**Meibomian gland dropout is associated with immunodeficiency at HIV diagnosis:
implications for dry eye disease**

Short title: Meibomian gland dropout in HIV individuals

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ABSTRACT

Aim: To characterize anterior eye health and tear film characteristics in individuals with human immunodeficiency virus (HIV) undergoing anti-retroviral therapy.

Methods: This cross-sectional study involved 35 adults, categorized as healthy controls (n=18) or as HIV-positive patients (n=17), with no history of opportunistic infection or current ocular fundus abnormalities. Participants underwent a comprehensive anterior eye assessment. Primary outcome measures were dry eye symptoms (Ocular Surface Disease Index survey), tear film osmolarity, and extent of meibomian gland dropout. Secondary outcomes measures were ocular redness, tear film stability, and ocular surface staining. Levels of 36 cytokines were assayed from basal tears using a multiplex bead array.

Results: The HIV-positive group showed more extensive meibomian gland dropout relative to controls (mean $\pm$ SD, controls: 29.6 ± 5.8 versus $37.0 \pm 13.9\%$, $p=0.045$). The extent of meibomian gland dropout was negatively correlated with blood CD4 T-cell count (a marker of immunodeficiency) at diagnosis ($r= -0.69$, $p=0.006$). All other tests of anterior ocular health, including dry eye symptom levels, were not significantly different between the groups. There were no significant inter-group differences for the 36 cytokines assayed in the tear film.

Conclusions: We find greater meibomian gland dropout in HIV-positive individuals that is related to disease severity at diagnosis. Given this feature predisposes to dry eye disease, it suggests the need for long-term studies of anterior eye health in people with HIV.

Keywords (5-7 words): Anti-retroviral therapy; cytokine; dry eye; HIV; human immunodeficiency virus; meibomian gland; eye; tear film

1.1 Introduction

Despite effective anti-retroviral therapy (ART), structural and functional eye abnormalities have been demonstrated in individuals with human immunodeficiency virus (HIV) in the absence of opportunistic infection [1, 2]. A spectrum of ocular changes have been documented, including reductions in contrast sensitivity [3, 4], visual field defects [3, 5-7], thinning of the retinal nerve fiber layer [7, 8], and retinal electrophysiological deficits [9]. In many cases these ocular abnormalities are subtle and regarded as subclinical, and have been associated with disease severity, as indexed by a low CD4 T-cell count [8, 9].

While much attention has been placed on posterior eye dysfunction in HIV-infected individuals, relatively few studies have specifically characterized anterior segment and external ocular disorders, which can still affect one-third of individuals with HIV in the era of ART [10-12]. The most common anterior segment manifestation is dry eye disease, which affects approximately 10% of people with HIV [10]. Whilst previous epidemiological studies have broadly reported on the prevalence of anterior ocular health conditions amongst individuals living with HIV [10, 11], a comprehensive and quantitative characterization of anterior ocular health in individuals on stable ART is currently lacking. In the present study, we sought to investigate whether people who have HIV infection, effectively managed with ART and without current symptomatic eye disease, demonstrate anterior eye abnormalities. Given the documented disease association of HIV infection with dry eye disease, we specifically aimed to examine meibomian gland architecture, and tear film integrity and composition.

2.1 Methods

The study was approved by the Alfred Hospital Ethics Committee (ID #191-17) and registered with The University of Melbourne Human Research Ethics Committee (ID #1749896). The study procedures adhered to the Declaration of Helsinki for research involving human participants, and written informed consent was obtained from each participant prior to performing any test procedures. Participants were recruited consecutively, in response to advertisements at The University of Melbourne and Melbourne Sexual Health Centre. All participants who expressed interest in volunteering were enrolled into the study. HIV-positive participants were enrolled exclusively from the Melbourne Sexual Health Centre; these participants were patients of the clinic and provided consent for their relevant medical records to be accessed to determine eligibility for the study (i.e. on effective antiretroviral therapy, taken as undetectable viral load at their most recent clinic visit). Control participants were HIV-negative by self-report and consisted of staff, research participants of the Melbourne Sexual Health Centre, or healthy volunteers who had previously participated in research at the Department of Optometry and Vision Sciences, with no known or detected eye disease.

Participants were required to be between the ages of 18 and 55 years. HIV-positive participants needed to be on effective antiretroviral therapy (i.e., viral load undetectable at their most recent clinic visit, at one to 14 weeks before testing). Participants underwent a brief ophthalmic screening to ensure normal visual acuity (i.e., at least 0.1 logMAR or 20/25 Snellen equivalent), measured monocularly using a distance ETDRS logMAR chart at 3m, and that subjective refractive error did not exceed $\pm 5.00\text{D}$ sphere and -2.50D cylinder. Ophthalmoscopic examination and retinal photography were used to confirmed

normal posterior ocular health and no history of prior or current opportunistic infection. In a subset of participants (11 controls, 17 HIV-positive), additional high-resolution optical coherence tomography (OCT) retinal imaging (Spectralis OCT, Heidelberg Engineering GmbH, Heidelberg, Germany) was conducted to quantify the structure of the peripapillary (optic nerve head) and macular regions.

Participants underwent comprehensive ocular surface health examinations using our previously established protocols [13]. Primary outcome measures were dry eye symptoms measured using the validated Ocular Surface Disease Index (OSDI) questionnaire [14], tear film osmolarity measured using the Tearlab system (Tearlab Corp., San Diego, CA, USA), and extent of meibomian gland dropout measured by meibography. Meibography was performed using infra-red illumination on the slit lamp biomicroscope (Topcon SL-D701 with DC4, Topcon, USA) to photodocument the meibomian gland architecture in the superior and inferior eyelids following lid eversion. A single examiner, masked to the participant group and study objectives, quantified the degree (%) of meibomian gland atrophy (dropout) using ImageJ software (version 1.47, National Institutes of Health, USA: <http://rsb.info.nih.gov/ij/index.html>). The average extent of gland dropout in the superior and inferior eyelids was averaged to derive a single representative measure of meibomian gland dropout for each eye.

Secondary outcomes measures were ocular redness, tear film stability, and ocular surface staining by generalised assessment of anterior segment health using slit lamp biomicroscopy. The severity of bulbar and limbal conjunctival redness were graded from 0.0 to 4.0, according to the four-step Efron scale [15] in 0.1 grading increments.

Tear break-up time, as a measure of tear stability, was measured after two gentle blinks using sodium fluorescein (involving the instillation of 1µl of sodium fluorescein using the Dry Eye Test strips, Amcon Laboratories, USA), 10x magnification, blue illumination and a Wratten 12 yellow-barrier filter. Tear break-up time measurements were repeated three times per eye, and averaged. Ocular surface staining scores of the cornea (fluorescein), nasal conjunctiva and temporal conjunctiva (lissamine green, using (GreenGlo test strips, Sigma Pharmaceuticals, USA) were graded separately using the five-step Oxford scale [16] in 0.1 grading increments. These staining scores were summed to give a total ocular surface staining score, ranging from 0.0 to 15.0, with higher grades indicative of more severe staining.

Basal (non-stimulated) tear samples (~10µl/eye) were collected by capillary flow from the inferior tear meniscus, as previously described [17], from a random subset of participants (11 controls, 17 HIV-positive) for cytokine analyses (Bio-Plex Pro™ Human Inflammation Panel 1, 37-Plex, BioRad). To confirm that the tear collection procedure was robust and did not represent reflex tears, secretory IgA levels (µg/mL) were assessed using an in-house enzyme-linked immunosorbent assay (ELISA) and total tear protein concentration (µg/mL) was measured by absorbance at 280nm (A280) on a NanoDrop (Thermo Scientific, Waltham, MA, USA) spectrophotometer. The same subset of participants (11 controls, 17 HIV-positive) also underwent high-resolution optical coherence tomography (OCT) retinal imaging to assess the structure of the peripapillary (optic nerve head) and macular regions (Spectralis OCT, Heidelberg Engineering GmbH, Heidelberg, Germany).

Statistical analyses were conducted using Prism (Version 5.0, GraphPad Software, San Diego, CA, United States). Data were tested for normality with a Kolmogorov-Smirnov test, and student t-tests or Mann Whitney rank sum tests were performed for normally distributed, or non-normally distributed data, respectively. An alpha level of 0.05 was considered the criterion for statistical significance. Effect sizes (Cohen's d) were calculated to represent the difference between the control and HIV-positive group in terms of numbers of standard deviations, using the equation:

$$d = (\mu_c - \mu_p) / \sigma_{pooled}$$

where

$$\sigma_{pooled} = \sqrt{[(\sigma_c^2 + \sigma_p^2) / 2]}$$

and μ_c and μ_p are the mean values for the control and HIV-positive groups, respectively, and σ_c and σ_p are the standard deviations.

3.1 Results

We prospectively recruited 35 individuals to undergo the ocular health testing procedures. One control participant was excluded from the study further to the ophthalmic screening identifying an anterior ocular pathology (keratoconus). The demographic and clinical data for the remaining participants, comprising 18 healthy male controls (aged 24 to 59 years) and 17 age-similar HIV-positive males (aged 19 to 55 years) are summarized in Table 1. Suppressive ART was being taken by all HIV-positive participants, who had been HIV-

positive for periods ranging from one to 29 years, and had confirmed undetectable viral load (i.e. effective ART) at one to 14 weeks before testing. Additional high-resolution OCT imaging in the subset of participants (11 controls, 17 HIV-positive) confirmed that there were no significant inter-group differences in retinal structure, with respect to central macular thickness ($p=0.29$) or global peripapillary retinal nerve fiber layer thickness ($p=0.36$).

The participants were generally asymptomatic of ocular surface symptoms; however, we surmised there may have been subtle differences in anterior eye health in the group of HIV-positive participants. We assessed objective signs of dry eye disease in each eye; however, here we report data from only one eye because the two eyes are not necessarily independent [18]. Tear osmolarity is the single best ocular marker for dry eye disease and severity [19] and cannot be averaged across the two eyes; hence, we chose to report data from the eye with the highest tear osmolarity (similar proportion of right and left eyes represented, 59% and 41%, respectively). Figure 1 summarizes the monocular anterior eye findings for bulbar and limbal redness, tear osmolarity, tear break-up-time and ocular surface staining. Despite a mean duration of HIV infection of seven years, there were no significant inter-group differences for any of these parameters ($p>0.05$ for all comparisons).

Given that the meibomian glands secrete lipids that are essential for maintaining tear film integrity, and thus supporting long-term anterior eye health, we performed infra-red meibography on all study participants. Figure 2 provides representative infra-red meibography images from control (Figure 2A&C) and HIV-positive (Figure 2B&D)

participants. Figure 3A shows that meibomian gland dropout (average of superior and inferior eyelids) was higher in the HIV-positive group (mean $\pm$ standard deviation; controls: 29.6 ± 5.8 versus $37.0 \pm 13.9\%$; $t_{33}=2.09$, $p=0.045$, medium effect size: $d = 0.61$). In the individuals with HIV, meibomian gland dropout was correlated with CD4 T-cell count at diagnosis (Figure 3B, Pearson $r = -0.69$, $R^2 = 0.48$, $p=0.006$). Meibomian gland dropout was not correlated with the estimated duration of HIV infection or viral load at diagnosis ($p>0.05$ for both comparisons).

Tear cytokine levels can be elevated in individuals with early-stage dry eye disease [17]. We obtained basal tear samples from all 17 HIV-positive subjects and 11 of the 18 HIV-negative controls, for protein and cytokine analyses. Secretory IgA levels (control: 583 ± 811 versus HIV-positive: 656 ± 912 $\mu\text{g/mL}$, $p=0.42$) and total protein concentration (control: 1085 ± 715 versus HIV-positive: 1234 ± 683 $\mu\text{g/mL}$, $p=0.30$) from basal tear samples were similar between the study groups. Although active dry eye disease is known to be associated with increased pro-inflammatory tear cytokine levels [17], we found no significant inter-group differences in the tear film levels of 36 key inflammatory markers from the tumor necrosis factor-superfamily proteins, interferon-gamma family proteins, T-regulatory cytokines or matrix metalloproteinases (Figure 4).

4.1 Discussion

We undertook a novel and comprehensive characterisation of anterior ocular health, with particular relevance to dry eye disease, in people with and without HIV infection. The major finding of this cross-sectional study is the presence of more extensive meibomian gland dropout in asymptomatic HIV-positive individuals relative to healthy control

participants, despite no active dry eye signs or symptoms. Although previous descriptive studies have reported the prevalence of posterior blepharitis and meibomian gland dysfunction in people treated for HIV [10], the present study is the first to conduct objective meibomian gland evaluation (meibography) and quantification in individuals with HIV infection.

Meibography is an established technique for quantifying the extent of meibomian gland loss (dropout). In terms of established physiological levels of meibomian gland dropout, Mathers and Lane (1998) used meibography to quantify meibomian gland dropout in 72 healthy adult participants [20]. These authors found that meibomian gland dropout remained, on average, below one gland per eight assessed, up to about 50 years of age. Based on the presence of 20-30 meibomian glands in the inferior lid [21], this finding corresponds to about 3-4 glands lost over the lower eyelid (or in percentage terms, about 12-15% gland dropout, which is similar to the degree of loss observed in the inferior eyelids in controls in the present study; see Figure 2C). Also consistent with the present study, Arita and colleagues found that most individuals under the age of 50 showed glandular loss over less than one-third of the total of the eyelid area [22]. The average extent of meibomian gland dropout in the HIV-positive individuals seen here (~40% dropout), is similar to that observed in contact lens wearers [23], and thus proposed to be of potential pathological significance.

Our finding of meibomian gland atrophy adds to recent literature with a focus on anterior ocular health and tear film parameters in HIV-infected individuals. In a small cross-sectional study, Han et al. (2011) reported the presence of HIV-1 virus in the tears of

HIV-positive individuals undergoing long-term ART. However, there were no clinical signs or symptoms suggestive of ocular health abnormality [24]. However, the clinical ophthalmic tests performed by Han et al. (2011) were not described and so it is unclear exactly how anterior eye health specifically was characterised. Cetin and colleagues (2018) undertook a comprehensive study of corneal and anterior chamber morphology but found no significant differences in a range of anterior ocular structural measures (e.g. corneal endothelial cell density, corneal thickness and volume, anterior chamber depth and volume) between HIV-positive individuals and healthy controls [25], despite a previous study demonstrating increased risk of corneal endothelial cell polymegathism in HIV-infected individuals [26].

In this study, the negative relationship between the extent of meibomian gland dropout and CD4 T-cell count at diagnosis, which reflects immune damage prior to diagnosis and treatment, was notable. These data suggest that meibomian gland dropout may reflect immune-driven damage to the meibomian glands that occurs early during HIV infection, and potentially other immune-mediated diseases. Further studies are required to corroborate these findings and dissect the mechanism of meibomian gland loss. A possible mechanism for immune-driven meibomian gland damage is via enhanced immune cell recruitment, which may be relevant to a range of immune-mediated conditions. Specifically, T-cell mediated meibomian gland obstruction has been recently demonstrated in a mouse model of chronic inflammation [27]. In this model, the lymphocytic response was associated with polymorphonuclear neutrophil recruitment, which is a key pathogenic factor in meibomian gland obstruction. Together, these findings

support a role for an immune-mediated mechanism underlying meibomian gland dropout in early HIV.

Other demographic variables, such as estimated duration of HIV infection and viral load at diagnosis, were not related to the degree of meibomian gland dropout. All of the HIV infected individuals who participated in this study were being effectively treated with ART; hence, measures such as the most recent CD4 count or viral load were not useful in the context of understanding associations with disease severity. Furthermore, the HIV cohort for this study was recruited from a sexual health clinic and attending for regular therapy and monitoring. We do not have clinical data describing the duration of low CD4 count or high viral load prior to initiating treatment, which may be an important factor in determining the extent of meibomian gland dropout. Other forms of ocular dysfunction, including delayed retinal electrophysiological responses, have been reported in HIV-positive people with prolonged low (<100) CD4 nadir counts [9]. Longitudinal data, including a comparison between pre- and post-treatment (if possible), are needed to confirm the proposed link between immune-mediated meibomian gland atrophy and early phase HIV infection.

We found no evidence for acute inflammation in the tear cytokine analysis, which is consistent with the absence of clinical indicators for active dry eye disease in the participant population. A previous study compared 41 tear cytokine profiles in HIV-positive individuals and healthy controls, all of whom had dry eye disease [28]. Epithelial growth factor and interferon gamma-induced protein 10 (IP-10) levels were reported to be higher in the tears of people with dry eye disease with HIV infection, whereas growth

related oncogene levels were lower, relative to healthy controls with dry eye disease [28]. It was proposed that the observed cytokine differences reflected HIV-mediated inflammation as the underlying pathogenic mechanism. Taken together, our results and those of others [28] suggest that dry eye disease is an important confound to consider when examining tear cytokine levels for evidence of ocular inflammation in HIV.

In both study groups, participants were asymptomatic, generally had similar ocular surface signs and had no apparent differences in tear inflammatory cytokine profiles. The extent of meibomian gland dropout was the most striking inter-group difference. This was somewhat unexpected, given that dry eye disease has been reported to be more prevalent in HIV populations, particularly where CD4 cell count is low [29]. These apparently divergent findings may be due to a range of potential inter-study differences, including the modest duration of disease, level of anti-viral control (our participants were stably treated with ART and relatively healthy) and parameters used to define dry eye status. We also acknowledge the possibility that a larger study population may have enabled the delineation of additional inter-group differences. Nevertheless, our finding of increased meibomian gland dropout is particularly significant in the context of the observed absence of any markers of active ocular surface inflammation, based upon the tear osmolarity and cytokine analyses. Meibomian gland dropout is a major predisposing factor for the eventual development of dry eye disease [30]. Dry eye disease is a chronic and progressive disorder of the ocular surface with a substantial impact on quality of life with increasing disease severity [31]. An estimated 37 million people are living with HIV worldwide [32] and lifelong ART is recommended for all, with an estimated 21.7 million currently on ART [33]. Since successful ART results in decades of healthy life, our results

highlight a need for clinicians to be alert to potential sub-clinical anterior eye pathology that may heighten the risk of longer-term ocular surface disease. In particular, our study supports the merit of undertaking a comprehensive examination of anterior eye health in people with HIV, especially upon diagnosis, to detect subclinical glandular changes and potentially institute preventive measures (e.g., blinking training) to aid in the long-term preservation of meibomian gland function.

5.1 Conclusions

In conclusion, we report greater loss of meibomian gland integrity that is related to CD4 cell count at HIV diagnosis. Earlier HIV diagnosis, and the prompt initiation of ART, may preserve meibomian gland integrity, and potentially reduce the long-term incidence of dry eye disease in HIV-positive individuals. Our findings support the need for future comprehensive studies of anterior ocular health during both early HIV infection and long-term ART.

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Declarations of interest

All authors report no direct competing interests in relation this work. Author LED reports grants from the Rebecca L Cooper Medical Research Foundation, Macular Disease Foundation Australia, Allergan Pty Ltd, Alcon Pty Ltd, CooperVision Pty Ltd and Azura Ophthalmics Pty Ltd, for research outside the submitted work. She has also acted as a consultant to Seqirus Pty Ptd outside the submitted work. Author SJK reports grants and personal fees from ViiV HealthCare and Gilead Sciences, and grants from Johnson and Johnson and Sanofi-Pasteur, outside the submitted work.

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Figure Legends

Figure 1. Anterior eye health parameters in controls and HIV-positive groups. (A) Bulbar conjunctival redness grade (median $\pm$ IQR); (B) Limbal conjunctival redness grade (median $\pm$ IQR); (C) Dry eye symptoms measured using the Ocular Disease Surface Index (OSDI) score (median $\pm$ IQR); (D) Tear film osmolarity (median $\pm$ IQR); (E) Fluorescein tear break-up time (mean $\pm$ SD); (F) Total ocular surface staining score (median $\pm$ IQR).

Figure 2. Representative infra-red meibography images from the superior (A&B) and inferior (C&D) eyelids of a control (A&C) and HIV-positive (B&D) participant. The degree of meibomian gland (%) dropout in each panel is: (A) 21%, (B) 34%, (C) 18%, (D) 65%. Overall, the HIV-positive participant demonstrates more extensive meibomian gland dropout than the control participant.

Figure 3. Meibography data. (A) Meibomian gland dropout (% average of the superior and inferior eyelids) in the control and HIV-positive groups. Error bars represent the standard deviation. (B) Negative correlation between the extent of meibomian gland dropout (%) and CD4 T-cell count at HIV diagnosis.

Figure 4. Tear cytokine analysis results. Tear cytokine concentrations, normalized by Z-score and presented as a heatmap (Morpheus, <https://software.broadinstitute.org/morpheus>). Cytokines are listed on the y-axis, while participant IDs and respective cohorts (HIV-positive or control) are listed on the x-axis. For each cytokine, a negative Z-score (blue) indicates a relatively lower concentration compared to the mean of the tested population.

Table 1. Participant demographic information and clinical characteristics. Mean $\pm$ standard deviations (and t-tests for between-group comparisons) are given where the data were normally distributed. Group differences in proportions were compared using a chi-square test of proportions.

	Controls (n=18)	HIV positive (n=17)	Inter-group comparison
Age, mean $\pm$ SD [range], years	39 $\pm$ 11 [24-59]	35 $\pm$ 10 [19-55]	$t_{33}=1.2$, $p=0.24$
Ethnicity, Asian:Caucasian (% Asian)	3:15 (17%)	5:12 (29%)	$X^2(1)=0.81$, $p=0.37$
Estimated duration of HIV infection, mean $\pm$ SD [range], years	—	7 $\pm$ 7 [1-29]	—
Viral load at diagnosis, mean $\pm$ SD [range], RNA copies/mL	—	65596 $\pm$ 81379 [5300-310154]	—
CD4 T-cell count at diagnosis, mean $\pm$ SD [range], cells/mm ³	—	410 $\pm$ 201 [44-787]	—

Table 2: Anterior eye health test results. Mean $\pm$ standard deviations and t-tests are given where the data were normally distributed. Median [interquartile range] and Mann-Whitney rank sum tests are given where the data were non-normally distributed.

	Controls (n=18)	HIV-positive (n=17)	Inter-group comparison
Ocular Surface Disease Index, score	8.7 [1.6 – 15.2]	6.3 [1.1 – 15.2]	Mann Whitney U=148, p=0.88
Tear osmolarity, mOsm/L	305 [298 – 314]	311 [303 – 316]	Mann Whitney U=120, p=0.28
Bulbar conjunctival redness, grade	0.4 [0.4 – 0.8]	0.7 [0.4 – 1.1]	Mann Whitney U=124, p=0.35
Limbal conjunctival redness, grade	0.3 [0.3 – 0.8]	0.7 [0.2 – 1.5]	Mann Whitney U=137, p=0.59
Ocular surface staining, score	1.2 [0.6 – 3.2]	1.5 [0.5 – 3.5]	Mann Whitney U=152.5, p=0.99
Tear break-up time, seconds	7.44 $\pm$ 3.82	6.26 $\pm$ 1.51	t ₃₃ =1.18, p=0.25
Meibomian gland dropout, %	29.6 $\pm$ 5.8	37.0 $\pm$ 13.9	t ₃₃ =2.09, p=0.045

Table 3: Cytokine analysis results. Mean $\pm$ standard deviations and t-tests are given where the data were normally distributed. Median [interquartile range] and Mann-Whitney rank sum tests are given where the data were non-normally distributed. The cytokine names (as appears in Figure 4) have been spelt out in full.

Cytokine (pg/mL)	Controls (n=11)	HIV-positive (n=17)	Inter-group comparison
APRIL (a proliferation inducing ligand)	345221 [284628 – 512898]	440758 [366383 – 683313]	Mann-Whitney U=61, p=0.13
BAFF (B cell activating factor)	6991 $\pm$ 1810	6672 $\pm$ 1647	t ₂₆ =0.48, p=0.63
sCD30 (soluble CD30)	131 [105 – 146]	118 [90 – 131]	Mann-Whitney U=70, p=0.28
sCD163 (soluble CD163)	4622 [3893 – 6060]	4368 [2986 – 5074]	Mann-Whitney U=74, p=0.37
CHI3L1 (Chitinase 3-like protein 1)	992 [816 – 1669]	978 [835 – 1451]	Mann-Whitney U=86.5, p=0.75
sIL-6R β (soluble interleukin-6 receptor β)	597 $\pm$ 197	663 $\pm$ 267	t ₂₆ =0.70, p=0.49
IFN- α 2 (interferon α 2)	193 $\pm$ 97	166 $\pm$ 72	t ₂₆ =0.85, p=0.40
IFN- β (interferon β)	444 [365 – 520]	359 [247 – 461]	Mann-Whitney U=55.5, p=0.08
IFN- γ (interferon γ)	356 $\pm$ 139	313 $\pm$ 116	t ₂₆ =0.89, p=0.38
IL-2 (interleukin-2)	144 [129 – 187]	124 [103 – 169]	Mann-Whitney U=67.5, p=0.23
sIL-6R α (soluble interleukin-6 receptor α)	578 $\pm$ 299	493 $\pm$ 225	t ₂₆ =0.85, p=0.40
IL-8 (interleukin-8)	724 [594 – 765]	581 [520 – 767]	Mann-Whitney U=66.5, p=0.21
IL-10 (interleukin-10)	73 $\pm$ 37	65 $\pm$ 29	t ₂₆ =0.62, p=0.54
IL-11 (interleukin-11)	28 $\pm$ 15	24 $\pm$ 10	t ₂₆ =1.02, p=0.32
IL-12(p40) (interleukin-12 p40 subunit)	723 $\pm$ 345	674 $\pm$ 303	t ₂₆ =0.40, p=0.70
IL-12(p70) (interleukin-12 p70 subunit)	73 [61 – 84]	55 [39 – 74]	Mann-Whitney U=54, p=0.06
IL-19 (interleukin-19)	529 [478 – 547]	492 [466 – 656]	Mann-Whitney U=88.5, p=0.83
IL-20 (interleukin-20)	14 [13 – 18]	13 [9 – 16]	Mann-Whitney U=66.5, p=0.21

IL-22 (interleukin-22)	283 [230 – 407]	239 [152 – 310]	Mann-Whitney U=71, p=0.31
IL-26 (interleukin-26)	762 [562 – 913]	669 [429 – 780]	Mann-Whitney U=73, p=0.35
IL-27(p28) (interleukin-27 p28 subunit)	549 ± 215	471 ± 198	t ₂₆ =0.98, p=0.33
IL-28A (interleukin-28A)	699 ± 304	618 ± 268	t ₂₆ =0.74, p=0.46
IL-29 (interleukin-29)	1666 [1502 – 2054]	1489 [1144 – 1851]	Mann-Whitney U=65, p=0.19
IL-34 (interleukin-34)	1601 [1015 – 2006]	1717 [1297 – 2410]	Mann-Whitney U=77, p=0.46
IL-35 (interleukin-35)	663 ± 421	580 ± 375	t ₂₆ =0.54, p=0.59
LIGHT/TNFSF14 (tumor necrosis factor superfamily member 14)	265 [237 – 330]	229 [166 – 292]	Mann-Whitney U=60.5, p=0.12
MMP-1 (matrix metalloproteinase-1)	4671 ± 2161	4252 ± 1810	t ₂₆ =0.56, p=0.58
MMP-2 (matrix metalloproteinase-2)	3870 [3191 – 4417]	3357 [2517 – 3947]	Mann-Whitney U=68.5, p=0.25
MMP-3 (matrix metalloproteinase-3)	29056 ± 4231	28325 ± 4146	t ₂₆ =0.45, p=0.65
Osteocalcin	169 [145 – 264]	168 [86 – 218]	Mann-Whitney U=74.5, p=0.38
Osteopontin	1907 ± 634	1716 ± 606	t ₂₆ =0.80, p=0.43
Pentraxin-3	208 ± 100	245 ± 170	t ₂₆ =0.68, p=0.50
sTNF-R1 (soluble tumor necrosis factor receptor 1)	219 [150 – 256]	218 [151 – 319]	Mann-Whitney U=86, p=0.75
sTNF-R2 (soluble tumor necrosis factor receptor 2)	305 ± 199	254 ± 157	t ₂₆ =0.76, p=0.46
TSLP (thymic stromal lymphopoietin)	484 ± 203	435 ± 167	t ₂₆ =0.68, p=0.50
TWEAK (tumor necrosis factor-like weak inducer of apoptosis)	182 ± 67	242 ± 133	t ₂₆ =1.39, p=0.18

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475 **Figure 1**

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478 **Figure 2**

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481 **Figure 3**

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484 **Figure 4**

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