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Original Research

Mass spectrometric identification of diagnostic markers for chronic prostatitis in seminal plasma by analysis of seminal plasma protein clinical samples

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Abstract: There are few specific diagnostic markers for chronic prostatitis. Therefore, we used mass spectrometry to evaluate differences in seminal plasma protein expression among patients with prostatitis and young and middle-aged healthy controls. We analysed pooled seminal plasma protein samples from four prostatitis patients (two pools), three young controls (one pool), and three middle-aged controls (one pool). The samples were analysed by liquid chromatography–tandem mass spectrometry. Of the 349 proteins identified, 16 were differentially expressed between the two control pools. Five proteins were up- or down-regulated in both of the prostatitis pools compared to middle-aged controls but not between young and middle-aged pools. Progestagen-associated endometrial protein (PAEP) was over-expressed in prostatitis samples compared to young and middle-aged controls. Our findings and those of previous studies indicate that PAEP is a potential seminal plasma marker for chronic prostatitis. In conclusion, we found age-related changes in seminal plasma protein expression. PAEP expression in seminal plasma should be investigated further to evaluate its potential as a diagnostic marker for chronic prostatitis.

Key words: Prostatitis; Semen; Mass spectrometry.

Introduction

Prostatitis syndromes comprise infectious forms (acute and chronic), the chronic pelvic pain syndrome (CPPS), and asymptomatic prostatitis. A causative uropathogenic organism can be detected in <10% of patients with prostatitis syndrome. CPPS accounts for most of the prostatitis-like symptoms in >90% of men. Chronic prostatitis/CPPS is subclassified as an inflammatory type (NIH category IIIA) and a noninflammatory type (NIH category IIIB) according to the presence of leukocytes in prostatic samples (1). Although the detection of leukocytes confirms chronic prostatis/CPPS type IIIA, in type IIIB no signs of inflammation are observed. The clinical value of this categorization has never been validated (2). Chronic prostatitis/CPPS is a common health issue in men. In Finland, the prevalence of prostatitis symptoms is 14.2% (3). Prostatitis affects quality of life, as psychological stress is common in men with prostatitis (4). Despite its high prevalence, controversy exists regarding the optimal diagnostic methods and therapeutics for chronic prostatitis (2). Bacterial analysis of seminal fluid is used as a diagnostic assay for chronic prostatitis (5-7), but a bacteriological etiology is relatively uncommon. Thus, definitive diagnoses usually can't be made based on seminal fluid bacteriological analyses (8). Sperm concentration, sperm progressive motility, and normal sperm morphology may be negatively affected by chronic prostatitis/CPPS (9).

There are several potential markers for prostatitis in seminal fluid. Interleukin-6 (10-12), interleukin-8 (10, 13, 14), and interleukin-10 (15) have been widely studied as potential markers; however, they are not yet used in clinical settings. Further, immunoglobulin-A (16), elastase (17), peroxidase-positive leukocytes (17), and nerve growth factor (18) are significantly altered in the seminal plasma of patients with prostatitis or chronic pelvic pain syndrome.

Previous studies have performed large-scale seminal fluid proteomic analyses of samples from healthy men (19), men with asthenozoospermia (20), asymptomatic men and after vasectomy (21), before and after varicocele repair (22), and men with prostatitis (23). In 2012, Kagedan et al. (23) identified 59 candidate biomarkers for prostatitis.

It may be useful to differentiate chronic pelvic pain from chronic prostatitis so that patients can be directed to the appropriate specialists with the most extensive knowledge of different treatment options. Patients with prostatitis commonly see urologists, while those with chronic pelvic pain may be best served by pain clinics, depending on local practice. The purpose of the present study was to use mass spectrometry to identify potential diagnostic markers for chronic prostatitis in seminal fluid. We compared the seminal protein expression profiles of four patients with prostatitis with those of asymptomatic young men and asymptomatic middle-aged men.

Materials and Methods

We enrolled four men with chronic prostatitis (aged 31–44 years), three asymptomatic medical students (aged 20–22 years), and three asymptomatic medical professionals (aged 40–49 years) in the study. All of the patients and controls provided written informed consent for participation in the study. All of the subjects were without known hepatitis or HIV infection. We performed seminal fluid bacterial, fungal, mycoplasma, and ureaplasma cultures to exclude infections with these microorganisms. Seminal fluid samples were obtained by masturbation after a minimum of 3 days of sexual abstinence. Routine semen analysis was not performed. The research plan was approved by the Ethics Council of Northern Ostrobothnia Hospital District, Finland (number 22/2007, 15.3.2007).

Spermatozoa were immediately spun down, and 1 ml of seminal plasma was frozen with 100 µl of protease inhibitor cocktail (Sigma-Aldrich) and sodium tartrate dehydrate at a final concentration of 40 mM (Sigma-Aldrich). Before storage at −70°C, the sample was centrifuged at 100,000 × *g* for 30 minutes at 4°C to remove cellular debris. Protein concentration was measured with the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories).

Mass spectrometry–based protein quantitation

Before protein quantitation by a mass spectrometry–based method, we pooled the seminal fluid protein samples together as follows: two prostatitis pools, each containing protein from two patients with prostatitis; one pool from the three young controls; and one pool from the three middle-aged controls. We precipitated 500 µg of protein from each of the four pooled samples with six volumes of cold acetone at −20°C for 2 h. Precipitated proteins were dissolved in 100 µl of dissolution buffer (0.5 M triethylammonium bicarbonate), and 100 µg of proteins were sampled for two parallel labellings with iTRAQ, one under denaturing conditions and one without SDS. Proteins were digested and labeled with the iTRAQ 4-plex Reagent Kit (Applied Biosystems). Each sample was digested with 10 µg of sequence-grade modified trypsin (Promega) at +37°C overnight and labeled with the iTRAQ reagents. SDS was removed with Pierce Detergent Removal Spin Columns (Thermo Scientific) according to the manufacturer's instructions. Before mass spectrometric analysis, samples were desalted with OMIX C18 tips (Varian) and peptides were evaporated to dryness in a SpeedVac.

Liquid chromatography–tandem mass spectrometry analysis

Labeled peptides were dissolved in 1% formic acid, and 5 µl (1 µg of peptide) samples were analysed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) on a nanoflow high performance liquid chromatography system (Ultimate 3000, Dionex) coupled to a QSTAR Elite mass spectrometer (Applied Biosystems/MDS Sciex) equipped with a nano-electrospray ionisation source (Proxeon). Peptides were first loaded onto a trapping column (0.3 × 5 mm PepMap C18, LC Packings) and subsequently separated inline on a homemade 15 cm C18 column (75 µm × 15 cm; Magic 5 µm 200 Å C_{18}, Michrom BioResources Inc.). The mobile phase consisted of water/acetonitrile (98:2 (v/v)) with 0.2% formic acid (solvent A) or acetonitrile/water (95:5 (v/v)) with 0.2% formic acid (solvent B). A linear 80 min gradient from 2% to 30% B was used to elute peptides.

MS data were automatically acquired by Analyst QS 1.1 software (Applied Biosystems/MDS Sciex). The information-dependent acquisition method consisted of a time-of flight mass spectrometric survey scan of mass range 350–1500 m/z and product ion scans of mass range 50–2000 m/z. The three most intense peaks over 20 counts with charge state 2–4 were selected for fragmentation.

Data analysis

ProteinPilot software (v. 3.0, Applied Biosystems, MDS-Sciex) was used to identify and quantify proteins with the Paragon™ search algorithm (24). The software compares the relative intensity of proteins present in samples based on the intensity of reporter ions released from each labeled peptide, and it automatically calculates protein ratios and *p* values for each protein. We merged data from the LC-MS/MS analyses and searched against the UniProtKB (v. 2010_09) protein sequence database. Merging data from several analyses usually improves probability values (*p* value). For protein identification, we used 95% confidence (i.e., at least one peptide matched with 95% confidence). Only those proteins for which the *p* value was <0.05 were accepted for quantitation, as recommended by the software manufacturer.

Results

We identified 349 proteins under nondenaturing conditions and 315 proteins under denaturating conditions. Sixteen proteins were differentially expressed between the age control pools (Table 1), and 14 proteins were differentially expressed in both prostatitis pools compared to either the young or middle-aged controls (Table 2). Of the 16 proteins differentially expressed between control pools, ALB, CST4, and SERPINA1 have been described previously as potential candidate biomarkers for prostatitis (23). Only five proteins, KLK3, CLU, AZGP1, PIP, and PAEP, were up- or down-regulated in both of the prostatitis pools compared to the young or middle-aged pool but not between the control pools (Table 2). Only one protein, PAEP, has been described previously as a potential candidate biomarker for prostatitis (23). We further identified three proteins that were opposite differentially expressed between prostatitis pools and the same control pool: SEMG1, KLK3, and ORM1 (Table 2).

Discussion

To identify potential diagnostic markers for chronic prostatitis, we characterised the seminal plasma protein expression profiles of patients with prostatitis and asymptomatic controls. Semen quality and fertility are known to decrease during ageing (25, 26); however, to our knowledge, the association between seminal plasma protein expression and age has not been evaluated pre-

Table 1. Proteins differentially expressed between middle-aged and young controls.

Accession No.	Protein name	Symbol	Sequence coverage (%)	*p* value	Expression fold change in middle-aged vs young controls
P04279	Semenogelin-1	SEMG1	80.5	<0.001	−7.2
P08118	Beta-microseminoprotein MSMB	MSMB	36.0	0.048	−6.6
P15309	Prostatic acid phosphatase	ACPP	44.8	<0.001	−4.5
Q53FJ5	Prosaposin	PSAP	24.2	0.005	−2.2
P15144	Aminopeptidase N	ANPEP	10.0	0.003	−2.1
P01009	Alpha-1-antitrypsin	SERPINA1	23.2	0.015	1.9
P02763	Alpha-1-acid glycoprotein 1	ORM1	25.9	0.041	2.1
Q6W4X9	Mucin-6	MUC6	11.5	0.004	2.2
P01036	Cystatin-S	CST4	39.0	0.029	2.7
P54107	Cysteine-rich secretory protein 1	CRISP1	31.3	0.047	3.1
P61916	Epididymal secretory protein E1	NPC2	38.8	0.001	3.3
Q16610	Extracellular matrix protein 1	ECM1	30.6	<0.001	4.6
P41222	Prostaglandin-H2 D-isomerase	PTGDS	25.8	0.004	10.8
P01833	Polymeric immunoglobulin receptor	PIGR	5.0	0.010	11.8
P02787	Serotransferrin	TF	31.9	<0.001	12.9
P02768	Serum albumin	ALB	74.9	<0.001	15.7

Positive values for expression fold change represent proteins that were more abundant in the midde-aged controls. SwissProt accession numbers are presented. Sequence coverage is the percentage of matching amino acids from identified peptides divided by the total number of amino acids in the sequence.

Table 2. Proteins differentially expressed between the two prostatitis pools and the young or middle-aged control pools.

Accession No.	Protein name	Symbol	Sequence coverage (%)	Expression fold change in prostatitis 1 vs young controls (*p*)	Expression fold change in prostatitis 2 vs young controls (*p*)	Expression fold change prostatitis 1 vs middle-aged controls (*p*)	Expression fold change in prostatitis 2 vs middle-aged controls (*p*)	Expression fold change in middle-aged vs young controls (*p*)
P04279	Semenogelin-1	SEMG1	80.5	−2.6 (0.014)*	1.9 (0.001)*	2.7 (0.12)	12.9 (<0.001)*	−7.2 (<0.001)*
P10909	Clusterin	CLU	39.0	−2.2 (0.003)*	−1.1 (0.25)	−2.6 (<0.001)*	−1.3 (0.014)*	1.1 (0.14)*
P15309	Prostatic acid phosphatase	ACPP	48.7	−1.8 (0.006)*	−4.7 (<0.001)*	2.5 (0.007)*	1.0 (0.86)	−4.5 (<0.001)*
P07602	Proactivator polypeptide	PSAP	24.2	−1.7 (0.017)*	−1.6 (0.038)*	1.3 (0.45)	1.4 (0.24)	−2.2 (0.005)*
P25311	Zinc-alpha-2-glycoprotein	AZGP1	52.0	−1.5 (0.049)*	−1.6 (0.018)*	−1.3 (0.067)	−1.4 (0.024)*	−1.2 (0.87)
P02763	Alpha-1-acid glycoprotein 1	ORM1	25.9	1.0 (0.81)	−4.1 (0.20)	−2.2 (0.031)*	−8.4 (0.007)*	2.1 (0.041)*
P07288	Prostate-specific antigen	KLK3	64.2	1.2 (0.71)	−4.5 (<0.001)*	1.8 (0.038)*	−3.0 (0.001)*	−1.5 (0.097)
P12273	Prolactin-inducible protein	PIP	65.8	1.9 (0.006)*	2.5 (0.002)*	1.6 (0.036)*	2.1 (0.013)*	1.2 (0.24)
Q16610	Extracellular matrix protein 1	ECM1	30.6	2.6 (0.001)*	1.9 (0.030)*	−1.8 (0.080)	−2.4 (0.001)*	4.6 (<0.001)*
P01036	Cystatin-S	CST4	39.0	3.6 (0.010)*	2.9 (0.017)*	1.3 (0.27)	1.2 (0.59)	2.7 (0.029)*
P09466	Glycodelin	PAEP	26.2	3.8 (0.014)*	11.4 (0.001)*	1.7 (0.088)	5.0 (0.002)*	2.4 (0.24)
P41222	Prostaglandin-H2 D-isomerase	PTGDS	25.8	6.1 (0.010)*	5.2 (0.043)*	−1.9 (0.11)	−2.1 (0.12)	10.8 (0.004)*
P02787	Serotransferrin	TF	31.9	9.1 (<0.001)*	4.5 (<0.001)*	−1.5 (0.011)*	−3.2 (<0.001)*	12.9 (<0.001)*
P02768	Serum albumin	ALB	74.9	9.5 (<0.001)*	3.5 (<0.001)*	−1.8 (<0.001)*	−5.4 (<0.001)*	15.7 (<0.001)*

Expression changes between prostatitis samples (two biological replicates) and young controls or between prostatitis samples and middle-aged controls are shown. Positive values for expression fold change represent proteins that were more abundant either in the prostatitis pooled samples compared to control pools or in the middle-aged control pool compared to the young control pool. SwissProt accession numbers are presented. Sequence coverage is the percentage of matching amino acids from identified peptides divided by the total number of amino acids in the sequence. *p*, p value. *, p<0.05.

viously by means of mass spectrometry. In the present study, most differences in semen protein profiles occurred between the young and middle-aged controls; only five proteins (KLK3, CLU, AZGP1, PIP, and PAEP) were expressed at similar levels in the control groups while being differentially expressed between both prostatitis samples compared to the young or middle-aged controls. This is a striking difference compared to a previous report (23), in which as many as 59 proteins were differentially expressed between control and prostatitis samples. When we compared our results to those of the previous report (23), we found that proteins ALB, CST4, and SERPINA1 were differentially expressed between our control groups, while Kagedan

et al. (23) reported these as potential prostatitis markers. Our results suggest that these proteins were false positives in the previous study (23). Our finding of opposite differential expression of SEMG1, KLK3, and ORM1 between prostatitis samples and other or both control group may be explained by variations in the expression of biological samples.

PAEP has an immunosuppressive function in embryo implantation and tumor development and also induces angiogenesis during embryogenesis and tumor development (27). An isoform of PAEP is found in seminal plasma, and it may contribute to the immunogenic activity of seminal plasma (28). PAEP expression in prostatitis samples was higher than in the young control samples. The functional significance of PAEP overexpression in prostatitis samples remains to be clarified.

Identifying markers for chronic prostatitis in seminal plasma is challenging. Our results found clear age-related changes in protein expression in seminal plasma. Further, clinical diagnosis of chronic prostatitis is not always definitive, because it is often difficult to distinguish between chronic prostatitis and chronic pelvic pain. This represents a considerable challenge when validating potential markers for chronic prostatitis (inflammatory NIH category IIIA chronic prostatitis/CPPS), since any large cohort will contain some men diagnosed with chronic prostatitis without prostatic inflammation (noninflammatory NIH category IIIB chronic prostatitis/CPPS) but with chronic pelvic pain and vice versa. Finally, with the several treatment options available at present, there is no reason to believe that providing patients with a specific diagnosis of chronic prostatitis will lead to them receiving more effective treatment compared to diagnosing them with suspected chronic prostatitis (29-31).

Limitations of our study included the limited number of samples and the use of pooled samples in mass spectrometric analyses. However, using pooled samples diminishes individual changes in protein expression profiles while allowing for the detection of the most prominent and common changes in expression levels, which is a clear benefit. Another limitation was the small number of proteins identified in the different groups, which may have been due to masking of low-abundance proteins by high-abundance proteins. Proteomic methods based on LC-MS/MS do not identify all biologically relevant components, and they exclude analysis of intact proteins. Our results should be validated by quantitative analysis of semen protein extracts by western blotting with specific antibodies.

In summary, we identified proteins in seminal plasma that appeared to be differentially expressed in young vs. middle-aged men. This observation suggests that seminal plasma protein expression analysis may be of limited diagnostic value, except in cases when a specific protein is exclusively expressed or absent in the disease condition. PAEP expression in seminal plasma merits further investigation to evaluate its significance as a diagnostic marker for chronic prostatitis.

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The authors report no conflicts of interest.

Author's contribution
Study design: AR, AM, PT, MV
Acquisition of material: AM, PT
Analysing and interpreting data: AR, MV
Writing the article: AR, MV
Revising of the article: AR, AM, PT, MV

References

1. Krieger JN, Nyberg L,Jr, Nickel JC. NIH consensus definition and classification of prostatitis. Jama 1999; 282:236-7.
2. Magistro G, Wagenlehner FM, Grabe M, Weidner W, Stief CG, Nickel JC. Contemporary Management of Chronic Prostatitis/Chronic Pelvic Pain Syndrome. Eur Urol 2016; 69:286-97.
3. Mehik A, Hellstrom P, Lukkarinen O, Sarpola A, Jarvelin M. Epidemiology of prostatitis in Finnish men: a population-based cross-sectional study. BJU Int 2000; 86:443-8.
4. Mehik A, Hellstrom P, Sarpola A, Lukkarinen O, Jarvelin MR. Fears, sexual disturbances and personality features in men with prostatitis: a population-based cross-sectional study in Finland. BJU Int 2001; 88:35-8.
5. Schaeffer AJ, Knauss JS, Landis JR, Propert KJ, Alexander RB, Litwin MS, et al. Leukocyte and bacterial counts do not correlate with severity of symptoms in men with chronic prostatitis: the National Institutes of Health Chronic Prostatitis Cohort Study. J Urol 2002; 168:1048-53.
6. Punab M, Loivukene K, Kermes K, Mandar R. The limit of leucocytospermia from the microbiological viewpoint. Andrologia 2003; 35:271-8.
7. Schneider H, Ludwig M, Hossain HM, Diemer T, Weidner W. The 2001 Giessen Cohort Study on patients with prostatitis syndrome--an evaluation of inflammatory status and search for microorganisms 10 years after a first analysis. Andrologia 2003; 35:258-62.
8. Korrovits P, Punab M, Turk S, Mandar R. Seminal microflora in asymptomatic inflammatory (NIH IV category) prostatitis. Eur Urol 2006; 50:1338-44.
9. Fu W, Zhou Z, Liu S, Li Q, Yao J, Li W, et al. The effect of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) on semen parameters in human males: a systematic review and meta-analysis. PLoS One 2014; 9:e94991.
10. Paulis G, Conti E, Voliani S, Bertozzi MA, Sarteschi ML, Menchini Fabris F. Evaluation of the cytokines in genital secretions of patients with chronic prostatitis. Arch Ital Urol Androl 2003; 75:179-86.
11. Korrovits P, Ausmees K, Mandar R, Punab M. Seminal interleukin-6 and serum prostate-specific antigen as possible predictive biomarkers in asymptomatic inflammatory prostatitis. Urology 2011; 78:442-6.
12. John H, Maake C, Barghorn A, Zbinden R, Hauri D, Joller-Jemelka HI. Immunological alterations in the ejaculate of chronic prostatitis patients: clues for autoimmunity. Andrologia 2003; 35:294-9.
13. Penna G, Mondaini N, Amuchastegui S, Degli Innocenti S, Carini M, Giubilei G, et al. Seminal plasma cytokines and chemokines in prostate inflammation: interleukin 8 as a predictive biomarker in chronic prostatitis/chronic pelvic pain syndrome and benign prostatic hyperplasia. Eur Urol 2007; 51:524-33.
14. Khadra A, Fletcher P, Luzzi G, Shattock R, Hay P. Interleukin-8 levels in seminal plasma in chronic prostatitis/chronic pelvic pain

syndrome and nonspecific urethritis. BJU Int 2006; 97:1043-6.
15. Miller LJ, Fischer KA, Goralnick SJ, Litt M, Burleson JA, Albertsen P, et al. Interleukin-10 levels in seminal plasma: implications for chronic prostatitis-chronic pelvic pain syndrome. J Urol 2002; 167:753-6.
16. Kastner C, Jakse G. Measurement of immunoglobulins in seminal fluid with modified nephelometry--an alternative diagnostic tool for chronic prostatitis. Prostate Cancer Prostatic Dis 2003; 6:86-9.
17. Ludwig M, Vidal A, Diemer T, Pabst W, Failing K, Weidner W. Chronic prostatitis/chronic pelvic pain pyndrome): seminal markers of inflammation. World J Urol 2003; 21:82-5.
18. Miller LJ, Fischer KA, Goralnick SJ, Litt M, Burleson JA, Albertsen P, et al. Nerve growth factor and chronic prostatitis/chronic pelvic pain syndrome. Urology 2002; 59:603-8.
19. Rolland AD, Lavigne R, Dauly C, Calvel P, Kervarrec C, Freour T, et al. Identification of genital tract markers in the human seminal plasma using an integrative genomics approach. Hum Reprod 2013; 28:199-209.
20. Wang J, Wang J, Zhang HR, Shi HJ, Ma D, Zhao HX, et al. Proteomic analysis of seminal plasma from asthenozoospermia patients reveals proteins that affect oxidative stress responses and semen quality. Asian J Androl 2009; 11:484-91.
21. Batruch I, Lecker I, Kagedan D, Smith CR, Mullen BJ, Grober E, et al. Proteomic analysis of seminal plasma from normal volunteers and post-vasectomy patients identifies over 2000 proteins and candidate biomarkers of the urogenital system. J Proteome Res 2011; 10:941-53.
22. Camargo M, Lopes PI, Del Giudice PT, Carvalho VM, Cardozo KH, Andreoni C, et al. Unbiased label-free quantitative proteomic profiling and enriched proteomic pathways in seminal plasma of adult men before and after varicocelectomy. Hum Reprod 2013; 28:33-46.
23. Kagedan D, Lecker I, Batruch I, Smith C, Kaploun I, Lo K, et al. Characterization of the seminal plasma proteome in men with prostatitis by mass spectrometry. Clin Proteomics 2012; 9:2,0275-9-2.
24. Shilov IV, Seymour SL, Patel AA, Loboda A, Tang WH, Keating SP, et al. The Paragon Algorithm, a next generation search engine that uses sequence temperature values and feature probabilities to identify peptides from tandem mass spectra. Mol Cell Proteomics 2007; 6:1638-55.
25. Kidd SA, Eskenazi B, Wyrobek AJ. Effects of male age on semen quality and fertility: a review of the literature. Fertil Steril 2001; 75:237-48.
26. Eskenazi B, Wyrobek AJ, Sloter E, Kidd SA, Moore L, Young S, et al. The association of age and semen quality in healthy men. Hum Reprod 2003; 18:447-54.
27. Song M, Ramaswamy S, Ramachandran S, Flowers LC, Horowitz IR, Rock JA, et al. Angiogenic role for glycodelin in tumorigenesis. Proc Natl Acad Sci U S A 2001; 98:9265-70.
28. Morris HR, Dell A, Easton RL, Panico M, Koistinen H, Koistinen R, et al. Gender-specific glycosylation of human glycodelin affects its contraceptive activity. J Biol Chem 1996; 271:32159-67.
29. Cohen JM, Fagin AP, Hariton E, Niska JR, Pierce MW, Kuriyama A, et al. Therapeutic intervention for chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS): a systematic review and meta-analysis. PLoS One 2012; 7:e41941.
30. Anothaisintawee T, Attia J, Nickel JC, Thammakraisorn S, Numthavaj P, McEvoy M, et al. Management of chronic prostatitis/chronic pelvic pain syndrome: a systematic review and network meta-analysis. Jama 2011; 305:78-86.
31. Thakkinstian A, Attia J, Anothaisintawee T, Nickel JC. Alpha-Blockers, Antibiotics and Anti-Inflammatories have a Role in the Management of Chronic Prostatitis/chronic Pelvic Pain Syndrome. BJU Int 2012; 110:1014-22.