position of the conserved cysteine residue (CC, CXC, CX3C, or C), and the chemokine receptor nomenclature follows a similar principle, that is, CC chemokines bind to CC receptors and CXC chemokines bind to CXC receptors.[1,4] However, the chemokine system is redundant, which means that some chemokines can bind to several different receptors, resulting in overlapping biological functions and making it challenging to target therapeutically.[1,4]

In cancer, chemokines can be produced by tumour cells and influence various oncogenic mechanisms, including immune cell trafficking, anti-tumour immunity, microenvironment modulation, angiogenesis, proliferation, activation of the epithelial-to-mesenchymal transition (EMT) pathway, resulting in increased cell migration and invasion, as well as in defining the response to oncological treatments.[1,4] The chemokine system has been extensively investigated in cancer, including endocrine-related cancers,[1,2,4–9] but little is currently known in pituitary neuroendocrine tumours (PitNETs).

PitNETs are common adenohypophyseal tumours, accounting for 15% of all intracranial neoplasms, and are usually benign. However, PitNETs can cause a significant burden due to hormone hypersecretion, compression of surrounding structures, such as the optic chiasm, and many are invasive and/or refractory to conventional treatments.[2,10] PitNETs express a wide range of chemokines, including CCL2, CCL3, CCL4, CCL17, CXCL8, CX3CL1, CXCL1, CXCL10, among others. The role of this chemokine network in determining the tumour microenvironment, aggressiveness and outcomes in PitNETs has been progressively unravelled, but knowledge concerning chemokine receptors is lacking.[2,11–15] Recent studies showed that increased CCL2 expression in PitNETs is associated with poorer outcomes and more difficult-to-treat PitNETs,[16] and invasive PitNETs have increased expression of epidermal growth factor (EGF), CXCL1 and CCL17.[11]

In contrast, the expression pattern and biological role of the respective chemokine receptors in PitNETs remain largely unknown, although previous studies showed that PitNETs can express chemokine receptors and these may also be involved in tumour proliferation and invasion, namely the receptors CXCR1/CXCR2 (binding mainly to CXCL8),[17,18] and CXCR4/CXCR7 (binding mainly to CXCL12).[19–22] Recent studies also showed that CXCR4 expression in corticotroph PitNETs predicts biological behaviour, hormone activity and

recurrence, and CXCR4-targeted functioning imaging may be useful in tumour localisation in Cushing's disease.[23,24] Hence, investigating chemokine receptors in PitNETs may allow to identify new diagnostic and prognostic biomarkers, as well as facilitate the development of new imaging modalities and treatments targeting chemokine receptors.

In this study, we aimed to study the role of various chemokines and chemokine receptors in defining the PitNET phenotype and clinical outcomes, including scarcely investigated chemokines (e.g., CCL3, CCL4, CX3CL1) and chemokine receptors (e.g., CCR2, CCR4, CCR5) in PitNETs, as well as to study their role in the regulation of tumorigenic mechanisms, particularly in the EMT activation, angiogenesis and tumour proliferation.

2 | MATERIALS AND METHODS

2.1 | Study population and PitNET samples

Our study population consisted of 96 patients with PitNETs who underwent a pituitary operation at Hospital Santa Maria between 2014 and 2020, and from whom snap-frozen tissues obtained at the time of surgery were available at our Biobank for the transcriptomic study. Of these, 86 cases previously characterised in Reference [16] had also paraffin blocks available for the immunohistochemical studies. This study was approved by the local Ethics Committee (No. 400/21) and written informed consent was obtained from each patient.

2.2 | Clinico-pathological outcome and immunohistochemical data of PitNET patients

Patients' demographic, clinical, biochemical, radiological, treatment and outcome data were retrieved from hospital medical records. Clinico-pathological features, clinical outcomes and treatment-related parameters were defined as previously described,[16,25] and are briefly explained in the legend of Table 1.

PitNET tissues were processed for routine histopathological diagnosis. Pathological reports were available for all cases and included immunohistochemistry for pituitary hormones; immunohistochemical assessment for transcription factors (TPIT, SF-1, and PIT-1) was performed in pituitary hormone-negative cases. Based on their clinico-biochemical profile and histological diagnoses, patients were grouped as clinically nonfunctioning-PitNETs (NF-PitNETs, $n = 68$), acromegaly (somatotroph tumours, $n = 19$), Cushing's disease (corticotroph tumours, $n = 6$), prolactinomas (lactotroph PitNETs, $n = 2$), and thyrotropinomas (thyrotroph PitNETs, $n = 1$).

Paraffin blocks with well-conserved and sufficient PitNET specimens for the immunohistochemical studies were available in 86 of the 96 cases with snap-frozen tissues. Immunohistochemistry protocol, list of antibodies and methods used to evaluate E-cadherin immunostaining and angiogenesis are described in detail in Reference [16]. Briefly, immunostains for E-cadherin (anti-E-cadherin, Ventana clone 36, RTU) and for CD31-positive endothelial cells (anti-CD31, DAKO, M0823, dilution 1:100) were performed on 4 μm formalin-fixed paraffin-embedded tissue sections using Automated Ventana BenchMark Ultra System (Roche).[16] E-cadherin immunostaining was analysed on the basis of plasma membrane expression, and reported as E-cadherin score, as described in Reference [16] and briefly explained in the legend of Table 2. Immunostaining analysis of CD31-positive vessels was conducted using the image software QuPath version 0.5.0. A script was created to ensure the reproducibility of the cross-sectional analysis of histological images selected by an experienced pathologist (20× magnification "hot spot" images), and an Artificial Intelligence algorithm based on training of pixel classification with positive annotations for CD31 staining in regions of interest and negative annotations in regions to be excluded was developed and used for automated analysis. Vessels were counted in three different ×20 high power fields (HPF) per case. The following angiogenic outputs were considered: number of vessels per selected sample area, vessel area (μm^2), vessel perimeter (μm) and vessel roundness (value comprised between 0 and 1, with 1 corresponding to perfect circle shape vessels and 0 to very elongated vessels). Further details about CD31 immunohistochemistry and angiogenic parameters, as well as representative images, can be found in Reference [16].

2.3 | RNA extraction and quantitative reverse transcription-polymerase chain reaction (RT-qPCR)

Pituitary frozen tissues were disrupted using a beadbeater (BioSpec, OKC, USA) after addition of lysis buffer and zirconia/silica beads (1 mm, BioSpec, OKC, USA) to each sample following the manufacturer's protocol. Total RNA isolation, cDNA synthesis and SYBR Green-based RT-qPCR were performed, as previously described.[16] Each amplification was performed in triplicate. Fold difference in mRNA expression was calculated using the efficiency corrected model of the ΔΔCt method. *CCL2*, *CCL3*, *CCL4*, *CXCL8*, *CX3CL1*, *CCR2*, *CCR4*, *CCR5*, *CXCR1*, and *CXCR2* mRNA levels were normalised to expression levels of the endogenous control gene TATA-binding protein (*TBP*) per sample, and normalised values were expressed relative to those of a normal human tissue pool reference sample (Quantitative PCR Human Reference Total RNA, Agilent). Expression values correspond to arbitrary units representing fold differences relative to the reference sample. Primer sequences used in the RT-qPCR experiments are shown in Supplemental Table 1.

2.4 | Statistical analysis

Clinico-pathological, outcome and immunohistochemical data were correlated with chemokine and chemokine receptors mRNA expression levels. Data are presented as absolute number or percentages for categorical variables, or as mean and standard deviation (SD) or standard error of the mean (SEM) for continuous variables. Comparisons involving categorical variables were performed using Chi-squared test, while continuous variables were tested for Gaussian distribution with

TABLE 1 Baseline characteristics and treatment-related outcomes of our PitNET cohort.

	Whole cohort of PitNETs ($n = 96$)	NF-PitNETs ($n = 68$)	Acromegaly ($n = 19$)	Cushing's disease ($n = 6$)	Prolactinomas ($n = 2$)	Thyrotroph PitNETs ($n = 1$)
Gender [*n* (%)]						
Male	45 (46.9%)	36 (52.9%)	8 (42.1%)	1 (16.7%)	0	0
Female	51 (53.1%)	32 (47.1%)	11 (57.9%)	5 (83.3%)	2 (100%)	1 (100%)
Age at diagnosis (years) [mean ± SD]	55.3 ± 15.2	59.7 ± 12.1	45.4 ± 19.0	43.3 ± 13.9	39.0 ± 12.7	49
PitNET subtypes [*n* (%)]						
NF-PitNETs	68 (70.8%)					
Acromegaly	19 (19.8%)					
Cushing's disease	6 (6.3%)					
Prolactinomas	2 (2.1%)					
Thyrotroph PitNETs	1 (1.0%)					
Pituitary apoplexy [*n* (%)]	5 (5.2%)	2 (2.9%)	1 (5.3%)	1 (16.7%)	1 (50.0%)	0
Headache at diagnosis [*n* (%)]	27 (28.1%)	17 (25.0%)	6 (31.6%)	3 (50.0%)	1 (50.0%)	0
Hypopituitarism at diagnosis [*n* (%)]	64 (66.7%)	52 (76.5%)	8 (42.1%)	3 (50.0%)	1 (50.0%)	0
Secondary hypogonadism	49 (51.0%)	38 (55.9%)	7 (36.8%)	3 (50.0%)	1 (50.0%)	0
Secondary hypothyroidism	37 (38.5%)	32 (47.1%)	2 (10.5%)	2 (33.3%)	1 (50.0%)	0
GH deficiency	21 (21.9%)	21 (30.9%)	0	0	0	0
Secondary adrenal insufficiency	29 (30.2%)	25 (36.8%)	4 (21.1%)	0	0	0
Hyperprolactinemia at diagnosis [*n* (%)]	45 (46.9%)	31 (45.6%)	10 (52.6%)	2 (33.3%)	2 (100%)	0
Number of pituitary deficiencies at diagnosis [mean ± SD]	1.5 ± 1.3	1.8 ± 1.3	0.7 ± 1.0	0.8 ± 1.0	1.0 ± 0.3	0
Macroadenoma [*n* (%)]	93 (96.9%)	68 (100%)	17 (89.5%)	5 (83.3%)	2 (100%)	0
Maximum tumour diameter (mm) [mean ± SD]	30.6 ± 12.1	32.3 ± 11.3	28.8 ± 13.7	18.2 ± 9.9	40.0 ± 11.5	17
Suprasellar extension [*n* (%)]	88 (91.7%)	67 (98.5%)	14 (73.7%)	4 (66.7%)	2 (100%)	1 (100%)
Cavernous sinus invasion [*n* (%)]	36 (37.5%)	25 (36.8%)	7 (36.8%)	2 (33.3%)	2 (100%)	0
Any treatment before first surgery [*n* (%)]	15 (15.6%)	5 (7.4%)	7 (36.8%)	1 (16.7%)	2 (100%)	0
Histological diagnosis [*n* (%)]						
Somatotroph tumour	14 (14.6%)	0	14 (73.7%)	0	0	0
Somatolactotroph tumour	5 (5.2%)	0	5 (26.3%)	0	0	0
Lactotroph tumour	1 (1.0%)	0	0	0	1 (50.0%)	0
Corticotroph tumour	6 (6.3%)	0	0	6 (100%)	0	0
Gonadotroph tumour	41 (42.7%)	41 (60.3%)	0	0	0	0
Silent corticotroph tumour	8 (8.3%)	8 (11.8%)	0	0	0	0
Silent thyrotroph tumour	2 (2.1%)	2 (2.9%)	0	0	0	0
Plurihormonal tumour	13 (13.5%)	12 (17.6%)	0	0	0	1 (100%)
Null cell tumour	6 (6.3%)	5 (7.4%)	0	0	1 (50.0%)	0
Ki-67 >3% [*n* (%)]	19 (19.8%)	9 (13.2%)	6 (31.6%)	2 (33.3%)	2 (100%)	0
Tumour remnant within 1-year post-operatively [*n* (%)]	41 (42.7%)	29 (42.6%)	8 (42.1%)	2 (33.3%)	2 (100%)	0
Number of total treatments [mean ± SD]	1.6 ± 1.1	1.3 ± 0.6	2.2 ± 1.2	2.0 ± 1.7	6.0 ± 2.8	1
Number of surgeries [mean ± SD]	1.1 ± 0.4	1.1 ± 0.3	1.1 ± 0.2	1.2 ± 0.4	2.5 ± 0.7	1
Need for re-operation [*n* (%)]	11 (11.5%)	6 (8.8%)	2 (10.5%)	1 (16.7%)	2 (100%)	0
Post-operative medical therapy [*n* (%)]	12 (12.5%)	1 (1.5%)	9 (47.4%)	1 (16.7%)	2 (100%)	0

TABLE 1 (Continued)

	Whole cohort of PitNETs ($n = 96$)	NF-PitNETs ($n = 68$)	Acromegaly ($n = 19$)	Cushing's disease ($n = 6$)	Prolactinomas ($n = 2$)	Thyrotroph PitNETs ($n = 1$)
Radiotherapy [*n* (%)]	19 (19.8%)	12 (17.6%)	3 (15.8%)	2 (33.3%)	2 (100%)	0
Multimodal treatment [*n* (%)]	31 (32.3%)	14 (20.6%)	13 (68.4%)	2 (33.3%)	2 (100%)	0
Multiple treatment [*n* (%)]	11 (11.5%)	2 (2.9%)	5 (26.3%)	2 (33.3%)	2 (100%)	0
Active disease at last follow-up [*n* (%)]	19 (19.8%)	11 (16.2%)	6 (31.6%)	1 (16.7%)	1 (50.0%)	0
Hypopituitarism at last follow-up [*n* (%)]	62 (64.6%)	52 (76.5%)	6 (31.6%)	2 (33.3%)	2 (100%)	0
Number of pituitary deficiencies at last follow-up [mean ± SD]	1.7 ± 1.4	2.0 ± 1.4	0.7 ± 1.2	0.7 ± 1.2	2.5 ± 0.7	0
Follow-up duration (months) [mean ± SD]	71.9 ± 35.5	69.9 ± 37.4	76.5 ± 29.4	64.3 ± 29.3	102.5 ± 47.4	105

Note: Hypopituitarism was defined as the presence of at least 1 pituitary hormone deficiency on basal pituitary function tests, and when necessary dynamic tests were performed. Invasion was assessed with the Knosp classification, with grades 3 and 4 regarded as presence of cavernous sinus invasion. Multimodal treatment was defined as the employment of 2 or more distinct forms of treatment, while multiple treatment was defined as the employment of 3 or more treatments in the patient's management. The need for re-operation subgroup involved patients who had at least 1 additional surgery following the first pituitary surgery. Active disease at last follow-up was considered in case of persistent or recurrent progressive tumour remnants in both hormone-secreting PitNETs and NF-PitNETs; small persistent remnants after surgery that remained stable over time and required no further intervention were regarded as not active; for acromegaly and Cushing's disease cases, biochemical remission at the last follow-up was interpreted in line with current guidelines. The mean follow-up duration was calculated from the date of first pituitary operation until the last follow-up observation date.
Abbreviations: GH, growth hormone; NF-PitNETs, nonfunctioning-pituitary neuroendocrine tumours; PitNETs, pituitary neuroendocrine tumours; SD, standard deviation.

Shapiro–Wilk test, and non-parametric and parametric data were analysed with Mann–Whitney U and Student's *t*-tests. Correlation between continuous variables were determined by the Spearman correlation coefficient *rho*. Statistical analysis was performed in SPSS version 20 (IBM, USA) and GraphPad Prism version 10.2. *p*-values <.05 were considered statistically significant.

3 | RESULTS

3.1 | Characteristics and treatment-related outcomes of our PitNET cohort

Of the 96 PitNET patients included in the study, 51 were females (53.1%), the mean age at PitNET diagnosis was 55.3 ± 15.2 years, and the mean follow-up was estimated at 71.9 ± 35.5 months. Five of the 96 patients presented with pituitary apoplexy (5.2%), 36 had cavernous sinus invasion (37.5%), and 19 cases had a Ki-67 >3% (19.8%). In terms of treatment-related outcomes, 31 of the 96 patients required a multimodal treatment approach (32.3%), with 11 patients needing a re-operation (11.5%), 19 had radiotherapy (19.8%), and 12 individuals required medical therapy post-operatively (12.5%). Nineteen patients (19.8%) had active disease at the last follow-up (Table 1).

3.2 | Chemokines and chemokine receptors expression in PitNETs

The mean relative mRNA expression fold change normalised to *TBP* and mRNA expression range of each chemokine and chemokine receptor we studied in the whole cohort and per PitNET subtype are shown in Table 2. We observed a wide difference between minimum and maximum mRNA expression values among the studied chemokine and chemokine receptors, reflecting a significant heterogeneity within the whole PitNET cohort and per each subgroup. In general, the absolute mean mRNA expression was higher for chemokines, particularly *CXCL8*, *CCL2*, and *CCL3*, in comparison to the chemokine receptors. *CX3CL1* and *CXCR2* expression significantly differed across PitNET subtypes ($p = .008$ and $p = .025$, respectively), being remarkably high in prolactinomas and low in acromegaly (Table 2). Overall, there was a remarkable positive correlation between practically all chemokines and chemokine receptors (Table 3).

Given the low number of patients with Cushing's disease ($n = 6$), prolactinomas ($n = 2$), and thyrotropinomas ($n = 1$), we performed a comparative gene expression subanalysis only between NF-PitNET and acromegaly subgroups. Compared to NF-PitNETs, somatotroph tumours had significantly higher expression of *CCL2*, *CCL4*, and *CCR2*, and lower expression of *CX3CL1* and *CCR4* (Figure 1).

3.3 | Chemokines and chemokine receptors expression in PitNETs and clinical characteristics/outcomes

Chemokine and chemokine receptor gene mRNA expression levels, shown in Table 4, were higher in females (*CCL2* [$p = .046$]), in patients who presented with pituitary apoplexy (*CCR2* [$p = .042$], *CXCR1* [$p = .009$] and *CCL4* [trend, $p = .076$]) and in patients who had hypopituitarism at diagnosis (*CCL2* [$p = .029$] and *CCR4* [trend, $p = .050$]). mRNA expression levels were also higher in PitNETs with cavernous

TABLE 2 Chemokines and chemokine receptors mRNA gene expression in the cohort of PitNETs and within each PitNET subtype, and correlation analysis with EMT pathway markers and angiogenesis parameters.

	CCL2	*CCL3*	*CCL4*	*CXCL8*	*CX3CL1*	*CCR2*	*CCR4*	*CCR5*	*CXCR1*	*CXCR2*
Mean value in the PitNET cohort [mean ± SEM]	6.87 ± 2.87	4.94 ± 0.58	0.30 ± 0.03	23.67 ± 8.70	1.32 ± 0.14	0.23 ± 0.06	1.07 ± 0.32	3.33 ± 1.11	1.34 ± 0.45	3.73 ± 0.85
Minimum and maximum mRNA gene expression values in the PitNET cohort [min–max]	0–221.60	0.13–28.01	0.04–2.05	0–694.60	0.05–5.30	0.001–4.68	0.001–22.47	0.13–84.63	0–29.37	0.035–44.86
Mean value per subgroup [mean ± SEM]										
NF-PitNETs ($n = 68$)	4.63 ± 3.29	4.50 ± 0.67	0.28 ± 0.04	15.99 ± 6.19	1.60 ± 0.17	0.14 ± 0.03	1.09 ± 0.41	3.50 ± 1.43	1.47 ± 0.61	3.60 ± 1.02
Acromegaly ($n = 19$)	15.49 ± 8.23	5.54 ± 1.48	0.30 ± 0.04	48.10 ± 36.44	0.60 ± 0.53	0.33 ± 0.10	0.54 ± 0.19	1.18 ± 0.28	0.52 ± 0.25	2.30 ± 0.60
Cushing's disease ($n = 6$)	8.25 ± 5.03	7.75 ± 2.58	0.57 ± 0.32	43.47 ± 37.34	0.89 ± 0.36	0.96 ± 0.75	0.93 ± 0.47	2.32 ± 1.14	1.66 ± 1.15	3.71 ± 1.53
Prolactinomas ($n = 2$)	0.10 ± 0.10	4.99 ± 0.67	0.22 ± 0.11	3.45 ± 0.35	2.72 ± 2.57	0.19 ± 0.02	6.20 ± 6.17	21.27 ± 20.99	4.18 ± 4.12	22.40 ± 20.83
Thyrotroph PitNETs ($n = 1$)	0.80 $p = .671$	6.68 $p = .702$	0.18 $p = .357$	3.50 $p = .634$	1.76 $\mathbf{p = .008}$	0.11 $\mathbf{p = .008}$	0.14 $p = .196$	0.45 $p = .167$	0.03 $p = .801$	0.68 $\mathbf{p = .025}$
Minimum and maximum mRNA gene expression values per subgroup [min–max]										
NF-PitNETs ($n = 68$)	0–221.60	0.13–28.01	0.04–1.49	0–321.80	0.06–4.57	0.001–1.30	0.001–22.47	0.01–84.63	0–29.37	0.04–44.86
Acromegaly ($n = 19$)	0–149.70	0.36–22.17	0.08–0.62	0.10–694.60	0.46–1.76	0.02–1.75	0.04–3.44	0.02–4.21	0.003–3.99	0.20–9.49
Cushing's disease ($n = 6$)	0.10–25.80	1.19–18.39	0.06–2.05	1.10–229.60	0.06–2.53	0.01–4.68	0.02–2.69	0.10–6.08	0.10–7.29	0.10–10.24
Prolactinomas ($n = 2$)	0–0.20	4.32–5.66	0.10–0.33	3.10–3.80	0.15–5.30	0.17–0.21	0.03–12.37	0.28–42.26	0.06–8.29	1.57–43.24
Thyrotroph PitNETs ($n = 1$)	0.80	6.68	0.18	3.50	1.76	0.11	0.14	0.45	0.03	0.68
Correlation analysis [rho; *p*-value]										
CDH1 mRNA expression	−0.276; **0.006**	−0.147; 0.156	−0.287; **0.005**	−0.121; 0.240	0.135; 0.259	−0.237; **0.021**	−0.217; **0.036**	−0.165; 0.112	−0.199; 0.054	−0.313; **0.002**
ZEB1 mRNA expression	0.038; 0.714	0.258; **0.012**	0.227; **0.028**	0.127; 0.218	0.398; **0.001**	0.145; 0.164	0.188; 0.070	0.165; 0.111	0.118; 0.258	0.154; 0.139
E-cadherin immunohistochemical score	−0.204; 0.055	0.116; 0.285	0.103; 0.343	−0.028; 0.795	0.077; 0.531	0.084; 0.441	0.128; 0.238	0.066; 0.546	0.015; 0.893	0.037; 0.736
Number of vessels/HPF	0.014; 0.898	0.075; 0.487	0.187; 0.080	0.139; 0.191	0.285; **0.017**	0.082; 0.448	0.194; 0.071	0.188; 0.079	0.208; 0.052	0.104; 0.333
Total vessel area (μm^2)	0.005; 0.963	0.085; 0.487	0.127; 0.238	0.126; 0.237	0.271; **0.024**	0.068; 0.529	0.197; 0.067	0.179; 0.096	0.221; **0.039**	0.092; 0.395
Total vessel perimeter (μm)	0.030; 0.780	0.046; 0.671	0.182; 0.090	0.151; 0.156	0.308; **0.010**	0.081; 0.455	0.157; 0.144	0.166; 0.122	0.135; 0.209	0.049; 0.652
Vessel roundness (0–1)	0.015; 0.891	0.023; 0.832	0.166; 0.121	0.144; 0.175	0.236; 0.051	0.044; 0.682	0.054; 0.616	0.081; 0.455	0.004; 0.968	−0.018; 0.870

Note: The E-cadherin immunohistochemical score was analysed on the basis of plasma membrane expression of E-cadherin, and reported as E-cadherin score according to the following criteria: score 0 assigned to cases where all tumour cells were negative for membranous E-cadherin; score 1 when <50% of tumour cells had a complete membrane E-cadherin and most remaining cells had absent E-cadherin expression; score 2 when <50% of tumour cells had a complete membrane E-cadherin staining, and most remaining cells had partial E-cadherin expression; score 3 when ≥50% of tumour cells had a complete membrane staining; score 4 when all tumour cells had a complete membrane staining. Data are shown as mean ± SEM. One-way ANOVA with Bonferroni Test was used for the comparisons between PitNET subgroups. In the correlation subanalysis, *p* values were determined by the Spearman correlation coefficient *rho*. *p*-values <0.05 are shown in bold.

Abbreviations: EMT, epithelial-to-mesenchymal transition; HPF, high power field; NF-PitNETs, nonfunctioning-pituitary neuroendocrine tumours; PitNETs, pituitary neuroendocrine tumours; SEM, standard error of the mean.

TABLE 3 Correlation analysis between chemokines and chemokine receptors gene expression data.

Whole cohort of PitNETs ($n = 96$)		*CCL2*	*CCL3*	*CCL4*	*CXCL8*	*CX3CL1*	*CCR2*	*CCR4*	*CCR5*	*CXCR1*	*CXCR2*
CCL2	Spearman correlation *rho*		0.284	0.336	0.433	0.071	0.260	0.252	0.226	0.264	0.137
	p-value		**.005**	**.001**	**<.001**	.553	**.011**	**.014**	**.029**	**.010**	**.186**
CCL3	Spearman correlation *rho*	0.284		0.740	0.555	0.380	0.436	0.590	0.628	0.569	0.523
	p-value	**.005**		**<.001**	**<.001**	**.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
CCL4	Spearman correlation *rho*	0.336	0.740		0.427	0.386	0.605	0.664	0.655	0.568	0.535
	p-value	**.001**	**<.001**		**<.001**	**.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
CXCL8	Spearman correlation *rho*	0.433	0.555	0.427		0.312	0.335	0.426	0.356	0.367	0.365
	p-value	**<.001**	**<.001**	**<.001**		**.008**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
CX3CL1	Spearman correlation *rho*	0.071	0.380	0.386	0.312		0.166	0.267	0.243	0.351	0.165
	p-value	.553	**.001**	**.001**	**.008**		.164	**.023**	**.039**	**.003**	.165
CCR2	Spearman correlation *rho*	0.260	0.436	0.605	0.335	0.166		0.529	0.515	0.425	0.405
	p-value	**.011**	**<.001**	**<.001**	**<.001**	.164		**<.001**	**<.001**	**<.001**	**<.001**
CCR4	Spearman correlation *rho*	0.252	0.590	0.664	0.426	0.267	0.529		0.848	0.758	0.747
	p-value	**.014**	**<.001**	**<.001**	**<.001**	**.023**	**<.001**		**<.001**	**<.001**	**<.001**
CCR5	Spearman correlation *rho*	0.226	0.628	0.655	0.356	0.243	0.515	0.848		0.722	0.714
	p-value	**.029**	**<.001**	**<.001**	**<.001**	**.039**	**<.001**	**<.001**		**<.001**	**<.001**
CXCR1	Spearman correlation *rho*	0.264	0.569	0.568	0.367	0.351	0.425	0.758	0.722		0.717
	p-value	**.010**	**<.001**	**<.001**	**<.001**	**.003**	**<.001**	**<.001**	**<.001**		**<.001**
CXCR2	Spearman correlation *rho*	0.137	0.523	0.535	0.365	0.165	0.405	0.747	0.714	0.717	
	p-value	**.186**	**<.001**	**<.001**	**<.001**	.165	**<.001**	**<.001**	**<.001**	**<.001**	

Note: In the correlation subanalysis, *p*-values were determined by the Spearman correlation coefficient *rho*. *p*-values < 0.05 are shown in bold.
Abbreviation: PitNETs, pituitary neuroendocrine tumours.

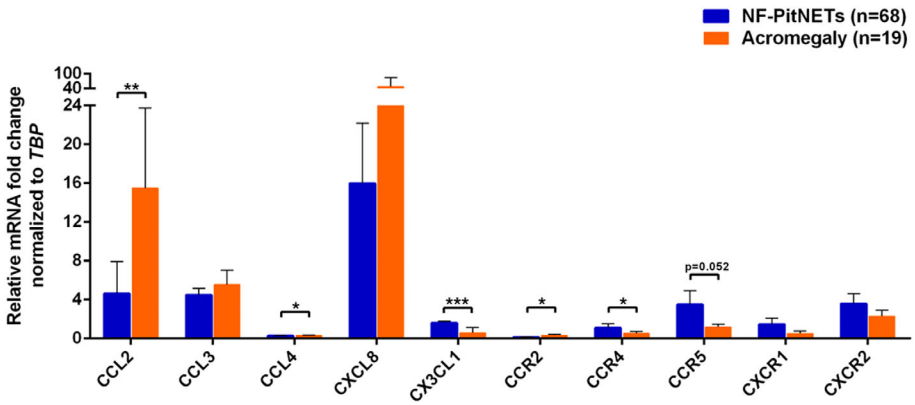

FIGURE 1 Chemokine and chemokine receptor genes mRNA expression in NF-PitNETs in comparison to acromegaly cases. Gene expression data are shown as relative mRNA fold change expression for each chemokine or chemokine receptor gene normalised to the house-keeping gene *TBP* and expressed as mean (graph columns) ± SEM (error bars). NF-PitNETs, nonfunctioning pituitary neuroendocrine tumours. SEM, standard error of the mean; *TBP*, TATA-binding protein. Mann Whitney U test was used for the comparisons per gene between NF-PitNET and acromegaly subgroups. *, <0.05; **, <0.01; ***, <0.001.

sinus invasion (*CCL3* [trend, $p = .087$]) and Ki-67 >3% (*CCL4* [$p = .037$] and *CXCR2* [$p = .041$]) (Figure 2, Table 4).

Chemokine and chemokine receptor expression were higher in patients who required re-operation (*CCL3* [$p = .007$], *CXCL8* [$p = .017$], and *CXCR2* [$p = .044$]), multimodal therapy (*CCL2* [$p = .017$]), and had active disease at last follow-up (*CCL2* [trend, $p = .085$]). In contrast, patients who received multimodal and multiple treatment regimens had lower expression levels of *CX3CL1*, and irradiated patients had lower expression of *CCR2* (Figure 3, Table 4).

In our PitNET cohort, there were positive correlation trends between the number of pituitary surgeries and mRNA expression levels of *CCL3* (rho = 0.262; $p = .012$), *CXCL8* (rho = 0.176; $p = .091$), *CX3CL1* (rho = 0.214; $p = .075$), *CXCR1* (rho = 0.173; $p = .099$), and *CXCR2* (rho = 0.194; $p = .064$). The total number of

TABLE 4 Correlation between chemokine and chemokine receptor gene expression and clinico-pathological features, clinical outcomes and treatment-related parameters in the whole cohort of PitNETs ($n = 96$).

Whole PitNET cohort ($n = 96$) mRNA expression (Mean ± SEM)		*CCL2*	*CCL3*	*CCL4*	*CXCL8*	*CX3CL1*	*CCR2*	*CCR4*	*CCR5*	*CXCR1*	*CXCR2*
Gender	Males	10.82 ± 5.95	4.01 ± 0.75	0.28 ± 0.05	23.08 ± 9.53	1.52 ± 0.22	0.15 ± 0.03	1.37 ± 0.61	4.28 ± 2.10	1.90 ± 0.89	4.22 ± 1.47
	Females	3.39 ± 1.18 **$p = .046$**	5.75 ± 0.85 $p = .217$	0.32 ± 0.05 $p = .666$	24.19 ± 14.14 $p = .513$	1.18 ± 0.18 $p = .235$	0.31 ± 0.10 $p = .220$	0.81 ± 0.28 $p = .190$	2.49 ± 0.97 $p = .225$	0.85 ± 0.30 $p = .275$	3.29 ± 0.96 $p = .596$
Pituitary apoplexy	Yes	2.66 ± 1.34	4.82 ± 1.16	0.43 ± 0.13	68.86 ± 63.25	2.03 ± 1.63	0.35 ± 0.18	5.25 ± 3.16	14.93 ± 9.01	7.76 ± 5.61	18.37 ± 10.49
	No	7.25 ± 3.13 $p = .331$	4.52 ± 0.53 $p = .288$	0.28 ± 0.04 $p = .076$	20.12 ± 8.76 $p = .138$	1.25 ± 0.13 $p = 0.906$	0.21 ± 0.06 **$p = .042$**	0.56 ± 0.13 $p = .347$	1.74 ± 0.47 $p = .143$	0.69 ± 0.19 **$p = .009$**	2.46 ± 0.42 $p = .158$
Headache at diagnosis	Yes	6.30 ± 2.71	4.53 ± 0.92	0.32 ± 0.08	25.99 ± 14.11	1.17 ± 0.27	0.33 ± 0.17	1.26 ± 0.66	3.40 ± 1.88	1.88 ± 1.13	4.84 ± 2.22
	No	7.41 ± 4.09 $p = .511$	4.59 ± 0.61 $p = .912$	0.28 ± 0.03 $p = .689$	21.69 ± 11.37 $p = .223$	1.30 ± 0.16 $p = .555$	0.17 ± 0.03 $p = .912$	0.65 ± 0.17 $p = .829$	2.10 ± 0.63 $p = .795$	0.74 ± 0.24 $p = .158$	2.71 ± 0.55 $p = .795$
Hypopituitarism at diagnosis	Yes	7.35 ± 3.62	4.66 ± 0.67	0.28 ± 0.04	28.96 ± 12.77	1.34 ± 0.16	0.22 ± 0.08	0.85 ± 0.27	2.51 ± 0.77	1.32 ± 0.52	3.11 ± 0.83
	No	6.73 ± 3.37 **$p = .029$**	4.66 ± 0.92 $p = .518$	0.30 ± 0.05 $p = .213$	9.98 ± 4.26 $p = .976$	1.07 ± 0.21 $p = .311$	0.28 ± 0.08 $p = .112$	0.45 ± 0.14 $p = .050$	0.99 ± 0.27 $p = .262$	0.33 ± 0.12 $p = .968$	2.47 ± 0.73 $p = .523$
Suprasellar extension	Yes	7.13 ± 3.12	5.07 ± 0.62	0.30 ± 0.04	25.45 ± 9.47	1.41 ± 0.15	0.23 ± 0.06	1.10 ± 0.35	3.44 ± 1.19	1.38 ± 0.48	3.82 ± 0.92
	No	4.66 ± 3.60 $p = .857$	3.24 ± 1.37 $p = .542$	0.26 ± 0.11 $p = .696$	4.64 ± 2.59 $p = .467$	0.61 ± 0.17 $p = .107$	0.36 ± 0.12 $p = .061$	0.75 ± 0.35 $p = .325$	2.13 ± 0.97 $p = .398$	1.07 ± 0.64 $p = .434$	2.81 ± 1.48 $p = .899$
Cavernous sinus invasion	Yes	9.90 ± 6.35	6.04 ± 1.08	0.28 ± 0.05	26.12 ± 19.21	1.48 ± 0.25	0.19 ± 0.05	1.13 ± 0.43	3.60 ± 1.53	1.21 ± 0.44	4.10 ± 1.35
	No	5.67 ± 2.92 $p = .701$	3.95 ± 0.57 $p = .087$	0.30 ± 0.05 $p = .856$	22.29 ± 8.76 $p = .496$	1.24 ± 0.17 $p = .802$	0.28 ± 0.09 $p = .703$	0.71 ± 0.28 $p = .646$	1.84 ± 0.67 $p = .959$	1.10 ± 0.59 $p = .246$	2.71 ± 0.93 $p = .121$
Ki-67 >3%	Yes	6.14 ± 3.44	7.11 ± 1.86	0.48 ± 0.12	59.08 ± 37.68	1.33 ± 0.31	0.41 ± 0.24	2.22 ± 1.30	7.74 ± 4.80	2.43 ± 1.41	6.78 ± 3.04
	No	7.24 ± 3.57 $p = .488$	4.43 ± 0.55 $p = .269$	0.26 ± 0.03 **$p = .037$**	15.04 ± 5.60 $p = .337$	1.32 ± 0.16 $p = .717$	0.17 ± 0.03 $p = .158$	0.79 ± 0.24 $p = .287$	2.25 ± 0.67 $p = .219$	1.10 ± 0.45 $p = .287$	3.02 ± 0.75 **$p = .041$**
Any treatment before first surgery	Yes	13.47 ± 9.92	4.89 ± 1.53	0.20 ± 0.04	12.09 ± 7.84	0.85 ± 0.38	0.28 ± 0.12	1.30 ± 0.82	3.65 ± 2.78	1.11 ± 0.59	4.73 ± 2.82
	No	5.78 ± 2.95 $p = .281$	4.70 ± 0.57 $p = .878$	0.31 ± 0.04 $p = .234$	24.82 ± 10.38 $p = .486$	1.44 ± 0.15 **$p = .009$**	0.22 ± 0.06 $p = .779$	0.76 ± 0.22 $p = .646$	2.25 ± 0.64 $p = .903$	1.09 ± 0.43 $p = .828$	3.05 ± 0.72 $p = .715$
Tumour remnant within 1-year post-operatively	Yes	9.94 ± 5.62	5.97 ± 0.98	0.29 ± 0.04	25.06 ± 16.84	1.45 ± 0.24	0.18 ± 0.05	1.18 ± 0.40	3.66 ± 1.36	1.20 ± 0.41	4.36 ± 1.29
	No	5.19 ± 3.16 $p = .277$	3.84 ± 0.61 $p = .059$	0.30 ± 0.06 $p = .627$	23.06 ± 9.66 $p = .234$	1.13 ± 0.17 $p = .505$	0.28 ± 0.10 $p = .117$	0.63 ± 0.29 $p = .973$	1.64 ± 0.70 $p = .568$	1.09 ± 0.64 $p = .568$	2.59 ± 0.97 $p = .652$
Need for re-operation	Yes	14.36 ± 13.54	9.58 ± 2.51	0.44 ± 1.13	89.73 ± 62.11	1.95 ± 0.51	0.16 ± 0.03	3.37 ± 2.20	11.87 ± 8.20	3.32 ± 2.36	10.11 ± 5.11
	No	6.02 ± 2.82 $p = .585$	4.27 ± 0.55 **$p = .007$**	0.28 ± 0.04 $p = .103$	15.43 ± 5.56 **$p = .017$**	1.19 ± 0.14 $p = .249$	0.24 ± 0.07 $p = .222$	0.77 ± 0.22 $p = .410$	2.22 ± 0.61 $p = .333$	1.10 ± 0.41 $p = .204$	2.90 ± 0.68 **$p = .044$**
Post-operative medical therapy	Yes	10.33 ± 5.43	6.41 ± 2.02	0.29 ± 0.05	64.56 ± 57.33	0.90 ± 0.42	0.34 ± 0.14	1.59 ± 1.02	4.62 ± 3.44	1.08 ± 0.69	5.74 ± 3.46
	No	6.51 ± 3.27 $p = .072$	4.68 ± 0.61 $p = .451$	0.30 ± 0.04 $p = .281$	18.21 ± 5.91 $p = .571$	1.39 ± 0.15 **$p = .014$**	0.22 ± 0.06 $p = .120$	1.01 ± 0.35 $p = .138$	3.19 ± 1.20 $p = .118$	1.41 ± 0.52 $p = .963$	3.46 ± 0.86 $p = .100$
Radiotherapy	Yes	17.04 ± 11.87	5.34 ± 1.52	0.28 ± 0.07	44.18 ± 36.32	1.24 ± 0.35	0.11 ± 0.04	1.51 ± 0.75	4.74 ± 2.50	1.48 ± 0.72	4.62 ± 2.29
	No	4.45 ± 2.09	4.79 ± 0.63	0.30 ± 0.04	19.05 ± 6.41	1.33 ± 0.15	0.27 ± 0.07	0.97 ± 0.37	3.02 ± 1.27	1.33 ± 0.55	3.54 ± 0.93

TABLE 4 (Continued)

Whole PitNET cohort ($n = 96$) mRNA expression (Mean ± SEM)		*CCL2*	*CCL3*	*CCL4*	*CXCL8*	*CX3CL1*	*CCR2*	*CCR4*	*CCR5*	*CXCR1*	*CXCR2*
		$p = .772$	$p = .973$	$p = .613$	$p = .550$	$p = .354$	$\mathbf{p = .045}$	$p = .534$	$p = .661$	$p = .626$	$p = .668$
Multimodal treatment	Yes	16.82 ± 8.55	5.32 ± 1.17	0.26 ± 0.04	33.27 ± 22.47	0.98 ± 0.23	0.23 ± 0.07	1.17 ± 0.47	3.40 ± 1.55	1.17 ± 0.46	3.76 ± 1.10
	No	2.16 ± 0.78	4.69 ± 0.66	0.32 ± 0.05	19.63 ± 7.42	1.54 ± 0.17	0.24 ± 0.08	1.04 ± 0.43	3.36 ± 1.52	1.46 ± 0.65	3.75 ± 1.44
		$\mathbf{p = .017}$	$p = .885$	$p = .683$	$p = .735$	$\mathbf{p = .008}$	$p = .763$	$p = .713$	$p = .859$	$p = .814$	$p = .683$
Multiple treatment	Yes	8.96 ± 5.79	6.14 ± 2.18	0.23 ± 0.06	68.55 ± 62.65	0.99 ± 0.47	0.22 ± 0.15	1.65 ± 1.11	4.73 ± 3.77	1.16 ± 0.75	5.30 ± 3.81
	No	6.74 ± 3.23	4.73 ± 0.60	0.31 ± 0.04	18.24 ± 5.84	1.36 ± 0.15	0.23 ± 0.06	1.01 ± 0.34	3.19 ± 1.19	1.39 ± 0.51	3.55 ± 0.85
		$p = .373$	$p = .643$	$p = .568$	$p = .948$	$\mathbf{p = .040}$	$p = .184$	$p = .643$	$p = .899$	$p = .976$	$p = .741$
Active disease at last follow-up	Yes	18.77 ± 11.80	3.10 ± 0.69	0.22 ± 0.05	9.16 ± 3.75	1.45 ± 0.39	0.18 ± 0.06	1.50 ± 0.75	4.18 ± 2.32	1.33 ± 0.56	5.62 ± 2.35
	No	4.07 ± 2.11	5.44 ± 0.72	0.32 ± 0.04	28.29 ± 11.20	1.26 ± 0.14	0.25 ± 0.07	0.99 ± 0.37	3.18 ± 1.32	1.39 ± 0.57	3.30 ± 0.93
		$p = .085$	$p = .154$	$p = .385$	$p = .721$	$p = .733$	$p = .675$	$p = .452$	$p = .458$	$p = .733$	$p = .249$
Hypopituitarism at last follow-up	Yes	6.52 ± 3.73	4.56 ± 0.65	0.26 ± 0.03	25.63 ± 12.77	1.40 ± 0.19	0.16 ± 0.03	1.00 ± 0.34	3.03 ± 1.03	1.30 ± 0.54	3.61 ± 1.08
	No	8.43 ± 5.05	5.89 ± 1.25	0.39 ± 0.08	22.52 ± 9.02	1.05 ± 0.19	0.40 ± 0.17	1.33 ± 0.77	4.25 ± 2.89	1.57 ± 0.91	4.19 ± 1.59
		$p = .556$	$p = .405$	$p = 0.115$	$p = .107$	$p = .361$	$p = .151$	$p = .856$	$p = .171$	$p = .503$	$p = .531$

Note: Gene expression data are shown as relative mRNA fold change expression for each chemokine or chemokine receptor gene normalised to the house-keeping gene *TBP* (TATA-binding protein) and expressed as mean ± SEM. Mann Whitney U test was used for all comparisons. *p*-values < 0.05 are shown in bold.

Abbreviations: PitNETs, pituitary neuroendocrine tumours; SEM, standard error of the mean.

treatments received also tended to correlate (rho = 0.180; $p = .082$) with *CCL2* mRNA expression levels (Supplemental Table 2).

3.4 | Chemokine and chemokine receptors expression in PitNETs and EMT pathway/angiogenesis

CDH1 (encoding E-cadherin, key epithelial marker) mRNA expression levels correlated negatively with *CCL2* (rho = −0.276; $p = .006$), *CCL4* (rho = −0.287; $p = .005$), *CCR2* (rho = −0.237; $p = .021$), *CCR4* (rho = −0.217; $p = .036$) and *CXCR2* (rho = −0.313; $p = .002$). In contrast, *ZEB1* (mesenchymal marker) mRNA expression positively correlated with *CCL3* (rho = 0.313; $p = .012$), *CCL4* (rho = 0.227; $p = .028$) and *CX3CL1* (rho = 0.398; $p = .001$) (Figure 4, Table 2).

PitNETs expressing higher levels of *CCL4*, *CX3CL1*, *CCR4*, *CCR5*, and *CXCR1* seemed to have more and bigger vessels, as the mRNA expression levels of these chemokine and chemokine receptors correlated with the number of vessels/HPF, total vessel area and/or total vessel perimeter (Table 2).

3.5 | Chemokines and chemokine receptors expression and pre-operative medical treatment

Pre-operative medical treatment was used in 15 of 96 (15.6%) patients (Table 1), and there were no significant differences in chemokine or chemokine receptor gene expression in the analysis encompassing the whole cohort (Table 4), except for *CX3CL1* (lower expression in pre-treated patients). However, as medical therapies vary across different PitNET subtypes, we further analysed the gene expression data among somatotropinomas and NF-PitNETs. Somatotroph tumours treated with somatostatin analogues (SSAs) before pituitary surgery (7 out of 19 cases) had higher expression levels of *CCL2* (28.50 ± 20.55 vs. 7.90 ± 5.18; $p = .038$), *CXCR1* (1.12 ± 0.59 vs. 0.14 ± 0.06; $p = .021$), *CXCR2* (3.32 ± 1.12 vs. 1.65 ± 0.66; $p = .033$) and *CCR4* (0.97 ± 0.45 vs. 0.27 ± 0.07; $p = .063$) than the untreated counterparts (Figure 5A). Conversely, NF-PitNETs pre-surgically treated with dopamine agonists (5 out of 68 cases) were associated with lower expression of *CCL3* (1.21 ± 0.49 vs. 4.44 ± 0.62; $p = .044$), *CCL4* (0.07 ± 0.02 vs. 0.28 ± 0.04; $p = .005$), *CX3CL1* (0.36 ± 0.014 vs. 1.72 ± 0.18; $p = .017$), *CCR5* (0.09 ± 0.04 vs. 2.49 ± 0.81; $p = .028$), *CXCR1* (0.04 ± 0.03 vs. 1.21 ± 0.54; $p = .019$) and *CXCR2* (0.55 ± 0.35 vs. 3.23 ± 0.90; $p = .068$) in comparison to untreated NF-PitNETs (Figure 5B).

4 | DISCUSSION

The role of various chemokines in the biological and clinical behaviour of PitNETs was investigated in previous studies and is further expanded in our study. The concomitant involvement of chemokine receptors in tumorigenic mechanisms, such as the EMT pathway, angiogenesis, tumour aggressiveness, and clinical outcomes in

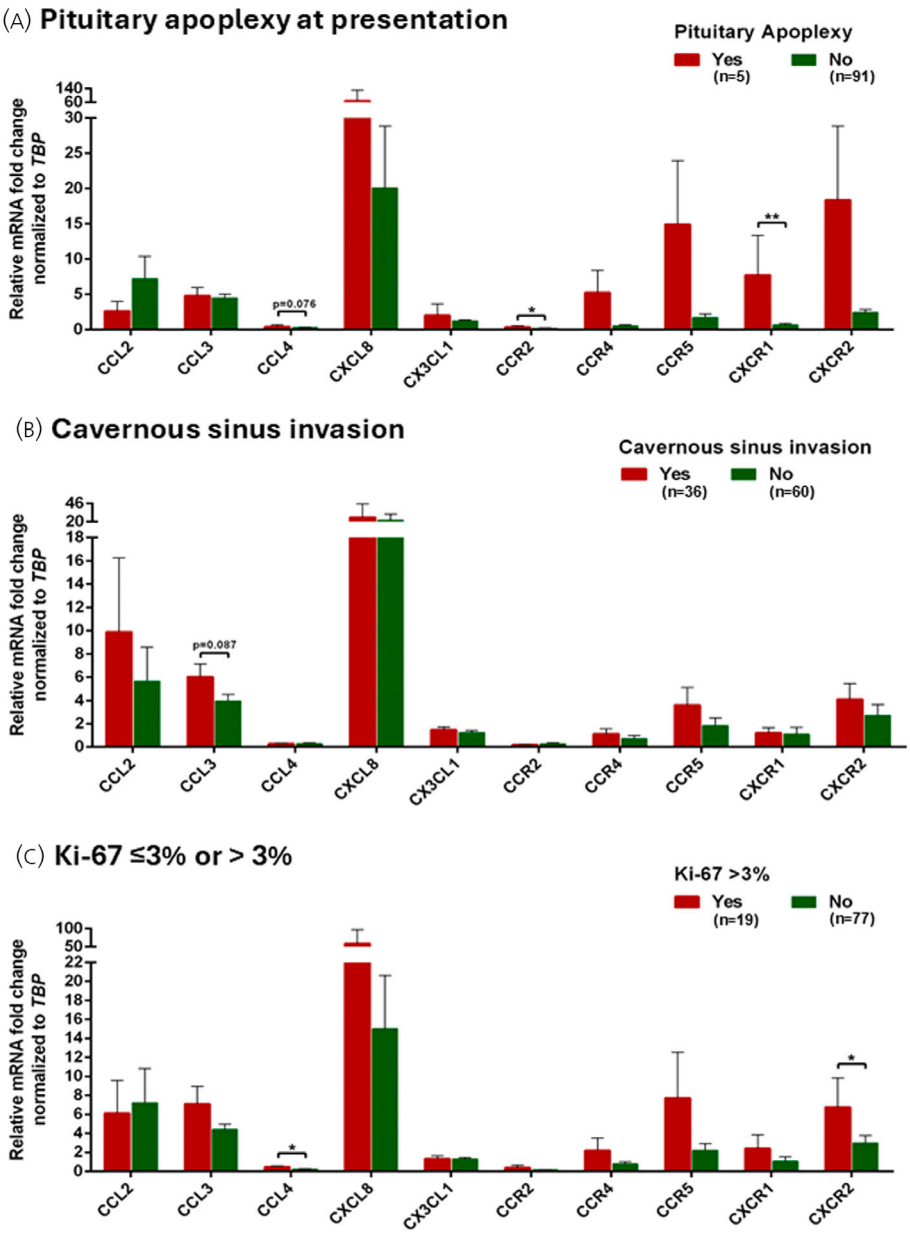

FIGURE 2 Chemokine and chemokine receptor genes mRNA expression in the whole cohort of PitNETs across different clinico-pathological variables. (A) Pituitary apoplexy at presentation: Yes (red) versus No (green); (B) Cavernous sinus invasion: Yes (red) versus No (green); C) Ki-67: ≤3% (green) or >3% (red). Gene expression data are shown as relative mRNA fold change expression for each chemokine or chemokine receptor gene normalised to the house-keeping gene *TBP* and expressed as mean (graph columns) ± SEM (error bars). SEM, standard error of the mean; *TBP*, TATA-binding protein. Mann Whitney U test was used for the comparisons. *, <0.05; **, <0.01; ***, <0.001.

PitNETs, was poorly investigated until now. In this study, we investigated the mRNA expression and their potential role of five chemokines and five chemokine receptors in the phenotype and clinical outcomes of PitNET patients and their potential regulatory effects in the EMT pathway and angiogenesis. Our findings suggest that chemokines and chemokine receptors are heterogeneously expressed in PitNETs and may be involved in the modulation of different tumorigenic mechanisms, including EMT and angiogenesis, as well as may be associated with more aggressive and difficult-to-treat PitNETs. We also observed gene expression differences between pre-surgically treated PitNETs and drug-naïve PitNETs, which implicate that various chemokine and chemokine receptors expression may be altered by PitNET-directed drugs.

Cytokines and chemokines are major key factors in tumour-related inflammation and important mediators in the growth, migration, invasion and survival of tumour cells.[4,26,27] We found a strong positive correlation between the expression of all five chemokines we studied. Our data, together with the positive correlation between the expressions of EGF, CXCL1, and CCL5 in PitNETs reported by Chiloiro et al.[11] and the association between the expression of chemokine-related and inflammatory-related genes described earlier,[15] indicates the presence of an integrated inflammatory cytokine network acting as a coordinated system to modulate growth, support tumour progression and potentially regulate tumorigenic mechanisms in PitNETs. These positive correlations between different cytokines and chemokines in PitNETs support the notion of an existing microenvironment-related inflammation, which may in turn trigger cytokine positive feedback loops and synergistic effects within the pro-inflammatory PitNET microenvironment, as high levels of one cytokine can stimulate the production of other cytokines and activate

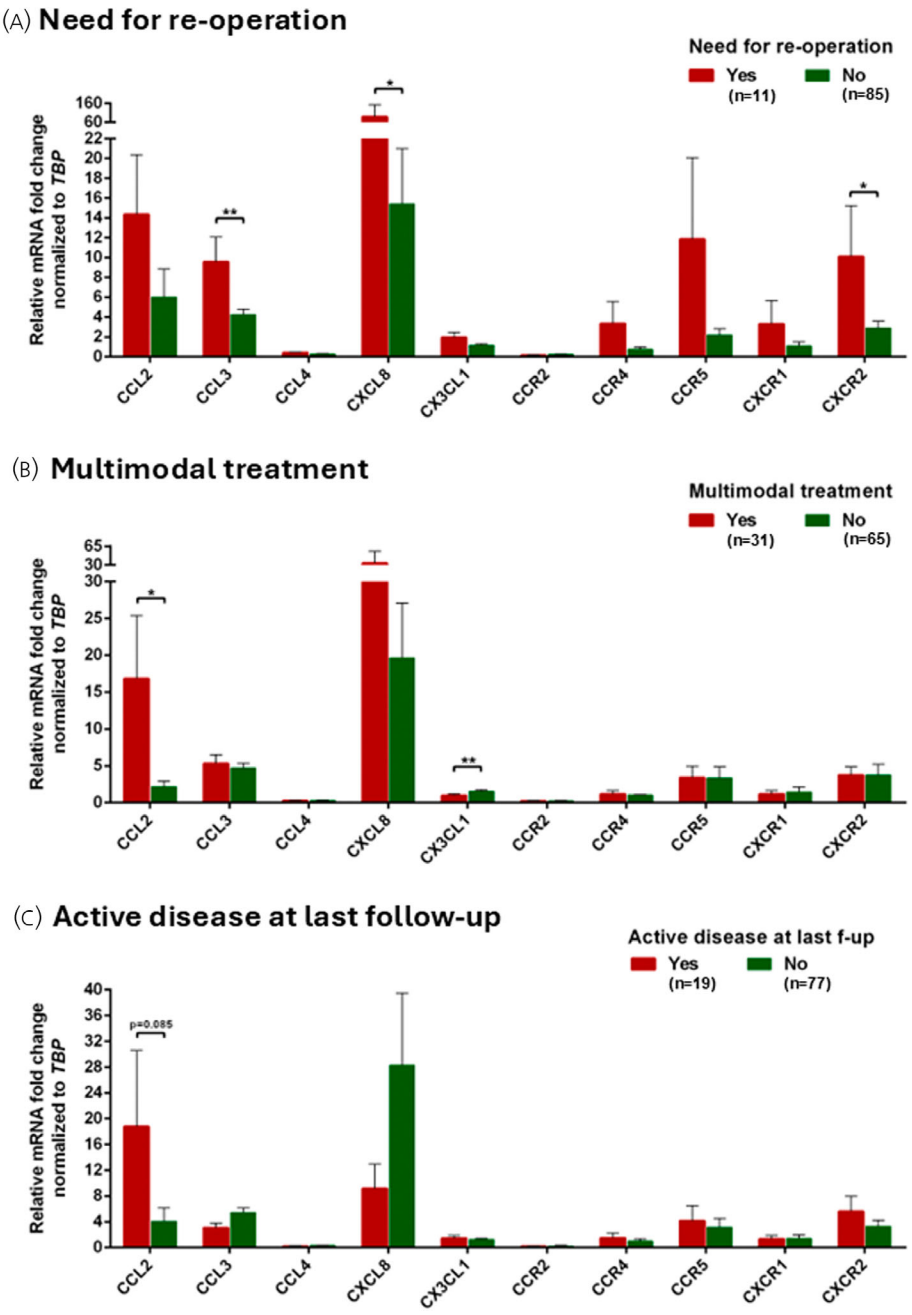

FIGURE 3 Chemokine and chemokine receptor genes mRNA expression in the whole cohort of PitNETs across different outcome and treatment-related variables. (A) Need for re-operation: Yes (red) versus No (green); (B) Multimodal treatment: Yes (red) versus No (green); (C) Active disease at last follow-up: Yes (red) versus No (green). Gene expression data are shown as relative mRNA fold change expression for each chemokine or chemokine receptor gene normalised to the house-keeping gene *TBP* and expressed as mean (graph columns) ± SEM (error bars). SEM, standard error of the mean; *TBP*, TATA-binding protein. Mann Whitney U test was used for the comparisons. *, <0.05; **, <0.01; ***, <0.001.

cytokine-related pathways (e.g., STAT3, NF-kβ), oncogenic pathways and other inflammatory-related genes. Moreover, the positive correlation among various cytokines often transpires a state of pro-tumour inflammation in the background of an immunosuppressive microenvironment created by increased concentrations of cytokines and recruitment of immune suppressive cells.[27–30]

Tumour cells may acquire the ability to express functional chemokine receptors because of autocrine and paracrine extracellular signals within the microenvironment.[4,26,27] We found a strong positive correlation between the expression of the studied chemokines and the 5 chemokine receptors, suggesting that chemokines and eventually other microenvironment factors may upregulate the expression of chemokine receptors in PitNET cells. In general, we observed chemokine receptors expression in our PitNET samples, but at lower levels than the chemokines we studied. This relatively low expression pattern of chemokine receptors in PitNETs is consistent with their benign nature and their lack of metastatic properties, as chemokine receptors contribute to tumour inflammation and metastasis.[1,4,27]

PitNET apoplexy occurrence may be related to the aberrant vascular architecture, altered hypoxia responses and dysregulated extracellular matrix remodelling pathways, as well as to the increased expression of angiogenic and growth factors, such as vascular endothelial growth factor, tumour necrosis factor-α (TNF-α), and hypoxia-inducing factor (HIF-1).[31,32] We observed an increased expression of *CCR2* and *CXCR1* in PitNET tissues from patients presenting with pituitary apoplexy. Chemokine receptors, such as CCR2 and CXCR1,

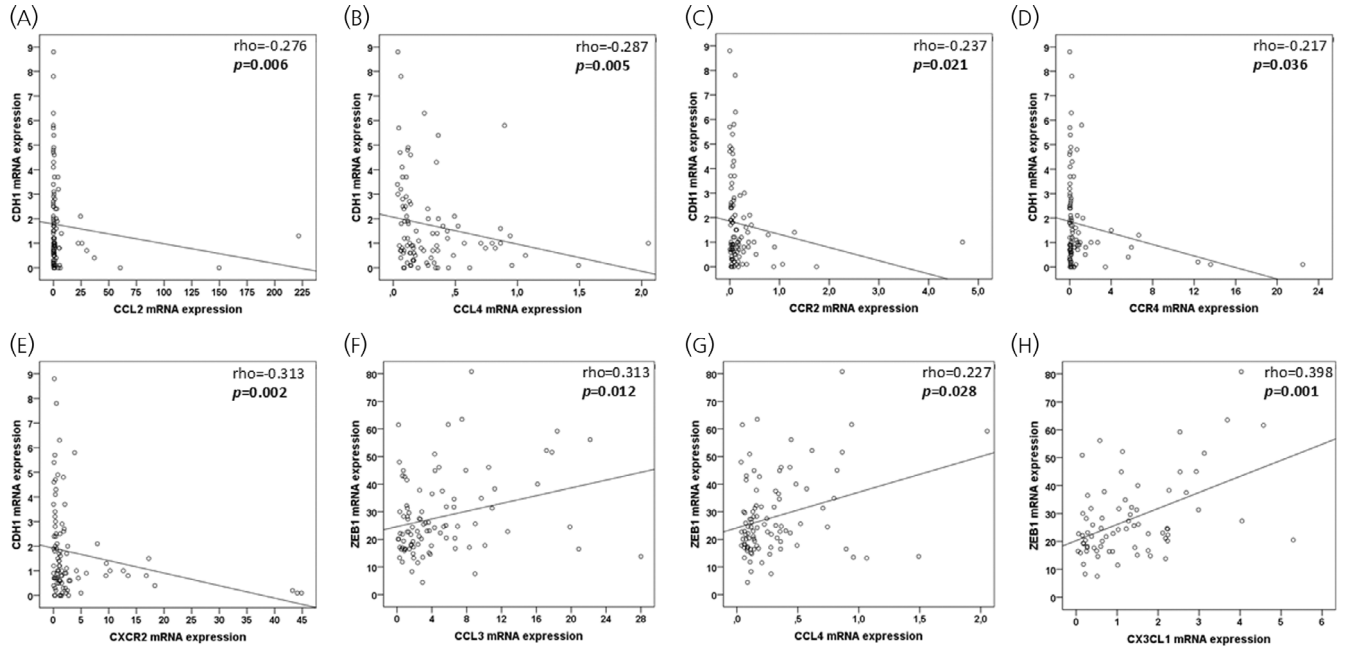

FIGURE 4 Significant correlations between *CDH1* or *ZEB1* mRNA expression levels (EMT pathway markers) and mRNA expression levels of *CCL2* (A), *CCL4* (B), *CCR2* (C), *CCR4* (D), *CXCR2* (E), *CCL3* (F), *CCL4* (G) and *CX3CL1* (H) in the whole PitNET cohort. *p*-values were determined by the Spearman correlation coefficient *rho*. EMT, epithelial-to-mesenchymal transition; PitNETs, pituitary neuroendocrine tumours.

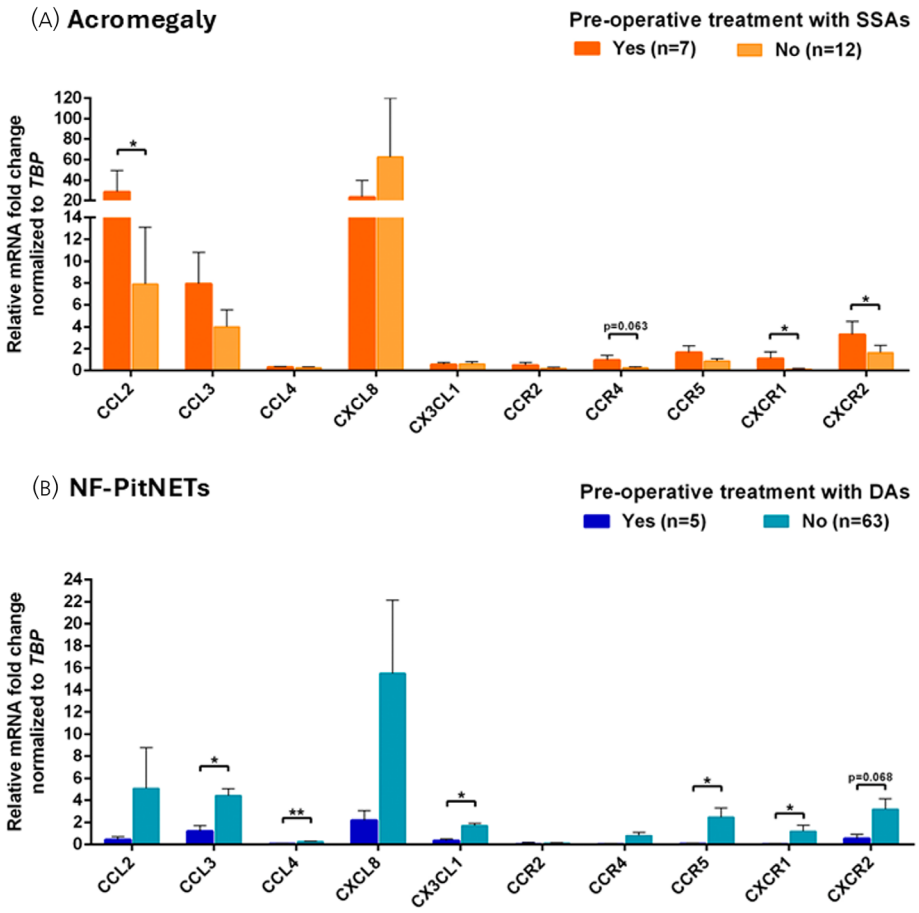

FIGURE 5 Chemokine and chemokine receptor genes mRNA expression in acromegaly (A) and NF-PitNET (B) patients who received pre-operative medical treatment versus untreated patients. Gene expression data are shown as relative mRNA fold change expression for each chemokine or chemokine receptor gene normalised to the house-keeping gene *TBP* and expressed as mean (graph columns) ± SEM (error bars). DAs, dopamine agonists; NF-PitNETs, nonfunctioning pituitary neuroendocrine tumours; SEM, standard error of the mean; SSAs, somatostatin analogues; *TBP*, TATA-binding protein. Mann Whitney U test was used for the comparisons. *, <0.05; **, <0.01; ***, <0.001.

are expressed in endothelial cells and mediate angiogenic responses in the presence of the respective binding chemokines.[33,34] CCR2 is the main binding receptor for CCL2, a chemokine with an angiogenic role in PitNETs,[16,35] and CXCR1 can be activated by CXCL8, a chemokine with angiogenic properties[36,37] and highly expressed in PitNETs,[11,13,17] thus, it is plausible that *CCR2* and *CXCR1* upregulation may contribute to the PitNET haemorrhage or infarction.

In a recent study using a 42-multiplex antibody array, Chiloiro et al. found increased expression of EGF in proliferative PitNETs, and increased expression of CXCL1, CCL17 and EGF in invasive tumours, confirming that the PitNET chemokine network may regulate tumour behaviour.[11] Other studies showed that PitNET proliferation may be increased by CXCL12-CXCR4[19,38] or CCL17,[39] while tumour invasiveness can be enhanced by increased expression of CCL5[14] or CXCL12-CXCR4.[20] Our study further expands the range of chemokines and chemokine receptors that may affect PitNET growth and invasiveness, as we observed increased expression levels of *CCL4* and *CXCR2* in PitNETs with Ki-67 >3%, and a trend for increased expression of *CCL3* in PitNETs with cavernous sinus invasion. We observed increased mRNA expression levels of chemokine and chemokine receptors in patients who required re-operation (*CCL3*, *CXCL8*, *CXCR2*), multimodal therapy (*CCL2*), and had active disease at last-follow-up (*CCL2*). We also found a positive correlation between the number of pituitary surgeries and expression levels of *CCL3*, *CXCL8*, *CX3CL1*, *CXCR1*, and *CXCR2*. Collectively, these data suggest that increased expression of chemokines and chemokine receptors, beyond CCL2,[16] may have the potential to influence clinical outcomes, response to treatment and recurrence in PitNET patients.

Chemokines are key drivers of the EMT pathway and can induce the loss of epithelial markers (notably, E-cadherin) and increase the expression of mesenchymal markers (such as *ZEB1*).[40–43] In our study, we observed that *CDH1* (encoding E-cadherin) mRNA expression levels correlated negatively with the chemokines *CCL2* and *CCL4*, while *ZEB1* (mesenchymal marker) expression correlated positively with *CCL3*, *CCL4* and *CX3CL1*. These data suggest that various chemokines, beyond CCL5 and CCL2,[16,44] may activate the EMT pathway in PitNETs, including CCL3, CCL4, and CX3CL1 (and potentially other chemokines not assessed in our study). Interestingly, we found a negative correlation between *CDH1* expression and three chemokine receptors (*CCR2*, *CCR4*, and *CXCR2*), suggesting that chemokine receptors may also be involved in the activation of the EMT in PitNETs. In fact, chemokine receptors in cancer, including CCR2,[45] CCR4[46] and CXCR2,[47] can activate EMT by triggering intracellular signalling cascades, notably through G-protein coupled receptor pathways (such as JAK/STAT, PI3K, MAPK), resulting in the downregulation of E-cadherin and upregulation of EMT-inducing factors (such as ZEB1, Snail, Slug), which in turn allow tumour cells to gain mesenchymal properties, lose adhesion, migrate and invade other tissues.[48]

Chemokines are key regulators of the stromal compartment and tumour angiogenesis, including in PitNETs,[16,35,49] as stromal cells and quiescent endothelial cells may be stimulated by angiogenic chemokines and growth factors within the microenvironment.[40,49–52] We found an association between PitNETs with more and bigger vessels and higher expression levels of *CCL4* and *CX3CL1*, chemokines with recognised angiogenic effects in cancer.[53,54] Additionally, PitNETs expressing higher levels of *CCR4*, *CCR5*, and *CXCR1* also tended to be associated with a higher number of vessels and increased total vessel area. The expression of chemokine receptors has not been previously linked with angiogenesis in PitNETs; however, such receptors promote angiogenesis in cancer by binding to angiogenic chemokines, inhibiting the apoptosis of endothelial cells and activating endothelial cells to proliferate and migrate, recruiting pro-angiogenic immune cells as well as by triggering extracellular matrix remodelling.[4,55]

Our study raised the hypothesis that different PitNET-directed drugs may modulate the expression of chemokine and chemokine receptors in PitNETs. We observed that NF-PitNETs pre-surgically treated with dopamine agonists were associated with lower expression levels of *CCL3*, *CCL4*, *CX3CL1*, *CCR5*, *CXCR1*, and *CXCR2*, which may be related to the dopamine inhibitory effects on the NF-kβ pathway and on the expression of pro-inflammatory cytokines.[56,57] In contrast, we found that somatotroph tumours treated pre-operatively with SSAs were associated with higher expression of *CCL2*, *CXCR1*, *CXCR2*, and *CCR4*. This finding was rather unexpected as SSAs inhibit the expression and secretion of pro-inflammatory cytokines in neuroendocrine neoplasms, PitNETs and tumour-associated fibroblasts.[58–62] However, the SSAs effect depends on the cell type and on the specific context; hence, it is possible that SSAs may instead lead to an increased expression of cytokines in some circumstances, as previously shown in intestinal neuroendocrine tumours[63] or peripheral blood mononuclear cells.[64] Pre-surgical medical treatment allocation was not randomised in our PitNET cohort and depended on patient and tumour characteristics; thus, mRNA expression differences may reflect confounding effects rather than drug effects. Further studies, preferably randomised and prospectively designed, encompassing a higher number of PitNET patients pre-surgically treated with medical therapy, and complemented with in vitro functional data, are needed to provide more insights and corroborate our association findings.

Our study has some limitations mainly related to its retrospective design and with the fact that our chemokine and chemokine receptor expression data relied solely on mRNA data generated by RT-qPCR from fresh-frozen PitNET tissues, thus reflecting chemokine and chemokine receptor gene expression rather than functional protein level. The strong post-transcriptional regulation of chemokines and chemokine receptors and the lack of protein validation studies thereby limit our possible conclusions about their biological role and functional activity in the PitNET microenvironment. Another important limitation derives from the fact that we extracted RNA from bulk fresh-frozen PitNET tissues, which was then used in RT-qPCR experiments. This means that the chemokine and chemokine receptor expression data do not refer exclusively to pituitary tumour cells, but also to other non-tumour cells such as immune or stromal cells present in PitNETs that may also express chemokines and chemokine receptors. Therefore, our association data require further validation in future studies with functional in vitro and/or in vivo experiments. Single cell sequencing and ligand-receptor inference may be

critical to help refine a better interception of low-expressed cytokines, chemokines, and respective receptors in PitNETs, as well as to further help to dissect the connection of the cytokine signalling pathways between pituitary tumour cells and non-tumour cells in the microenvironment of PitNETs.[49] Nevertheless, our series comprises a unique well-characterised cohort of PitNETs with wide available microenvironment-related mRNA and immunohistochemical data,[16] and is exploring a wide range of PitNET chemokine network elements that have been poorly assessed, and can now be further investigated in future studies.

In summary, our exploratory transcriptomic study suggests that various chemokines and chemokine receptors may be eventually involved in the modulation of different tumorigenic mechanisms in PitNETs, including tumour cell proliferation, EMT pathway, and angiogenesis, and may be associated with poorer clinical outcomes and more difficult-to-treat PitNETs. Our study also raised the possibility that PitNET-directed drugs may alter the chemokine and chemokine receptors' expression in PitNET cells, which in turn may determine the responsiveness to such medical therapies. Future studies assessing the expression of chemokines and chemokine receptors at both RNA and protein levels, and employing functional in vitro and/or in vivo experiments, are needed to validate some of the association findings reported in our study.

AUTHOR CONTRIBUTIONS

Ema Nobre: Methodology; validation; writing – review and editing. **Dolores López-Presa:** Investigation; methodology; validation; formal analysis; writing – review and editing. **Claudia C. Faria:** Methodology; validation; writing – review and editing; resources; supervision; funding acquisition; investigation. **Mariana de Griné Severino:** Investigation; methodology; validation; writing – review and editing. **Gonçalo Borrecho:** Investigation; methodology; validation; writing – review and editing; formal analysis; software. **Ana Hipólito:** Investigation; methodology; validation; formal analysis; writing – review and editing. **Francisco Tortosa:** Methodology; validation; formal analysis; writing – review and editing; investigation. **Márta Korbonits:** Methodology; validation; writing – review and editing; resources; funding acquisition; investigation. **Sayka Barry:** Validation; methodology; investigation; writing – review and editing; formal analysis. **Ana Luísa Silva:** Investigation; methodology; validation; formal analysis; writing – paper writing, review and editing. **Rita Joaquim:** Investigation; methodology; validation; formal analysis; writing – review and editing. **Charlotte Hall:** Investigation; validation; writing – review and editing. **Tiago Oliveira:** Investigation; methodology; validation; formal analysis; writing – review and editing. **Pedro Marques:** Investigation; methodology; validation; formal analysis; writing – paper writing, review and editing; resources; funding acquisition.

ACKNOWLEDGMENT

Open access publication funding provided by FCT (b-on).

FUNDING INFORMATION

P.M. was supported by a Pilot Project Award from the Neuroendocrine Tumor Research Foundation (NETRF), and by the Maria de Sousa Prize awarded by the Fundação BIAL (BIAL Foundation) and the Ordem dos Médicos (Portuguese Medical Order). A.L.S. was supported by a research grant from the Portuguese Society of Endocrinology (Sociedade Portuguesa de Endocrinologia, Diabetes e Metabolismo (SPEDM)).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

For transparency, the peer review documents associated with this article are available at https://doi.org/10.1111/jne.70184.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved by the local Ethics Committee (No. 400/21).

PATIENT CONSENT STATEMENT

Written informed consent was obtained from each patient.

ORCID

Márta Korbonits https://orcid.org/0000-0002-4101-9432
Pedro Marques https://orcid.org/0000-0002-4959-5725

REFERENCES

1. Balkwill FR. The chemokine system and cancer. *J Pathol*. 2012;226(2):148-157.
2. Marques P, Grossman AB, Korbonits M. The tumour microenvironment of pituitary neuroendocrine tumours. *Front Neuroendocrinol*. 2020;58:100852.
3. Xu H, Lin S, Zhou Z, et al. New genetic and epigenetic insights into the chemokine system: the latest discoveries aiding progression toward precision medicine. *Cell Mol Immunol*. 2023;20(7):739-776.
4. Mantovani A, Savino B, Locati M, Zammataro L, Allavena P, Bonecchi R. The chemokine system in cancer biology and therapy. *Cytokine Growth Factor Rev*. 2010;21(1):27-39.
5. Yapa S, Mulla O, Green V, England J, Greenman J. The role of chemokines in thyroid carcinoma. *Thyroid*. 2017;27(11):1347-1359.
6. Grizzi F, Borroni EM, Vacchini A, et al. Pituitary adenoma and the chemokine network: a systemic view. *Front Endocrinol*. 2015;6:141.
7. Chen CX, Chen DN, Sun XL, et al. Identification of vital prognostic genes related to tumor microenvironment in pheochromocytoma and paraganglioma based on weighted gene co-expression network analysis. *Aging*. 2021;13(7):9976-9990.
8. Marchesi F, Locatelli M, Solinas G, Erreni M, Allavena P, Mantovani A. Role of CX3CR1/CX3CL1 axis in primary and secondary involvement of the nervous system by cancer. *J Neuroimmunol*. 2010;224(1–2):39-44.
9. Merecz K, Hikisz P, Tarasiuk-Zawadzka A, Fichna J, Jacenik D. Chemokines with CXC motif and their receptors in neuroendocrine neoplasms. *Endocr Relat Cancer*. 2025;32(6):e240190.
10. Melmed S, Kaiser UB, Lopes MB, et al. Clinical biology of the pituitary adenoma. *Endocr Rev*. 2022;43(6):1003-1037.
11. Chiloiro S, Mattogno PP, Angelini F, et al. Cytokines and chemokines modulate the growth of pituitary adenoma/neuroendocrine tumors:

preliminary results of a monocenter prospective pilot study. *Pituitary*. 2025;28(2):37.
12. Guo X, Yang Y, Qian Z, et al. Immune landscape and progress in immunotherapy for pituitary neuroendocrine tumors. *Cancer Lett*. 2024;592:216908.
13. Marques P, Barry S, Carlsen E, et al. Chemokines modulate the tumour microenvironment in pituitary neuroendocrine tumours. *Acta Neuropathol Commun*. 2019;7(1):172.
14. Principe M, Chanal M, Ilie MD, et al. Immune landscape of pituitary tumors reveals association between macrophages and Gonadotroph tumor invasion. *J Clin Endocrinol Metab*. 2020;105(11):3459-3473.
15. Zhou W, Zhang C, Zhang D, et al. Comprehensive analysis of the immunological landscape of pituitary adenomas: implications of immunotherapy for pituitary adenomas. *J Neurooncol*. 2020;149(3):473-487.
16. Silva AL, Barry S, Lopes-Pinto M, et al. CCL2 expression predicts clinical outcomes and regulates E-cadherin and angiogenesis in pituitary tumours. *Endocr Relat Cancer*. 2025;32(5):e240293.
17. Salomon MP, Wang X, Marzese DM, et al. The epigenomic landscape of pituitary adenomas reveals specific alterations and differentiates among acromegaly, Cushing's disease and endocrine-inactive subtypes. *Clin Cancer Res*. 2018;24(17):4126-4136.
18. Tecimer T, Dlott J, Chuntharapai A, Martin AW, Peiper SC. Expression of the chemokine receptor CXCR2 in normal and neoplastic neuroendocrine cells. *Arch Pathol Lab Med*. 2000;124(4):520-525.
19. Barbieri F, Bajetto A, Stumm R, et al. Overexpression of stromal cell-derived factor 1 and its receptor CXCR4 induces autocrine/paracrine cell proliferation in human pituitary adenomas. *Clin Cancer Res*. 2008;14(16):5022-5032.
20. Xing B, Kong YG, Yao Y, Lian W, Wang RZ, Ren ZY. Study on the expression levels of CXCR4, CXCL12, CD44, and CD147 and their potential correlation with invasive behaviors of pituitary adenomas. *Biomed Environ Sci*. 2013;26(7):592-598.
21. Yoshida D, Koketshu K, Nomura R, Teramoto A. The CXCR4 antagonist AMD3100 suppresses hypoxia-mediated growth hormone production in GH3 rat pituitary adenoma cells. *J Neurooncol*. 2010;100(1):51-64.
22. Yoshida D, Nomura R, Teramoto A. Signalling pathway mediated by CXCR7, an alternative chemokine receptor for stromal-cell derived factor-1alpha, in AtT20 mouse adrenocorticotrophic hormone-secreting pituitary adenoma cells. *J Neuroendocrinol*. 2009;21(5):481-488.
23. Wu Y, Wu Y, Yao B, et al. Diagnostic accuracy and value of CXCR4-targeted PET/MRI using (68)Ga-Pentixafor for tumor localization in Cushing disease. *Radiology*. 2024;313(3):e233469.
24. Yao B, Liu Y, Wu Y, et al. The value of targeting CXCR4 with (68)Ga-Pentixafor PET/MRI for Cushing's disease: a retrospective cohort study. *Eur J Nucl Med Mol Imaging*. 2026.
25. Peixe C, Alexandre MI, Gomes AR, et al. Usefulness of a clinicopathological classification in predicting treatment-related outcomes and multimodal therapeutic approaches in pituitary adenoma patients: retrospective analysis on a Portuguese cohort of 129 patients from a tertiary pituitary center. *Pituitary*. 2023;26(4):352-363.
26. Balkwill FR, Mantovani A. Cancer-related inflammation: common themes and therapeutic opportunities. *Semin Cancer Biol*. 2012;22(1):33-40.
27. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. 2008;454(7203):436-444.
28. D'Orazi G, Cordani M, Cirone M. Oncogenic pathways activated by pro-inflammatory cytokines promote mutant p53 stability: clue for novel anticancer therapies. *Cell Mol Life Sci*. 2021;78(5):1853-1860.
29. Kartikasari AER, Huertas CS, Mitchell A, Plebanski M. Tumor-induced inflammatory cytokines and the emerging diagnostic devices for cancer detection and prognosis. *Front Oncol*. 2021;11:692142.
30. Kureshi CT, Dougan SK. Cytokines in cancer. *Cancer Cell*. 2025;43(1):15-35.
31. Biagetti B, Simo R. Pituitary apoplexy: risk factors and underlying molecular mechanisms. *Int J Mol Sci*. 2022;23(15):8721.
32. Gupta P, Dutta P. Landscape of molecular events in pituitary apoplexy. *Front Endocrinol*. 2018;9:107.
33. Kiefer F, Siekmann AF. The role of chemokines and their receptors in angiogenesis. *Cell Mol Life Sci*. 2011;68(17):2811-2830.
34. Geindreau M, Bruchard M, Vegran F. Role of cytokines and chemokines in angiogenesis in a tumor context. *Cancers*. 2022;14(10):2446.
35. Marques P, Barry S, Carlsen E, et al. The role of the tumour microenvironment in the angiogenesis of pituitary tumours. *Endocrine*. 2020;70(3):593-606.
36. Hebert CA, Baker JB. Interleukin-8: a review. *Cancer Invest*. 1993;11(6):743-750.
37. Matsushima K, Yang D, Oppenheim JJ. Interleukin-8: an evolving chemokine. *Cytokine*. 2022;153:155828.
38. Lee Y, Kim JM, Lee EJ. Functional expression of CXCR4 in somatotrophs: CXCL12 activates GH gene, GH production and secretion, and cellular proliferation. *J Endocrinol*. 2008;199(2):191-199.
39. Zhang A, Xu Y, Xu H, et al. Lactate-induced M2 polarization of tumor-associated macrophages promotes the invasion of pituitary adenoma by secreting CCL17. *Theranostics*. 2021;11(8):3839-3852.
40. Izhak L, Wildbaum G, Jung S, Stein A, Shaked Y, Karin N. Dissecting the autocrine and paracrine roles of the CCR2-CCL2 axis in tumor survival and angiogenesis. *PLoS One*. 2012;7(1):e28305.
41. Yoshimura T. The production of monocyte chemoattractant protein-1 (MCP-1)/CCL2 in tumor microenvironments. *Cytokine*. 2017;98:71-78.
42. de Craene B, Berx G. Regulatory networks defining EMT during cancer initiation and progression. *Nat Rev Cancer*. 2013;13(2):97-110.
43. Jin J, Lin J, Xu A, et al. CCL2: an important mediator between tumor cells and host cells in tumor microenvironment. *Front Oncol*. 2021;11:722916.
44. Barry S, Carlsen E, Marques P, et al. Tumor microenvironment defines the invasive phenotype of AIP-mutation-positive pituitary tumors. *Oncogene*. 2019;38(27):5381-5395.
45. Zhang Y, Fan B, Yang H, et al. CCL2/CCR2 signaling pathway in tumorigenesis and metastasis (review). *Oncol Lett*. 2025;30(6):598.
46. Zhang L, Tian S, Chang J, et al. Activation of the CCL22/CCR4 causing EMT process remodeling under EZH2-mediated epigenetic regulation in cervical carcinoma. *J Cancer*. 2024;15(19):6299-6314.
47. Korbecki J, Kupnicka P, Chlubek M, Goracy J, Gutowska I, Baranowska-Bosiacka I. CXCR2 receptor: regulation of expression, signal transduction, and involvement in cancer. *Int J Mol Sci*. 2022;23(4):2168.
48. Tian X, Wang J, Jiang L, Jiang Y, Xu J, Feng X. Chemokine/GPCR signaling-mediated EMT in cancer metastasis. *J Oncol*. 2022;2022:2208176.
49. Batchu S, Diaz MJ, Patel A, Reddy A, Lucke-Wold B. Transcriptome-derived ligand-receptor Interactome of major PitNET subgroups. *J Neurol Surg B Skull Base*. 2024;85(4):340-346.
50. Arendt LM, McCready J, Keller PJ, et al. Obesity promotes breast cancer by CCL2-mediated macrophage recruitment and angiogenesis. *Cancer Res*. 2013;73(19):6080-6093.
51. Ma W, Ou T, Cui X, et al. HSP47 contributes to angiogenesis by induction of CCL2 in bladder cancer. *Cell Signal*. 2021;85:110044.
52. Pausch TM, Aue E, Wirsik NM, et al. Metastasis-associated fibroblasts promote angiogenesis in metastasized pancreatic cancer via the CXCL8 and the CCL2 axes. *Sci Rep*. 2020;10(1):5420.
53. Lu CC, Tsai HC, Yang DY, et al. The chemokine CCL4 stimulates Angiopoietin-2 expression and angiogenesis via the MEK/-ERK/STAT3 pathway in Oral squamous cell carcinoma. *Biomedicine*. 2022;10(7):1612.

54. Szukiewicz D. CX3CL1 (Fractalkine)-CX3CR1 Axis in inflammation-induced angiogenesis and tumorigenesis. *Int J Mol Sci*. 2024;25(9):4679.
55. Mollica Poeta V, Massara M, Capucetti A, Bonecchi R. Chemokines and chemokine receptors: new targets for cancer immunotherapy. *Front Immunol*. 2019;10:379.
56. Beck G, Brinkkoetter P, Hanusch C, et al. Clinical review: immunomodulatory effects of dopamine in general inflammation. *Crit Care*. 2004;8(6):485-491.
57. Yoshioka Y, Sugino Y, Yamamuro A, Ishimaru Y, Maeda S. Dopamine inhibits the expression of proinflammatory cytokines of microglial cells through the formation of dopamine quinone in the mouse striatum. *J Pharmacol Sci*. 2022;148(1):41-50.
58. Marques P, Barry S, Carlsen E, et al. Pituitary tumour fibroblast-derived cytokines influence tumour aggressiveness. *Endocr Relat Cancer*. 2019;26(12):853-865.
59. Susini C, Buscail L. Rationale for the use of somatostatin analogs as antitumor agents. *Ann Oncol*. 2006;17(12):1733-1742.
60. Thiele JO, Lohrer P, Schaaf L, et al. Functional in vitro studies on the role and regulation of interleukin-6 in human somatotroph pituitary adenomas. *Eur J Endocrinol*. 2003;149(5):455-461.
61. Vindelov SD, Hartoft-Nielsen ML, Rasmussen AK, et al. Interleukin-8 production from human somatotroph adenoma cells is stimulated by interleukin-1beta and inhibited by growth hormone releasing hormone and somatostatin. *Growth Horm IGF Res*. 2011;21(3):134-139.
62. Moatassim-Billah S, Duluc C, Samain R, et al. Anti-metastatic potential of somatostatin analog SOM230: indirect pharmacological targeting of pancreatic cancer-associated fibroblasts. *Oncotarget*. 2016;7(27):41584-41598.
63. Sciammarella C, Luce A, Riccardi F, et al. Lanreotide induces cytokine modulation in intestinal neuroendocrine tumors and overcomes resistance to everolimus. *Front Oncol*. 2020;10:1047.
64. ter Veld F, Rose B, Mussmann R, Martin S, Herder C, Kempf K. Effects of somatostatin and octreotide on cytokine and chemokine production by lipopolysaccharide-activated peripheral blood mononuclear cells. *J Endocrinol Invest*. 2009;32(2):123-129.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Silva AL, Barry S, Hipólito A, et al. The emerging role of chemokines and chemokine receptors in the biological and clinical behaviour of pituitary neuroendocrine tumours: An exploratory transcriptomic study. *J Neuroendocrinol*. 2026;38(4):e70184. doi:10.1111/jne.70184