Regarding “Transcriptional and Cytokine Profiles Identify CXCL9 as a Biomarker of Disease Activity in Morphea”


TO THE EDITOR

In Volume 137, Issue 8, we read with interest the paper by O’Brien et al. (2017) reporting that CXCL9 and CXCL10 are present at increased concentrations in serum from morphea patients, and that CXCL9, but not CXCL10, serum concentrations correlate with measures for disease activity. In this letter, we confirm increased CXCL9 and CXCL10 serum concentrations in a cohort of 80 morphea patients. However, in contrast to O’Brien et al. (2017), we report similar correlations between measures for disease activity and both CXCL9 and CXCL10 serum concentrations. This observation suggests equal biomarker capabilities for both chemokines. In addition, high-throughput transcriptome sequencing of morphea skin samples demonstrated increased gene expression of both CXCL9 and CXCL10 in morphea skin. Lastly, we did not observe altered CXCL9 and CXCL10 gene expression in circulating monocytes, supporting the theory postulated by O’Brien et al. (2017), that morphea may result from skin-directed immune dysregulation rather than a systemic dysregulation as in systemic scleroderma.

Eligible patients were ≥4 years old and visited the outpatient clinics of dermatology or rheumatology of the University Medical Center Utrecht or Radboud University Medical Center. This study was conducted in accordance with principles of the Declaration of Helsinki and approved by the ethical board of the University Medical Center Utrecht. All patients or legal guardians signed informed consent previous to study participation. In total, serum from 80 patients and 23 age- and gender-matched healthy controls were included for the analyses of CXCL9 and CXCL10 levels. Cohort details are displayed in Supplementary Table S1 (online). Median modified Localized Scleroderma Skin Severity Index (mLoSSi) (Arkachaisri et al., 2009) was 13 (interquartile range [IQR] 6–25) and median Localized Scleroderma Damage Index (Arkachaisri et al., 2010) was 7 (IQR 4–15). More than two-thirds of the patients (n = 51 [64%]) were treatment-naïve at inclusion. The majority of the remaining patients (n = 29 [36%]) received methotrexate with or without systemic corticosteroids.

CXCL9 and CXCL10 serum concentrations were determined via Enzyme-Linked Immuno Sorbent Assay (Human CXCL9/MIG Quantikine ELISA Kit, R&D Systems, Minneapolis, MN) and Luminex assay (Supplementary Materials and Methods online), respectively. Cytokine values below the lower limit of detection were set to half of the lower limit of detection for analysis. Median values for CXCL9 were substantially higher in morphea patients (57.81 pg/ml, IQR 15.63–232.2 pg/ml), compared to controls, where CXCL9 levels were below threshold of detection in 22 of 23 individuals (median CXCL9 15.63 pg/ml, IQR 15.63–15.63 pg/ml; P < 0.0001, Figure 1a). Median CXCL10 concentrations were also elevated in morphea patients (424.2 pg/ml, IQR 245.0–74.3 pg/ml) versus controls (153.3 pg/ml, IQR 110.2–223.8 pg/ml; P < 0.0001, Figure 1b). CXCL9 serum concentrations strongly correlated with CXCL10 serum concentrations (Spearman’s ρ rS = 0.715; P < 0.0001). Both CXCL9 and CXCL10 concentrations significantly correlated with the mLoSSi (CXCL 9: rS = 0.4904, P < 0.0001, Figure 1c, CXCL10: rS = 0.4065, P = 0.0006, Figure 1d), but not with Localized Scleroderma Damage Index (rS = 0.0443, P = 0.710, rS = 0.09, P = 0.449). Next, normal ranges of both chemokine serum concentrations were defined as mean plus 2 standard deviations observed in controls and patients with concentrations above the...
Figure 1. Serum concentrations of CXCL9 and CXCL10. (a, b) CXCL9 and CXCL10 are elevated in morphea serum. (c, d) Spearman’s rank correlation coefficient ($r_s$) between modified Localized Scleroderma Severity Index (mLoSSI) and CXCL9 and CXCL10. (e, f) Proportion of patients with elevated CXCL9 and CXCL10 levels in active and inactive disease. (g, h) Longitudinal serum CXCL9 and CXCL10 concentrations during active and inactive disease.
normal range were termed positive. The proportion of positive patients was investigated in patients with active (mLoSS1 > 5, n = 65) and inactive (mLoSS1 ≤ 4, n = 28) disease. The proportions of CXCL9- and CXCL10-positive patients were both increased at active disease (55% and 52%, respectively) compared to the proportions at inactive disease (21% and 24%, respectively) (Figure 1e, 1f). Lastly, longitudinal serum samples were available for 18 patients and showed significantly lower values for both CXCL9 (P = 0.0007, Figure 1g) and CXCL10 (P = 0.0001, Figure 1f) at inactive disease compared to the serum concentration at disease onset, underlining the relevance of these markers in measuring disease activity.

To assess tissue expression of CXCL9 and CXCL10, high-throughput sequencing was performed on RNA collected from skin samples of eight morphea patients and five controls (Supplementary Materials and Methods). In morphea patients, skin biopsies were taken from the inflammatory border (I) and the sclerotic center (II) of affected skin and from unaffected skin (III) (Figure 2a). CXCL9 and CXCL10 gene expression was significantly increased at the inflammatory border and, to a lesser extent, at the sclerotic center of affected tissue when compared to healthy controls, whereas unaffected tissue from morphea patients did not show any differences in gene expression for both the genes (Figure 2b, 2c). Further investigation revealed very strong correlation between CXCL9 and CXCL10 gene expression (r_s = 0.902, P < 0.0001, Figure 2d). Lastly, both CXCL9 and CXCL10 gene expression strongly correlated with CD68 expression (Figure 2e, 2f), suggesting the involvement of myeloid cells in the high expression of these chemokines.

Next, we investigated CXCL9 and CXCL10 gene expression in monocytes from the circulation of 45 adult morphea patients and 25 controls. For this purpose, peripheral blood mononuclear cells were isolated from whole blood using Ficoll separation (Radstake et al., 2004). Subsequently, monocytes, among other cell subsets, were isolated by positive selection from freshly isolated peripheral blood mononuclear cells using immunomagnetic labeling (CD14 MicroBead kits; Miltenyi Biotec, Bergisch Gladbach, Germany) and cells were lysed consecutively. RNA was isolated and used for real-time PCR (Supplementary Materials and Methods). In monocytes, CXCL9 and CXCL10 gene expression did not differ significantly between patients and controls (CXCL9 median fold change = 1.12, P = 0.513, CXCL10 median fold change = 0.884, P = 0.778).
Our study confirms increased concentrations of CXCL9 and CXCL10 in morphea serum and correlation of CXCL9 to mLoSSI, a validated measure for disease activity. In addition to that, we also observe correlation between CXCL10 and mLoSSI. Our analysis demonstrates no relations between the presence of disease damage, captured by the Localized Scleroderma Damage Index score, and both CXCL9 and CXCL10. Torok et al. (2015) reported a similar correlation ($r_s = 0.34$) between CXCL10 and mLoSSI and no significant correlation between CXCL10 and Localized Scleroderma Damage Index scores in cohort of 69 pediatric morphea patients (Torok et al., 2015). The strong correlation between CXCL9 and CXCL10 serum concentrations combined with the similar trends in correlations between clinical measures and both chemokines, suggest equal biomarker capabilities for both CXCL9 and CXCL10.

Furthermore, we demonstrate increased CXCL9 and CXCL10 gene expression at both the inflammatory border and the sclerotic center of affected morphea tissue, with normal gene expression at unaffected tissue of morphea patients. A strong correlation between CD68 and CXCL9 and CXCL10 gene expression, together with the lack of an association between circulating monocytes, and these chemokines not only suggests a relationship between the presence of local macrophages and CXCL9/CXCL10 production, it also underscores that morphea is a disease confined to the skin where inflammatory markers are measured in the circulation as the result from “leakage” from the inflammatory sites.

In conclusion, we confirm the potential of CXCL9 as biomarker for disease activity in morphea. Interestingly, CXCL10 serum concentrations showed similar biomarker capabilities. Lastly, increased CXCL9 and CXCL10 gene expression in morphea skin, and absence of increased gene expression in monocytes, supports the hypothesis, postulated by O’Brien et al. (2017), that morphea may result from skin-directed immune dysregulation rather than the systemic presence of inflammation on contrast to that observed in systemic scleroderma.

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

**REFERENCES**


TO THE EDITOR

We read with interest the recent publication by Li et al. (2017) in the *Journal of Investigative Dermatology*. The authors comment on our finding of heterozygous truncating PSENEN mutations in six patients and families with Dowling-Degos disease (DDD), approximately half of whom also presented with acne inversa (AI) (Ralsen et al., 2017). Recently, we and others have identified mutations in three other genes that cause clinically and histopathologically divergent DDD subtypes: KRT5, encoding intermediate filament protein keratin 5...