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# Neutrophil Extracellular Traps and High Presence of NETotic Cells in Vaginal Discharges of Women with Vaginitis: An Exploratory Study

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## Abstract

Infectious vaginitis is a microbiological syndrome of great importance in public health that affects millions of women worldwide. However, no studies have explored the phenomenon of the production of the neutrophil extracellular traps (NETs) that are released into the female reproductive tract in these pathologies. This study aimed to determine the presence of NETosis in vaginal discharges of women with bacterial vaginosis, candidiasis, and trichomoniasis by characterizing NETs. Extracellular DNA with neutrophil elastase and citrullinated histones was identified to confirm the NET components (n = 10). The concentration, phenotypes of NETs, and number of NETotic cells were determined. The results showed an increase in NETotic cells in women with Candida albicans (CA) and Trichomonas vaginalis (TV) and an increase in NETs in TV-induced vaginitis. Samples of Women with CA- and TV-infections displayed several NET phenotypes (diffNETs, sprNETs, and aggNETs); diffNETs were present in high numbers in samples with CA infections and were elevated in three types of NET infections with TV infections. In contrast to samples with bacterial vaginosis, those with intermediate microbiota displayed higher concentrations of NETs and more NETotic cells. Therefore, changes in the microbiota and the presence of fungal and parasite infections are key factors in the activation and induction of NETosis, and their cytotoxic actions may increase tissue damage

## Introduction

Due to the fact that it affects millions of women globally, infectious vaginitis is a microbiological syndrome of significant public health significance. The most prevalent is bacterial vaginosis (BV), a vaginal dysbiosis caused by a number of bacteria and present in over 30% of reproductive-age women worldwide. With a frequency ranging from 12.1% to 57.3% in symptomatic women, vulvovaginal candidiasis (VVC) is the second most frequent infectious cause of vaginitis after BV. Candida albicans (CA) is the most typical causal agent of VVC [1]. Trichomonas vaginalis, a flagellated protozoan parasite, is what causes trichomoniasis, the most prevalent non-viral sexually transmitted infection in the world, in addition to bacterial and fungal diseases (TV). 173 million people worldwide are infected by TV each year; 32 million of these Epidemiological data in Latin America indicate a prevalence of 3.9% for these diseases, which also occur in sub-Saharan Africa. The host's innate immune system's first line of defence against invasive pathogens is represented by the recruitment of polymorphonuclear neutrophils (PMN) and tissue infiltration to infection sites [2].

Variable PMN concentrations have been observed during vaginal infections with CA, BV and TV according to in vitro studies. In vitro, CA damages tissue by causing edoema, vacuolization, and keratinocyte separation. Phagocytosis, degranulation, and the creation of reactive oxygen species (ROS) are all part of the PMN effector arsenal [3]. Neutrophil extracellular traps (NETs), which are extracellular chromatin structures released by PMN, are of particular interest; These are at the centre of the resurgence of interest in PMN biology.

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For the purpose of capturing and eliminating fungal filaments, the release of NETs from PMN is very crucial. As shown in male urogenital infections, where the presence of bacteria like Ureaplasma urealyticum, Chlamydia trachomatis, and E. coli had a negative impact on sperm quality and was associated with an increase in extracellular traps, this may therefore be a crucial defence mechanism in the physiopathology and outcome of vaginal infections [4].

## Description

In Temuco, Chile, 10 women (n = 10) between the ages of 18 and 51 who were in the follicular phase of the ovarian cycle provided the samples during obstetric consultations at various primary healthcare facilities. The Universidad of La Frontera's Scientific Ethics Committee approved the research protocol (file #045-2017). Each volunteer who took part in the study gave their informed consent [5]. Women over the age of 18 who consulted on their own because of an abnormal vaginal discharge or who were clinically diagnosed at the time of the usual consultation in the examination met the inclusion criteria. Women under the age of 18, those who had received antibiotic treatment within the previous 30 days, and those who were using immunosuppressive medications were excluded from the study Females who displayed immunosuppression, females who were in their period at the time the sample was collected, and females who had sexual activity within the previous 48 hours [6].

Except for the women in the control group, all of the women who participated in this study had symptoms, including abnormal vaginal discharge, itching, and erythema on the vaginal walls and external genitalia. The following slices with samples of TV+ and CA (n = 3), samples of CA+ and TV (n = 3), and samples from the control group that were negative for CA and TV with normal vaginal microbiota according to Nugent's criteria (n = 10) met the eligibility criteria. A total of 125 archival samples were considered for this study [7]. In response to a second scenario, we suggested analysing the samples from the perspective of the microbiota. To do this, an additional sample of TV and CA with bacterial vaginosis was added in order to have a minimum number of samples necessary to do the quantitative analyses.

The control-group samples were taken from archival samples of women who underwent a routine Pap smear collection appointment and displayed clinically normal flows (physiological flows), no external vaginal symptoms (such as erythema or edoema), and no abnormal speculoscopy findings.

According to Nugent's criterion classification, they belonged to samples from healthy patients without an abnormal vaginal discharge and with normal microbiota. The definition of controls was TV and CA negative (as verified by PCR) and Gram staining and the use of Nugent's classification criteria for microbiota help identify the normal microbiota [8].

Nuclear staining and immunofluorescence of H4cit3 were carried out as previously described in order to reveal the various NET phenotypes. An Olympus IX81 inverted fluorescent microscope with an Olympus XM10 digital camera was used to examine the samples [9]. Five randomly chosen photos from each of the three NET types—diffNETs, sprNETs, and aggNETs—were used for microscopical analysis and morphological classification (I) diffNETs were made up of a decondensed extracellular chromatin network stained with compact globular antimicrobial proteins, measuring 15–20 m in diameter; (II) sprNETs were made up of structures in the form of smooth, extended bands of decondensed chromatin with antimicrobial proteins made of fine fibres, measuring 15–17 m in diameter; and (III) aggNETs were made up of a very high density Large DNA-derived aggregates covered with global histones and antimicrobial components are simultaneously formed by PMN. All phenotypes were counted in each sample and compared to those in the control group that didn't have an infection. The average of the diffNETs, sprNETs, and aggNETs computed for each group is used to express data [10].

## Conclusion

In conclusion, ongoing study into the pathogenic processes and early host innate immune responses associated with trichomoniasis, BV, and candidiasis is necessary given the impact these gynaecological infections have on public health. To fully comprehend the role that NETs play in the physiopathology of many infections, it is imperative to understand how they can trap microorganisms. However, because NETotic cells and NETs have cytotoxic properties that can enhance tissue damage, there is growing interest in their potential to be pathogenic. A crucial stimulus for the beginning of PMN recruitment into the vagina, activation of the NETotic process, and ultimate release of NETs appears to be changes in the vaginal microbiota and the presence of pathogens. A large rise in NETs brought on by bacterial vaginosis in the female reproductive system may have an impact.

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