Published in final edited form as:

J Reprod Immunol. 2009 January ; 79(2): 174–182. doi:10.1016/j.jri.2008.11.003.

Cervical cytokine network patterns during pregnancy: the role of bacterial vaginosis and geographic ancestry

Kelli K. Ryckman^{1,2}, Hyagriv N. Simhan³, Marijane A. Krohn³, and Scott M. Williams^{1,2,*}

¹ Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA

² Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA

³ Department of Obstetrics, Gynecology, and Reproductive Sciences, Division of Maternal-Fetal Medicine and Reproductive Infectious Diseases and Immunology, University of Pittsburgh School of Medicine, Magee-Women's Research Institute, Pittsburgh, PA, USA

Abstract

Few studies have examined the coordinated regulation of the extensive network of cytokines, chemokines, and growth factors involved in the immune response to bacterial vaginosis (BV) during pregnancy. We compared these patterns between women with (BV⁺) and without (BV⁻) bacterial vaginosis and between women of African and of European ancestry. This cohort included 83 whites (28 BV⁺ and 55 BV⁻) and 80 blacks (41 BV⁺ and 39 BV⁻). Pair-wise correlations were determined for 28 factors that included cytokines, chemokines, and growth factors. In whites, there were significantly more correlations involving immunoregulatory cytokines in BV⁻ compared with BV⁺ women. In blacks, there were no significant differences in the correlation patterns between BV⁺ and BV⁻ women. Overall, in BV⁻ women, there were no significant differences in the correlation patterns between whites and blacks. Conversely, in BV⁺ women, blacks have a stronger correlated response to infection than whites. This indicates that whites and blacks have different correlated immune responses to BV that may at least partially explain the disparity observed in the prevalence of this disease.

Keywords

Bacterial vaginosis; inflammatory response; correlation patterns

Introduction

Bacterial vaginosis (BV) is a vaginal disorder characterized by a significant decrease in Lactobacilli and an overgrowth of anaerobic bacteria including *Gardnerella vaginalis*, *Prevotella*, and *Mobiluncus* species (Koumans and Kendrick, 2001; CDC, 2004). BV is classified as a condition where a malfunctioning innate or adaptive immune response can lead to colonization of microorganisms in the upper genital tract (Romero et al., 2004). This can result in adverse pregnancy outcomes such as amniotic fluid infection, preterm delivery, and premature rupture of the membranes (Eschenbach, 1993; McGregor and French, 2000;

Corresponding Author: Scott M. Williams, CHGR, Vanderbilt University, 519 Light Hall, Nashville, TN 37232, USA, Telephone: +1-615-322-8036, Fax: +1-615-343-8619, E-mail: E-mail: smwilliams@chgr.mc.vanderbilt.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Goldenberg and Culhane, 2003). The regulation of the response to microorganisms in the genital tract involves a network of cytokines, chemokines, and growth factors, and local innate immunity is critical to this response (Fidel, 2003; Russell et al., 2004). Local protein expression and the coordination of multiple factors are therefore likely to be important indicators of key responses in the innate immune system, and play a critical role in the regulation of immune and inflammatory responses to microorganisms.

The study of inflammation in the setting of lower genital tract infection has focused on a handful of inflammatory molecules, most often considered individually. However, investigating cytokines individually does not provide insight into their *holistic* function as members of a system or network, and it is probable that structured correlations among key cytokines, chemokines, and growth factors that represent networked responses are more important in understanding both susceptibility and response to BV. The coordination of this expression remains unclear, particularly with respect to BV. A few studies have examined correlations among vaginal pro-inflammatory cytokine concentrations and found stronger correlations in BV-positive (BV⁺) compared with BV-negative (BV⁻) women (Cauci et al., 2003; Wasiela et al., 2005; Ryckman et al., 2008a). These studies suggest that there is a strong, correlated response to BV, particularly among pro-inflammatory cytokines.

It is well documented that blacks and whites can exhibit different inflammatory responses to similar stimuli (Myslobodsky, 2001; Hoffmann et al., 2002; Blake and Ridker, 2003; Ness, 2004). In a recent study, we demonstrated that for 28 pro-inflammatory, immunoregulatory, and growth factors, there were no statistically significant differences in cervical cytokine concentration between black and white BV⁺ women (Ryckman et al., 2008b). However, in BV⁻ women, interleukins (IL-) 1 α , 6, and 10 and platelet-derived growth factor beta (PDGF-BB) concentrations were higher in whites than in blacks (Ryckman et al., 2008b). Additionally, blacks are at greater risk of BV than whites, and this risk remains elevated after adjusting for socio-demographic differences such as education and income (Ness et al., 2003). Therefore, it is possible that, even though individual cytokine concentrations were not different between blacks and whites in the presence of BV, the interrelationships of the components of the cytokine network might be different among blacks with BV than among whites. If this is true, then such a difference may contribute to the disparity observed in BV prevalence between black and white women.

To more completely examine the network of cervical cytokine expression, we measured cervical levels of pro-inflammatory cytokines, immunoregulatory cytokines, and growth factors. We assessed the patterns of correlations among these immune factors between BV⁺ and BV⁻ women. We also compared whites with blacks to determine if the correlation patterns of cervical immune factors are different between black and white women with healthy vaginal flora, and whether or not these differences occur as a response to infection.

Materials and Methods

Study participants

A cross-sectional study was performed from a prospective cohort collected at Magee-Women's Hospital in Pittsburgh, PA, from 2003 to 2007. Inclusion criteria for the cohort study were singleton intrauterine pregnancy of less than 13 weeks' gestation. Exclusion criteria included: vaginal bleeding, fetal anomalies, known thrombophilia, pre-gestational diabetes mellitus, chronic hypertension requiring medication, current or planned cervical cerclage, immune compromise (HIV-positive, use of systemic steroids within six months, use of post-transplant immunosuppressive medication), and autoimmune disease (inflammatory bowel disease, systemic lupus erythematosus, rheumatoid arthritis, scleroderma). These exclusions were developed prior to study enrollment because they are believed to be associated with preterm

delivery or an alteration in immune status that would confound the associations we proposed to examine. All women provided demographic, medical, and clinical information through standardized, closed question research interviews administered by research personnel. This study was approved by the University of Pittsburgh Institutional Review Board.

A total of 372 women were initially enrolled in this study. Two hundred and eight women (55.9%) were excluded from this analysis. Exclusion criteria included: absence of a BV score (1.1%), no cytokine measurements (16.1%), presence of *Trichomonas vaginalis* (11.0%), presence of *Neisseria gonorrhoeae* (0.3%), presence of *Chlamydia trachomatis* (4.3%), antibiotic use three months prior to pregnancy (18.0%), and ancestry other than self-identified white or black (14.0%). Women with an intermediate BV score (Nugent score of 4–6, 15.3%) were also excluded because of small numbers. These exclusion criteria were chosen before data analysis because these variables could bias the cytokine measurements being examined. A total of 163 women were included in the analysis. Two vaginal swabs were collected for culture and identification of vaginal flora. BV⁺ women were identified by a vaginal pH $\geq$ 4.7 and a score of 7 through 10 from a Gram-stained vaginal smear interpreted using the Nugent method (Nugent et al., 1991). BV⁻ women were identified by a Nugent score of 0–3. *Trichomonas vaginalis*, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* were evaluated as described in detail previously (Ryckman et al., 2008b). There were 83 white (28 BV⁺ and 55 BV⁻) and 80 black (41 BV⁺ and 39 BV⁻) women included in this study.

Cytokine Characterization

Twenty-eight cytokines were assayed using the Luminex LabMAP® multiplex system and a BeadLyte bead kit (Upstate, Lake Placid, NY, USA). Each assay was run with an intra- and inter-assay variation of <10%. These factors were specifically chosen to represent a broad collection of early response cytokines as well as molecules important in the downstream cascade of inflammatory events. Cytokines were loosely categorized as pro-inflammatory, immunoregulatory or growth factors based on reference materials (Supplemental Table 1). Ten pro-inflammatory cytokines were included: chemokine CC motif ligand 11 (EOTAXIN), interleukins (IL-) 1 α , 1 β , 6, and 8, interferon gamma-inducible protein 10 (IP10), macrophage inflammatory protein 1-alpha (MIP1 α), monocyte chemotactic protein 1 (MCP1), regulated upon activation, normally T-expressed and presumably secreted (RANTES), and tumor necrosis factor alpha (TNF- α). Nine immunoregulatory cytokines were examined: interferon gamma (IFN- γ), interleukins (IL-) 2, 4, 5, 10, 12 subunit p40, 12 subunit p70, 13, and 15. Also, nine growth factors were examined: epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF2), fms-related tyrosine kinase 3 (FLT3), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukins (IL-) 3 and 7, platelet-derived growth factor (PDGF- $\alpha\alpha$ and PDGF- $\alpha\beta/\beta\beta$), and vascular endothelial growth factor (VEGF).

Statistical Analysis

Spearman's rank correlation was calculated to determine correlations for all pairwise combinations of the 28 cytokines, chemokines, and growth factors using JMP-IN® (Sall et al., 2005). The overall correlation structure was assessed with two analyses: 1) testing for differences in the number of correlations present in each group and 2) testing for differences (heterogeneity) in correlation coefficients between each pair of cytokines among groups. The first method determines if the global pattern of cytokine correlations is different between BV statuses or races for any group of cytokines or for an individual cytokine. The second method examines individual cytokine pairs to determine exactly what cytokine correlations are different between groups. McNemar's chi-squared test was performed with Stata statistical software version 9.2 (StataCorp) to determine if the number of correlations for each cytokine and group of cytokines differed by BV status or between blacks and whites. McNemar's exact test was used if there were less than five observations per group. McNemar's test determines

the dependence of categorical data that are matched or paired. For this study, the categorical data are defined as the number of correlations that were significant in both groups, neither group, or either group but not the other. A *p* value of less than 0.05 was considered significant. The results for differences in the number of correlations between BV⁺ and BV⁻ women and between ancestral groups were corrected for multiple testing separately, using false discovery rate (FDR) with a significance of 0.2 (Benjamini and Hochberg, 1995). Because of the large number of factors being studied, only the cytokines significant after FDR correction are presented in the results and discussion.

To test for heterogeneity of correlation coefficients between BV⁺ and BV⁻ women and between ancestral groups, a *t*-test on the Fisher *r*-to-*z* transformations of the Spearman correlation coefficients was performed. This analysis is similar to previous work by both Ryckman *et al.* (2008a) and Velez *et al.* (2008). Owing to the exploratory nature of this analysis these results were not corrected for multiple testing.

Results

Three-hundred and seventy-eight correlations were examined for each of the following four groups: whites, BV⁺ and BV⁻; blacks, BV⁺ and BV⁻ (Supplemental Table 2). In whites there are 180 significant correlations in BV⁺ and 199 in BV⁻ women (Table 1A, Fig. 1A and B). In blacks, 225 correlations in BV⁺ and 206 correlations in BV⁻ women are significant (Table 1B, Fig. 1C and D).

Correlation and heterogeneity patterns by BV status

In whites, there are significantly more correlations involving at least one immunoregulatory cytokine in BV⁻ than BV⁺ women (Table 1A). This is especially true for correlations with IL-4 (Fig. 1A and B, Supplemental Table 3A). Twenty-four correlations between BV⁺ and BV⁻ women are heterogeneous and 20 of these are more correlated in BV⁺ women (Fig. 2A, Supplemental Table 4). This heterogeneity is driven by correlations involving pro-inflammatory cytokines, particularly MIP-1 α (Fig. 2A).

In contrast, there are no significant differences in correlation patterns between BV⁺ and BV⁻ women for any functional group or individual cytokine in blacks (Fig. 1C and D, Table 1B, Supplemental Table 3B). Also, only 13 out of 23 heterogeneous correlations between BV⁺ and BV⁻ women are more correlated in BV⁺ women (Fig. 2B, Supplemental Table 4). This heterogeneity is driven by correlations involving pro-inflammatory cytokines, particularly IL-1 α , which has eight heterogeneous correlations (Fig. 2B).

Correlation and heterogeneity patterns by ancestral groups

In BV⁺ women, blacks have significantly more correlations than whites (Table 2A). This is not due to any one set of factors, but involves correlations with pro-inflammatory cytokines, immunoregulatory cytokines, and growth factors. Specifically, the pro-inflammatory cytokine RANTES, the immunoregulatory cytokines IL-4, IL-5, and IL-10, and the growth factors FGF2, FLT3, and PDGF-BB are driving this significance; however, only immunoregulatory cytokines are significant after correction for multiple testing (Supplemental Table 5A). Eleven out of seventeen correlations that showed heterogeneity between whites and blacks are more correlated in whites (Fig. 3A, Supplemental Table 4). However, this does not appear to be driven by any particular group of cytokines (Fig. 3A).

In contrast, the only significant difference observed between blacks and whites in BV⁻ women is between pro-inflammatory cytokines and growth factors; however, no individual cytokine drives this effect (Table 2B, Supplemental Table 5B). The majority of heterogeneous

correlations between whites and blacks are more significant in blacks; however, no particular group of cytokines drives this effect (Fig. 3B, Supplemental Table 4).

Correlations in individuals without *C. albicans*

Because of reports that cytokine production in vaginal epithelial cells is affected by the presence of *Candida albicans* (Steele and Fidel, 2002), we performed a secondary correlation structure analysis excluding 38 individuals with *C. albicans* (whites: 24 BV⁺ and 44 BV⁻, blacks: 31 BV⁺ and 26 BV⁻). The results are generally very similar to the previous ones; however, there are more significant results, especially involving growth factors, in this subset of the data (Supplemental Tables 6 and 7). We are, however, cautious about over-interpreting these results as the sample size is small.

Discussion

The response to infection requires a delicate balance among pro-inflammatory cytokines, immunoregulatory cytokines, and growth factors. It is likely that the expression of these factors is tightly coordinated and this can be detected using correlation analyses; however, few studies have examined networks of correlations with respect to infection with BV. To fully evaluate the pattern of coordination in different groups of cytokines, chemokines, and growth factors, we determined if the number of significant correlations differs between BV⁺ and BV⁻ women and between blacks and whites. Additionally, we examined individual correlation coefficients to determine if they are heterogeneous between BV⁺ and BV⁻ women or between blacks and whites.

In comparisons of BV⁺ with BV⁻ white women we found fewer correlations in BV⁺ samples involving immunoregulatory cytokines, specifically IL-4. It is important to note that for these immunoregulatory cytokines, we previously did not find any significant differences in cervical concentrations between BV⁺ and BV⁻ women (Ryckman et al., 2008b). This indicates that while immunoregulatory cytokine levels are not individually suppressed in BV⁺ women, the functional networks involving these cytokines are less prominent in BV⁺ than in BV⁻ women. The absence of strong correlations among immunoregulatory cytokines in BV⁺ women suggests that these cytokines are not being regulated in a coordinated fashion; if these cytokines, specifically IL-4, IL-10, and IL-13, are not being produced at sufficient levels, overproduction of pro-inflammatory cytokines may occur, which has been observed in women with BV (Platz-Christensen et al., 1993; Sturm-Ramirez et al., 2000; Cauci et al., 2003; Ryckman et al., 2008b). This trend is not observed in blacks; in fact, there are no significant differences in the number of correlations between BV⁺ and BV⁻ women for any group of cytokines, indicating a disparity in the correlated response of cervical cytokines to infection between blacks and whites.

While in whites the number of significant correlations between BV⁺ and BV⁻ women differs only for immunoregulatory cytokines, the differences in specific correlation coefficients is driven by pro-inflammatory cytokines, specifically MIP-1 α . All of the correlations with MIP-1 α are more significant in BV⁺ women. Previously, we found that in whites, cervical concentrations of several pro-inflammatory cytokines, including MIP-1 α , were lower in BV⁻ than in BV⁺ women (Ryckman et al., 2008b). This suggests a hypo-responsiveness to infection that appears to be strongly coordinated.

In blacks, differences in specific correlation coefficients between BV⁺ and BV⁻ women are driven by the pro-inflammatory cytokine IL-1 α . The majority of these are more significantly correlated in BV⁻ women. Previously, we found that in blacks, cervical levels of IL-1 α were elevated in BV⁺ compared with those in BV⁻ women (Ryckman et al., 2008b). This indicates that while there is an increase in cervical levels of IL-1 α in BV⁺ women, this is not correlated

with other cytokine levels, which is particularly interesting, since IL-1 induces and is induced by several other cytokines and growth factors (Dinarello, 1996). However, it is possible that strong correlations are absent in BV⁺ women because the median cervical IL-1 α levels are twice as high as levels in BV⁻ women and other cytokines do not vary to that same degree. As with all correlation analyses, it is unclear if this effect causes BV or is caused by BV. However, if one assumes that the baseline state is the absence of infection, it is reasonable to argue that the changes in correlation patterns that we observed are in fact the result of infections and not the cause of them. This argument is consistent with others made previously, that changes in cytokine levels are a result of BV and not a cause of it (Cauci and Culhane, 2007). In any case, the correlated response involving IL-1 α , an important cytokine for response to infection, is a critical factor in blacks.

It is evident that whites and blacks have different immune responses to infection and have differing susceptibilities to BV (Myslobodsky, 2001; Hoffmann et al., 2002; Blake and Ridker, 2003; Ness, 2004); however, few studies have focused on differences between these groups with respect to the cervical immune response to BV. The number of significant correlations between white and black BV⁻ women did not differ significantly in most comparisons. The one exception was that blacks have more significant correlations between growth factors and pro-inflammatory cytokines. However, in BV⁺ women blacks have more significant correlations than whites for pro-inflammatory cytokines, immunoregulatory cytokines and growth factors. In particular, the immunoregulatory cytokines IL-4, IL-5, and IL-10 exhibit more significant correlations in blacks than in whites. In a previous study examining the same cytokines, IL-1 α , IL-6, IL-10, and PDGF-BB were elevated in white compared with black BV⁻ women (Ryckman et al., 2008b). However, there were no cervical cytokine concentration differences between black and white BV⁺ women (Ryckman et al., 2008b). While in BV⁺ women, individual cytokine levels did not significantly differ between blacks and whites, the correlated response to local infection is stronger in blacks. This hyper-responsive immunity, particularly involving immunoregulatory cytokines, could be a plausible explanation for why blacks with BV are at greater risk of developing adverse reproductive outcomes, such as preterm birth, than their white counterparts.

Few studies have examined the coordinated regulation of an extensive network of cytokines, chemokines, and growth factors involved in the immune response to BV. Our results have identified key factors involved in the coordinated immune response to BV in whites and blacks. These coordinated responses do not differ much between black and white women without BV; however, the coordinated regulation is very different between whites and blacks with BV, an aspect very few studies have addressed. Based on our data, we hypothesize that the coordination of inflammatory factors in the cervix is important in understanding the immune response to BV as well as differences between black and white women with respect to the host consequences of bacterial vaginosis during pregnancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Stat Soc B* 1995;57:289–300.
- Blake GJ, Ridker PM. C-reactive protein and other inflammatory risk markers in acute coronary syndromes. *J Am Coll Cardiol* 2003;41:37S–42S. [PubMed: 12644339]

- Cauci S, Culhane JF. Modulation of vaginal immune response among pregnant women with bacterial vaginosis by *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and yeast. *Am J Obstet Gynecol* 2007;196:133.e1–7. [PubMed: 17306653]
- Cauci S, Guaschino S, De AD, Driussi S, De SD, Penacchioni P, Quadrifoglio F. Interrelationships of interleukin-8 with interleukin-1beta and neutrophils in vaginal fluid of healthy and bacterial vaginosis positive women. *Mol Hum Reprod* 2003;9:53–58. [PubMed: 12529421]
- CDC. STDs and Pregnancy. Centers for Disease Control and Prevention fact sheet. 2004
- Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood* 1996;87:2095–2147. [PubMed: 8630372]
- Eschenbach DA. History and review of bacterial vaginosis. *Am J Obstet Gynecol* 1993;169:441–445. [PubMed: 8357040]
- Fidel PL. Immune regulation and its role in the pathogenesis of candida vaginitis. *Curr Infect Dis Rep* 2003;5:488–493. [PubMed: 14642190]
- Goldenberg RL, Culhane JF. Infection as a cause of preterm birth. *Clin Perinatol* 2003;30:677–700. [PubMed: 14714919]
- Hoffmann SC, Stanley EM, Cox ED, DiMercurio BS, Koziol DE, Harlan DM, Kirk AD, Blair PJ. Ethnicity greatly influences cytokine gene polymorphism distribution. *Am J Transplant* 2002;2:560–567. [PubMed: 12118901]
- Koumans EH, Kendrick JS. Preventing adverse sequelae of bacterial vaginosis: a public health program and research agenda. *Sex Transm Dis* 2001;28:292–297. [PubMed: 11354269]
- McGregor JA, French JI. Bacterial vaginosis in pregnancy. *Obstet Gynecol Surv* 2000;55:S1–19. [PubMed: 10804540]
- Myslobodsky M. Preterm delivery: on proxies and proximal factors. *Paediatr Perinat Epidemiol* 2001;15:381–383. [PubMed: 11703688]
- Ness RB. The consequences for human reproduction of a robust inflammatory response. *Q Rev Biol* 2004;79:383–393. [PubMed: 15669771]
- Ness RB, Hillier S, Richter HE, Soper DE, Stamm C, Bass DC, Sweet RL, Rice P. Can known risk factors explain racial differences in the occurrence of bacterial vaginosis? *J Natl Med Assoc* 2003;95:201–212. [PubMed: 12749680]
- Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol* 1991;29:297–301. [PubMed: 1706728]
- Platz-Christensen JJ, Mattsby-Baltzer I, Thomsen P, Wiqvist N. Endotoxin and interleukin-1 alpha in the cervical mucus and vaginal fluid of pregnant women with bacterial vaginosis. *Am J Obstet Gynecol* 1993;169:1161–1166. [PubMed: 8238178]
- Romero R, Chaiworapongsa T, Kuivaniemi H, Tromp G. Bacterial vaginosis, the inflammatory response and the risk of preterm birth: a role for genetic epidemiology in the prevention of preterm birth. *Am J Obstet Gynecol* 2004;190:1509–1519. [PubMed: 15284723]
- Russell, M.; Sparling, F.; Morrison, R. Mucosal immunology of sexually transmitted diseases. In: Mestecky, J.; Bienenstock, J.; Lamm, M., editors. *Mucosal Immunology*. Elsevier; Oxford: 2004.
- Ryckman KK, Williams SM, Kalinka J. Correlations of selected vaginal cytokine levels with pregnancy-related traits in women with bacterial vaginosis and mycoplasmas. *J Reprod Immunol* 2008a;78:172–180. [PubMed: 18436310]
- Ryckman KK, Williams SM, Krohn MA, Simhan HN. Racial differences in cervical cytokine concentrations between pregnant women with and without bacterial vaginosis. *J Reprod Immunol* 2008b;78:166–171. [PubMed: 18336917]
- Sall J, Creighton L, Lehman A. JMP® Start Statistics. 2005
- Steele C, Fidel PL Jr. Cytokine and chemokine production by human oral and vaginal epithelial cells in response to *Candida albicans*. *Infect Immun* 2002;70:577–583. [PubMed: 11796585]
- Sturm-Ramirez K, Gaye-Diallo A, Eisen G, Mboup S, Kanki PJ. High levels of tumor necrosis factor-alpha and interleukin-1beta in bacterial vaginosis may increase susceptibility to human immunodeficiency virus. *J Infect Dis* 2000;182:467–473. [PubMed: 10915077]

- Velez DR, Fortunato SJ, Morgan N, Edwards TL, Lombardi SJ, Williams SM, Menon R. Patterns of cytokine profiles differ with pregnancy outcome and ethnicity. *Hum Reprod* 2008;23:1902–1909. [PubMed: 18487217]
- Wasiela M, Krzeminski Z, Kalinka J, Brzezinska-Blaszczyk E. [Correlation between levels of selected cytokines in cervico-vaginal fluid of women with abnormal vaginal bacterial flora]. *Med Dosw Mikrobiol* 2005;57:327–333. [PubMed: 16494210]

Figure 1.

Significant cytokine correlation patterns for (A) BV⁻ whites, (B) BV⁺ whites, (C) BV⁻ blacks, and (D) BV⁺ blacks. A shaded cell denotes a significant correlation for a given pair of cytokines, with the darker shade representing stronger correlation coefficients, as indicated in the key. White boxes denote that the correlation between the two cytokines was not significant. A sideways slash denotes a negative correlation.

Figure 2.

Significant heterogeneity between BV⁺ and BV⁻ women for (A) whites and (B) blacks. GF = Growth Factors, IR = Immunoregulatory cytokines, PI = Pro-inflammatory cytokines.

Significant heterogeneity between BV statuses is represented by the presence of a line, with the thickness of the line indicating the significance of heterogeneity. The type of line (solid, dotted, dashed or solid and dotted) indicates the direction of the heterogeneity.

Figure 3.

Significant heterogeneity between whites and blacks for (A) BV⁺ women and (B) BV⁻ women. GF = Growth Factors, IR = Immunoregulatory cytokines, PI = Pro-inflammatory cytokines. Significant heterogeneity between ancestral groups is represented by the presence of a line, with the thickness of the line indicating the significance of heterogeneity. The type of line (solid, dotted, dashed or solid and dotted) indicates the direction of the heterogeneity.

Table 1
Differences in the number of significant correlations between BV⁺ and BV⁻ white (A) and black (B) women

A)					
First Factor	Second Factor	Significant In Both Groups	Significant In BV ⁺ Only	Significant In BV ⁻ Only	Significant In Neither Group
<u>Pooled Comparisons</u>					
ALL	ALL	128	52	71	127
PI	ALL	52	39	30	104
IR	ALL	79	19	44	65
GF	ALL	75	31	39	62
<u>Individual Comparisons</u>					
PI	PI	12	8	9	16
PI	IR	21	11	13	45
PI	GF	19	20	8	43
IR	IR	20	2	10	4
IR	GF	38	6	21	16
GF	GF	18	5	10	3
<u>p value</u>					
					0.10
					0.28
					<0.01*
					0.34
					1.00
					0.84
					0.04
					0.04
					0.01*
					0.30
B)					
First Factor	Second Factor	Significant In Both Groups	Significant In BV ⁺ Only	Significant In BV ⁻ Only	Significant In Neither Group
<u>Pooled Comparisons</u>					
ALL	ALL	159	66	47	106
PI	ALL	65	51	34	75
IR	ALL	100	31	19	57
GF	ALL	93	36	26	52
<u>Individual Comparisons</u>					
PI	PI	11	12	8	14
PI	IR	28	18	9	35
PI	GF	26	21	17	26
IR	IR	27	0	4	5
					0.09
					0.06
					0.12
					0.20
					0.50
					0.12
					0.63
					0.13

B)

First Factor	Second Factor	Significant In Both Groups	Significant In BV ⁺ Only	Significant In BV ⁻ Only	Significant In Neither Group	p value
IR	GF	45	13	6	17	0.17
GF	GF	22	2	3	9	1.00

The first factor denotes the group (GF = Growth Factors, IR = Immunoregulatory cytokines, PI = Pro-inflammatory or ALL= All 28 cytokines) of one of the cytokines in the pairwise correlation. The second factor represents the group of the other cytokine in the pairwise correlation. For example, the correlation between IL-1 α and EOTAXIN would be counted in three groups in the above table: 1) ALL-ALL 2) PI-ALL, and 3) PPI. Each value represents the number of correlations that were significant in both BV⁺ and BV⁻, significant in BV⁺ but not BV⁻, significant in BV⁻ but not BV⁺ or not significant in BV⁺ and BV⁻. The p value tests for the differences between these groups.

* indicates significant after correction for multiple testing.

Table 2
Differences in the number of significant correlations between white and black BV⁺ women (A) or BV⁻ women (B)

A)					
First Factor	Second Factor	Significant In Both Groups	Significant In Whites Only	Significant In Blacks Only	Significant In Neither Group
<u>Pooled Comparisons</u>					
ALL	ALL	140	40	85	113
PI	ALL	68	23	48	86
IR	ALL	81	17	50	59
GF	ALL	81	25	48	53
<u>Individual Comparisons</u>					
PI	PI	15	5	8	17
PI	IR	25	7	21	37
PI	GF	28	11	19	32
IR	IR	19	3	8	6
IR	GF	37	7	21	16
GF	GF	16	7	8	5
					p value
					<0.01*
					<0.01*
					<0.01*
					0.01*
					0.58
					0.01*
					0.20
					0.23
					0.01*
					1.00
B)					
First Factor	Second Factor	Significant In Both Groups	Significant In Whites Only	Significant In Blacks Only	Significant In Neither Group
<u>Pooled Comparisons</u>					
ALL	ALL	154	45	52	127
PI	ALL	56	26	43	100
IR	ALL	100	23	19	65
GF	ALL	89	25	30	63
<u>Individual Comparisons</u>					
PI	PI	11	10	8	16
PI	IR	24	10	13	43
PI	GF	21	6	22	41
					p value
					0.54
					0.04
					0.54
					0.50
					0.81
					0.68
					<0.01*

B)						
First Factor	Second Factor	Significant In Both Groups	Significant In Whites Only	Significant In Blacks Only	Significant In Neither Group	p value
IR	IR	30	0	1	5	1.00
IR	GF	46	13	5	17	0.10
GF	GF	22	6	3	5	0.51

The first factor denotes the group (GF = Growth Factors, IR = Immunoregulatory cytokines, PI = Pro-inflammatory cytokines or ALL= All 28 cytokines) of one of the cytokines in the pairwise correlation. The second factor represents the group of the other cytokine in the pairwise correlation. For example, the correlation between IL-1 α and EOTAXIN would be counted in three groups in the above table: 1) ALL-ALL, 2) PI-ALL, and 3) PIPI. Each value represents the number of correlations that were significant in both whites and blacks, significant in whites but not blacks, significant in blacks but not whites or not significant in blacks and whites. The p value tests for the differences between these groups.

* indicates significant after correction for multiple testing.