# Inflammatory and Tissue Remodeling Urinary Biomarkers before and after Mid Urethral Sling Surgery for Stress Urinary Incontinence

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**Purpose:** Urinary biomarkers were measured in women at baseline and 1 year after surgery for stress urinary incontinence, and associations with clinicodemographic covariates and outcomes were analyzed.

**Materials and Methods:** Preoperative and postoperative urine specimens from 150 women were assayed for inflammatory biomarkers (tumor necrosis factor-α, interferon-γ, interleukin-1β, interleukin-6, interleukin-10, interleukin-12p70, interleukin-17 and nerve growth factor) and tissue remodeling biomarkers (collagenase activity, matrix metalloproteinases-1, 2, 9 and 13, and NTx [N-telopeptide cross-linked collagen], epidermal growth factor and heparin-binding epidermal growth factor-like growth factor). Paired t-tests were used to compare changes in biomarkers during 1 year (significance $p < 0.05$). Linear regression models correlated baseline and changes in biomarker levels with covariates (significance $p \leq 0.001$). Logistic regression models, controlling for age, were used to analyze associations of baseline and changes in biomarker levels with surgical failure (significance $p < 0.05$).

**Results:** During 1 year interleukin-12p70 decreased (mean ± SD 0.53 ± 1.4 to 0.28 ± 0.62 pg/mg creatinine, $p = 0.04$) and nerve growth factor increased (0.034 ± 0.046 to 0.044 ± 0.060 pg/ml/mOsm, $p = 0.03$). Baseline NTx level per mg creatinine was positively associated with age and postmenopausal status ($p = 0.001$), and negatively associated with current estrogen use ($p = 0.0001$). Baseline collagenase activity per mg creatinine was positively associated with age ($p = 0.001$). Epidermal growth factor per mOsm, NTx per mOsm and interferon-γ per mOsm were negatively correlated with age, current estrogen use and UDI (Urogenital Distress Inventory)-irritative subscale score, respectively ($p \leq 0.001$). Subjects with lower baseline NTx per mg creatinine were less likely to experience surgical failure (OR 0.49, 95% CI 0.26–0.93, $p = 0.03$). Changes in biomarker levels were not associated with any covariates or surgical failure.

**Abbreviations and Acronyms**

BMI = body mass index
Cr = creatinine
EGF = epidermal growth factor
HB-EGF = heparin-binding epidermal growth factor-like growth factor
IFN = interferon
IL = interleukin
LUTS = lower urinary tract symptoms
MMP = matrix metalloproteinase
mOsm = milliosmole
NGF = nerve growth factor
OAB = overactive bladder
SUI = stress urinary incontinence
TNF = tumor necrosis factor
UI = urinary incontinence
UTI = urinary tract infection

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**Conclusions:** Stress urinary incontinence surgery was significantly less likely to fail in women with lower baseline NTx levels. Studies are needed to validate NTx as a possible independent biomarker for stress urinary incontinence surgery outcomes.

**Key Words:** biological markers; urinary incontinence, stress; surgical procedures, operative; prognosis; treatment outcome

STRESS urinary incontinence is a highly prevalent and bothersome condition. While there has been increasing interest in the identification and use of biomarkers for lower urinary tract dysfunction, few studies of biomarkers have been performed in women with SUI.[1–3] The identification of clinically useful biomarkers in women with SUI may aid in diagnosis, elucidate its pathophysiology and improve counseling regarding the prognosis of this condition including treatment.

Urinary nerve growth factor has been considered a marker for LUTS secondary to bladder outlet obstruction,[4] OAB[5] and interstitial cystitis/painful bladder syndrome.[6,7] HB-EGF, EGF and antiproliferative factor have been proposed as biomarkers for interstitial cystitis/painful bladder syndrome.[8] Ultimately urinary biomarkers may help to understand the pathophysiology of LUTS, to provide an objective measure of symptom severity and to predict the likelihood of a successful treatment outcome. Although SUI is not generally regarded as a functional bladder problem, sling surgery often increases bladder outlet resistance,[9] which could result in changes in the urinary biomarkers of tissue remodeling and inflammation.

In this report we analyzed urinary inflammatory and tissue remodeling biomarkers measured before and 1 year after continence surgery in a subsample of women participating in the ValUE (Value of Urodynamics Evaluation) trial.[10] The primary hypothesis was that increased urethral resistance after mid urethral sling may result in measureable changes in the amount of certain urinary biomarkers after 1 year. Secondary goals included determining whether preoperative and changes in biomarker levels were associated with baseline clinicodemographic variables, surgical outcomes (success/failure), and baseline LUTS measured with validated patient reported instruments.

## MATERIALS AND METHODS

This UMACS (Urinary Markers after Continence Surgery) trial was an ancillary study to the ValUE trial and received institutional review board approval at each participating institution. UMACS was initiated approximately 8 months after ValUE enrollment began. The details and outcomes of ValUE have been published.[10] Of the UMACS subjects 95% received a mid urethral sling. Successful outcomes at 12 months were defined as a 70% or greater reduction on the UDI,[11] a response of much better or very much better on the PGI-I (Patient Global Impression of Improvement),[12] and a negative provocative stress test. Clean catch urine specimens were obtained just before urodynamics randomization (preoperatively) and at 1 year postoperatively.

Standardized urine specimen processing was performed. All specimens were immediately chilled in ice and centrifuged at 4C, and the supernatant was stored at −80C until assayed. All assays were performed in triplicate as described, and normalized to urinary creatinine (mg/dl) and osmolality (mOsm). Matched specimens (preoperative and postoperative specimens) were always assayed on the same experimental assay plates to minimize effects of inter-test variability. Standard curves were obtained to ensure quality for every biomarker measurement experiment. Tissue remodeling markers included total collagenase activity, MMP-1, MMP-2, MMP-9, MMP-13, NTx, HB-EGF and EGF. Inflammatory markers included TNF-$\alpha$, IFN-$\gamma$, IL-1$\beta$, IL-6, IL-10, IL-12p70, IL-17 and NGF. Personnel performing assays were masked to the clinical information of the subjects.

### Urinary Biomarker Measurements

For HB-EGF measurement a noncommercial ELISA (enzyme-linked immunosorbent assay) using anti-HB-EGF antibody (R&D Systems, Minneapolis, Minnesota) and a goat anti-mouse IgG secondary antibody were used. EGF and MMP-2 were measured by ELISA using kits from R&D Systems. MMP-1 was measured using an ELISA kit from Calbiochem (Billerica, Massachusetts). ELISA multiplex assay kits (Millipore, Billerica, Massachusetts) were used to measure inflammatory markers (NGF, IL-6, IL-1$\beta$, TNF-$\alpha$, IFN-$\gamma$, IL-10, IL-17 and IL-12p70). The amounts of active MMP-9 and MMP-13 were quantified using a Fluorokine E enzyme assay (human active MMP-9 or MMP-13, R&D Systems). The concentration of urinary NTx was measured using an ELISA kit (Osteomark NTx Urine, Wampole Laboratories, Princeton, New Jersey). Total collagenase activity was measured using a collagenase activity assay (Chondrex Inc., Redmond, Washington) as previously described.[13]

### Urinary Cr and mOsm

We decided a priori to normalize to Cr and mOsm based on the lack of clear evidence that one method was superior. Cr was measured by ELISA using a kit from Arbor Assays (Ann Arbor, Michigan). Urinary mOsm was measured using an osmometer per standardized protocol.

### Self-Report Questionnaires and Clinicodemographic Characteristics

Frequency of stress and urge incontinence symptoms was obtained from the MESA (Medical, Epidemiological