1 ## *Risk factors associated with IgG seropersistence to Chlamydia trachomatis*

2 ## *and Mycoplasma genitalium*

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Summary

Sexually transmitted infections caused by *Chlamydia trachomati*s *(Ct)* and *Mycoplasma genitalium (Mg)* have significant implications both at the individual and societal levels. Our study evaluated various co-factors associations with persistent serum IgG-antibodies to *Ct* and *Mg.* 329 pregnant women and 135 men from the Finnish Family HPV study were analysed for serum IgG-antibodies of pGP3 for *Ct* and MgPa and rMgPa for *Mg* using multiplex serology. Seropersistence to both *Ct* and *Mg* was more common in women (30.4% and 13.3%) than in men (17.4% and 5.3%). The number of lifetime sexual partners above 10, practice of anal sex, and history of diagnosed *Ct* were associated with seropersistence to *Ct* in women, adjusted ORs 5.6 (95%CI 1.39–22.29), 15.3 (95%CI 1.18–197.12) and 18.0 (95%CI 5.59–57.92), respectively. The increasing number of partners before the age of 20 was the main risk factor for seropersistence among women with *Mg,* adjusted OR range from 5.0 to 12.3 (95%CI range 1.17–100.90) and in men only with 6 to 10 partners for *Ct*, adjusted OR 12.6 (95%CI 1.55–102.49). To conclude, persistent *Ct* antibodies were associated with various sexual activities, and *Mg* seropositivity was mainly associated with increased sexual activity in early adulthood.

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Introduction

The outcome of sexually transmitted *Chlamydia trachomatis* (*Ct*) and *Mycoplasma genitalium* (*Mg*) infections can be severe because of their potentially serious sequels on reproductive health, including pelvic inflammatory disease (PID), salpingitis, and tubal infertility [1,2]. Globally, infections caused by *Ct* are the most prevalent sexually transmitted infections (STIs), second only to the parasite *Trichomonas vaginalis* (*Tv*) infections [2]. According to global estimates, there are nearly 129 million new cases of *Ct* infections worldwide each year [3]. To date, comprehensive global studies assessing the prevalence of *Mg* remain absent. However, a recent meta-analysis concluded the prevalence of *Mg* to be 1.3% and 3.9% in developed and developing countries, respectively [4]. Importantly, both pathogens can cause asymptomatic infections, which are challenging in terms of diagnosis and treatment.

Ct and *Mg* present with analogous clinical behavior, causing similar clinical manifestations or being asymptomatic [1,2,5,6]. Case-control studies have shown that clinical carriage of *Ct* and *Mg* can be independent of one another [6,7]; however, a study of female sex workers showed women with *Ct* and *N. gonorrhea* infection had an increased risk of *Mg* [8]. This correlation is understandable as they share the same route of transmission. Additionally, a recent study at the National Sexual Health Clinic in Singapore found that *Mg* was strongly associated with *Ct* infection, present in 8.1% of cases compared to 2.4% of *Ct*-negative cases [9]. Nucleic Acid Amplification Tests (NAATs) or Polymerase Chain Reaction tests (PCRs) are reliable in the diagnosis of *Ct* and *Mg,* and in recent years, serological assays, among other methods, have been exploited to better understand the epidemiology of these infections [10].

Quantification of different *Ct* and *Mg* serum antibodies can provide an accurate means to evaluate past or ongoing infections [10–13]. Plasmid Gene Protein 3 (known as pgp3 or pGp3) is a highly

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63 immunogenic antigen during chlamydial infection [14–17], and notably, the antibodies produced

64 against pGp3 exhibit a strong dependence on its protein conformation. In a UK-based study [13,18]

65 that compared several assays in the same population, pGp3 serology was the most sensitive to detect

66 a previously known *Ct* infection.

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68 Mycoplasma genitalium protein of adhesion (MgPa) is the major adhesin protein and the primary

69 virulence factor of *Mg,* playing an essential role in mediating the attachment of the bacteria to host

70 cells, thus facilitating their subsequent invasion [11,19,20]. Recombinant MgPa (rMgPa) is the C-

71 terminal part of MgPa. Serological assays using MgPa and rMgPa antigens have shown promise in

72 detecting *Mg* antibodies without cross-reactivity to *M. pneumoniae* [11,21]. The interactions between

73 *Mg* antigens and the host immune responses, however, remain largely unexplored.

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75 The primary objective of this study was to cast further light on understanding the serological outcomes

76 of *Ct* and *Mg* antibodies in young women and men and to exploit the possible co-factors role in *Ct* and

77 *Mg* seropersistence.

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Material and methods

Subjects

This study is based on the prospective Finnish HPV Family Study (FFHPV) cohort study jointly conducted at the Department of Obstetrics and Gynecology, Turku University Hospital (TUH), and the Institute of Dentistry, Faculty of Medicine, University of Turku, Finland [22]. The participants comprised 329 pregnant women and 135 fathers-to-be and their newborns who were enrolled (between 1998 and 2001) during the index pregnancy, at 36 weeks of pregnancy [22], and subsequently followed up for three years. The adult participants comprised spouses, however, the spouses of 194 women opted not to take part in the study, thus accounting for the difference in the number of male and female participants. All study participants were of Caucasian descent and shared the same ethnic background [23]. The Research Ethics Committee of Turku University Hospital has approved this study's design (#3/1998 and 2/2006, with amendments 45/1801/2018).

The participants were requested to complete a structured questionnaire at baseline and repeated as shorter versions at 3-year and 6-year visits. It encompassed over 60 meticulously designed questions, including information about demographics, sexual behavior, smoking habits, history of STIs, and overall health. Responses about the childbirth experience were collected from the mothers at approximately two months post-partum. To minimize the potential bias, the spouses participating in this study were deliberately kept uninformed regarding the content of his/her spouse's questionnaires [22].

Serology

Blood samples were collected at the baseline and subsequently at 12-, 24- and 36-month follow-up visits from both spouses. The serological assays were performed in collaboration with the German Cancer Research Center (DKFZ), Heidelberg, Germany. For *Ct,* the serum IgG-antibodies to highly

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immunogenetic antigen pGp3 were assayed [14,15]. Serology to *Mg* was analysed using the primary

virulence factor of this bacterium, MgPa, in two protein fragments, MgPa N-Term and rMgPa [19,20].

Studies about *Mg* serology are scanty, but antibodies targeting MgPa and rMgPa have been previously

described [11,24]. The quantitative multiplex serology assay was used as previously described [24,25].

This method is based on glutathione S-transferase (GST) capture ELISA combined with fluorescent-

bead technology. The median fluorescence intensity (MFI) of at least 100 beads per antigen was

measured, and the cut-off values for *Ct* (pGp3) seropositivity was MFI> 500 as well as for *Mg* MFI>

1000 for both MgPa N-Term and rMgPa. Assay development and validation are described in detail

earlier [24].

Statistical analysis

The flowchart of the study design is illustrated in **Supplementary Figure 1**. Overall, 280 women and

115 men in the *Ct* antibody analysis and 264 women and 113 men in the *Mg* antibody analysis were

included in the final statistical analyses. They all had at least two visits during the follow-up. Subjects

with only one blood sample or no visits were excluded (50 women and 20 men). Additionally, we

excluded participants with fluctuating serological results, e.g., seropositive at baseline, negative at the

next follow-up, and then again positive (1 woman and 4 men from the *Ct*- and 17 women and 6 men

from the *Mg* analyses).

The final cohorts were divided into subgroups according to the seropersistence to *Ct* and/or *Mg*. The

always negative subgroup consisted of participants whose antibody levels remained below the defined

cut-off value at every follow-up visit. The seropersistent subgroup consisted of the participants, who

remained constantly seropositive to the given antigen(s) during the entire follow-up. The

seroconversion group included individuals whose antibody levels transitioned from seronegative to

seropositive, with an additional criterion of at least a two-fold increase over their previous serum

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129 measurement, after which all subsequent antibody levels for these individuals remained consistently

130 seropositive throughout the follow-up period. Respectively, the serological decay subgroup consisted

131 of those participants whose antibody titers were falling at least 50% from the previous serum titer

132 below the cut-off values and remained negative until the end of the follow-up. For *Mg*, both antigens

133 (Mg-Pa and rMgPa) had to increase/decrease the above amount to be included in the final serology

134 outcomes groups.

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136 Frequency tables were analysed using the $\chi 2$ test, employing the likelihood ratio or Fisher's exact test

137 as appropriate for categorical variables. For the examination of differences in means of continuous

138 variables, nonparametric tests (Mann-Whitney U test or Kruskal-Wallis) test were used. Crude and

139 adjusted odds ratios (ORs) and their 95% confidence intervals (95%CI) were calculated by using

140 logistic regression. In the adjusted model, age and all baseline co-factors that were statistically

141 significant in the crude analysis were simultaneously included for mutual adjustment. All statistical

142 analyses were performed using STATA MP17.0 (Stata Corp., College Station, TX, USA). All tests were

143 run two-sided, and, in all analyses, probability values (p-values) of <0.05 were considered statistically

144 significant.

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Results

147 *Ct* seroprevalence at baseline was 32.4% among all women and 20.3% among all men. The

148 corresponding results for *Mg* were 16.4% and 8.3%, respectively. The mean MFI antibody levels of

149 pGp3 (*Ct*) and MgPa and rMgPa (*Mg*) among the seropersistent women and men stratified by their

150 FU-visit are shown in **Figure 1**. The mean MFI levels of all three antibodies remained stable during

151 the whole FU period of the seropersistent women. In men, however, variation of the levels of two IgG

152 antibodies (MgPa and rMg) of *Mg* was seen, although the number of *Mg* seropositive men was much

153 lower (only 5 to 6 men at each follow-up point).

155 The majority of both women and men remained seronegative to *Ct,* 65.7% and 81.7%, respectively.

156 The same was true for *Mg,* to which 85.2% of women and 92.0% of men tested constantly seronegative.

157 Persistent seropositivity to both *Ct* and *Mg* was more common in women (30.4% and 13.3%) than in

158 men (17.4% and 5.3%), respectively. Seroconversion or serological decay were rare events.

159 Additionally, there were only 20 women and 3 men who were persistently seropositive to *Ct* and *Mg*

160 during the 3-year follow-up. Regarding the serological outcomes between couples, there were 72

161 couples in *Ct-* and 92 couples in *Mg* analyses with corresponding serological profiles, either

162 seropositive or negative. These data are summarized in detail in **Supplementary Table 1**.

165 The demographic data stratified by the different serological outcomes of *Ct* and *Mg* among women are

166 shown in **Supplementary Table 2**. The mean age of women varied between 23 to 27 years among the

167 serological outcome groups. Women with persistent *Ct* antibodies were older than those always

168 seronegative (26 years vs 25 years, *p=0.016*).

170 The potential co-factors associated with seropersistence for *Ct* and *Mg* in women during the follow-up

171 were first evaluated with crude ORs (**Supplementary Table 3)**, followed by **Table 1,** which has all

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significant baseline co-factors adjusted with each other. The increasing number of lifetime sexual partners was associated with persistent (pGp3) seropositivity to *Ct, as* the OR increased in parallel with the increasing number of lifetime sexual partners, up to crude OR 12.1 (95%CI 1.29–32.79) in those who reported more than 10 lifetime sex partners. This association stayed significant also with the adjusted model with the 6–10 and >10 partners, with an OR of 5.5 and 5.6 (95%CI range of 1.39 to 22.29). Seropersistence to *Mg* was significantly associated only with more than 10 lifetime sex partners, crude OR 15.5 (95%CI 3.36–59.71). However, this did not stay significant after adjustment. Sexual debut at the age of 16 years or later was associated with lower odds for seropersistence to *Ct* and *Mg* separately or in combination, but after adjustment, none of these remained their significance. Women reporting more than 10 sexual partners before the age of 20 years had relations to becoming persistently seropositive to *Ct:* crude OR 5.2 (95%CI 1.82–14.72) or *Mg:* crude OR 14.6 (95%CI 3.57–59.71) or both: crude OR 27.7 (95%CI 4.18–182.92). Interestingly, after adjustment, only persistent *Mg* remained significant with an adjusted OR 12.3 (95%CI 1.52–100.90).

In women, occasional anal intercourse was connected to *Ct* seropositivity (crude OR 2.1, 95%CI 1.07–4.04) and with the *Mg* and *Ct* combination seropositivity (crude OR 3.4, 95%CI 1.14–10.12), while oral sex had no such association. After adjustment, the occasional and regular anal intercourse remained significant with *Ct* only, adjusted OR 2.1 (95%CI 1.00–4.58) and 15.3 (95%CI 1.18–197.12), respectively. As expected, reported STIs were associated with *Ct* seropersistence in both crude and adjusted models but did not influence *Mg* seropersistence. In our study, only 28.2% of the women and 45.0% of the men seropersistent to *Ct* reported a history of diagnosed infection.

Also, when evaluating factors at the end of the study follow-up, a higher number of deliveries seemed to be protective against seropersistence to *Ct.* There was nearly a 15-fold increased likelihood (95%CI 1.88–112.17) for seropersistence to *Ct* when the use of contraceptive pills was started at the age of 14

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197   to 16 years. The association was also evident among women who had changed their partner since

198   entering the cohort, with OR ranging from 2.7 (95%CI 1.24–6.05) to 4.9 (95%CI 1.44–16.52).

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200   **Table 2** and **Supplementary Table 4** summarize the corresponding data of the men. For men, no

201   separate adjusted OR table was done due to only one significant finding after adjustment, as further

202   clarified under. The mean age varied between 27 and 36 years among the different serogroups of *Ct*

203   and *Mg*. Men with 6–10 sex partners by the age of 20 years were more likely seropersistent to *Ct* (<5

204   as the reference group) crude OR of 3.4 (95%CI 1.05–11.14), but this did not stay significant after

205   adjustment with overall sex partners. Men reporting >5 lifetime sexual partners were significantly

206   associated with *Ct* seropersistence, which remained significant also after adjustment, adjusted OR 12.6

207   (95%CI 1.55–102.49). Importantly, men who reported having had several partners during the index

208   pregnancy were more likely to have simultaneous seropositivity to both bacteria, crude OR 36.0

209   (95%CI 1.61–805.20). Interestingly, none of the co-factors included in our analysis showed any

210   significant association with *Mg* seropersistence in men.

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212   As the female and male subjects of our study represent marital couples, we also made a pair-wise

213   analysis of the serological outcomes among the seropersistent couples. However, only a limited

214   number of couples demonstrated co-existent seropersistence for *Ct* (n=10) or *Mg* (n=4). As measured

215   by the mean seropositivity among these couples, we could not disclose any significant correlations

216   between the couples at any time point (using a scatterplot). As expected, the scatterplot yielded a

217   limited number of measurements with notable dispersion. The same limitation hampered the analysis

218   of the potential co-factors in these couples, including the meaningful calculation of odds ratios.

219   Noteworthy, however, is the fact across all three categories (*Ct, Mg,* and antibody co-occurrence), none

220   of the couples with seropersistence reported a history of five or fewer lifetime sexual partners (data

221   not shown).

Discussion

Our family study focused on the serological outcomes of *Ct* and *Mg* and also assessed the potential co-factors associated with their seropersistence, followed up for three years. The persistence of *Ct* antibodies was associated with a variety of sexual practices among women but less significantly among men. Among the women in our cohort, both adolescent and lifetime number of sexual partners were associated with both *Ct* and *Mg* seropersistence. Among the men in our study, no such associations were disclosed with *Mg* seropersistence. Until now, the majority of the risk factors for *Ct* and *Mg* have been focused on seropositivity, not seropersistence. However, it is important to understand i) whether the acquired IgG antibody levels remain stable, ii) which are the potential co-factors that predict the persistent seropositivity in a longitudinal setting, and iii) which are the possible consequences that might be attributed to seropersistence.

Previous studies have shown that both *Ct* and *Mg* are usually contracted during young adulthood. *Ct* usually peaks under the age of 25 in both genders [26] while *Mg* peaks in males at 25–34 years and in females at 16–19 years [4]. Microbe-specific serum IgG antibodies signify past or ongoing infection, though factors like antibody specificity, assay type, time since infection, and patient characteristics can affect IgG response and serological assay efficacy, particularly for prior *Ct* infections [18,27]. It is known that mucosal *Ct* infections typically elicit an observable rise of IgG responses when suitable serological assays are used. Thus, antibody levels can reflect both past or ongoing *Ct* infection [13,18,27,28], and in a clinical setting to confirm the infection, additional characterizations of the pathogen's nucleic acids are needed.

In a Finnish study, *Ct* IgG antibodies to MOMP (chlamydial major outer membrane protein) were detected in 65.5% of women within three months of infection onset, with approximately one-third remaining seropositive for 3–10 years after the initial infection [29]. Another study of a female cohort

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247 using the same antigen as in the present study reported that pGp3 antibodies persisted for up to 12

248 years, and the antibody prevalence in the female cohort was higher than in their male counterparts [18].

249 Another study based on 9.695 biobank samples with a wider age distribution showed that pGp3

250 seroprevalence was 25.7% among women and 15.9% in men [30]. Our study confirms these

251 observations on the stability of *Ct* antibodies and reports a similar prevalence of these antibodies (being

252 higher in women), although the participants in our study were significantly younger.

253

254 As both *Ct* and *Mg* are STIs, we analysed sexual practices, but also non-sexual co-factors as predictors

255 of seropersistence. The present analysis disclosed that an increasing number of lifetime sexual partners

256 and early onset of sexual activities were significantly associated with seropersistence to both bacteria.

257 Previous studies have confirmed the association between an increased number of sexual partners and

258 the prevalence of *Ct* and *Mg*. In the study by Horner and colleagues [13], the OR increased in parallel

259 with an increasing number of sexual partners, and in participants who reported having had 20 or more

260 partners, OR for pGp3 seropositivity ranged from 49.1 to 58.7, depending on whether the participants

261 had reported past *Ct* infection. Early sexual debut was also associated with seropositivity, consistent

262 with our results. As to *Mg* infections [31], it was reported that their prevalence was more common in

263 women reporting two or more sexual partners in the previous year (adjusted OR 2.2). In another survey,

264 a strong association of *Mg* with sexual risk behaviors was disclosed in both genders [32]. Noteworthy

265 is the fact that these studies focused on NAAT detection, being different from our approach.

266

267 The prevalence of *Mg* infection closely parallels the prevalence of *Ct* among women with high-risk

268 sexual behavior, such as multiple partners and inconsistent condom use [33]. Studies on simultaneous

269 serology of *Ct* and *Mg* are scanty, and studies on seropersistence measured with different *Mg* antigens

270 are nearly lacking. In our cohort, seropersistence to both pathogens was more common among women

271 (11.1%) than men (3.5%). Persistence of *Mg* infection has been demonstrated before [8,31], although

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272 those studies used NAATs and had shorter follow-up periods compared to our serological approach.

273 Additional adequately powered studies are needed to acquire a comprehensive view of *Mg* infections

274 and their clinical implications. Given their shared STI pathogenesis, it is likely that the factors driving

275 *Mg* transmission and serological persistence parallel those identified for *Ct*.

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277 *Ct* infections are extensively studied due to their serious reproductive health consequences when

278 untreated, including increased risks of adverse pregnancy and fertility outcomes [2,26]. Recently, it

279 was shown that *Mg* seropositivity was also more common among women of infertile couples (5.4%)

280 than fertile couples (1.6%) with an OR of 3.5 (95%CI 1.10–10.75) [12]. However, no such association

281 was observed in male partners [12]. In our study, all women were pregnant at baseline, and only one

282 fertility-associated co-factor could be identified; a higher parity was a protective factor against *Ct*. This

283 likely reflects more stable relationships and reduced exposure to *Ct* due to fewer lifetime sexual

284 partners and lower engagement in high-risk behaviors. Notably, among women with 10 or more

285 lifetime sexual partners, having a parity of three or more was associated with a stronger protective

286 effect, adjusted OR 0.3 (95%CI 0.09–0.89).

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288 It can be speculated that protective factors against *Mg* seropersistence, including a lower number of

289 sexual partners, could be similar to those of *Ct*. However, our study did not substantiate this hypothesis.

290 A limitation of this study is the relatively small cohort size, which restricted our ability to explore the

291 causes of seroconversion or decay in greater depth. Additionally, reliance on self-reported data for

292 variables such as STI history, partner count, and sexual behaviors may have introduced potential non-

293 differential misclassification, adding a degree of vulnerability to our findings. An important strength

294 of our study is its longitudinal design, with repeated serum sampling throughout the follow-up period,

295 allowing for a detailed analysis of changes over time.

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297   Taken together, persistent *Ct* antibodies exhibit a strong association with well-defined high-risk sexual

298   practices among women, less significantly in males. It can be concluded that appropriate preventive

299   measures are of vital importance in combating STIs, irrespective of gender. Notably, seropositivity to

300   *Mg* was predominantly correlated with the early sexual behavior of women but not those of men.

301   Further studies of both genders are warranted to comprehensively assess the factors associated with

302   *Mg* infection and the development of serological persistence.

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Declaration of interest

The authors declare no conflict of interest.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Table 1. Age and all potential significant baseline co-factors* adjusted with each other of persistent seropositivity versus seronegativity to *C. trachomatis* and/or *M. genitalium* among *women* in the Finnish Family HPV study.

	Chlamydia trachomatis	Mycoplasma genitalium	Combination [a]
		Adjusted OR (95%Cl)	
Questionnaire at baseline visit			
Age	1.1 (0.96–1.19)	**1.2 (1.01–1.36)**	1.2 (0.94–1.45)
Smoking	0.8 (0.41–1.57)	1.6 (0.67–3.98)	1.2 (0.32–4.47)
Number of lifetime sex partners			
0–2	1.0	1.0	…
3–5	2.5 (0.88–7.37)	1.7 (0.31–9.41)	…
6–10	**5.5 (1.65–18.58)**	1.3 (0.21–8.75)	…
>10	**5.6 (1.39–22.29)**	2.8 (0.39–20.32)	…
Age at first sexual intercourse ≥ 16 years	0.6 (0.28–1.27)	0.8 (0.26–2.44)	0.5 (0.08–3.23)
Number of sex partners before the age of 20			
0–2	1.0	1.0	1.0
3–5	1.3 (0.53–2.30)	**5.0 (1.23–20.17)**	4.7 (0.42–43.03)
6–10	0.7 (0.21–2.34)	**6.2 (1.17–32.85)**	3.1 (0.25–39.08)
> 10	1.2 (0.25–6.04)	**12.3 (1.52–100.90)**	6.0 (0.25–147.95)
Practise of anal sex			
Never	1.0	1.0	1.00
Occasionally	**2.1 (1.00–4.58)**	1.9 (0.74–4.83)	3.0 (0.73–11.92)
Regularly	**15.3 (1.18–197.12)**	11.4 (0.80–162.57)	**168.8 (3.22–8845.50)**
History of reported STIs			
None	1.0	1.0	1.00
Chlamydia	**18.0 (5.59–57.92)**	1.2 (0.37–4.12)	**16.1 (2.24–115.93)**
Genital herpes	1.9 (0.46–7.75)	1.2 (0.25–6.23)	4.0 (0.43–36.85)
Other / several STIs	**3.7 (1.15–11.90)**	1.2 (0.27–5.13)	1.4 (0.10–20.87)

* *Crude ORs shown in Supplementary Table 3.*

[a] *Participants consistently testing negative/positive to both Chlamydia trachomatis and Mycoplasma genitalium.*

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Table 2. Potential co-factors of persistent seropositivity versus seronegativity to *C. trachomatis* and/or *M. genitalium* among men during the three-year follow-up. Significant crude association highlighted in bold and those that remained significant after adjustment are underlined.

	Chlamydia trachomatis	*Mycoplasma genitalium*	*Combination* [a]
	Crude OR (95%Cl)		
Questionnaire at baseline visit			
Smoking	1.1 (0.40–3.27)	1.2 (0.19–7.63)	NC
Infertility	NC	NC	NC
> 5 sex partners overall	<u>**13.7 (1.75–107.05) ***</u>	NC	NC
≥ 5 sexual intercourses a month	0.9 (0.31–2.52)	1.0 (0.16–6.27)	0.6 (0.04–0.71)
Age at first sexual intercourse ≥ 16 years	0.4 (0.14–1.12)	0.6 (0.09–3.67)	NC
Number of sex partners before the age of 20			
0–5	1.0	1.0	1.0
6–10	**3.4 (1.05–11.14)**	1.6 (0.16–16.91)	…
> 10	1.8 (0.33–9.77)	3.1 (0.29–33.24)	…
Oral sex at least occasionally	0.6 (0.16–2.61)	…	…
Anal sex occasionally	1.5 (0.47–4.74)	6.2 (0.96–39.48)	4.4 (0.26–75.18)
History of reported STIs			
None	1.0	1.0	…
Chlamydia	**15.3 (2.46–95.19)**	1.7 (0.10–29.18)	…
Other	…	…	…
Allergies	1.8 (0.64–4.76)	2.2 (0.34–13.50)	1.7 (0.10–28.36)
Atopy	0.9 (0.10–8.08)	NC	NC
More than one sex partner during this pregnancy	2.5 (0.21–28.81)	11.6 (0.86–156.63)	**36.0 (1.61–805.20)**

[a] *Participants consistently testing negative/positive to for both Chlamydia trachomatis and Mycoplasma genitalium*

* *Adjusted OR 12.6 (1.55–102.49).*

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Figure Legends
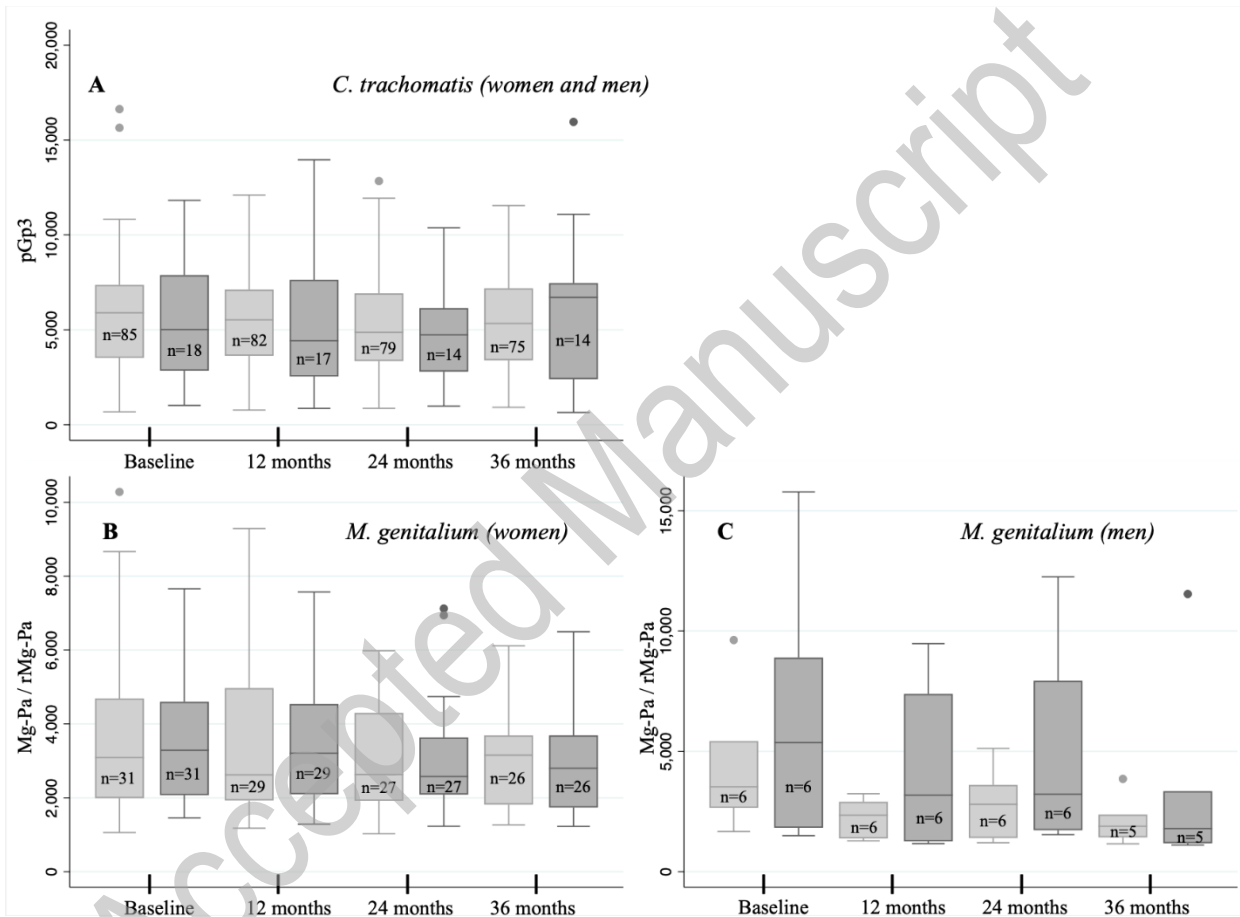**Figure 1.** Mean IgG-antibody levels to *C. trachomatis* antigen pGp3 and *M. genitalium* antigens MgPa and rMgPa

among the seropersistent women and men followed for 3 years. A) Mean antibody levels to *C. trachomatis* in the

seropersistent subgroup stratified by follow-up visit. The light gray bars represent women, and the darker gray bars

represent men; B) Mean antibody levels to *M. genitalium* in women (lighter gray for MgPa and darker gray for rMgPa)

and C) Mean antibody levels to *M. genitalium* in men (lighter gray for MgPa and darker gray for rMgPa). n= stands for

the number of seropositive individuals at each visit.

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