ELSEVIER

International Journal of Antimicrobial Agents 19 (2002) 451–456

INTERNATIONAL JOURNAL OF
Antimicrobial
Agents

www.isochem.org

Prevention of recurrent urinary tract infections with immuno-active *E. coli* fractions: a meta-analysis of five placebo-controlled double-blind studies

Hartwig Wilhelm Bauer [a,*], Volker W. Rahlfs [b], Pierre A. Lauener [c], Gabriela S.S. Bleßmann [d]

[a] *Maximilianstraße 31, 80539 München, Germany*
[b] *idv-Datenanalyse und Versuchsplanung, Wessobrunner Straße 6, 82131 Gauting, München, Germany*
[c] *Healthcare & Pharma Consulting SA, Genéve, 8, av. Des Grandes-Communes, CH-1213 Petit-Lancy, Geneva, Germany*
[d] *Sanofi Winthrop GmbH. Augustenstraße 10, 80333 München, Germany*

Abstract

A meta-analysis was performed on five studies conducted over the last decade to demonstrate a positive effect for the drug Uro-Vaxom® compared with Placebo in double-blind studies in patients with urinary tract infection (601 women), with special reference to the prevention of recurrences over an observation period of 6 months, the treatment being given for the first 3 months. The five studies were similar in design. The analysis by means of the Wilcoxon–Mann–Whitney test showed superiority of Uro-Vaxom in all five studies, ($P < 1\%$). The summarising Mann–Whitney (MW) statistics also indicated superiority with the Mann–Whitney value being 0.684. In all studies, the Uro-Vaxom group was statistically significant and clinically relevant superior to control with respect to the reduction of the frequency of UTIs and to dysuria, bacteriuria and leucocyturia. The confidence intervals (CI)s were small (0.64–0.72). The drug was well tolerated and compliance of patients was excellent in all studies. Oral immunotherapy with the Uro-Vaxom *Escherichia coli* (*E. coli*) extract is an effective prophylactic approach in the prevention of UTIs. © 2002 Elsevier Science B.V. and International Society of Chemotherapy. All rights reserved.

Keywords: Uro-Vaxom; E. coli; Dysuria

1. Introduction

A meta analysis was performed to give an overview of studies from 1984 dealing with the efficacy of Uro-Vaxom in patients suffering from recurrent urinary tract infections [1,2]. The efficacy of Uro-Vaxom has been investigated by 12 studies [3–14]. Five placebo-controlled randomised double-blind studies [5–7,12,13] with a similar study design were chosen for performing the meta-analysis. Open studies [9–11,14] or studies with special patients like children [4,8] or paraplegics [3] were not included.

The primary criterion in all studies was the number of recurrences per patient. The double-blind, randomised parallel-group studies were basically identical: with 3 months treatment with observation and a further observation period of 3 months without treatment. Despite differences, in data analysis and type of report, the basic design was identical and the primary criterion essentially the same so that all studies concerning efficacy could be pooled.

Uro-Vaxom is a bacterial extract consisting of immunostimulating components derived from 18 uropathogenic *Escherichia coli* strains [15].

2. Immunological activities of Uro-Vaxom [15]

2.1. Mechanism of action in vitro

Uro-Vaxom has been shown to induce a marked dose-dependent proliferation of lymphocytes taken from mouse spleens [16]. It also increased oxidative metabolism in bone marrow-derived macrophages as measured

* Corresponding author. Tel.: +49-89-22-0051; fax: +49-89-228-9653.
E-mail address: mail@praxis-bauer.de (H.W. Bauer).

0924-8579/02/$ - see front matter © 2002 Elsevier Science B.V. and International Society of Chemotherapy. All rights reserved.
PII: S0924-8579(02)00106-1

by monitoring hexose monophosphate shunt activity. In addition, the generation of the oxygen metabolites, superoxide anion and hydrogen peroxide was increased by Uro-Vaxom upon stimulating with phorboll myristate acetate. This generates toxic free radicals that destroy invading pathogens.

In a further experiment, the killing of the intracellular parasite *Leishmania enriettii* by macrophages isolated from two different mouse strains and exposed to Uro-Vaxom and/or IFN-gamma was studied [16]. Treatment with Uro-Vaxom or IFN-gamma alone failed to stimulate macrophage microbicidal activity but simultaneous incubation with Uro-Vaxom and IFN-gamma led to intracellular destruction of the microorganisms within 24 h in macrophages from both mouse strains. Uro-Vaxom was shown to activate macrophages to kill bacteria in a dose dependent manner. Similar results were obtained against *Candida albicans*.

Uro-Vaxom increased the secretion of the cytokines IL-6 and TNF by mouse peritoneal macrophages in a dose-dependent manner, although IL-1 secretion was not stimulated by Uro-Vaxom. The effect of Uro-Vaxom on the production of cytokines has also been studied in human peripheral blood mononuclear cells [17] and showed that Uro-Vaxom significantly enhanced the production of IL-1, but only at certain concentrations and in the presence of LPS, a B-cell mitogen which induces IL-1. Uro-Vaxom significantly increased the production of TNF-alpha and IL-2 independently of PHA, but IFN-gamma was only produced in the presence of PHA. Furthermore, Uro-Vaxom enhanced the natural killer activity by approximately 40%.

2.2. Activity in vivo

Uro-Vaxom administration significantly increased the levels of sIgA in intestinal secretions [18]. Uro-Vaxom protected against infection with *E. coli* and *Pseudomonas aeruginosa* [18]. Uro-Vaxom compensated for immunosuppression induced by antibiotics or mycotoxins [19]. The immunogenicity of Uro-Vaxom after repeated oral administration has been studied in mice. Sedelmeier and Bessler [14] found that multiple i.p. injections of Uro-Vaxom led to the production of Uro-Vaxom-specific antisera, mainly IgG and IgM antibodies. The maximum serum antibody content was achieved after six immunisations. The effect was dose-dependent, but even the lowest dose had a marked effect. Uro-Vaxom - specific antisera, which were obtained after nine immunisations with 0.05 mg Uro-Vaxom, were found to bind to each of the bacterial strains used for preparation of Uro-Vaxom. The antisera recognised typical bacterial cell wall components of Gram-negative and positive bacteria such as murein-lipoprotein and protein I (G−) and murein (G+ and −).

Repeat oral administrations of Uro-Vaxom to mice resulted in a Uro-Vaxom-specific serum Ig response, which led to increased levels of bacteria-specific serum IgA and IgG, and a total serum IgA [20]. Furthermore, the sera bound to thel *E. coli* strains used for the preparation of Uro-Vaxom. The sera also recognised the bacterial cell wall components, muramyl dispeptide, protein I and lipopeptide. This study confirmed and extended the results from a previous study [14].

Nauck et al. [10] have determined the ability of Uro-Vaxom to stimulate the killing activity of rabbit PMN leucocytes against *E. coli* or *Staphylococcus aureus*. PMNs from Uro-Vaxom-treated animals were markedly more active than cells from control animals. In similar experiments performed in mice, Uro-Vaxom stimulated the clearance of *E. coli* from the blood stream at a higher rate than unstimulated controls. These data indicate that Uro-Vaxom functionally upregulates the activity of PMNs in vivo, and could account for an increase in overall survival rates following bacterial infection.

2.3. Data quality of the studies

The data for the first three studies were taken for the data analysis as they were available in the final reports. For the case of the study of [21] this was more or less an intention-to-treat analysis, for the studies of Tammen [22] and Magasi [9] it was more of a per-protocol patient population. For the two newer studies, Pisani (in press [23]) and Schulman [24], the raw data were available, so that the analysis could be performed with the intention-to-treat patient population.

2.3.1. Frey et al. [21]

A total of 108 patients were enrolled but only 27 (Uro-Vaxom group) and 31 (placebo group), a total of 58, were evaluated for efficacy. Seven of 108 patients did not have the required bacterial count at the beginning, or were non-compliant or dropout cases. Of the remaining 101 cases, two subgroups were removed, one of 22 patients who were treated with a different dose because of a change in the protocol and 15 patients with permanent catheters and, thus, with a different risk for recurrence of urinary tract infection. Thus, 64 patients remained, a well-defined population, and, because of the nature of selection, not afflicted with a bias. The number of real dropouts was small and the remaining data set was more or less an intention-to-treat patient population.

2.3.2. Tammen [22]

A total of 150 patients were enrolled, but only 120 (61/59) were included in the analysis. Of the 30 patients not evaluated, 15 were those with poor compliance. The analysis is in principle a 'per protocol' analysis and not

the currently required intention-to-treat analysis but there is no reason to discard the data.

2.3.3. Magasi [9]

A total of 122 patients were enrolled; 10 patients were excluded, one for pregnancy, the other nine for poor compliance. The remaining 112 patients were distributed as follows, 58 in the Uro-Vaxom group and 54 in the placebo group.

2.3.4. Pisani [23]

Enrolled in the study were 86 patients (Uro-Vaxom) and 85 patients (placebo). Six and seven cases did not fulfil the selection criteria, respectively; four and one had only one baseline observation, and two patients in the Uro-Vaxom group dropped out because of adverse drug reactions. They included 74 and 77 patients in the two groups. Of these there were only seven and six dropout cases with at least one observation. These were included in two alternative evaluations, 'data as available' and 'worst case', which, however, lead to nearly identical results.

A listing of the recurrences was available and could be used with due consideration of the dropout cases. Recurrences at mandatory monthly visits were counted as well as a recurrences noted at one of the optional visits at intermediate times.

2.3.5. Schulman [24]

A total number of 166 patients (85/81) were enrolled; 82 (Uro-Vaxom) and 78 (placebo) could be evaluated as the intent-to-treat population. In addition there were 18 dropout cases (8 and 10), with at least one observation. Thus, the percentage was 11.3% of the 160 and the bias introduced by these cases, if any, was small. Two of the dropouts in each group were because of inefficacy so the groups are balanced with respect to these problem cases.

The study design: was multi-centre, placebo-controlled, randomised and double-blind.

Treatment schema in all studies was 1 capsule/d × 90 days and the study duration covered 6 months therapeutical success was measured as the number of UTIs, defined as bacteriuria $> 10^4$ per ml urine, or in some instances bacteriuria $> 10^5$ per ml urine.

2.4. Inclusion criteria

Patients with recurrent UTIs and without anatomical abnormalities of the urinary tract.

2.5. Exclusion criteria

These were: pregnancy, patients with anatomical abnormalities of the urinary tract, urolithiasis, vesicoureteral reflux, urological procedures (e.g. catheter), nephropathy of any kind, positive urine culture persisting after antibiotics for 10 days, antibiotics during the 15 days proceeding the study, taking of drugs that influence the immune system (e.g. steroids), drug or alcohol addiction, honeymoon-cystitis, retention of urine.

2.6. Statistical methods of data evaluation

The number of recurrences is distributed as a Poisson distribution and, thus, evaluable with a Wilcoxon–Mann–Whitney (WMW) test with the number of recurrences of each patient as the data value [21–29]. Counting the number of recurrences per group or per time unit is not the correct analysis method.

The P-value for a two-sided test for difference was calculated and also the Mann–Whitney statistics and their confidence interval (CI). The Mann–Whitney statistics are a measure of superiority of the test group. They give the probability that a randomly selected patient from the Uro-Vaxom group is superior to a randomly selected patient from the placebo group. The benchmarks for the relevance of superiority are: 0.5 no difference, 0.56 small difference, 0.64 medium difference, 0.71 large difference [30].

The summarising MW-statistics, which is a measure for all studies combined, were calculated according to the method of Colditz, et al. [30]; it is an average value weighed with the reciprocal variance of the study, which in turn depends on the sample size of the study.

As there was no CI available for the pooling MW-statistic another analysis with a Cochran–Mantel–Haenszel pooling was performed for the same data, just as a supportive analysis. It should be noted that the difference is now described as an Odds ratio where 1.0 is no difference, 1.27 small difference, 1.78 medium difference, 2.45 large difference.

The Cox–Mantel variance used in this procedure for calculating CIs is not as good as that of the Wilcoxon–Mann–Whitney test because the latter is more efficient for this Poisson-type data (see the difference for the study Pisani [23] and Schulman [24]. Nevertheless, this additional analysis gives a hint concerning the power, as expressed in the width of the CI, of a summary value over all single studies [31–33].

3. Results

Results of the calculations for each centre are given in Table 1. All P-values are statistically significant and all differences are in favour of Uro-Vaxom (MW > 0.5). Considering the benchmarks of 0.64 (-medium) and 0.71 (-large) one notices that there is only one study which just misses the medium-sized difference [21]. All other differences are larger than medium ($= 0.64$), three of them larger than a large difference (-0.71).

Table 1
Statistical results of each centre. P-value (two sided), Mann–Whitney statistics (MW) with 95% CI

		N	P-value	MW-statistic	95% CI
Frey et al. [21]	10^4	27/31	0.0257	0.663	0.525–0.800
	10^5	27/31	0.0009	0.730	0.608–0.852
Tammen [22]	10^4	61/59	< 0.0001	0.714	0.621–0.808
Magasi [9]	10^5	58/54	< 0.0001	0.755	0.666–0.843
Pisani[a] [23]		74/77	0.0025	0.621	0.545–0.696
Schulman[a] [24]	10^4	78/82	< 0.0001	0.679	0.596–0.761
	10^5	78/82	< 0.0001	0.687	0.609–0.765

[a] Given is intention-to-treat population result with data-as-available analysis; the worst case imputation gives nearly identical results.

The precision of the studies as judged by the width of the CI, increases with the sample size. For two studies Pisani [21] and Schulman [31] the precision is very good, the width being about 0.15 on the scale 0 to 1. Fig. 1 shows the Mann–Whitney statistics of all studies in one graph. All results of the five studies are homogeneous and show a relevant difference. The appropriate Wilcoxon–Mann–Whitney analysis shows that for all five studies the superiority is statistically relevant. Fig. 1 shows also the summarising Mann–Whitney statistic, pooled according to the procedure of Colditz et al. [8]. The value is 0.684 which shows an effect size between medium and large, thus, being medically relevant.

In addition to this analysis with the MW-statistics the Odds ratio was taken as a measure of relevance using a Cochran–Mantel–Haenszel pooling. The pattern of effect sizes is basically the same (data not shown). The studies of Pisani [21] and Schulman [31] show, however, a slightly smaller group difference. The pooled Odds ratio (OR-2.28) is again between medium and large. The lower CI does not cover the reference line of a medium effect size. Thus, there is a statistical proof for a relevant drug effect, of medium size at least.

The results of both analysis procedures agree. All results of the five studies are homogeneous and show a relevant difference. The appropriate Wilcoxon–Mann–Whitney analysis shows that for all five studies the superiority is statistically relevant (Table 2). The summarising MW-statistic and odds ratio indicate a relevant difference between medium and large, if these well-known benchmarks are to be used in the description. The pooling of all five looks at results in a very small CI. This helps to describe the observed superiority as at least medium-sized and shows that the precision of the summarised result is high.

All investigators assessed the safety and tolerability of Uro-Vaxom as good. Patients treated with Uro-Vaxom experienced minor adverse events (skin reactions and gastrointestinal discomforts) as often as the patients in the placebo group. Serious adverse events were not experienced by the patients treated with Uro-Vaxom. The agent was well tolerated and compliance of patients was excellent across the studies.

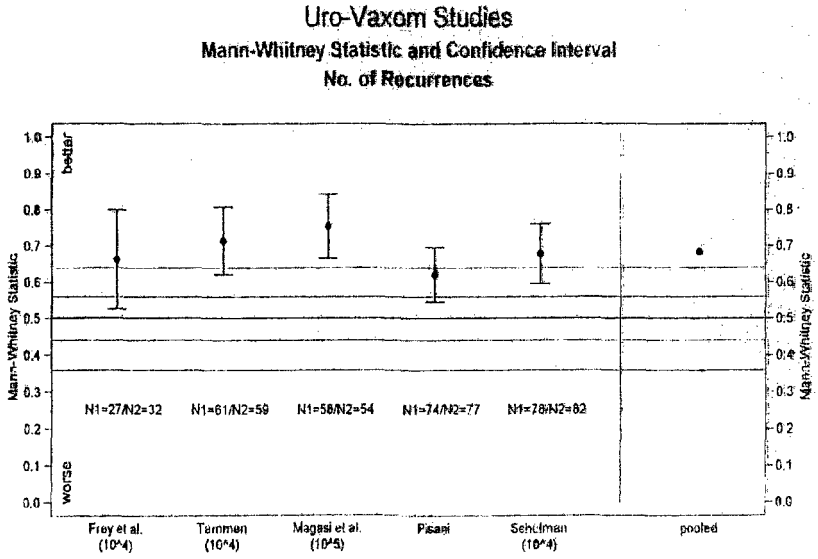

Fig. 1. Uro-Vaxom studies. Mann–Whitney statistic and CI.

Table 2
Mann–Whitney statistical analysis of the five studies

Study name		Point estimator	Conf. Int. LB	Conf. Int.UB	Standard Error	N1	N2	N total	Significance level	One- or two-sided
Frey et al. [21]	10^4	0.6630	0.5255	0.8005	0.0702	27	31	58	95.0	2
Tammen [22]	10^4	0.7140	0.6205	0.8075	0.0477	61	59	120	95.0	2
Magasi et al. [9]	10^5	0.7550	0.6665	0.8435	0.0452	58	54	112	95.0	2
Pisani [23]	10^5	0.6210	0.5455	0.6965	0.0385	74	77	151	95.0	2
Schulman [24]	10^4	0.6790	0.5965	0.7615	0.0421	82	78	160	95.0	2
Combined results										
Fixed effect		0.6834	0.6431	0.7236	0.0205	302	299	601	95.0	2
Random effect		0.6850	0.6364	0.7337	0.0248	302	299	601	95.0	2

Test for homogeneity: $\chi^2 = 5.6456$; df = 4.

Table 3
Antimicrobial prophylaxis regimens for women with recurrent UTIs

Continuous Prophylaxis	UTIs per year
Trimethoprim-sulphamethoxazole 40/200 mg daily	0–0.2
Trimethoprim-sulphamethoxazole 40/200 mg thrice weekly	0.1
Trimethoprim 100 mg daily	0–1.5b
Nitrofurantoin 50 mg daily	0–0.6
Nitrofurantoin 100 mg daily	0–0.7
Cefaclor 250 mg daily	0
Cephalexin 125 mg daily	0.1
Cephalexin 250 mg daily	0.2
Norfloxacin 200 mg daily	0
Ciprofloxacin 125 mg daily	0
Uro-Vaxom ®	0.15–0.82

Oral immunotherapy with the Uro-Vaxom *E. coli* extract is an effective therapy in the prevention of UTIs. and is a serious alternative to antibiotic low-dose prophylaxis of recurrent UTIs.

A comparison of the the results of Uro-Vaxom and of chemoprophylaxis in reducing the recurrence rate of UTIs per year is shown in Table 3 [16].

References

[1] Ascher AW. The challenge of urinary tract infections. London/ Grunea Stratton, New York: Academic Press, 1980:14.

[2] Bauer HW, Kempel A, Carrascosa Nerger A, Lühr-Giernalczyk C. Orale Immuntherapie als Präventionsmaßnahme bei rez:idivierenden Harnwegsinfektionen; Kosten-Nutzen-Analyse. 3, Symposium Disease Management, 1996;7:34–43.

[3] Fontanges R. Immunopharmacological investigation with orally administered immunostimulant OM-89 in the mouse. adv. in immunomodul. Rome: Pythagora Press, 1988:139.

[4] Hachen HJ. Oral immunotherapy in paraplegic patients with chronic urinary tract infections; a double-blind, placebo-controlled trial. J Urol 1990;143:759.

[5] Hasselblad V, Hedges LV. Meta-analysis of screening and diagnostic tests. Psychol Bulletin 1995;117:167–78.

[6] Hedges LV, Olkin I. Statistical methods for meta-analysis. London: Academic Press, 1985.

[7] Hooton TM. Recurrent urinary tract infection in women. Int J Antimicrob Agents 2001;17:259–68.

[8] Lettgen B. Prophylaxe rezidivierender Harnwegsinfektionen bei Mädchen. Curr Ther Res 1996;57:463–75.

[9] Magasi P, Pánovics J, Illés A, Nagy M. Uro-Vaxom and the management of recurrent urinary tract infection in adults: a randomized multicenter double-blind trial. Eur Urol 1994;26:137–40.

[10] Nauck M, Matthys H, Emmons LR, Perruchoud A, Reichel H, Plfeger S, Breyer S, Roedler S, Georgopoulos A, Block LH. The immunomodulators broncho-vaxom and Uro-Vaxom stimulate the bacterial killing and oxidative metabolism of ploymorphonuclear leukocytes by the activation of phosphatidylinositol turnover. Int J Exp Clin Chemother 1991;4:1–11.

[11] Popa G, Lauber K-D, Rothe H, Rugendorff E. Rezidivierende Harnwegsinfektionen in der Postmenopause. MMV 1996;43:713–6.

[12] Ruckdeschel M, Altwein JE. Rezidivprophylaxe des weiblichen Harnwegsinfektes. ZFA 1992;25:832–6.

[13] Rugendorff EW. Präventionsmaßnahme bei rezidivierender bakterieller Zystitis der Fau: orale Immuntherapie und low-dose-Antibiotikagabe als Langzeitmaßnahme. Urologe B 1997;37 :134–9.

[14] Sedelmeier EA, Bessler WG. Biological activity of bacterial cell-wall components: immunogenicity of the bacterial extract OM-89. Immunopharmacology 1995;29:29–36.

[15] Bauer HW, Bleßmann GS, Pitrow DB, Rahlfs VW. Prevention of recurrent urinary tract infections with immunoactive *E. coli* fractions. Eur J Obstet Gyn 1999;86:33.

[16] Pham TV, Kreis B, Corradin-Betz S, Bauer J, Mauel J. Metabolic and functional stimulation of lymphocytes and macrophages by an *Escherichia coli* extract (OM-89): in vitro studies. J Biol Response Mod 1990;9:231–40.

[17] Wybran J, Libin M, Schandene L. Enhancement of cytokine production and natural killer activity by an *Escherichia coli* extract. Onkologie 1989;12:22–5.

[18] Bosch A, Benedi VJ, Pares R, Jofre J. Enhancement of the humoral immune response and resistance to bacterial infection in mice by the oral administration of a bacterial immunomodulator (OM-89). Immunopharmacol Immunotoxicol 1988;10:333–43.

[19] Bottex C, Boyer G, Fontanges R. Efficacy of an immunomodulator in compensating antibiotic-induced immunosuppression. Int J Immunopathol Pharmacol 1989;2:41–8.

[20] Baier W, Sedelmeier EA, Bessler WG. Studies on the immunogenicity of an *Escherichia coli* extract after oral application in mice. Arzneim Forsch Drug Res 1997;47:980–5.

[21] Frey C, Obolensky W, Wyss H. Behandlung von rezidivierenden Harnwegsinfektionen: Wirksamkeit eines oral verabreichten Immunbiotherapeutikums. Urol Int 1986;41:444–6.

[22] The German Urinary Tract Infection Study Group, Tammen H. Immunbiotherapy with Uro-Vaxom® in recurrent urinary tract infection. Br J Urol 1990;65:6–9.

[23] Pisani G, Klinische Studie zur Wirksamkeit und Verträglichkeit eines *E.coli* Fraktionen-Präparates bei der Prävention rezidivierender Harnwegsinfektionen, Urology, in press.

[24] Schulman CC, Corbusier A, Michiels H, Taenzer HJ. Orale Immuntherapie rezidivierender Harnwegsinfekte: eine placebo-kontrollierte muitizentrische Doppelblindstudie. J Urol 1993;150:917–21.

[25] DerSimonian R, Laird N. Meta-analysis in clinical trials, Controlled Clinical Trials 1986;3:177–88.

[26] Geller NL, Proschan M. Meta-analysis of clinical trials: a consumer's guide. J Biopharm Stat 1996;6:377–94.

[27] Riedasch G. Harnwegsinfektionen bei Kindern:-Immuntherapeutikum beugt Rezidiven vor -. T & E Urologie Nephrologie *1998;10:345–6.*

[28] Sukhatme S. Stratification in nonparametric ROC studies. Biometrics 1994;50:49–163.

[29] Schemper M. Non-parametric analysis of treatment-covariate interaction in the presence of censoring. Stat Med 1988;7:1257–66.

[30] Colditz GA, Miller JN, Mosteller F. Measuring gain in the evaluation of medical technology: The probability of a better outcome. Int J Technol Assess Health Care 1988;4:637–42.

[31] Schneider HJ. Neuer Therapieansatz bei rezidivierenden Harnwegsinfekten. Allgemeinarzt 1990;12:626–33.

[32] Schmid JE, Koch GG, LaVange LM. An overview of statistical issues and methods of meta-analysis. J Biopharm Stat 1991;1:103–20.

[33] Tammen H, Frey C. Treatment of recurrent urinary tract infections with Uro-VAXOM®, open multicenter study with 521 patients. Urologe B 1988;28:294–6.