PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Intravesical administration of combined hyaluronic acid (HA) and chondroitin sulphate (CS) for the treatment of female recurrent urinary tract infections: a European multicenter nested case-control study
AUTHORS	Ciani, Oriana; Arendsen, Erik; Romancik, Martin; Lunik, Richard; Costantini, Elisabetta; Di Biase, Manuel; Morgia, Giuseppe; Fragala', Eugenia; Tomaskin, Roman; Bernat, Marian; Guazzoni, Giorgio; Tarricone, Rosanna; Lazzeri, Massimo

VERSION 1 - REVIEW

REVIEWER	C. van Nieuwkoop Haga Teaching Hospital Dept. of Internal Medicine The Hague, The Netherlands
REVIEW RETURNED	25-Sep-2015

GENERAL COMMENTS	This is an interesting study on a non-antimicrobial preventive treatment for recurrent UTI in women. The authors are complimented with their work as this is by far the largest study to evaluate the clinical and microbiological outcome of combined hyaluronic acid (HA) and chondroitin (CS). There are however some issues, listed in the following, that need to be addressed. **Abstract** -The authors are encouraged to point their conclusions to one or two sentences. Part of the current text belongs to the results section; e.g. data upon total incidence rates and hazard rates. -I disagree with the conclusion that HA + CS reduces the risk of bacteriologically confirmed recurrence of UTI by 49%. The 49% reduction is just a point estimate after adjustment for potential confounders whereas residual bias and confounding by indication still remains and influences the results. The time to first UTI was indeed shorter in the HA+CA group though after adjustment there was no difference. Furthermore, total incidence rate of UTIs was higher in the HA+CS group and after adjustment there was no difference. This is in contrast to the conclusion that HA+CS prevents UTI. Definite conclusions may therefore be drawn more cautiously. The authors are encouraged to discuss these discrepant results more thoroughly. **Methods** Follow-up and data collection. -The schedule of follow-up is unclear. Were there any routine checks

(including urine cultures) during several predefined time points and what were the standard instructions to the patients in case symptoms of UTI occurred? If so, was the routine follow-up similar in both groups.

Study outcome.
-The definition of the primary outcome is somewhat misleading. Is it the percentage of patients who have had at least one symptomatic UTI during 12 months of follow-up? As the inclusion criterion is patients with recurrent UTI, from a patient perspective a reduction of recurrences might be a more realistic goal instead of absence of UTI. The authors are encouraged to clarify why they chose this primary endpoint.

Statistical analysis.
-As it is a retrospective study the authors should describe how was dealt with missing data and lost to follow-up. The assumption that missing data, as stated in the discussion, occurred randomly is questionable. I rather assume that the patients in HA+CS were followed more closely as they received an experimental treatment; thus missing data and lost to follow-up are more likely to occur in the group of standard care.
The authors are encouraged to discuss to what extent this may have influenced their results.
-In addition is should also be described in the Methods section for which potential confounders they adjusted for.

Ethical approval and registration.
-Was the study also approved locally at all the participating centers?
-The study was registered at clinicaltrials.gov in October 2013 whereas the inclusion period was 2009-2013. The collaborating centers were informed about data collection during a workshop held in July 2013. Please comment on the statement in the methods section that is was a retrospective case-control study using prospectively collected data. Based on the above, I'll tend to conclude there no prospective element at all.

Results
-As all women with recurrent uncomplicated UTI were included during the study period, it is interesting that 181 women were treated with HA+CS and just 95 women were treated according to standard care. It is hard to believe that in routine urologic practices, women with recurrent UTI are preferably (twice as much women) treated experimentally; e.g. the guideline on urological infections of the European Association of Urology 2015 does not recommend the use of HA+CS to treat recurrent UTI because convincing evidence is lacking.
The authors should clarify this and explain which selection criteria were used to start with HA+CS instead of standard care.

-There are no data on uropathogens and the presence of antimicrobial resistance within the groups. It would be helpful to know these results.

-Details upon the treatment strategy in the standard care group are lacking. Please indicate in Table 1 how many patients were on antimicrobial prophylaxis (and specify which regimen), immunoactive prophylaxis, postcoital prophylaxis cranberries or a combination of those, etc. It case it was allowed for patients on HA+CS to use additional treatment for UTI (e.g. cranberry use), this should also be

	stated in Table 1. -Table 1. The patients in the standard care group were statistically significantly different; more of them were employed, though younger more of them were postmenopausal, they more frequently suffered postcoital UTI, less had dyspareunia and on the contrary they had a lower female sexual function index. Though 63% of the women in both groups were sexually active, these data might suggest that the sexually active women in the standard group did have sexual intercourse more frequently. The authors are encouraged to perform a subgroup analysis including only the 37% women who were not sexually active. -Table 1. The median number of UTIs in the preceding year before inclusion in the study should be added in this table. -A flowchart of the screened, included and analyzed patients is missing. Please add a Figure 1 with a complete overview of the selected patients at each stage according to CONSORT. -The primary and secondary outcome measures are only presented as ratios (OR, IRR, HR). Please specify in detail what happened with the patients during the 12 month follow up in a Table. The following data will be of interest: total number of UTIs per patient, time to first UTI, total number of antibiotic prescriptions per patient and if available mortality, number of hospitalizations per patient and number of doctor's visit. -Table 4. As there are so many missing values, no conclusion can be drawn from these results. I therefore suggest to present this table as a supplement. -Table 5. These data are of specific interest as it suggests a dose related response. Please indicate how many patients actually received ≥5, ≥6 and ≥7 instillations. **Discussion** -The discussion should begin with the main findings of the study and what this adds to our current knowledge on this topic. -The authors are encouraged to speculate upon the reasons for the discrepancy between the observation that more patients (how many?) were free of UTI while there was no effect on the incidence and hazard rates of recurrent UTI. -The limitations of the study should be discussed more thoroughly; in detail it should be discussed how residual confounding factors (e.g. differences in frequency of sexual intercourse) and the problem of confounding by indication may have influenced their results. -The conclusions section is too long.

REVIEWER	Robert J Evans Wake Forest University Department of Urology Winston-Salem NC USA
REVIEW RETURNED	05-Oct-2015

GENERAL COMMENTS	This is an intriguing study describing the use of GAG replacement as an alternative treatment option for management of recurrent UTIs.

	This is a significant clinical problem and current therapies are not consistently satisfactory. I have several questions that I would like to see addressed.1)How do you explain the findings that the use of HA plus CS instillations seems to lower the risk of UTI recurrence within 12 months but there was no difference in the total incidence rate or the hazard rate 2) in table 3 it looks as if the incidence of UTIs in the group treated with instillations was higher in the 0-90 day group, the 90-180 group, the 180-240 group but much less in the 240-365 group. There seems to be an implication that over time the instillations continue to improve resistance to infection but could there be another reason for this finding? What happened to the patients who developed an infection while on the instillation protocol? Where they treated and then dropped from the study or did they restart the protocol at the beginning ? If there were a lot of UTIs in the first three time points could this have been due to UTIs induced by catheterization ? I would like to see some description of the treatment of those who did have a UTI and how they were addressed in terms of the statistical analysis 3) if this type of instillation treatment proves effective in reducing the incidence of UTIs compared to standard prophylaxis protocols then the extra cost associated with the treatments may be worthwhile but in this time of cost constraints the extra cost may be prohibitive. Is there any role for oral agents to replace the GAG such as oral chondroitin and glucosamine? If oral therapy also helped lower the incidence of UTIs and eliminated the cost of catheterization as well as the risk of UTI caused by the catheterization it might it be a more cost effective option I think this is an excellent concept with important clinical implications with exploring in a randomized trial . I would like some additional discussion to explain these results

VERSION 1 – AUTHOR RESPONSE

Reviewer 1			
1.1	[…] Abstract: The authors are encouraged to point their conclusions to one or two sentences. Part of the current text belongs to the results section; e.g. data upon total incidence rates and hazard rates.	The conclusions in the abstract have been shortened.	*Abstract* "Our results show that bladder instillations of combined HA + CS reduces the risk of bacteriologically confirmed recurrences compared to the current standard management of RUTIs. Total incidence rates and hazard rates were instead non significantly different between the two groups after adjusting for unbalanced factors. In contrast to what happens with antibiotic prophylaxis, the effectiveness of the HA + CS reinstatement therapy improves over time."
1.2	I disagree with the conclusion that HA + CS reduces the risk of bacteriologically confirmed recurrence of UTI by 49%. The 49% reduction is just a point estimate after adjustment for potential confounders whereas	The abstract has been revised with explicit report of results of total number of recurrences and time to first recurrence. Article summary has been updated as well. In the discussion session additional explanation is given on the meaning of these findings.	*Abstract* "181 patients treated with HA + CS and 95 patients treated with standard of care from 7 centers were included. The crude and adjusted OR (95% CI) for the primary endpoint were 0.77 (0.46 to 1.28) and 0.51 (0.27 to 0.96), respectively. However no evidence of improvement in

	residual bias and confounding by indication still remains and influences the results. The time to first UTI was indeed shorter in the HA+CA group though after adjustment there was no difference. Furthermore, total incidence rate of UTIs was higher in the HA+CS group and after adjustment there was no difference. This is in contrast to the conclusion that HA+CS prevents UTI. Definite conclusions may therefore be drawn more cautiously. The authors are encouraged to discuss these discrepant results more thoroughly.	Also see reply to 2.1 and 2.2.	terms of total number or recurrences (incidence rate ratio (95%CI), 0.99 (0.69 to 1.43)) or time to first recurrence was seen (hazard ratio (95%CI), 0.99 (0.61 to 1.61)). The benefit of intravesical HA + CS therapy improves when the number of instillations is ≥ 5."
1.3	Methods: Follow-up and data collection. The schedule of follow-up is unclear. Were there any routine checks (including urine cultures) during several predefined time points and what were the standard instructions to the patients in case symptoms of UTI occurred? If so, was the routine follow-up similar in both groups.	The follow up was reflective of the current clinical practice at the participating centers and clinical guidelines that, for non-pregnant women, recommend urine culture in symptomatic patients only. We have clarified this aspect in the manuscript. At the time of the protocol design, we discussed on the inclusion of clinical confirmed recurrences instead of bacteriologically confirmed ones, however we agreed the latter represented a more objective endpoint for this study.	*Methods, Study Outcomes* "According to current clinical guidelines, in non-pregnant women, urine culture is recommended in symptomatic patients only."
1.4	Study outcome: The definition of the primary outcome is somewhat misleading. Is it the percentage of patients who have had at least one symptomatic UTI during 12 months of follow-up? As the inclusion criterion is patients with recurrent UTI, from a patient perspective a reduction of recurrences might be a more realistic goal instead of absence of UTI. The authors are	The reviewer is right, the primary outcome reflect the probability of undergoing a first recurrence within 12 months. We believe the clinical outcomes identified for this study (i.e. bacteriologically confirmed recurrence, total number of recurrences and time to first recurrence) are comprehensive and allow to capture a broad effectiveness profile of the HA + CS reinstatement therapy vs the standard treatment in terms of, not	-

	encouraged to clarify why they chose this primary endpoint.	only probability of relapse, but also time to first recurrence and total number of recurrences.	
1.5	Statistical analysis: As it is a retrospective study the authors should describe how was dealt with missing data and lost to follow-up. The assumption that missing data, as stated in the discussion, occurred randomly is questionable. I rather assume that the patients in HA+CS were followed more closely as they received an experimental treatment; thus missing data and lost to follow-up are more likely to occur in the group of standard care. The authors are encouraged to discuss to what extent this may have influenced their results.	As stated in the manuscript, we assumed data missing at random (MAR) (i.e. given the observed data, data are missing independently of unobserved data, that is missing data does not depend on the level of their outcome). This is an assumption less restrictive than "missingness completely at random" (MCAR) (i.e. data are missing independently of both observed and unobserved data). Under the MAR assumption, it could be that there are more missing in the treated than in the controls (or viceversa), however since missingness does not depend on the value of the variables left unobserved, the analysis is unaffected. Nonetheless, we decided to perform additional checking on the data: 1) We restricted the primary analyses to all-complete-cases (i.e. no missing in both outcomes or adjusting factors) and observed that the results were similar to those in the whole sample; 2) For all outcomes and adjusting variables we tested through Fisher's exact test whether proportions of missing was different between HA + CS and Standard of Care groups. No significant difference was observed. As regards the resource consumption, there is a higher number of missing in the HA + CS group. In terms of loss to follow-up, all patients (N = 276) were followed until 12 months after the start of the treatment.	*Discussion* "The issue of missing data was dealt with by assuming they were missing at random (i.e. given the observed data, data are missing independently of unobserved data, that is missing data does not depend on the level of their outcome) and applying pairwise deletion. In this regard, we performed two additional analyses, first by restricting the primary analyses to all-complete-cases (i.e. no missing in both outcomes or adjusting factors). That provided similar results to those presented here (data not shown). Second, for all outcomes and adjusting variables we tested through Fisher's exact test whether proportions of missing was different between HA + CS and Standard of Care groups. No significant difference was observed with the exception of the resource consumption where the number of missing was higher in the HA + CS group."
1.6	In addition it should also be described in the Methods	Agreed. A sentence has been added to the methods	*Methods, Statistical Analyses* "Adjusting variables were age,

	section for which potential confounders they adjusted for.	section.	body mass index (BMI), employment and menopause status, postcoital infections, dyspareunia, Female sexual function index (FSFI) and severity of RUTI."
1.7	Ethical approval and registration: Was the study also approved locally at all the participating centers?	The study was reviewed and approved at the coordinating center (Dept. of Surgical and Biomedical Science, Urology and Andrology Clinic at the University of Perugia) by an Independent Ethics Committee.	-
1.8	The study was registered at clinicaltrials.gov in October 2013 whereas the inclusion period was 2009-2013. The collaborating centers were informed about data collection during a workshop held in July 2013. Please comment on the statement in the methods section that is was a retrospective case-control study using prospectively collected data. Based on the above, I'll tend to conclude there no prospective element at all.	"Prospectively" collected data refers to the fact that data were routinely collected at the centers whilst patients were seen at their clinics. However, we realize this might cause misunderstanding hence we deleted this word.	*Methods, Study Design* "This was a EU-based, multicenter, retrospective nested case-control comparison of individual patient data collected from electronic medical records and/or administrative databases available at the participating institutions."
1.9	Results: As all women with recurrent uncomplicated UTI were included during the study period, it is interesting that 181 women were treated with HA+CS and just 95 women were treated according to standard care. It is hard to believe that in routine urologic practices, women with recurrent UTI are preferably (twice as much women) treated experimentally; e.g. the guideline on urological infections of the European Association of Urology 2015 does not recommend the use of HA+CS to treat recurrent UTI because convincing evidence is lacking. The authors should clarify this and explain which	We believe the imbalance is due to the fact that the participating centers represent highly specialized organizations where usually patients refer to after standard first line management of RUTIs is rejected. Also please see our replies to comments 0.1 and 0.3 from the editor.	*Results* "The numerical imbalance was probably due to the participating organizations being tertiary referral centers for patients who are not satisfied with standard management of RUTIs."

	selection criteria were used to start with HA+CS instead of standard care.		
1.10	There are no data on uropathogens and the presence of antimicrobial resistance within the groups. It would be helpful to know these results.	We agree this information would be useful, however it is not available in our dataset. We have acknowledged this as a limitation in the discussion.	*Discussion* "Data on uropathogens and antimicrobial resistance within the groups was unfortunately not available from this database, although we know most commonly prescribed antibiotics were Ciprofloxacin (13.2% of all prescriptions), Cefuroxime (6.9%), Fosfomycin (6.9%), Nitrofurantoin (6.4%) and E. Coli bacterial extract (OM-89, 4.8%)."
1.11	Details upon the treatment strategy in the standard care group are lacking. Please indicate in Table 1 how many patients were on antimicrobial prophylaxis (and specify which regimen), immunoactive prophylaxis, postcoital prophylaxis cranberries or a combination of those, etc. In case it was allowed for patients on HA+CS to use additional treatment for UTI (e.g. cranberry use), this should also be stated in Table 1.	Thanks for the suggestion. We have updated Table 1 with this additional information.	Table 1 includes now details on treatment strategies for the control group.
1.12	Table 1. The patients in the standard care group were statistically significantly different; more of them were employed, though younger more of them were postmenopausal, they more frequently suffered postcoital UTI, less had dyspareunia and on the contrary they had a lower female sexual function index. Though 63% of the women in both groups were sexually active, these data might suggest that the sexually active women in the standard group did have sexual intercourse more frequently. The authors are encouraged to perform a subgroup analysis including only the 37% women who	We followed the reviewer's suggestion and performed a subgroup analysis on non-sexually active patients (see Table below). Results similar to those obtained from the overall sample, although significance is lost in all cases. We have included a sentence in the manuscript to introduce the results of this *post hoc* analysis.	*Results, Sensitivity Analyses* "As a post hoc subgroup analysis, we repeated primary analyses in non-sexually active patients only and obtained similar patterns of results as in the whole sample although with loss of statistical significance."

	were not sexually active.		
1.13	Table 1. The median number of UTIs in the preceding year before inclusion in the study should be added in this table.	We believe this information is captured by the variable "Severity of RUTI", which is defined according to the European Association of Urology Guidelines on Urological Infections. The number of urinary tract infections in the last year is essential to identify whether the severity is 1 (i.e. low severity cystitis) or 6 (i.e. extreme severity including organ failure).	-
1.14	A flowchart of the screened, included and analyzed patients is missing. Please add a Figure 1 with a complete overview of the selected patients at each stage according to CONSORT.	According to what is suggested by the STROBE statement a flow diagram has been included.	Figure 1 added.
1.15	The primary and secondary outcome measures are only presented as ratios (OR, IRR, HR). Please specify in detail what happened with the patients during the 12 month follow up in a Table. The following data will be of interest: total number of UTIs per patient, time to first UTI, total number of antibiotic prescriptions per patient and if available mortality, number of hospitalizations per patient and number of doctor's visit.	In the HA + CS group 55.7% of patients showed bacteriologically confirmed recurrences, whereas 62.1% had such recurrence in the standard of care group (p = 0.313). In the HA + CS group there were 121 bacteriologically confirmed recurrences in 61.5 person-year whereas in the standard treatment group there were 59 bacteriologically confirmed recurrences in 51.1 person-year (p = 0.001). The time to first UTI (median (IQR)) is 169.5 days (72.5-341.5) in HA + CS vs 320 days (179-369) (p < 0.001). All patients were alive at 12 months follow up. There were 14 all-cause hospitalizations in the HA + CS and 1 in the control group. In terms of antibiotic prescriptions, (median (IQR)) was 0 (0-1) (mean (SD), 0.99 (1.85)) in HA + CS vs 1 (0-1) in standard of care (mean (SD), 1.31 (2.47)) (p = 0.001). Most commonly prescribed antibiotics were	*Results, Primary Analyses* "In the HA + CS group, 55.7% of patients showed bacteriologically confirmed recurrences, whereas 62.1% had such recurrence in the standard of care group (p = 0.313). […] When the number of recurrences is considered, in the HA + CS group there were 121 bacteriologically confirmed recurrences in 61.5 person-year whereas in the standard treatment group there were 59 bacteriologically confirmed recurrences in 51.1 person-year (p = 0.001). […] All patients were alive at 12 months follow up. There were 14 all-cause hospitalizations in the HA + CS and 1 in the control group."

		Ciprofloxacin (13.2% of all prescriptions), Cefuroxime (6.9%), Fosfomycin (6.9%), Nitrofurantoin (6.4%) and E. Coli bacterial extract (OM-89, 4.8%). This information has been included in the results. Number of visits is reported in Supplementary Table 1.	
1.16	Table 4. As there are so many missing values, no conclusion can be drawn from these results. I therefore suggest to present this table as a supplement.	Agreed.	Table 4 moved to the supplementary material.
1.17	Table 5. These data are of specific interest as it suggests a dose related response. Please indicate how many patients actually received ≥5, ≥6 and ≥7 instillations.	Patients with ≥5, ≥6, ≥7 instillations are 156, 134 and 82 respectively. This information has been added in Table 5.	New Table 4 updated.
Reviewer 2			
2.1	This is an intriguing study describing the use of GAG replacement as an alternative treatment option for management of recurrent UTIs. This is a significant clinical problem and current therapies are not consistently satisfactory. I have several questions that I would like to see addressed.1) How do you explain the findings that the use of HA plus CS instillations seems to lower the risk of UTI recurrence within 12 months but there was no difference in the total incidence rate or the hazard rate.	We thank the reviewer for his feedback. The results seem to discriminate between "responders" and "non-responders". Whilst, after adjusting for confounding, there seems to be a benefit for HA + CS treated patients in terms of avoiding the first bacteriologically confirmed recurrence ("responders"), once the first recurrence occurs, there is no evidence of differential courses of the condition, either in terms of number of infections or time-to-first recurrence, for patients treated with antibiotic or non-antibiotic therapies. The different time and mechanism of actions play also a role in explaining these findings. Initially the antibiotic prophylaxis is certainly more effective, however whilst it decreases with time, the benefits of the GAG reinstement therapy emerge (Table 3). We believe this is an interesting finding that we are able to observe and	*Discussion* " […] The different mechanism of action could explain the apparent reduction in the incidence of UTIs in the group treated with HA + CS instillations compared with standard care when considering later time intervals (Table 3). Whilst antibiotics are immediately effective, although subject and conducive to resistance, GAG layer administration is progressively restoring the epithelium that will protect women from future uropathogen infections."

		discuss because of the choice made around the study outcomes at the time of the study design.	
2.2	In table 3 it looks as if the incidence of UTIs in the group treated with instillations was higher in the 0-90 day group, the 90-180 group, the 180-240 group but much less in the 240-365 group. There seems to be an implication that over time the instillations continue to improve resistance to infection but could there be another reason for this finding?	We believe this finding is reflecting the different mechanism of action of the two treatments. Whilst antibiotics are immediately effective, although subject and conducive to resistance, GAG layer administration is progressively restoring the epithelium that will protect women from future uropathogen infections. Hence in selected "responders" (see above) this effect will appear stronger over time.	*Discussion* "[…] The different mechanism of action could explain the apparent reduction in the incidence of UTIs in the group treated with HA + CS instillations compared with standard care when considering later time intervals (Table 3). Whilst antibiotics are immediately effective, although subject and conducive to resistance, GAG layer administration is progressively restoring the epithelium that will protect women from future uropathogen infections."
2.3	What happened to the patients who developed an infection while on the instillation protocol? Where they treated and then dropped from the study or did they restart the protocol at the beginning? I would like to see some description of the treatment of those who did have a UTI and how they were addressed in terms of the statistical analysis.	Patients who developed a UTI whilst on the HA + CS instillation protocol were treated according to clinical guidelines with antibiotics but could continue the instillations afterward. After the first bacteriologically confirmed recurrence, not only the time to first recurrence was registered, but also the number of additional UTIs.	*Methods, Study outcomes* "Patients who developed a UTI whilst on the HA + CS instillation protocol were treated according to clinical guidelines with antibiotics but could continue the instillations afterward. After the first bacteriologically confirmed recurrence, not only the time to first recurrence was recorded, but also the number of additional UTIs."
2.4	If there were a lot of UTIs in the first three time points could this have been due to UTIs induced by catheterization?	We believe this is unlikely. Catheterisation is performed under sterile condition by nurses trained in the procedure. If we consider two RCTs of similar follow-up on the topic, Damiano et al. recorded no serious adverse events over 12 months follow up, only 3/28 patients on HA+CS reported moderate storage urinary symptoms in the absence of infection, with one requiring anti-inflammatory medication for symptom relief. Also De Vita and Giordano did not record any adverse effect with the procedure over 1 year follow up.	*Discussion* "On the other hand, catheterization-induced UTIs might represent an unintended consequence of this procedure. Previous reports (29, 30) have highlighted good tolerability and safety of the intervention that must be performed in under sterile condition by nurses trained in the procedure."
2.5	If this type of instillation	This is an interesting point.	*Discussion*

	treatment proves effective in reducing the incidence of UTIs compared to standard prophylaxis protocols then the extra cost associated with the treatments may be worthwhile but in this time of cost constraints the extra cost may be prohibitive. Is there any role for oral agents to replace the GAG such as oral chondroitin and glucosamine? If oral therapy also helped lower the incidence of UTIs and eliminated the cost of catheterization as well as the risk of UTI caused by the catheterization it might be a more cost effective option. I think this is an excellent concept with important clinical implications worth exploring in a randomized trial. I would like some additional discussion to explain these results.	We plan to explore also the cost-effectiveness of the two alternative strategies in the future and provide in this manuscript a summary of direct resource consumptions in both groups. As regards the oral non-antibiotic therapy, it seems to be an attractive option although currently available in few countries. Exploratory studies are still ongoing in order to establish the safety and efficacy of such promising therapy.	"As regards the economic profile of the two alternative approaches, it has been reported that the cost of the HA + CS could be even five times higher than the cost for a 6-month antibiotic prophylaxis. […] Future methodologically sound economic evaluation studies are recommended to compare the societal or payer value of the two treatment strategies."

Subgroup analysis - Bacteriologically confirmed recurrence, total number of recurrences and time to first recurrence between HA + CS vs standard of care in non sexually active patients

Outcome	**OR (95% CI)**	**Adjusted* OR (95% CI)**
Bacteriologically confirmed recurrence	0.37 (0.12 to 1.1)	0.27 (0.07 to 1.03)
	IRR (95% CI)	**Adjusted* IRR (95% CI)**
Total number of bacteriologically confirmed recurrence	1.16 (0.76 to 1.76)	0.65 (0.39 to 1.08)
	HR (95% CI)	**Adjusted* HR (95% CI)**
Time to first bacteriologically confirmed recurrence	1.27 (0.70 to 2.33)	0.77 (0.36 to 1.63)

VERSION 2 – REVIEW

REVIEWER	Robert J Evans Wake Forest Baptist Health Winston-Salem NC
REVIEW RETURNED	05-Jan-2016

GENERAL COMMENTS	I enjoyed reviewing this paper and think this is an important topic of great clinical significance. What I have a hard time deciding based on the data presented is whether I would utilize this treatment in practice as it is not at all clear to me whether the patients received

	any real benefit from the instillations . The difference in the recurrence rate between the two groups (55.7 versus 62.1) ic not clinically significant and the total number of recurrences in the treatment group seems to be higher at 61.5 suggesting to me that there is very little benefit to the instillation protocol . I understand that QOL data is not available for all participants but I would be interested in seeing whatever data is available to determine if this treatment was perceived to be helpful and the regimen acceptable in terms of bother, discomfort etc. One concern I have is the statement that it seems to work better the longer one stays on it. is it possible that this is a selection bias as patients will stick with something to which they respond? I have had a long standing interest in GAG replacement therapy as a treatment for IC/BPS with oral replacement and instillation therapy both listed as treatment options in the current AUA guidelines. What is fascinating to me is that much of the original work done in this area at the University of Pennsylvania by Dr. Bob Levin in the 1970's and 1980's was initiated to understand the role of GAG deficiencies in chronic cystitis and the IC/BPS work was a later offshoot. It seems that this concept of GAG replacement as a treatment for recurrent UTIs and not just IC/BPS has come full circle I think this is a topic of great importance and would like to see this information in print but agree that prospective studies are needed . I am simply not convinced based on the data presented that GAG replacement is of any significant benefit for prevention of recurrent UTI

VERSION 2 – AUTHOR RESPONSE

	Reviewers' Comments	Authors' reply	Modifications made on the manuscript
2.1	I enjoyed reviewing this paper and think this is an important topic of great clinical significance. What I have a hard time deciding based on the data presented is whether I would utilize this treatment in practice as it is not at all clear to me whether the patients received any real benefit from the instillations. The difference in the recurrence rate between the two groups (55.7 versus 62.1) is not clinically significant and the total number of recurrences in the treatment group seems to be higher at 61.5 suggesting to me that there is very little benefit to the instillation protocol.	We thank the reviewer for his interest and useful comments. In his first observation he refers to one of the findings whereby 55.7% of patients in the HA + CS group showed bacteriologically confirmed recurrences, vs 62.1% in the standard of care group (p = 0.313). However, these results are unadjusted for confounding and unbalanced characteristics between the two groups. The adjusted result in terms of risk of developing a bacteriologically confirmed recurrence becomes instead positive for the HA + CS treatment (OR 0.51 (0.27 to 0.96)). Given the differences observed in this non-experimental sample, it is important to consider not only the crude but also the	-

		adjusted results provided by all the analyses performed, in order to be able to make a comparison of the relevant outcomes other things being equal. Although we agree with the reviewer about the need of RCT to confirm our observational data, as we have clearly stated in the manuscript, we believe the results of this study are useful to the scientific and clinical community because they report a situation where a non-antimicrobial strategy seems at least comparable to the standard antimicrobial management strategy for the prevention of RUTIs. The availability of an alternative is of great value to counteract the current threat posed by the fast development of antimicrobial resistance.	
2.2	I understand that QOL data is not available for all participants but I would be interested in seeing whatever data is available to determine if this treatment was perceived to be helpful and the regimen acceptable in terms of bother, discomfort etc.	We agree with the reviewer on the importance of patient reported outcomes and patient perspectives around the treatment profile and choice. For this reason we decided to include health related quality of life as one of the secondary outcomes in this study, although routinely collection of this information is still rare. In addition to the results of validated health related quality of life questionnaires (EQ5D, SF-36, supplementary table 1), according to our experience and informal evidence collected during clinics, HA + CS patients do not raise any particular issue with the treatment but are willing to overcome a longstanding and bothersome RUTIs condition. Sometimes they may develop storage urinary symptoms that are however easy to handle.	-
2.3	One concern I have is the statement that it seems to work better the longer one stays on it. Is it possible	We thank the reviewer for this comment. This could be a possibility and we have decided to highlight this in	*Discussion* "Although patients who benefit from the treatment in first place might decide

	that this is a selection bias as patients will stick with something to which they respond?	the discussion.	to undertake higher number of instillations compared to patients who do not benefit immediately, the different mechanism of action could explain the apparent reduction in the incidence of UTIs in the group treated with HA + CS instillations compared with standard care when considering later time intervals (Table 3)."
2.4	I have had a longstanding interest in GAG replacement therapy as a treatment for IC/BPS with oral replacement and instillation therapy both listed as treatment options in the current AUA guidelines. What is fascinating to me is that much of the original work done in this area at the University of Pennsylvania by Dr. Bob Levin in the 1970's and 1980's was initiated to understand the role of GAG deficiencies in chronic cystitis and the IC/BPS work was a later offshoot. It seems that this concept of GAG replacement as a treatment for recurrent UTIs and not just IC/BPS has come full circle. I think this is a topic of great importance and would like to see this information in print but agree that prospective studies are needed. I am simply not convinced based on the data presented that GAG replacement is of any significant benefit for prevention of recurrent UTI.	We agree with the reviewer that innovation paths, in medicine as well as in other disciplines, are not always linear! We also fully agree that further prospective evidence is needed to confirm our observational data.	-

VERSION 3 – REVIEW

REVIEWER	Robert J Evans MD FACS Wake Forest University Winston-Salem NC 27455 USA
REVIEW RETURNED	29-Feb-2016

GENERAL COMMENTS	Thanks for your additional work on this manuscript. I believe you are addressing a very important clinical issue and the results indicate that bladder instillations with a GAGH substitute may be of benefit in preventing or lowering the risk for recurrent UTIs I look forward to the proposed randomized trial