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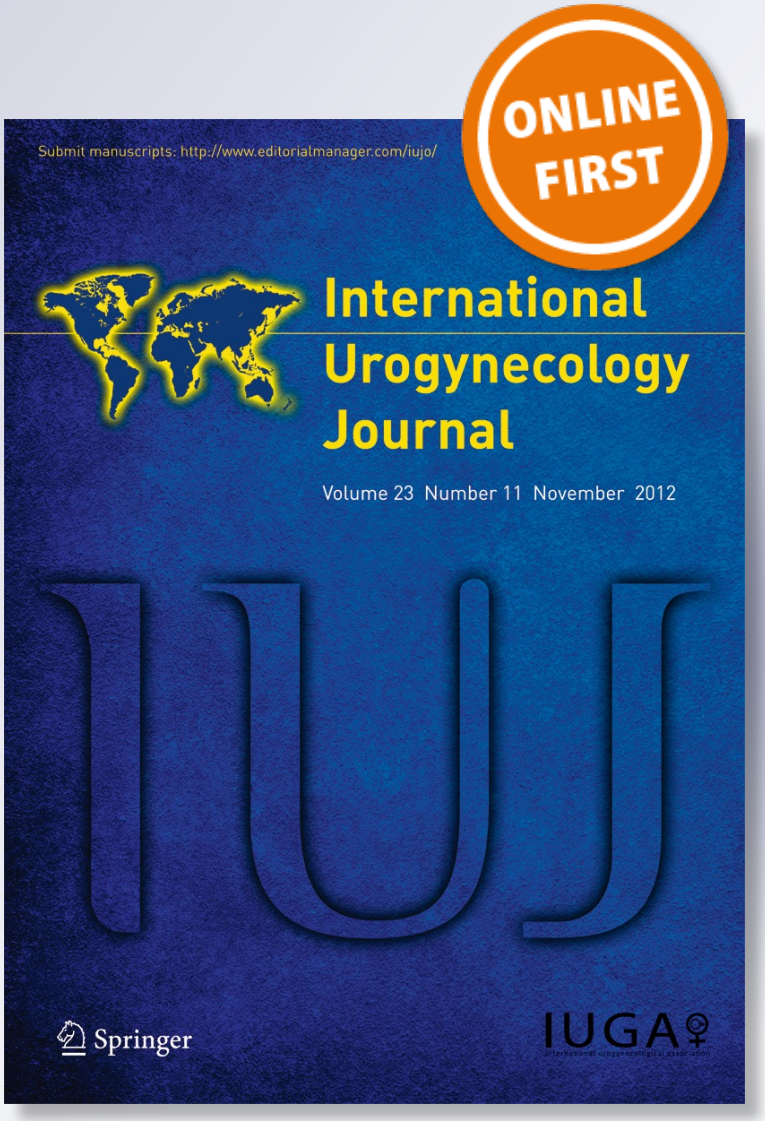
International Urogynecology Journal
Including Pelvic Floor Dysfunction

ISSN 0937-3462

Int Urogynecol J
DOI 10.1007/s00192-012-1957-y



Springer

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REVIEW ARTICLE

Effectiveness of intravesical hyaluronic acid with or without chondroitin sulfate for recurrent bacterial cystitis in adult women: a meta-analysis

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Received: 12 July 2012 / Accepted: 25 September 2012

Abstract
Introduction and hypothesis Glycosaminoglycan hyaluronic acid (HA) and chondroitin sulphate (CS) protect the urothelium. Damage to the urothelium may increase bacterial adherence and infection risk. This meta-analysis evaluated the effect of intravesical HA and HA and CS (HA-CS) combination therapy in recurrent bacterial cystitis (RBC) in adult women.
Methods A systematic literature search was performed. Primary outcomes were urinary tract infection (UTI) rate per patient-year, and UTI recurrence time (days). Secondary outcomes were 3-day voids and Pelvic Pain and Urgency/Frequency (PUF) symptom scale total score.
Results Four studies involving a total of 143 patients were retrieved and assessed in this analysis. Two were randomized, and two were nonrandomized. A significantly decreased UTI rate per patient-year [mean difference (MD) −3.41, 95 % confidence interval (CI) −4.33 to −2.49, $p<0.00001$) was found. Similarly, pooled analysis showed a significantly longer mean UTI recurrence time (days) using either HA or HA-CS therapy (MD 187.35, 95 % CI 94.33–280.37, $p<0.0001$). Two studies using HA and HA-CS therapy reported outcomes on 3-day voids, which were not significantly improved after therapy (MD −3.59, 95 % CI −8.43–1.25, $p=0.15$), but a significantly better PUF total score (MD −7.17, 95 % CI −9.86 to −4.48, $p<0.00001$) was detected in HA-CS groups.
Conclusions Intravesical HA and HA-CS in combination significantly reduced cystitis recurrence, mean UTI recurrence time, and PUF total score. Study limitations include the small number of patients and possible bias. Further studies are needed to validate this promising treatment modality.

Keywords Recurrent urinary tract infections · Chondroitin sulfate · Hyaluronic acid · Intravesical instillation · Cystitis

Introduction

Urinary tract infections (UTIs) are highly common and affect women much more frequently than men [1–3]. Most acute uncomplicated UTIs are caused by a single pathogen, usually *Escherichia coli* (80 %) or *Staphylococcus saprophyticus* (10–15 %) [3]. Less frequent pathogens are *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Proteus* spp., or *Enterococcus* spp. Acute UTIs are traditionally managed by intermittent or prolonged antibiotic therapy [2, 3]. There is a high level of UTI recurrence, and 25–35 % of initial UTI episodes will be followed by a recurrent infection within 3–6 months [2, 4]. Therefore, UTI prevention should be considered for various reasons, especially for patients at risk for UTI recurrence.

The conventional prophylaxis consists of intermittent or prolonged antibiotic therapy. However, the prevalence of *E. coli* resistant to antimicrobial agents is increasing; thus, novel nonantibiotic alternative therapies have gained increasing interest. Novel agents include estrogen cream, cranberry juice, and immunostimulatory vaccines such as Uro-Vaxom® (extract of 18 *E. coli* strains), and SolcoUrovac® (ten heat-killed uropathogens), but their effects have

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Published online: 06 November 2012

Table 1 Summary of data retrieved from studies on intravesical instillation of hyaluronic acid for recurrent bacterial cystitis

Authors	Study type	Number of patients (HA vs CG)	Age (years)	Product	HA and CS dose	Regimen	Control treatment	Follow-up	UTI rates per patient-year (HA vs CG)	Mean UTI recurrence time (days, HA vs CG)	3-day voids (HA vs CG)	Total PUF score (HA vs CG)
Constantinides 2004	Retrospective nonrandomized	40[a]	35	Cystistat ® (Bioniche Life Sciences Inc., Belleville,Ontario, Canada)	HA 40 mg	Four weekly plus 4 monthly	None	12.4 months	0.3 vs 4.3	498 vs 96	–	–
Lipovac 2007	Retrospective nonrandomized	20[a]	27	Cystistat ® (Bioniche Life Sciences Inc., Belleville,Ontario, Canada)	HA 40 mg	Four weekly plus 5 monthly	None	47 weeks	0.56 vs 4.99	178.3 vs 76.7	–	–
Damiano 2011	RCT	28/29	34	IALURIL® (IBSA, Lugano, Switzerland)	50 ml of sterile sodium HA 1.6 % and CS 2.0 % solution	Once weekly for 4 weeks, then monthly or 5 months	Bladder instillations of placebo (50 ml saline)	12 months	0.67 vs 4.19	185.2 vs 52.7	11.91 vs 13.33	14.8 vs 20.44
De Vita 2012	RCT	12/14	60	IALURIL® (IBSA, Lugano, Switzerland)	50 ml of sterile sodium HA 1.6 % and CS 2.0 % solution	Once weekly for 4 weeks, then every 2 weeks two times more	Sulfamethoxazole 200 mg, rimethoprim 40 mg once a week for 6 weeks	12 months	1.0 vs 2.3	181.5 vs 56.5	17.8 vs 24.2	11.2 vs 19.6

RCT randomized controlled trial, *PUF* Pelvic Pain and Urgency/Frequency symptom scale, *SD* standard deviation, *UTI* urinary tract infection, *HA* hyaluronic acid group, *CS* chondroitin sulphate, *CG* control group.

[a] Internal control.

not yet been proven [5–9]. A significant population of bacteria is able to invade the bladder epithelial cells and persist in the bladder after an acute UTI [10]. It has been speculated that this quiescent intracellular bacterial reservoir may contribute to UTI recurrence and act as a stimulus in interstitial cystitis [11], suggesting that damage to the glycosaminoglycan layer (GAG) may be related to both UTI recurrence and interstitial cystitis pathogenesis. It has been shown that the GAG layer has a role in protecting bladder epithelial cells from injury by toxic components of urine, as well as blocking bacterial adhesion [12, 13]. Impairment or partial disruption of the GAG layer exposes the epithelial cells to toxic or infectious urine components and may increase bacterial adherence and risk of infection [14]. Damage to the GAG layer has been postulated as a causative factor in the development of not only interstitial cystitis and common UTI but also bladder carcinoma [12, 14, 15]. Hyaluronic acid (HA) is a major mucopolysaccharide component of the extracellular matrix of most tissues and constitutes an important proportion of bladder surface GAGs. HA allows repair of the protective urothelial coating, reduces urothelial permeability, and is indicated in any clinical situation originating in damage of the GAG layer. HA blocks the intercellular adhesion molecule-1 (ICAM-1) receptor and prevents leukocyte activation, presumably alleviating the inflammatory processes [16]. HA targets bacterial adherence to the bladder mucosa; this is in contrast to antibiotic therapy, which aims to eradicate bacterial infection. HA influences the mechanism of protection against the invasion of *E. coli*, and the duration of this effect is about 7 days [16].

Intravesical treatment of interstitial cystitis with HA was shown in different studies to be beneficial, with rates varying from 71 % to 30 % [16, 17]. The benefit of HA for adult women with recurrent UTI has been supported by a few small clinical studies [18, 19] in which intravesical instillation of HA has dramatically reduced recurrent UTI. Chondroitin sulphate (CS) is another natural proteoglycan present in the GAG layer of the bladder epithelium. Intravesical instillation of this molecule, as with HA, has been proposed as a treatment for patients with interstitial cystitis in order to promote GAG regeneration in the bladder urothelium [20]. Hence, it is key to clarify this relevant issue by summarizing the existing evidence in a meta-analysis, as no previous systematic review is available on this topic. Therefore, the aim of this meta-analysis was to assess the

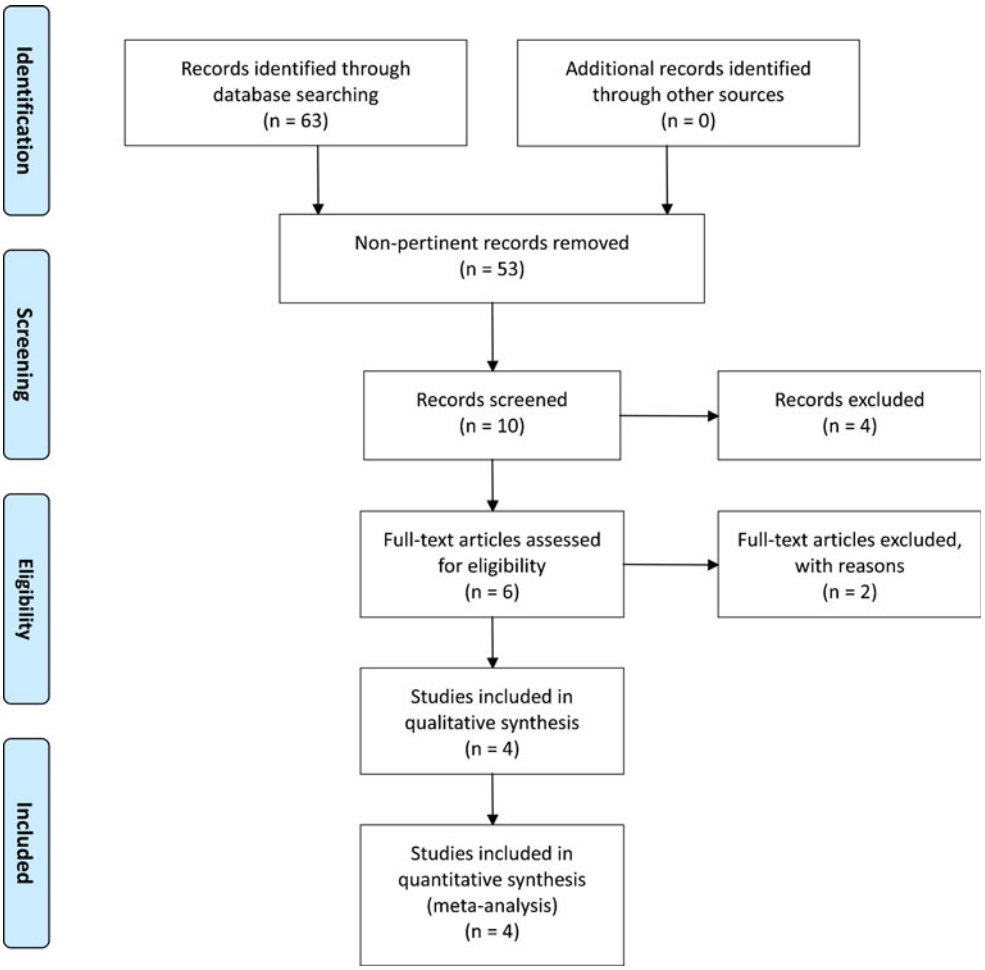

Fig. 1 Literature search results

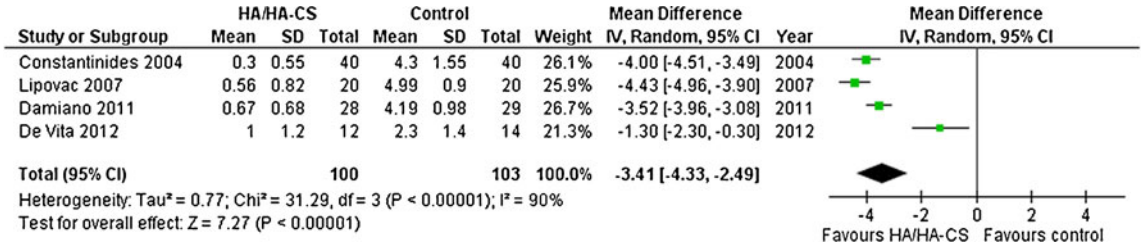

Fig. 2 Significantly decreased urinary tract infection (UTI) rate per patient-year

value of HA and HA-CS treatment in reducing the occurrence of recurrent bacterial cystitis (RBC).

Materials and methods

We performed a search through MEDLINE, Cochrane Library, Embase, Scopus, and Google Scholar for any study on use for intravesical instillations of HA for RBC. The search was performed in August 2012 and targeted studies comparing outcomes of intervention versus control groups. The search was limited to articles investigating this modality of treatment only for RBC. The following search string was used: ("hyaluronic acid" OR "hyaluronate") AND intravesical instillation AND chondroitin sulfate AND ("cystitis" OR "recurrent urinary tract infections" OR "UTI" OR "rUTI" OR "bladder infection"). The search was further limited to clinical studies, human subjects, and articles with an abstract available in the search engine. No language restriction was applied. The abstracts obtained by the search were reviewed for suitable articles. In addition, the "related article" function of PubMed was applied to suitable articles and the reference lists of the retrieved articles were handsearched to identify further articles. Only full-length articles were considered for this analysis. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement for reporting this meta-analysis [21], and the language of the articles was defined as reported in MEDLINE. The retrieved studies were included if they compared outcomes of HA administered for RBC through intravesical instillations in either randomized or nonrandomized fashion. Therefore, we excluded noncomparative studies. Data were retrieved only from the articles, and no attempt to attain missing data from the authors was made. The primary outcome measures were the number of UTIs per patient-year and the mean time to UTI recurrence at the reported longest follow-up. The secondary outcome measures were 24-h urinary frequency (number of voids in one day) [22] and the Pelvic Pain and Urgency/Frequency (PUF) symptoms assessed using the PUF symptom scale [23] at 12 months' follow-up. A subgroup analysis was performed assessing randomized studies only. All outcomes obtained from the studies are reported using the measures retrieved from the articles. Two authors (SG and DD) searched and identified published articles potentially dealing with this topic. Two reviewers (SG and DD) independently assessed the methodological quality of the randomized controlled trials using the Jadad scale [24]. It was decided a priori that an article would be considered high quality if it received a score of three or higher out of a maximum of five on the Jadad scale [24]. Two investigators (SG and DD) abstracted data from all eligible studies using a standardized Excel file. Missing data were dealt with according to previously validated estimations [25–27].

Statistical analysis

Statistical analysis was performed using Review Manager 5.1 software [28]. Differences in continuous variables were expressed as mean difference (MD) with 95 % confidence intervals (CI). Heterogeneity was assessed using the I^2 statistic, which describes the percentage of total variation across studies that is due to heterogeneity rather than chance [29]. Usually, values of the I^2 statistic <25 % are indicative of low heterogeneity, those ranging between 25 % and 75 % of moderate heterogeneity, and those >75 % of high heterogeneity. I^2 <75 % was considered as unimportant heterogeneity. To perform the meta-analysis, the inverse variance statistical method was used for continuous outcome variables. However,

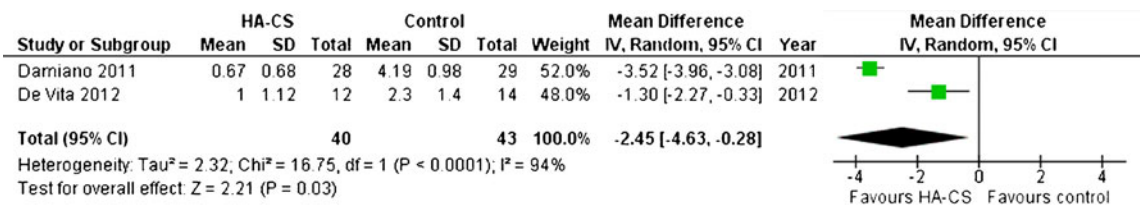

Fig. 3 Significantly decreased urinary tract infection (UTI) rate per patient-year using hyaluronic acid–chondroitin sulphate (HA-CS) therapy

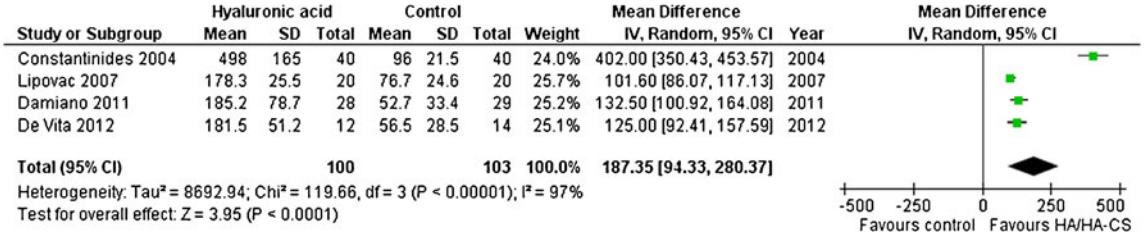

Fig. 4 Significantly longer mean urinary tract infection (UTI) recurrence time

in all cases, we performed random-effect analysis, which considers both within-study and between-study variation [30] because of the different nature of most studies included in this analysis. A p value <0.05 was considered statistically significant. Finally, we conducted sensitivity analyses omitting each study in turn to determine whether the results were influenced excessively by a single study.

Results

Literature search yielded 67 articles, four [18–20, 31] of which were pertinent to this issue and sources of information on effectiveness of HA in reducing UTIs (Table 1). The literature search flowchart is shown in Fig. 1.

Two articles were observational studies with HA therapy [18, 19] with internal controls, and two were prospective randomized studies with HA and CS combination therapy [20, 31] (Table 1). The two randomized studies [20, 31] had a mean Jadad score of 3.5, and no assessment of study quality was performed for the other two articles, as they were observational studies [18, 19]. All four studies [18–20, 31], involving a total of 143 patients, reported results on UTI rates per patient-year. Pooled analysis showed a significant difference between the two groups (MD −3.41, 95 % CI −4.33 to −2.49, p<0.00001; Fig. 2), and this beneficial effect is consistent among all the studies. Similarly, subgroup analysis of the two randomized studies [20, 31] using HA-CS reported a significantly decreased UTI rate per patient-year (MD −2.45, 95 % CI −4.63 to −0.28, p=0.03; Fig. 3).

We found a significantly longer mean UTI recurrence time (days) in all four studies [18–20, 31] using HA or HA-CS therapy (MD 187.35, 95 % CI 94.33–280.37, p<0.0001; Fig. 4). Similarly, subgroup analysis of the two randomized studies [20, 30] using HA-CS reported a significantly longer mean UTI recurrence time (MD 128.86, 95 % CI 106.15–151.57, p<0.0001; Fig. 5).

Two randomized studies using HA and CS combination therapy [20, 31] reported outcomes on 24-h urinary frequency measured as 3-day voids (number of voids in 3 days), which were not significantly improved after therapy (MD −3.59, 95 % CI −8.43 to 1.25, p=0.15; Fig. 6), but a significantly better PUF total score (MD −7.17, 95 % CI −9.86 to −4.48, p<0.00001; Fig. 7) was detected in HA-CS study groups.

Finally, the exclusion of any study from the analysis did not materially change the summary estimates, and absence of significant asymmetry in the funnel plots was observed.

Discussion

The meta-analysis reported here includes four studies and 143 patients and provides compelling evidence that HA and HA-CS are effective in reducing the incidence of UTI recurrence in adult women with a history of recurrent UTI (Fig. 2) [18–20, 31]. Recurrent UTI in women is common, resulting in considerable morbidity and expense, and can be a management problem for physicians. Nonantimicrobial prevention strategies are desirable given the possible adverse effects associated with antimicrobials and increasing antimicrobial resistance, especially in elderly women. Bladder epithelium, also known as transitional epithelium, is not just a simple barrier and a nonspecific defense against infections; it is a specialized tissue that regulates complex bladder function and plays a fundamental and active role in cystitis pathogenesis. HA allows repair of the protective urothelial coating, reduces urothelial permeability, and influences the mechanism of protection against the invasion of *E. coli*; the duration of this effect is about 7 days [32]. Thus, repeated instillations are needed to maintain response

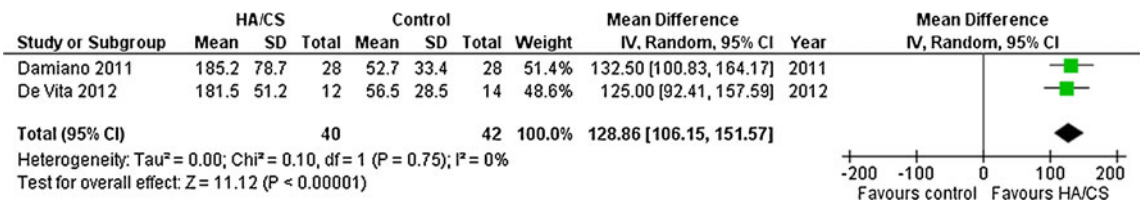

Fig. 5 Significantly longer mean urinary tract infection (UTI) recurrence time using hyaluronic acid–chondroitin sulphate (HA-CS) therapy

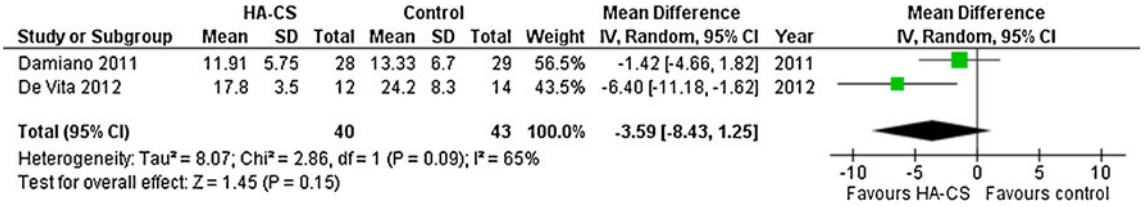

Fig. 6 Outcomes of 3-day voids

[32, 33]. Moreover, GAGs block C-fiber activation and consequent smooth-muscle contraction and reduce pain, neurogenic inflammation, and hypersensitivity, thus decreasing symptoms of urgency, frequency, overactive bladder (OAB), and bladder pain syndrome (BPS) [34]. Hypersensitivity-exaggerated perception of normal stimuli, unresolved acute trauma, chronic hypoxia, and tissue inflammation lead to sensory nerve sensitization, which is mediated by adenosine receptors. GAGs decrease chronic pelvic inflammation and pain due to visceral hypersensitivity not only by inhibiting peripheral C-fiber nociceptors and blocking smooth-muscle contraction, but also by preventing immune-cell migration and mast-cell degranulation and extravasion. This could explain the significant increase in maximum cystometric capacity at 12 months' follow-up, shown by only, in patients treated with intravesical instillations of HA-CS [20]. Different *noxa patogena* may underlie the early stage of GAG injury; consequently, a defect in the GAG layer could be the first step in the genesis of chronic inflammatory bladder diseases. Therefore, GAG therapy could be a protective-barrier strategy for recurrent UTI, interstitial cystitis, and BPS. Urothelial dysfunction seems to contribute to different clinical conditions, including interstitial cystitis (IC), idiopathic detrusor overactivity (IDO), stress urinary incontinence (SUI), and UTI [35]. The combination of HA and CS bound together by calcium chloride (important for better interaction with the different GAG classes and restoration of cell junctions) significantly reduces inflammatory cytokines and permits stabilization and functional improvement of the apical part of the urothelium (urothelial coating) [36, 37]. Efficacious treatment of recurrent cystitis may prevent chronic urinary tissue damage, which otherwise could lead to interstitial cystitis or BPS [38]. Possible GAG diseases are infection, recurrent UTI, interstitial cystitis, and OAB.

Despite showing encouraging results, previous prospective studies evaluating HA instillation only [18, 19] included no appropriate control group and used only an internal control (Table 1). The highest level of evidence was provided by Damiano et al. [31], who reported a prospective, randomized, double-blind, placebo-controlled study on the use of combined intravesical HA and CS. De Vita et al. [20] also performed a randomized study, but their control group received a long-term antibiotic prophylaxis. In all but one study, patient age was similar [18, 19, 30]; De Vita et al. [20] evaluated an elderly population. Only one study [18] showed side effects, which were limited to nine patients who reported mild bladder irritation, but no patient interrupted treatment. Although the studies in this review had a relatively small total number of patients, we found excellent results among studies reporting treatment with intravesical instillations of HA or HA-CS, as well as in subgroup analyses and secondary outcomes (Figs. 2, 3, 4, 5, 6, 7). The two randomized studies [20, 30] reported secondary outcomes on 3-day voids. Although not statistically significant, pooled results showed a trend toward improvement (Fig. 6). Pooled PUF total scores, however, were significantly better with HA-CS therapy (Fig. 7).

Some concerns might be raised by economic issues: The cost of a course of intravesical HA-CS instillations over a 12-month period is considerably higher than that of continuous 6-month treatment with nitrofurantoin or quinolone (total costs approximately 1,500 euros versus 30 euros). This does not take into account any additional costs associated with the possible side effects of antibiotics or, in particular, the impact of increased antimicrobial resistance [31]. Indeed, some women do not want to take antimicrobials over an extended period of time [39].

The results of this meta-analysis must be viewed in light of a number of limitations and potential bias influencing our

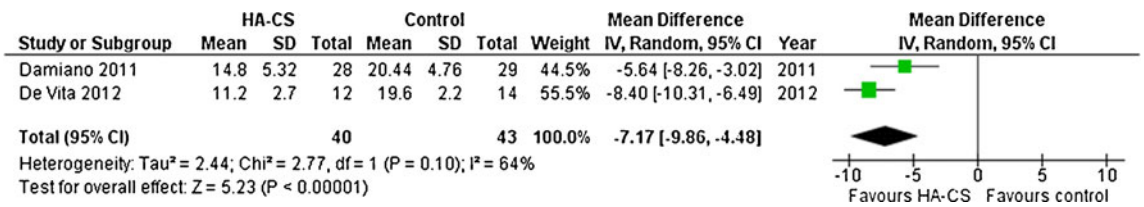

Fig. 7 Significantly improved Pelvic Pain and Urgency/Frequency (PUF) total score with hyaluronic acid (HA) and chondroitin sulphate (CS) therapy

findings. Four studies were used for this pooled analysis, only two of which were randomized controlled trials [20, 31]; two were observational studies with clear heterogeneity in methods and settings (Table 1), and did not provide an appropriate control group [18, 19]. We decided to pool all data from these four studies in relation to primary outcomes because, to the best of our knowledge, these are the only articles available in the literature on this topic. However, for a more rigorous assessment, we performed a meta-analysis of the two randomized studies, as well. Indeed, the number of patients considered was relatively small, and there were differences in patients' ages and their control treatment, which might be confounding factors. However, the exclusion of any study from the analysis did not materially change the summary estimates, and together with the absence of significant asymmetry in the funnel plot, this indicates that publication bias is unlikely to have appreciably influenced our results.

In conclusion, bladder instillation of HA and HA-CS in combination can be a promising and feasible treatment option and might be well tolerated by patients of different ages. Such treatment significantly reduces the incidence of recurrent lower UTIs, possibly through a protective effect on the GAG layer, and may offer an alternative to the widespread use of antibiotics, which are not always successful or well accepted by patients and can result in therapeutic resistance. Further research using more appropriate methods, with larger series of patients, different age groups, and long-term outcomes is clearly needed to obtain sustained evidence, especially compared with standard treatment modalities.

Conflicts of interest None.

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