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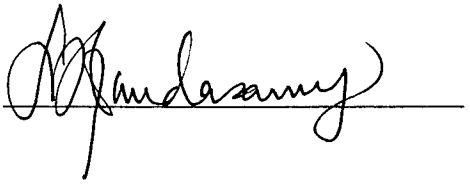

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13. ABSTRACT ***(Maximum 200 Words)***

Lower genital tract infections occur commonly among 17-25 year old women and pose a significant problem for military women especially on deployment. This project is to develop a rapid "self-test kit" for common, treatable cervical/vaginal and urinary tract infections. We have completed the developmental phase of the test kit and tested the performance in 486 women with genital complaints. We have also evaluated women's ability to self test and evaluate their own results in 289 subjects. Additional modifications have been made to correct suboptimal sensitivity, specificity and predictive value. This test exceeded the performance of clinical/wet mount evaluation and syndromic management schemes. A number of additional modifications were made and tested during this year based on sensitivity/specificity and quality assurance testing. Among the 289 women in the self-test phase, all women were successfully able to obtain specimens and perform testing. The patient interpretation of dipstick results was equally successful with 95% agreement between patient and clinician interpretation of results for lactoferrin dipstick and 84% for the pH/amine test. The self-test kit results suggested appropriate treatment in 80% of the cases. Thus, we remain optimistic that this project will result in a self-test kit for use on deployment and/or in other resource poor environments.

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Dan Anderson 10/15/99

PI - Signature Date

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APPENDIX

Introduction

The primary goal of this proposal is to develop a "self-test kit" to be used by military women in the rapid diagnosis of the common, treatable cervical/vaginal and urinary tract infections. Testing is performed on self-collected vaginal (introital) swabs (Q tips) and a urine sample. The secondary goal is to confirm the effectiveness of treating these infections with currently available, effective, single dose, low toxicity agents that could be included in a "self-care kit" (self-test kit plus single dose treatment packs) or administered by medical personnel in the field. The specific technical objectives of this proposal are:

1. To adapt the vaginal lactoferrin test to a simple, easily readable dipstick test to identify infection with *Trichomonas vaginalis, Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*;

2. To evaluate a vaginal amine and pH testing in a simple, easily readable, test for the diagnosis of bacterial vaginosis and *Trichomonas vaginalis*;

3. To combine the vaginal lactoferrin, amine/pH test and urine leukocyte esterase/nitrite dipstick into a simple to use and understand "self-test kit;"

4. To develop a simple and reliable algorithm for military women that combines symptomatology with rapid dipstick testing of vaginal fluid and urine which accurately predicts the presence of cervical/vaginal or urinary tract infections;

5. To test subjects' ability to select appropriate single dose treatment based on symptom/testing algorithm;

6. To demonstrate successful identification and eradication of infections predicted by "self-test kit", verified by "gold standard" diagnostic testing and treated with single dose, low toxicity antimicrobial agents;

Infections of the urogenital tract, particularly by sexually transmitted organisms, are a common and important health related problem to military women. These infections not only affect the mental and physical health of women, they may adversely affect the ability of military women to perform their duties. These conditions and symptoms may also cause embarrassment to women working and living in close quarters. Additionally, these conditions lead to decreased productivity and time off from the workplace for evaluation, diagnosis and treatment. All of these factors may significantly impact the ability and readiness of military women to perform their assigned tasks and duties. Furthermore, the adequately trained health care providers, laboratories and advanced technology required for rapid diagnosis and treatment of these conditions may not always be readily available to deployed military women especially while in remote areas or developing countries. Speculum examination requiring special tables, stirrups, directed lighting and other specialty equipment may not be easily accessible in many deployment situations.

Cervicitis, vaginitis and urinary tract infections occur in upward of 20 million women each year in the United States.[1-4] These infections occur most commonly in the 2nd, 3rd and 4th decade of life. The prevalence of these infections is highest in the 17-25 year old age group particularly the STDs. Thus, these infections will commonly occur among women in the U.S. Armed Services by virtue of their age range alone. Additional considerations including socioeconomic background, increased frequency of sexual activity, numbers of partners and prevalence of STDs in their partner pool will enhance the risk. In one study, of 1,744 military men deployed aboard ship for six months to South America, West Africa and the Mediterranean, 49% reported prior sexual contact with a commercial sex-worker and 22% reported a history of a STD before deployment. During the subsequent six-month deployment, 42% reported sexual contact with a commercial sex-worker, 10% acquired a new STD and 10% reported inconsistent condom use.[5]

Recent preliminary reports from a survey of Army personnel indicate that 18% of women respondents report having had at least one STD over a 2 year period and this may be an underestimate especially if women with an STD history were less likely to respond to the survey.[6] In another study of 476 asymptomatic active duty army women presenting for routine pap smears, 39(8.2%) tested positive for chlamydia. This is a high rate of asymptomatic chlamydia infection. These statistics are further compounded by the facts that only about 50% of all unmarried military personnel report using a condom during last intercourse and women under the age of 25, the age group at highest risk for acquiring an STD, account for two-thirds of the enlisted women that are pregnant at any given time.

There is additional accumulating evidence that other, less obvious, factors may influence the high rate of STDs among military women. Statistics show that 31% of women on active duty in the U.S. Army smoke cigarettes and 17% are heavy smokers.[6] This is significantly higher than the number of smokers in the general population.[7] Several recent studies have demonstrated that smoking is a significant risk factor in the acquisition of numerous STDs including *Chlamydia trachomatis, Neisseria gonorrhoeae* and pelvic inflammatory disease and its sequelae.[8-10]

Delayed diagnosis and treatment of STDs and urinary tract infections may well lead to significant, even life threatening long-term sequelae. Serious renal infections, permanent infertility and life-threatening ectopic pregnancies are all recognized and well documented sequelae of lower urogenital tract infections in women.[1-4] Recent studies also indicate that the presence of these cervical/vaginal STDs significantly increase the risk of HIV acquisition.[11,12]

The most common forms of lower urogenital tract infections in women are cervical and vaginal infections (cervicitis and vaginitis) and bladder or urethral infections (cystitis or urethritis). The sexually transmitted organisms *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are responsible for most cases of cervicitis while *Trichomonas vaginalis,*

Candida species, and bacterial vaginosis account for nearly all cases of infectious vaginitis/vaginosis.[2-4,13,14]

Chlamydial infections are the most common bacterial STDs in the developed world. There are an estimated 4 million chlamydial infections annually in the United States alone with over 2 million occurring in women.[2,15,16] Over a million cases of gonorrhea occur in the United States each year.[2] Presenting complaints include vaginal discharge, dysuria and abnormal uterine bleeding. Both gonorrhea and chlamydia can and often do present with minimal or very subtle symptoms necessitating screening and/or testing for minimal symptomatology in the "at risk" populations. Sequelae of these infections include pelvic inflammatory disease, ectopic pregnancy, permanent infertility and chronic, often debilitating pelvic pain.[2,16]

Infectious vaginitis and vaginosis account for some 8-10 million outpatient visits a year in the United States.[17] The three conditions accounting for the vast majority of these cases are trichomonas vaginitis, candida vaginitis and bacterial vaginosis.

Vaginal yeast infections commonly occur in women. It has been estimated that 75% of women will have at least one episode of yeast vulvovaginitis, with 40-45% having two or more episodes.[18] The predominant organism causing these infections is *Candida albicans,* and occasionally non-albicans candidiasis species (*Candida tropicalis, Candida (Torulopsis) glabrata* or other Candida species). The most common presenting complaint is vaginal and/or vulvar pruritis with or without vaginal discharge, however some 30% of women with yeast infections may present with discharge alone.[19]

An estimated 3 million cases of trichomoniasis occur in the United States annually. This infectious form of vaginitis is caused by *Trichomonas vaginalis*, a sexually transmitted motile protozoan.[14] It accounts for approximately 10-15% of all cases of clinically evident vaginal infections. Infection with this organism is most often characterized by a copious, foul smelling discharge but the clinical presentation can be quite variable including a significant number of women without specific vaginal complaints.

Bacterial vaginosis (formerly known as Gardnerella vaginitis, Haemophilis vaginitis and nonspecific vaginitis) is the most common cause of malodorous vaginal discharge in women.[18] It has been estimated to be the etiology in as many as 45% of women with vaginitis/vaginosis.[14] Bacterial vaginosis (BV) is caused by a shift in the vaginal flora from the normal high concentrations of hydrogen peroxide-producing lactobacilli to a mixed flora consisting of high concentration of anaerobic organisms, *Gardnerella vaginalis* and *Mycoplasma hominis*.[20] This shift in flora is associated with a homogenous, white vaginal discharge, elevated pH (>4.5), the production of amines and the presence of clue cells.

Urinary tract infections, especially bladder infections (cystitis), are the most common bacterial infection in adult women accounting for over 7 million office visits per year in the

United States.[2-4] Lower urinary tract infections may involve the urethra or the bladder. The usual presentation is internal dysuria (not external dysuria which is more associated with vulvar or vaginal infection). Acute urethritis is most often due to *Chlamydia trachomatis* or *Neisseria gonorrhoeae*. The vast majority of lower urinary tract infections in women are cystitis rather than urethritis. Acute, uncomplicated cystitis in young women is caused by *Escherichia coli* 80-90% of the time. The remaining 10-20% are caused by a variety of other organisms usually Gram negative bacteria including *Klebsiella, Proteus, Enterobacter* and *Pseudomonas* spp. and less commonly the Gram positive *Staphylococcus saprophyticus*, group B streptococci and enterococci. Pyelonephritis generally a sequelae of cystitis, is recognizable by fever and lower back pain in addition to dysuria. This condition can require hospitalization and even lead to sepsis.

In summary, urogenital infections are common among military women as in the civilian population, but the nature of deployment may complicate the diagnosis and treatment of these infections. Rapid diagnostic test that could be self-administered in the field without the need for special medical facilities would be logistically and economically advantageous. Single dose treatments are now available and within the standard of care. The 1998 Centers for Disease Control guidelines for the Treatment of Sexually Transmitted Diseases (STDs) were recently released and provide additional single dose treatment regimens for these infections and may further facilitate treatment success against the urogenital infections targeted by this proposal.[18]

Body

The first year of this project was divided into two phases in the original proposal. Phase I, aimed primarily at development of the self-test kit and the data form and Phase II, aimed at collecting specimens from 100 women for evaluation and refinement of the self-test kit. Each "Statement of Work" task listed in the original proposal is printed in italics and addressed separately below.

The nature of this study required that Phase I and II be carried out simultaneously to optimize the available time in accomplishing our stated goals. The overall goal of Phase I and II was to develop our proposed self-test kit, and to compare its sensitivity and specificity to gold standard testing. The intent is to develop a self-test kit for treatable, lower genital tract infections that can direct women to treatment regimens that will result in cure. The primary goal of Phase II was to assess and modify the self-test kit to optimize diagnostic accuracy and treatment efficacy before recruiting more women to test the kit (Phase III).

As outlined in the "Statement of Work" in our original proposal and in accordance with the specific objectives of this project we are now in Phase III and recruiting women for evaluation of the self-test kit. The progress for each task in the "Statement of Work" is described below.

Phase I Tasks:

1. *Determine optimal test format for the Lactoferrin dipstick including establishment of cutoff and appropriate threshold for sensitivity.*

Status: Completed

Lactoferrin dipsticks were provided by TechLab (Blacksburgh, VA) and have highly correlated with enzyme-linked immunosorbant assay (ELISA) values. We have now studied over 200 samples to determine the cutoff value (400ng/ml) that will optimize sensitivity and specificity for identification of STDs. We spent significant time optimizing this test by collecting specimens and performing lactoferrin testing in an additional 134 women (total 234). Studies of receiver-operator curves (ROC) indicated that the optimal cutoff value for distinguishing STDs from BV, yeast, UTI or no infection was 400ng/ml. We also determined that lactoferrin dipsticks read at 90 seconds will reflect as positive all values of 400 and greater.

Lactoferrin results of $\geq$ 400ng/ml have been studied on 483 women and this data for analyzed sensitivity and specificity in predicting the presence of one or more of the three targeted STDs (*Trichomonas vaginalis, Chlamydia trachomatis* and *Neisseria gonorrhoeae*). These results are described below under Phase II, Task 2.

2. *Combine the vaginal lactoferrin, amine/pH test and urine leukocyte esterase/nitrite dipstick into a simple to use and understand self test kit.*

Status: Completed.

This work was completed during Years 1 with the exception of determining the optimal cutoff value for the dipstick (400ng/ml). The lactoferrin dipstick is able to detect levels above 400 ng/ml with a high degree of accuracy and is included in the self-test kit. As we stated in last years progress report, defensins (another soluble product of polymorphonuclear leukocytes recruited to the lower female genital tract in women infected with *Trichomonas vaginalis, Chlamydia trachomatis* or *Neisseria gonorrhoeae*) were studied extensively over the past year for the purpose of improving detection of the STDs. We are now developing a dipstick capable of detecting optimal cutoff level of defensins in vaginal fluid (1100 ng/ml). The defensin data is shown below in Phase II, Task 2. If the defensin dipstick is developed and improves the sensitivity and specificity of the test kit then it will be included.

The pH/Amine test (FemExam card from Litmus Concepts, Calif., USA) is now FDA approved for the diagnosis of bacterial vaginosis and as reported in last year's report is included in the test kit. New cards have been produced to rectify the quality control issue discussed in last year's progress report. The new card (including a foil-wrapped swab is now being employed (see results in Phase II, Task 2).

We are currently collaborating with Litmus Concepts in the development of a yeast detection card able to identify the presence of Candida species, which would significantly enhance the sensitivity and specificity of our test kit. Preliminary results on this new rapid test card were encouraging and a prototype card was developed for preliminary testing. Thirty patients were included in the preliminary trial and the prototype proved to be user-unfriendly, being cumbersome even for the clinician to interpret. Dr. Lawrence of Litmus Concepts is now reconfiguring the test to a more user-friendly format since the *in vitro* data on the Candidal proteins that serve as the basis for the test are very encouraging.

The leukocyte-esterase dipstick has been commercially available for some time now from two different companies the Bayer Multistix and Boehringer Mannheim Chemstrip. We have compared these two tests for detection of leukocytes and nitrites and found that the Chemstrip to have lower numbers of false positives for predicting urinary tract infections. Although neither test was very highly sensitive in this population of women. Since our prevalence of urinary tract infection was so low in women with genital complaints this is not a fair assessment of the test's capacity to identify urinary tract infection. The Chemstrip dipstick has been included in the test kit and with larger numbers of women with urinary tract infection it will be possible to calculate the predictive value of this test in women with genital complaints.

3. *Prepare IRB application and create patient consent forms for IRB approval and patient enrollment.*

Status: Completed.

The IRB application has been prepared and approved at our institution and has been reviewed and approved by The Surgeon General's Human Subject Review Board. This board will require, and be provided with, ongoing information to maintain our approved status.

4. *Establish data collection instruments for patient demographics and relevant specimen information.*

Status: Completed.

Detailed data collection instruments have been created and tested in the first 100 patients. These forms were included in the appendix. They have been further tested in another 132 patients (total=232) and require no additional modification except as will be needed to add new fields for new tests (i.e. defensin dipstick, yeast card etc.).

5. *Develop a database for this information.*

Status: Completed.

An extensive database has been developed and is currently being used in our evaluations. The database contains 349 variables. Their variables include information of demographic and behavioral characteristics, symptoms results of physical examination, and laboratory testing. The data are written onto scannable forms, scanned, verified, labeled and coded, and imported to statistical package (SPSS for Windows) for descriptive analysis.

6. *Develop patient instruction sheets for sample collection and performance of the rapid test kit.*

Status: Completed.

Patient instruction sheets have been created for the collection of vaginal swabs. These sheets have been tested in over 300 patients collecting vaginal swabs for Chlamydia PCR testing and have assisted women in self-collecting specimens that yielded results similar to those obtained on simultaneous clinician-collected samples. These instruction sheets, provided in last years progress report, are successfully being used in Phase III of this study.

Phase II Tasks:

Status: Completed.

1. *Begin recruitment and patient sampling for the self-test kit development phase.*

Women presenting to the study sites with complaints of dysuria or vaginal discharge, itching, burning or irritation, between the ages of 18-40 were recruited as study participants. The exclusion criteria for the study were the use of antibiotics or other treatment for urogenital infections in the past two weeks and age outside the specified age range. During the clinic visit a complete medical history was taken. Upon completion, a pelvic exam was performed on each woman. The clinician collected three simultaneous vaginal (introital) swabs and performed the pH/amine test card, the lactoferrin dipstick, the leukocyte-nitrite dipstick, a wet mount form microscopic examination and recorded the results of each. A clean, unlubricated speculum was be placed into the vagina, and 6 sterile dacron swabs were used to obtain vaginal material from the posterior vaginal fornix. The following tests were performed to evaluate the self-test results and to determine the exact infectious agents present: Swab #1: Lactoferrin and defensin dipstick and ELISA, Swab #2: PCR for *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis*, Swab #3:pH/amine test, bacterial vaginosis Gram stain, Swab #4: wet mount, *Trichomonas* culture, Swab #5: Yeast culture, and Swab #6: *N. gonorrhoeae* culture (cervical). As of July 30,

1999, 486 women were recruited. Ongoing improvements are now tested on samples collected during Phase III recruitment.

The demographics of enrolled women are displayed in Table 1. All women recruited had at least one urogenital complaint including vaginal discharge in 48%, pruritus in 29%, abnormal vaginal odor in 37% and burning or pain in 18%. The results of gold standard testing are shown in Table 2. Among the first 486 patients, gold standard testing indicated that 225(46.3%) women had bacterial vaginosis (BV), of which, 50 women had a concurrent sexually transmitted disease (N. *gonorrhoeae*, *C. trachomatis*, or *T. vaginalis*), 131 women had BV alone and 44 had BV and yeast. There were an additional 46 women with one of the STDs that did not have BV. Overall, 96(20%) of women had one or more of the STDs, specifically 55 had *T. vaginalis,* 34 had *C. trachomatis,* and 20 had *N. gonorrhoeae.* Yeast was cultured from 87 women with no other infection. Pruritis was a presenting complaint in only 36% of these women. In 128(26%) women studied, none of the above pathogens were identified despite symptoms. These results are similar to our overall population of women with genital complaints. In addition to the organisms included in gold standard testing, visual and bimanual examinations did not reveal evidence of any other vaginal disease such as genital herpes or human papilloma virus that might account for their vaginal complaints.

Table 1. Demographics of Women Enrolled

N=486		**Age Mean = 25.2 yr.**		**S.D. = 6.3 yr.**	
Race		**Marital Status**			
African-American	60%	Single	75%		
European-American	38%	Married/Cohabiting	17%		
Multiethnic	1%	Separated/Divorce	8%		
Other	1%				
Employment Status		**Tobacco Use**		**Alcohol Use**	
Employed	67%	Any smoke	61%	Any Use	51%
Full-Time	41%	Heavy Smokers		Heavy Use	
Part-Time	26%	≥1ppd	26%	(Daily)	0.1%
Unemployed	33%	Non-Smokers	39%	None	49%
Douching Habits					
Ever	76%				
Never	24%				

Table 2. Gold Standard Testing

DIAGNOSIS	NUMBER
STD +/- BV	96(20%)
STD, No BV ----------	41
BV+/- YEAST	175(36%)
BV Only ----------	131
YEAST Only	87(18%)
No Organism (neg.)	128(26%)
TOTAL	**486**

2. *Analyze test kit performance compared with "gold standard test results and evaluate the kit's accuracy in predicting the presence of cervical/vaginal infections.*

   Status: Completed

Lactoferrin Testing for STDs

Lactoferrin levels were determined using the Leuko-ELISA Kit (TechLab, Blacksburg, VA.). The vaginal sample is diluted 1:10 in kit diluent and a 100ul aliquot is added to an antibody coated 96 well microtiter plate. The plates are incubated at 37°C for 10 min., washed, conjugate is added and the plate is incubated at 37°C for 10 min. The wash step is repeated and 1 drop of substrate is added, the plate is incubated at room temperature for 5 minutes. Following the substrate incubation, 1 drop of color intensifier is added and the plate is read at 450 nm. A positive test result is an absorbance reading >0.400. The lactoferrin ELISA was performed in Dr. Phillip Heine's laboratory.

Lactoferrin levels have now been determined on clinician-collected vaginal swabs obtained from 483 women. The data on these women was analyzed using several different cutoff values to optimize sensitivity and specificity. The optimal cutoff value was found to be 400ng/ml. This value was determined using receiver-operator curves to identify optimal levels of sensitivity and specificity. The sensitivity/specificity and predictive value of the lactoferrin test using this 400ng/ml cutoff are shown in Table 3.

Table 3. **Sensitivity, specificity and predictive value of lactoferrin test.**

	STD (TV, CT or GC) (+)	STD (TV, CT or GC) (-)	
Lactoferrin > 400 mg/mL	73	201	274
Lactoferrin ≤ 400 mg/mL	23	186	209
	96	387	483

Sensitivity	=	76%
Specificity	=	48%
PPV	=	26%
NPV	=	89%

The fact that 76% of women with an STD had a positive lactoferrin test (sensitivity = 76%) will identify more than three/fourths of infected patients prior to making treatment decisions. While this does not provide the sensitivity of gold standard testing such as PCR, treatment decisions for these three STDs are now made on the basis of wet mount (*T. vaginalis*) which has an estimated sensitivity of 50% and risk assessment and symptoms (*C. trachomatis, N. gonorrhoeae*) which have a sensitivity of 42%. The specificity of lactoferrin was 48% and the positive predictive value 26%. Thus, our data (sens=76%/spec=48%/ppv=26%/npv 89% compares favorably with recently published data on using risk assessment, symptoms and signs as predictors of STDs. In that study, Ryan and colleagues showed a sensitivity of 42%, a specificity of 74% and a positive predictive value of 34% in women with vaginal discharge for predicting the presence of *C. trachomatis* or *N. gonorrhoeae* using risk assessment and symptoms only[23]. The addition of speculum and bimanual examination and microscopy improved these results to a sensitivity of 52%, a specificity of 66% and a positive predictive value of 33%. Since our self-test kit involves no expertise, speculum or examination and is more sensitive and only slightly less specific, there is a great motivation to continue with our test development especially if improved sensitivity and specificity is possible. In this direction, we began last year and continued this year to evaluate other soluble white blood cell (WBC) products that can be measured in vaginal fluid and are potential candidates for colorimetric card or dipstick testing. We have begun ELISA testing for defensins, WBC products that have been highly associated with infection where there is a significant local neutrophil response. The mean defensin level measured from vaginal swabs in 26 women with an STD in our Phase I/II patients was 17,682 ng/ml compared with the 8,899 ng/ml mean value among 28 women without identifiable pathogens by gold standard tests. We have continued to evaluate the remaining women to determine sensitivity/ specificity of defensin in predicting the presence of TV, CT and/or NG in vaginal swabs. We have looked

at various cutpoints to obtain the point at which specificity is optimal. Table 4 shows sensitivity/specificity data on 428 women tested with both lactoferrin and defensin with a cutoff value of 400ng/ml for lactoferrin and 1,100 ng/ml for defensin. Although the addition of defensin measurements enhanced our specificity from 48% to 68%, it did so at the expense of sensitivity (76% to 55%). For this reason we have not continued on with the development of a defensin dipstick at this time.

Table 4. **Sensitivity, specificity and predictive value of combined lactoferrin and defensin tests with and without an STD.**

	STD (TV, CT or GC)		
	(+)	(-)	
Lactoferrin >400 and Defensin > 1,100	46	109	155
One or both below cutpoints	37	236	273
	83	345	428

Sensitivity	=	55%
Specificity	=	68%
PPV	=	30%
NPV	=	86%

pH/Amine Testing for BV

The rapid diagnosis of BV is based on pH and volatile amines (trimethylamines) using the FDA-approved (as of February 1997) FemExam card (Litmus Concepts, Calif., USA). As indicated in last years progress report, improvements in the barrier thickness of the pH/amine card improved sensitivity and specificity among the 180 patients tested with appropriate thickness cards. Among the 479 women tested with the pH/amine card the sensitivity remained greater than 90% and the specificity 62%. (Table 5) There seemed to us to be an inordinate number of false positive tests and further quality assurance investigations were undertaken. The card performed extremely well under laboratory conditions indicating that our sensitivity should be well over 95%, however, it was performing at 62% in the clinical setting. We then investigated each clinical site, evaluating the swabs obtained from these sites. Two significant findings resulted from these on site investigations. The first was that these clinical exam rooms were cleaned (as are most hospital and clinical sites) with an ammonia based cleaning solution. The second finding was that if the swabs in the drawers and open cabinets of these sites were not replaced after room cleaning with new swabs, the

swabs themselves, even though sterile and sealed in paper, could absorb ammonia and turn the amine test card positive when wet. Fresh swabs not exposed to cleaning reagents consistently tested negative. After discussion with Litmus Concepts representatives, it was agreed that all cards would now come with foil, sealed swabs used for testing. If this modification can restore the pH/amine test card to its previously reported sensitivity (>90%), this would reduce the number of false positives in Table 5 from 97 to approximately 10. This would not affect sensitivity of 91% or negative predictive value of 89%, but would enhance specificity from 62% to 96% and positive predictive value from 68% to 95%.

Table 5. **BV diagnosis by Gram stain**

		BV(score ≥7)	Normal/intermed.(<7)	
pH/Amine TESTCARD	Pos	203	97	300
	Neg	19	160	179
		222	257	479

Sensitivity	203/222 = 91%
Specificity	160/257 = 62%
PPV	203/300 = 68%
NPV	160/179 = 89%

Yeast Diagnosis

Our original algorithm depended on women having symptoms of pruritus or burning in the absence of positive lactoferrin and pH/amine testing. Yeast was identified by culture in 87(18%) of women in this study, however, only 31(36%) had symptoms of pruritus. In our collaboration with Dr. Paul Lawrence at Litmus Concepts we are continuing to work on developing an accurate Yeast Card to detect vaginal candida and we are continuing ongoing testing of prototype cards for this purpose. The Candida protein moieties used in the test perform well in *in vitro* studies and will remain the basis for the card development for yeast identification in vaginal swabs.

Urinary Tract Infections by Leukocyte /Esterase Testing

As we previously reported, urinary tract infections were detected by culture in 27/220 (12%) of women. Leukocyte/esterase/nitrites dipsticks identified 11/27(40%) of the women with positive cultures. Since only 2 women had a positive urine culture and nothing else, the majority of positive urine cultures were in women with other infections and it was not clear whether they had symptomatic urinary tract infections or asymptomatic bacteriuria and symptoms from their cervical/vaginal infection. In many cases treatment of their cervical/vaginal infection would also provide efficacy for urinary tract infection. Because so few women had urinary complaints we have subsequently focused our study toward vaginal complaints.

Overall Assessment of Self Test Kit

There were 128/486(26%) women presenting with urogenital tract symptoms that were found to have all negative testing by gold standard tests. This is not unexpected and is consistent with our outpatient clinics and many other populations of symptomatic women. In the typical clinical setting these women are treated empirically, based on symptoms until the results of cultures or other type of diagnostic testing become available. Our rapid tests selected for the self-test kit correctly ruled out infection in 36(29%) of these women obviating the need for unnecessary antimicrobial therapy.

Table 6. **Treatment Algorithm**

pH/Amine	Lactoferrin	Leukocyte Esterase/Nitrite	Proposed Treatment
+	+	+	Azithromycin, Ciprofloxacin, Metronidazole & Fleroxacin
+	+	-	Azithromycin, Ciprofloxacin & Metronidazole
+	-	-	Metronidazole
+	-	+	Metronidazole & Fleroxacin
-	+	+	Azithromycin, Ciprofloxacin & Fleroxacin
-	-	+	Fleroxacin
-	-	-	If pruritis is present treat with Fluconazole

Among 486 patients tested, 96 had one or more of the STDs, the most prevalent of which was TV (55/96). Concurrent BV was identified in 50 women with an STD and our treatment algorithm (see Table 6) for BV is metronidazole 2g, which is also the treatment for TV. Thus, in many cases, women with TV would be cured because of their BV treatment even when their self-test doesn't identify TV. This may further enhance the ability of self-test results to guide selection of curative single dose treatment. Overall, of the 352 women tested with self-test kit that had an infection, 280(80%) would have been directed by the self-test kit to take an antimicrobial agent able to affect a cure.

3. *Analysis of patients' ability to select appropriate single dose treatment based on symptom/testing algorithm.*

Status: Studies ongoing.

We have analyzed data now on 269 women who collected their own specimens and performed the self-test kit on themselves. They were able to perform and accurately read the lactoferrin dipstick test results in 223/235(95%) cases, which was extremely encouraging in this regard. In the case of the pH/amine card test, there was 229/274 (84%) agreement between clinician reading and patient reading. Moreover, compared to gold standard testing clinicians rather than patients tended to over read or over interpret the pH/amine card resulting in decreased sensitivity.

4. *Make any and all modifications to the test kit based on findings from the developmental phase data and make a final form of the kit.*

Status: Completed but subject to ongoing modification.

The test kit currently uses the lactoferrin dipstick, the pH/amine card and the leukocyte esterase/nitrite dipstick and clinical symptoms to determine treatment selection. Defensin dipstick will not be developed, since this test did not significantly improve sensitivity /specificity over that achieved by lactoferrin alone. Yeast card will be added when test development reaches an acceptable level of accuracy.

5. *Refine and finalize instruction sheets as needed to improve the efficiency and scope of the data collection process.*

Status: Completed.

Interview forms and instructions are reviewed on an ongoing basis and modifications will be made as is deemed appropriate. As patients begin to use self-test kit and interpret results this

will become a very important task. As noted above this tool has been developed and used very successfully in the first 269 women tested.

6. *Revise and finalize data collection sheets as needed to improve the efficiency and scope of the data collection process.*

Status: Completed.

The data collection sheets are also reviewed on an ongoing basis to insure the validity and accuracy of collected and entered data. We have to date entered complete data on 486 women enrolled during Phase I, II and III of this project.

Phase III Tasks

1. *Recruitment and enrollment of patients into the study.*

Status: Ongoing.

We have begun recruitment of women to self-collect specimens, perform and interpret the self-test kit results and to choose therapy. Thus far we enrolled 486 women to begin evaluating this phase of the study and the women have shown a remarkable ability to collect specimens, perform the test and interpret results as described above in Phase II, Task 3. We are actively enrolling women in order to meet the goal of 900 women by month 43 of the study.

2. *Sample collection and patient use of the self-test kit.*

Status: Ongoing.
As described above in Task 1, patients are being enrolled and patients are successfully able to collect the samples and perform and interpret the test. In this group of women performing their own self-test we noted an improved sensitivity to 86%, specificity of 76%, ppv of 33% and npv 93% by reading dipstick at 30 seconds rather than 90 seconds. This is likely due to the fact that most true positive values of lactoferrin are well above 400ng/ml and thus turn positive in the first 30 seconds.

3. *Continuous monitoring of patient treatment selection based on symptom/testing algorithm.*

Status: Ongoing.

Patient monitoring of treatment selection is an ongoing part of patient enrollment.

4. *Data collection and entry into the patient database.*

Status: Ongoing.
All data is collected and entered into the database on an ongoing basis.

5. *Laboratory testing and reporting of all patient samples.*

Status: Ongoing.
As with the other tasks in Phase III, this is being done in an ongoing fashion as the patient are enrolled and tested according to protocol.

6. *Respond to all progress inquiries.*

Status: Ongoing.
Responses to all progress inquiries are prepared expeditiously as requested.

Conclusions

The first three years of this project are completed with significant progress being made in developing a rapid self-test kit for symptomatic, treatable lower genital tract infections in women. A number of problems were encountered as described above, including suboptimal specificity of the lactoferrin and pH/amine tests. Troubleshooting and modifications have been or are being made to address each of these problems. We are well into Phase III studies with the kit although minor modifications or additions will be included as they become available (i.e. Yeast Card). We have had no problem recruiting patients for this study and therefore anticipate meeting our recruitment goals by Month 46, three months after that stated in the original statement of work despite additional time being spent optimizing our self-test kits.

It is notable that despite the difficulties outlined above; the self-test kit results would have directed women to appropriate treatment in the vast majority of cases. Specifically, 90% of women with BV alone, 84% of women with an STD and 87% of women with BV and/or an STD would have been directed to appropriate therapy based on lactoferrin and pH/amine testing. Planned improvements in the sensitivity/specificity of these tests will significantly enhance these results. Overall, including all 99 women with self-test results, 63% of women would have received the appropriate treatment decision. If 90% of women with yeast had been identifiable using a yeast card test, then 87% of women with disease by gold standard testing would have been directed to take appropriate therapy. This number may well exceed the number treated appropriately in fully equipped clinical settings. Thus, we remain

optimistic that a successful self-test kit can be developed for women with symptomatic urogenital infections.

We have submitted one manuscript on investigations of SLPI as a potential test to add to the self-test kit in the future (see Appendix). We have three additional manuscripts in preparation. One on the self-test kit itself, which we will complete when we have reached 500 patients (we are at 486 now). A second manuscript is on lactoferrin and defensins as a STD diagnostic tool, and the third compares the self-test kit with standard methods for diagnosis of lower genital tract infections.

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Office of Research Administration
Institutional Review Board

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September 29, 1999

Daniel Landers, M.D.
Dept. of OB/GYN/RS
Magee-Womens Hospital
Pittsburgh, PA 15213

RE: Self-Test Kit: Rapid Diagnosis of Urogenital Infections in Military Women. MWH-95-129

Dear Dr. Landers:

On September 27, 1999, the Institutional Review Board of Magee-Womens Hospital approved your progress report for the above-listed protocol.

Current approval date: September 27, 1999
Next renewal date: September 27, 2000

The requested continuation involves the addition of Christine Donahue, CRNP, as an additional person authorized to give informed consent, and that the Family Health Council - Downtown will be an additional source from which to recruit patients. This is to confirm that your request for continuation is approved.

Please be advised that this protocol has a one year approval period. This approval interval may have changed from your original approval due to modifications in the IRB guidelines concerning risk level definitions and corresponding renewal requirements. By the above renewal date, you must submit a progress report to the committee detailing the number of patients studied, any results and side effects occurring during the study, and a request for continuation, or for closure. You must report any proposed changes in your protocol to the committee prior to their implementation. Any serious or life-threatening complications of the study must be reported to my designate or me by telephone within twenty-four hours of their occurrence. All adverse consequences must be reported in writing to the committee within 14 days of their occurrence. If your study utilizes a consent document, please furnish a copy of the consent to each patient, place one copy in your files, and in Medical Records, if appropriate. The consent document must have the current approval date in the right upper corner. Copies of your research records should be maintained for a minimum of three years after your study is completed.

Sincerely,

Judy Knapp, Ph.D.
Chair, Institutional Review Board
(DHHS Assurance #M-1399, 01)

WAH/mmp

VAGINAL SLPI LEVELS ARE SIGNIFICANTLY DECREASED IN WOMEN WITH SYMPTOMATIC LOWER REPRODUCTIVE TRACT INFECTIONS.

by

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Running title: SLPI in STDs submission to Am J OB-GYN

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Abstract

VAGINAL SLPI LEVELS ARE SIGNIFICANTLY DECREASED IN WOMEN WITH SYMPTOMATIC LOWER REPRODUCTIVE TRACT INFECTION. Deborah L. Draper PhD, Daniel V. Landers MD, Maryjane A. Krohn PhD, Sharon Hillier PhD, Harold C. Wiesenfeld MD, R. Phillips Heine MD.

Secretory leukocyte protease inhibitor (SLPI) is secreted by epithelial cells and contributes to resistance to primary HIV infection in the oral cavity. Sexually transmitted diseases (STDs) are linked to enhanced urogenital transmission of HIV. However, the levels of SLPI in the female genital tract of women with STDs are not well studied.

Objective: To determine if SLPI levels are decreased in vaginal fluids of women presenting with symptomatic or asymptomatic infections.

Methods: We enrolled 202 women and tested for *N. gonorrhoeae* (GC), *T.vaginalis* (TV), *Chlamydia trachomatis* (CT), *Candida spp.* (CA), and bacterial vaginosis (BV) by PCR or standard methods. A asymptomatic pregnant women (n=157) were also screened for TV and bacterial vaginosis (BV). SLPI was measured by ELISA and results compared by non-parametric methods.

Results: SLPI levels were significantly lower in the vaginas of women with any STDs compared to those without infection in both groups ($p<.0001$). Patients with BV, or BV with yeast infection, it was also decreased ($p<.025$).

Conclusions: Decreased SLPI levels during STDs may represent a common mechanism of increasing susceptibility to HIV infection.

Key words: SLPI, STDs, mucosal defenses, HIV infection,

Condensation

Vaginal SLPI levels are significantly decreased in women with symptomatic lower reproductive tract infections including Chlamydia, gonorrhoeae, trichomoniasis, and bacterial vaginosis and may represent a common mechanism of increasing susceptibility to HIV infection.

Introduction

Secretory leukocyte protease inhibitor (SLPI) is a serine protease inhibitor produced by epithelial cells (1), and is a major controller of damage resulting from the inflammatory process (2). It localizes the destructive protease activity to the site of inflammation. SLPI has also been described with antimicrobial function against bacteria, viruses, and fungi (3, 4, 5). It is found in many secretions of the body and in high concentration in saliva (6). More recently SLPI was described as the factor in saliva which inhibits HIV transmission through the oral cavity (7). Inhibition experiments demonstrated that SLPI prevents primary HIV infection of target cells and by a mechanism which may involve inhibition of the uncoating process of the virus (4,8).

Increased HIV transmission has also been linked to co-infection with other sexually transmitted diseases (STDs) which include the ulcerative and non-ulcerative STDs (9). In a position paper, the NIH reviewed many epidemiology studies which linked STDs to a 2-5 fold increased risk of HIV acquisition and transmission by the non-ulcerative STDs including gonorrhea, Chlamydia infection, and trichomoniasis (10). The effect in the presence of ulcerative diseases such as herpes or syphilis can be as high as 8-fold (10). The mechanism(s) of enhancement are unknown. Suggestions include that these diseases have an inflammatory response in common which draws HIV effector cells into the genital tract and microulcerations which allow viral penetration of the mucosal barrier (10). We suggest that these pathogenic processes also may have mucosal effects which alter SLPI concentrations and thus, limit its protective functions.

SLPI has been detected in the genital tract of women (11, 12), but seems to occur in much less quantities there than in saliva (about 33 to 50 fold) (6).

SLPI is also found 1000-fold less in plasma than secretions and it is suggested as the predominant serine protease inhibitor of the mucosal surfaces (13). The role of SLPI in the genital tract is less well studied and the effect of STDs on its epithelial production is unknown. We have recently reported that SLPI is significantly decreased in the vaginal fluids of pregnant women with Trichomoniasis (14). In this report, we extend those studies to included different kinds of STDs and non-pregnant women with symptomatic lower reproductive tract infections. We find that SLPI levels are decreased in vaginal fluids of women with genital tract infections.

Material and Methods

Patient Selection and Specimen Collection.

The first group of patients (n=202) were identified from non-pregnant women attending the Allegheny County Health Department clinics from January 1997 to January 1998 with lower reproductive tract complaints. The patients were screened for urinary tract infection and STDs including trichomoniasis, gonorrhoea, Chlamydia, yeast vaginitis, and bacterial vaginosis. Evidence of infection was determined during pelvic exam and by investigation of vaginal fluids by KOH, wet mount microscopy, Whiff amine test, and pH. Standard culture methods were used to identify gonorrhoeae and yeast (15). PCR techniques were used to identify Chlamydia (Amplicor PCR, Roche Diagnositcs, Raway, NJ) and *Trichomonas vaginalis* infections (16). A vaginal fluid Gram stain score (17) greater than or equal to 7, the presence of "clue" cells, vaginal pH greater than 4.5, and a positive Whiff amine test were used to diagnosis bacterial vaginosis. HPV was not analyzed for beyond inspection for warts.(true???? should this statement be included?)

The second group of patients (n=231) were identified from pregnant women entering prenatal care at Magee-Women's Hospital antenatal clinics between January 1996 and January 1998. Patients were enrolled in the NIH Fetal Maternal Medicine Network study of bacterial vaginosis and trichomoniasis in pregnancy (18). All patients were enrolled under hospital IRB approved protocols. The women in this group were in the first trimester of pregnancy (≤ 20 weeks) and primarily asymptomatic. Women having more than one infection were eliminated from this study group. *T. vaginalis* infections were diagnosed by positive wet mount or culture after 5 days in modified Diamond's medium (Remel, Lenexa, KS). Discrepant results were confirmed by PCR. BV was diagnosed as described above.

The normal, pregnant patients were selected from the same clinic population and underwent the same screening procedures. Pregnant women subsequently found not to have BV or TV (n=78) were matched to the most recent infected patient of the same race and similar gestational age (±2 weeks age difference). This group served as the uninfected, vaginal secretions control.

Swab samples for SLPI analysis were collected from the vaginal posterior fornix on two dacron swabs and frozen at -80°C for batch analysis. Fluids were eluted from swabs into 1 ml of 25 mM Tris-phosphoric acid buffer with 12 mM Tween-80, pH 7.0, and further diluted 1:10 in the same buffer. The final test sample dilutions ranged approximately from 1:100 to 1:1000.

SLPI ELISA assay. Vaginal fluid concentrations of native SLPI were determined using a commercially available ELISA kit (R and D Systems, Minneapolis, MN) based on the methods of Kramps et al (6) and the following manufacturer's protocol. Samples were tested in duplicate and concentrations

were determined by optical densities of colorimetric endpoints read at 450 nm wavelength in a Ceres 900 hdi miniplate spectrophotometer (Biotek Instruments, Winooski, VT). Purified, recombinant SLPI (R and D Systems) was used to construct a standard concentration curve and the assay standards ranged from 0.0625 to 4.0 ng/ml. Sample concentrations were calculated using DeltaSoft 3.0 software for microplate analysis (BioMetallics, Inc., Princeton, NJ) Samples values which were above the standard curve on first assay were further diluted and reassayed. Median values for each group were expressed in nanograms or pg/ml +/- standard error and SLPI differences between the groups were compared using the Mann-Whitney U test. Linear regression analysis was used to assess the impact of gestational age on SLPI levels in pregnant women.

RESULTS

To determine if STDs had any effect on genital SLPI levels, we measured the concentration in vaginal fluids of infected women. A total of 207 women were enrolled through the STD clinic and the distribution of race was 57% African-American, 40% Caucasian of European or Hispanic decent, and 3.0% Asian and bi-racial. Forty patients (20%) were found to have STDs, and the distribution of infections was TV (11%), CT (6.5%), and GC (2.5%) (Table 1). Five patients had two or more STDs (2.5%) and were eliminated from the final analysis. 24 patients (12%) had BV alone. Twenty-two patients (11%) had urinary tract infections, some with STDs.

For the purpose of comparisons, patients were grouped into the following categories. Group I: any STD; Group II : BV alone or BV with yeast; Group III: yeast vaginitis only; and Group IV: no detectable infection. The median SLPI concentrations are seen in Table 1. There was a significant drop in vaginal

levels during STDs ($p<.0001$) and BV ($p<.0245$) but not with yeast infection when values were compared to control patient values. When the median levels were graphed and compared for the various STDs (Figure 1), all STDs had low levels of SLPI. Concentrations were extremely low and frequently below the level of detection of the ELISA, e.g., less than 62 pg/ml. Patients with yeast infection alone had a tendency towards decreased levels but the difference did not reach significance.

A total of 231 pregnant patients were studied for vaginal SLPI levels. (Table 2). Patients ranged in gestational age from 8 to 21 weeks, with a mean age of 14 weeks. The distribution of race was 17% white, 2% Asian and 81% African-American. We were unable to match one Asian patient with a control due to the low incidence of Asians in the clinic population that met the gestational age criteria. Patients with TV (n=79) or BV (n=74) were compared to control patients (n=78) and the results are seen in Figure 2. SLPI levels were also significantly decreased in pregnant patients with these two genital conditions. When regression analysis was performed for SLPI concentration vs. gestational age, there was no correlation (data not shown).

The striking difference between the pregnant and non-pregnant patients was the level of SLPI in vaginal fluids. Uninfected pregnant patients generally had 11 to 12 times as much SLPI in the vaginal vault than non-pregnant controls (Figure 3) suggesting an increased production of SLPI during pregnancy. Even with vaginal or cervical infection during pregnancy, SLPI levels were still higher than those in uninfected, non-pregnant patients.

DISCUSSION

In this series of studies, we have determined the levels of SLPI in vaginal fluids of pregnant and non-pregnant women attending an urban hospital antenatal clinic and inner city STD clinic, respectively. We found that there is a significant reduction in vaginal SLPI levels in both groups of patients during STDs. Our previous work demonstrated that asymptomatic trichomoniasis in pregnant women, significantly decreased SLPI concentrations (14). These studies presented here confirm and extend those studies to pregnant women with BV and to non-pregnant women with other STDs.

SLPI is believed to be produced by cervical epithelial cells and to coat the vaginal epithelial mucosa (6, 12). Our combined results indicate that processes of vaginal and cervical infection alter SLPI concentration. This may be due to altered production by epithelial cells or local pathogenic mechanisms of infection which destroy SLPI structure and/or function. An increase in microbial proteases may be an enzymatic source of such degradative activity (14) or, the increase in inflammatory proteases which destroy mucosal epithelial cells may result in decreased SLPI production (19).

Our knowledge of SLPI gene regulation comes from invitro studies of macrophage and monocyte production (20). The studies indicate that inflammatory cytokines and bacterial LPS both up-regulate SLPI production (20, 21). Studies of SLPI production by lung epithelium indicate that there is a transient increase of SLPI concentration during bacterial pneumonia (22). These increased concentrations are important in protecting the larger airways and bronchi from inflammatory damage of neutrophile elastase, rather than the smaller airspaces where alpha-1-antitrypsin is most important (23). In the genital tract, one might also expect to find increased levels of SLPI

intravaginally during infection but this is not the case. Genital infection correlates to decreased SLPI levels. Further studies will be needed to determine the contribution of pathogenic processes to the regulation of SLPI production in the genital tract.

SLPI has recently been described to have antimicrobial activity against several kinds of microorganisms (3,4,5). It has been suggested that SLPI has an antimicrobial activity which helps to maintain mucosal and microbial floral homeostasis (3). This aspect of function is not destroyed during inflammation; even when cleaved into peptide N- and C-terminal fragments which destroys protease inhibitory activity (24). SLPI retains about 50% of its bacterial antibiotic activity (3). It is during oxidative damage that SLPI loses function (25). These interesting findings suggests that SLPI has multifunctional roles on epithelial surfaces and that any infectious process which disturbs the concentrations of SLPI, will alter its protective functions.

In our previous study, we demonstrated that *Trichomonas vaginalis* cysteine proteases could cleave SLPI into two peptide fragments and destroyed its function as a serine protease inhibitor (14). The studies presented here, indicating loss of measureable SLPI, also suggest that other STDs may destroy SLPI structure. Further studies focused on SLPI structure during infection will sort out the mechanisms of altering SLPI concentrations on this mucosa.

One significant extension of our studies is that STDs may alter SLPI concentration enough to allow HIV infection to be established. The required amount is calculated at about 1 to 2.5 ug/ml as determined from in vitro HIV infectivity studies (4). The amount in cervical mucus is about 50-60 ug/ml and the amount released into vaginal secretions about 60 ng/ml. At first this low vaginal concentration might seem to be a non-significant protective amount, except that SLPI is concentrated at the surface of squamous and columnar

epithelial cells by immunochemical localization studies (26, 27). Although the role of SLPI in genital tract during HIV infection has not been directly established in vivo, there is mounting evidence that it may play some role. For example, women who are HIV seropositive are found to have decreased vaginal levels of SLPI compared to seronegative ones (28). Saliva with high concentrations of SLPI can block primary HIV infection of human peripheral blood mononuclear cells in culture (4). When the saliva is stripped of SLPI and retested, about 80% of the inhibitory activity is lost. Also immunohistochemical co-localization studies by electron microscopy for SLPI antibodies and HIV particles demonstrate that in sublingual and parasalivary glands, virus is never found in cells with high concentrations of SLPI (29).Unfortunately, evidence also exists that SLPI does not prevent HIV replication once the infection has been established (30).

There is also a large body of epidemiological evidence that STDs are linked to increased risk of HIV acquisition (9,10). The mechanism(s) for this increase are unknown but our studies demonstrate that SLPI is decreased and this suggests that it could alter susceptibility. Decreases in SLPI concentration during STDs could be one of the common factors which predisposes the genital tract to HIV transmission.

These and other studies suggest that SLPI is an epithelial cell product which may play an important role in the homeostasis of the mucosal surface in health and disease. Little is known currently about its regulation, secretion and function at the epithelial surface in the genital tract. The regulation here may be unique to this genital site. It seems likely however, that SLPI could serve as a therapeutic anti-inflammatory, a potential topical antimicrobial, and as a preventative strategy for HIV infection. It warrants further study as an example of a new emerging paradigm for HIV prevention and control of STDs.

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TABLE AND FIGURE LEGENDS

Table 1. median SLPI concentrations in vaginal fluids from non-pregnant women attending an urban STD clinic with symptomatic lower reproductive tract infections.

Table 2. A comparison of median SLPI levels in pregnant women with bacterial vaginosis (BV) or Trichomonas vaginalis infection (TV) to uninfected controls. Patients were matched to controls by race and gestational age.

Figure 1. Median SLPI levels in non-pregnant women attending an urban STD clinic with symptomatic lower reproductive tract complaints. STDs included infections with *Trichomonas vaginalis* (TV), *Neisseria gonorrhoeae* (GC), or *Chlamydia trachomatis* (CT). Vaginal concentrations are significantly decreased in patients with STDs, yeast infection, and bacterial vaginosis BV).

Figure 2. Comparison of vaginal SLPI levels in uninfected, pregnant patients in the first trimester of pregnancy and non-pregnant patients. Pregnant women had significantly increased ($p<.0001$) vaginal levels of SLPI and concentrations differed by as much as 11 fold. SLPI may provide one mechanism of protection against infection.

Table 1.
Vaginal SLPI levels in non-pregnant women with infection.

Group	Infection	N	%	SLPI pg/ml	25%, 75%	p value*
I	any STD	40	20	0.0	0 10025	<.0001
II	BV, BV+yeast	65	32	15517	0 79365	0.0245
III	yeast only	38	19	37044	0 100168	0.48
IV	none	59	29	59126	356 121058	

*compared to Group IV

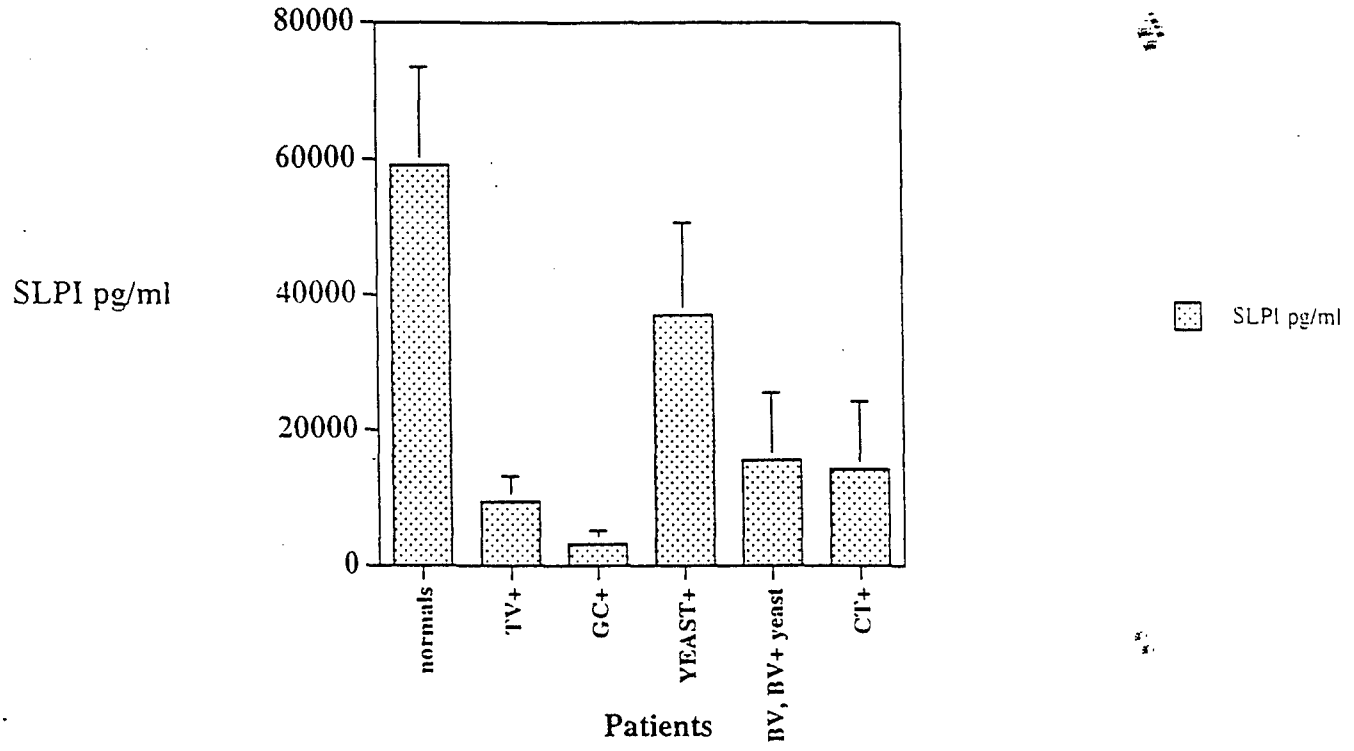
Table 2.
Vaginal SLPI levels in pregnant women with infection.

Group	Infection	N	SLPI ng/ml	p value*
I	BV	74	243	0.025
II	TV	79	167	<.0001
III	none	78	641	

* compared to Group III

Figure 1

SLPI.DODpts.Alldata



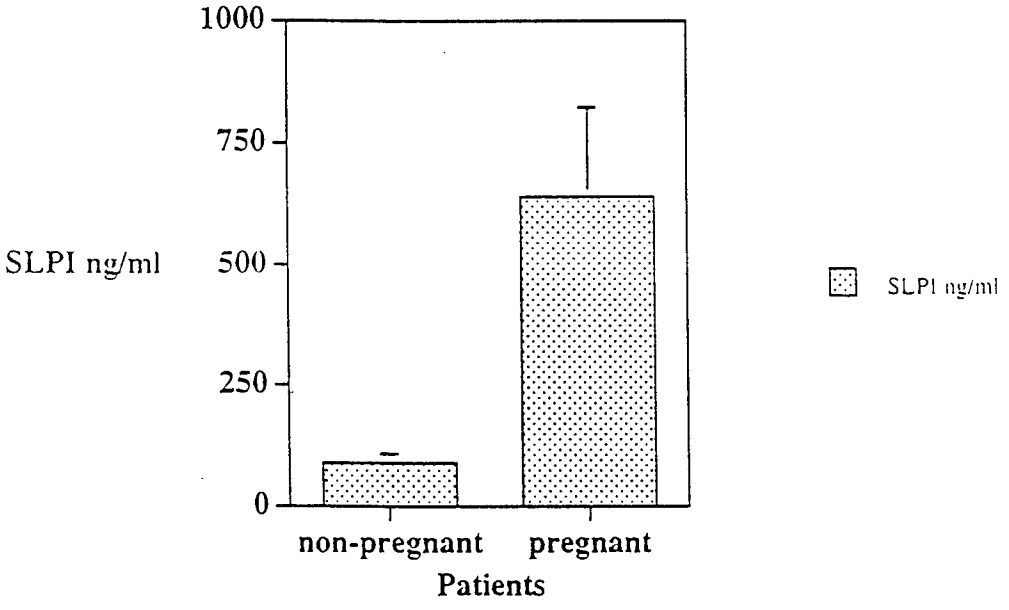

1000
750
500
250
0
SLPI ng/ml
non-pregnant
pregnant
Patients
SLPI ng/ml

Figure 2

DEPARTMENT OF THE ARMY
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REPLY TO
ATTENTION OF:

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FOR THE COMMANDER:

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