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Correlation Between *Chlamydia trachomatis* IgG and Pelvic Adherence Syndrome

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1. Introduction

Chlamydia trachomatis genital tract infections are prevalent worldwide (Stamm 2008), with 92 million new *chlamydia* cases occurred every year: 3–4 million new cases occur every year in the US, 5 million in Western Europe, and 16 million in Sub-Saharan Africa (World Health Organization 2001; Weinstock et al., 2004). *Chlamydia* prevalence has been reported to range from 3%–7% among asymptomatic populations in men, and in women range from 3.0% in the general population to 9.5% among university students (World Health Organization 2001; Stamm 2008; Patel et al., 2008; Forhan et al., 2009; Imai et al., 2010; Satterwhite et al., 2010).

Chlamydia is a pathogenic obligate intracellular bacterium with a biphasic developmental cycle that takes place inside a *parasitophorous vacuole* termed an inclusion. This implies cell types adapted for extracellular survival (elementary bodies, EBs) and intracellular multiplication (reticulate bodies, RBs). Within 2 hours after entry into host cells, *Chlamydia trachomatis* EBs are trafficked to the perinuclear region of the host cell and remain in close proximity to the Golgi apparatus, where they begin to fuse with a subset of host vesicles containing sphingomyelin, demonstrating that *chlamydial* migration from the cell periphery to the peri-Golgi region resembles host cell vesicular trafficking (Grieshaber et al., 2003).

Due to their obligate intracellular nature, the detection and manipulation of *Chlamydia* have proved challenging. Novel techniques such as real-time PCR facilitate the diagnosis of infections due to these pathogens, but in the absence of organized screening programs, asymptomatic infections are not treated, leading to several serious diseases. The development of tests based on nucleic acid amplification technology represented an important advance in the field of STD (sexually transmitted disease) diagnosis. Nucleic acid amplification detection technique is more sensitive and specific and offers the

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opportunity to screen for infections in asymptomatic individuals, using noninvasive sampling.

Generally, in female genital tract *Chlamydia trachomatis* produce asymptomatic infections in approximately 80% of women (Zimmerman et al., 1990), but is associated with serious reproductive morbidity causing cervicitis (Marazzo & Martin, 2007; Stamm et al., 2008; Falk 2010) and is also associated with urethritis. (Dieterle, 2008), PID (Howie et al., 2011), infertility (Forti G. & Krausz C., 1998).

The pathogenic processes causing the sequelae are thought to be partly immunological (Beatty et al., 1994) When initiation and propagation of infection occurs, both humoral and cellular immune response are triggered. In damaged area an influx of immune cells (lymphocytes, macrophages, dendritic cells) occurs. At the site of infection there is a strong inflammatory reaction characterized by a mucopurulent vaginal discharge and formation of immune complexes, a process that may contribute to the immunopathology of the disease. However, the absence of tools for genomic manipulation has limited the understanding of factors involved in host cell interactions.

However, all factors that induce inflammatory reaction (intrauterine devices for contraception, sexually transmitted diseases, etc) increase the risk of pelvic inflammatory disease (PID). Generally, the *Chlamydia trachomatis* infection resolves without sequelae, but occasionally it spreads from the lower to the upper genital tract and pelvic inflammatory disease may develop (Paavonen et al., 1985; Morre et al., 2002), leading to scarring of the fallopian tube, causing occlusion, resulting in ectopic pregnancy or tubal factor infertility. The diagnosis of the adherential syndrome is performed tardily using laparoscopy, an invasive method. In order to find markers that would impose the needing to perform laparoscopy from the beginning our aim was to evaluate possible correlations between various microbiological agents involved in infertility caused by pelvic adherence syndrome. We also evaluate a possible correlation between the presence of Ig G for *Chlamydia Trachomatis* and the adhesion syndrome.

2. Methods and materials

2.1 Patients

174 infertile women (with a mean age of 31.38 ± 4.3) were enrolled in the study between 2008- 2010. The study received the approval of ethical committee of Grigore T. Popa University of Medicine and Pharmacy.

2.2 Methods

Anamnestic data included: history of surgery in pelvic-abdominal area, obstetric history (pregnancies, births, abortions, SEU). All women performed an analysis set: assessment of ovarian reserve by second day hormonal dosage, ultrasonographic monitoring ovulation, spermogram and sperm culture of the male partner, uterotubal assessment component of infertility using the HYCOSY, HSG and hysteroscopy coupled with laparoscopy with Dye Test, considered to be the gold standard technique.

Hysteroscopies coupled with laparoscopies with blue methylene test, were carried out under general endotracheal anesthesia, in the operating room, using Karl Storz®

Tuttingen Germany equipment. We always started by a diagnostic hysteroscopy with the intention to visualise the entire cavity, the mucosal aspect and the tubal proximal ostia. After the cannulation of the uterus, we continued with the laparoscopic time, consisting in a peritoneal CO₂ insufflation and a transabdominal insertion of a 10mm laparoscope. We performed the visual inspection of the pelvic area, recording the aspect of the uterus and both adnexes (ovaries and tubes), the presence of the intraperitoneal adhesions and the foci of endometriosis. Adherence syndrome was framed according to the AFS classification (1998) in the following categories: minimal 1-5 points, mild 6-10 points, medium 11-20 points, severe 21-32 points. After completion of the pelvic inspection, the right upper quadrant was evaluated for the presence of perihepatic adhesions (Fitz Hugh Curtis Syndrome).

During pelvic surgery, attention was given to minimise the tissue handling, excision of all adhesions when possible and constant irrigation of tissue with warm saline solution during adhesiolysis. We used the electro surgery in bipolar or monopolar mode for dissection and only bipolar mode for coagulation. Adnexa adherent to the uterus were separated and tubes were detached from the ovaries and other pelvic organs. The ovary was separated from the underlying peritoneum of the ovarian fossa or uterosacral ligament. Omental adhesions to the anterior parietal peritoneum or bowel adhesions to pelvic structures were detached. In some patients, both adnexa were completely frozen with dense adhesions to bowel and vital organs. In these cases, operative surgery was not performed and patients were referred to AMP (Assisted Medical Procreation) techniques. Endometriosis foci of the peritoneum were inspected and if superficial, coagulated. Ovarian cysts (serous or endometriotic) were operated by cystectomy. At the end, patency of fallopian tubes was assessed by injecting dilute solution of methylene blue into the uterine cavity through the uterine canula. The passage was considered to be present if the methylene blue was visualised through the external tubal orifice and negative if no methylene blue passage was seen. The tubal obstruction was considered proximal if the tube did not change in volume or colour during the injection of the dye solution and distal, if the external end of the tube dilated and became blue. We performed for these last cases a neosalpingostomy (a new orifice into the external extremity of the tube) if the obstruction was ampullar, or a fimbrioplasty (the dilatation of the fimbrial end of the tube when fimbrial plis were conserved but agglutinated).

2.3 Sample collection and storage

Endocervical samples have been collected in duplicate from all patients using *Chlamydia* Swab/Brush Collection Kit (Bio-Rad Laboratories, France). The process of the samples was performed immediately after collection or samples were stored at 2-8 °C for 24 hours, or freeze at -20/80°C. Serum samples were obtained from each patient. Sera were aliquoted, stored at -80°C and thawed only once.

2.4 DNA isolation

For DNA isolation was used the recommended DNA-Sorb-A (Sacace, REF K-1-1/A) kit. DNA was extracted according to the manufacturer's instructions. 10 µl of Internal Control was added in each isolation mixture. Internal Control was the same for all urogenital infectious kits.

2.5 Real Time PCR for bacteria detection in cervical smear

Bacteria detection was performed using STD Real-TM (Saccace) Kit that detects the most important bacterial infections with *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma genitalium/hominis*, *Neisseria gonorrhoeae*.

2.6 Serological assay

Serological assay was done using NovaLisa™ Chlamydia trachomatis IgA/IgG/IgM ELISA kits (NovaTec Immundiagnostica GmbH, Dietzenbach - Germany).

2.7 Statistical calculations

Statistical calculations were performed using SPSS software (version 16), and P values of 0.05 or less being considered significant.

3. Results

Patients: From 174 women enrolled in our study, 97 cases were primary infertility and 77 cases presented secondary infertility.

In assessing the laparoscopic diagnostic with Dye test 71 adherence syndrome patients were classified according to American Fertility Society, within the following categories: minimal-39 cases, mild-28cases, medium-4 cases, severe-0 cases 7 patients had absent tubes (salpingectomy for ectopic pregnancy in history), (4 right, 3 left).



Fig. 1. Laparoscopic aspect: a) Fitz Hugh Curtis Syndrome; b) Left distal tube obstruction-hydrosalpinx; c) Dye test negative-hydrosalpinx



Fig. 2. Another laparoscopic aspect of diffuse adhesions, involving: a) right adnexa; b) left adnexa; c) perihepatic adhesions (Fitz Hugh Curtis)

Impairment was bilateral in 12 cases and unilateral in 27. Proximal localization of obstruction was observed in 37 patients, and distal in 12. In 2 cases, Fitz Hugh Curtis syndrome was present, one case in correlation with pelvic adhesions and the other with apparently intact internal genitalia, negative PCR results for all the germs tested and Ig G *Chlamydia trachomatis* (IgGCT) negative. Figure 1 show some laparoscopic aspect.

3.1 Bacterial diagnostic

In order to prove bacterial presence, real time PCR was performed. *Chlamydia trachomatis* DNA was detected in two smears, *Mycoplasma hominis* in 7, *Ureaplasma urealyticum* in 54 and only one was positive for *Neisseria gonorrhoeae* according to table 2.

	Positive
<i>Chlamydia trachomatis</i>	2/174 (1.15%)
<i>Mycoplasma hominis</i>	7/174 (4.02%)
<i>Ureaplasma urealyticum</i>	54/174 (31.03%)
<i>Neisseria gonorrhoeae</i>	1/174 (0.57%)

Table 1. Detection of bacterial DNA.

Serological detection of IgG specific for *Chlamydia trachomatis* was associated with presence of adhesions in 40 cases from 52 positive cases (76.9%). However, 38 cases that test negative for IgG (31.1%) presented adherence, but only 12 cases that didn't show adherence have IgG positive test (table 3).

		adherence	
		present	absent
IgG CT	present	40/52 (76.9%)	12/52 (23.07%)
	absent	38/122 (31.1%)	84/122 (68.9%)
	total	78/174 (44.8%)	96/174 (55.2%)

Table 2. Association of *Chlamydia trachomatis* IgG with presence of adherence,

Evaluating CT IgG positivity as possible marker for tubal obstructions (distal and proximal summed), we found:

- For tubal passage: sensitivity 36.5%, specificity 73.8%, PPV (positive predictive value) 44.2%, NPV (negative predictive value) 67.2%;
- For pathological tubal aspect: sensitivity 37.1%, specificity 79.2%, PPV 69.2%, NPV 50%.

		left and/or right tubal passage		tubal aspect	
		present	absent	present	absent
IgG CT	present	23/52 (44.2%)	29/52 (55.8%)	36/52 (69.2%)	16/52 (30.8%)
	absent	40/122 (32.79%)	82/122 (67.21%)	61/122 (50%)	61/122 (50%)
	total	63/174 (36.2%)	111/174 (63.8%)	97/174 (55.75%)	77/174 (44.25%)

Table 3. Association of *Chlamydia trachomatis* IgG with tubal disorders

Evaluation of outbreaks of endometriosis as a marker of adherent pelvic syndrome gave the following results:

adherences		endometriosis	
		present	absent
adherences	present	4/78 (5.22%)	74/78 (94.87%)
	absent	2/96 (2.08%)	94/96 (97.92%)
Total		6/174 (3.45%)	168/174 (96.55%)

Table 4. Association of endometriosis with presence of adherence

Regarding the classification performance of endometriosis with adhesions, we found the following values: sensitivity 66%, specificity 56%, PPV 5.2%, NPV 97.9%

adherences	Previous surgical interventions	
	present	absent
present	25/78 (30.05%)	53/78 (67.95%)
absent	18/96 (18.75%)	78/96 (81.25%)
Total	43/174 (24.71%)	131/174 (75.29%)

Table 5. Association of previous surgical pelvic interventions with presence of adhesions

The statistical measures of classification performance are: sensitivity 58%, specificity 59%, PPV 32%, NPV 81%.

We also used logistic regression with adherence the dichotomous dependant variable and the predictors defined by endometriosis, previous pelvic surgery and IgG CT. Hosmer and Lemeshow test concludes if the model adequately describes the data. We had obtained a "p" value greater than 0.9 which confirms the applicability.

Not all the variables have a consistent contribution to the model. Thus the procedure is selecting at each step the predictor with the highest score that has statistical significance (stepwise regression).

			Score	df	Sig.
Step 0	Variables	endometriosis	1.198	1	.274
		IgCT	30.890	1	.000
		Previous surgery	4.092	1	.043
	Overall Statistics		34.937	3	.000

Table 6. Statistical significance of endometriosis, IgG CT, and previous surgery in relation with pelvic adhesions

Next table shows that only two of the predictors appear to be important in prediction:

- For endometriosis, we got a significance of $p=0.274$, so no statistical relevance
- For IgG CT and previous surgery, $p<0.001$ and $p=0.043$, therefore statistically significant means to be added to the model in adhesions prediction.

We have calculated the usefulness of the predictors by means of the B coefficients with the Wald statistic for the two predictors selected in the model.

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
								Lower	Upper
Step 1	Surgical intervention	.786	.391	4.036	1	.045	2.196	1.019	4.729
	Ig G CT	2.024	.388	27.151	1	.000	7.567	3.535	16.202
	Constant	-.999	.227	19.402	1	.000	.368		

Table 7. Wald evaluation for IgG CT, and previous surgery.

The B coefficients meanings have not straightforward interpretation of the logistic regression. Therefore an exponentiation operation of the coefficients is applicable for a better and easier interpretation. The Exp(B) represents the ratio of the odds (odd=risk/(1-risk)) of the dependent variable for an unit increase of the predictor that is in charge. For example the odds of having adherence are 2.196 times bigger for a person with pelvic surgery compared with one that has no pelvic surgery. Similarly the odds ratio of having adherence by Ig G CT may be interpreted.

Both variables have statistical significance, but the largest Exp (B) value expresses that the odds ratio increases 7.56 times for a person with Ig G CT in order to get adherence, compared with no Ig CT presence.

4. Discussions

The attempt of using Ig G to *Chlamydia trachomatis* as a marker for pelvic adherence syndrome, in order to establish the need of performing laparoscopy from the beginning, we obtained a low sensitivity (36%), but a good specificity (76%). That means that there is not a good correlation established between *Chlamydia trachomatis* IgG positivity and tubal obstructions, and some women with disease may be lost in this screening. However, in our study, it seems that most adhesions were given by *Chlamydia*, while in the majority of studies coming from countries with screening programmes for *Chlamydia trachomatis* and for the prevention of STD (sexual transmitted diseases), endometriosis is far on the first place.

Several methods currently used for diagnosis of *chlamydia* infection have its own advantages and limitations. Cytological method (Romanovski-Giemsa staining of smear) is almost not used any more due to its low sensitivity and specificity. Culturing remain the golden tool of diagnosing as it has the highest specificity, and good sensitivity (aprox. 80%), but this method is very labor intensive as *chlamydiae* do not grow on artificial nutrient and isolation of *chlamydiae* need a monolayer of McCoy, L-929 or HeLa cells. Serological detection remain a usefull tool in detecting chronic and acute infections with *Chlamydia trachomatis*. Generally, there is an immunoassay test used to detect different classes of circulating antibodies IgG, IgA, IgM. However, their clinical utility is burdened by heterogeneity of humoral immune response, individual factor being especially important for proportion of false negative results. In superficial genital infections (cervicitis, urethritis), serological test did not have diagnosis relevance because of the superficial location of infection and low circulating antibodies titer subsequent infection. However, IgM is detected early after infection and persists aproximately one month, so it is transient and rises in Ig M titres are infrequently found and IgG have low titer. In contrast, in severe infection, like pelvic inflammatory syndrome, detection of a high titre of IgG indicates an older or evolving infection. However, in order to capture the antibodies dynamics, it is recommend a new sampling and retest. Therefore, according to our results IgG serological detection can be used for evaluate older infections that can cause infertility.

In our study sensitivity for IgG *Chlamydia trachomatis* in adhesion and tubal obstruction was lower than in other studies. Land et al.,(2003) reports a Ig G antibodies for *Chlamydia trachomatis* sensitivity of about 60% for tubal pathology with a 85-90% specificity.

Veenemans et al.,(2002) evaluated HSG and IgG for *Chlamydia Trachomatis* testing in predicting tubal factor infertility and found for HSG a sensitivity of 57% with a specificity of 66% and for IgG CT, a sensitivity of 80% with a specificity of 55%. The authors concluded that both tests have poor predictive value, but because both tests cause minimal inconvenience to the patient, both should be maintained in the infertility primary examination.

There is evident difference in results between published studies and the clinical significance of the the igG CT however has its limitations due to false positive and false negative test results (Land 2003). Patients with false negative CT antibody test results may have not *chlamydia* related causes of adhesions or tubal occlusions or no antibodies may be found after a previous CT infection.

Between other causes of adhesions or tubal occlusions, the most frequent are: pelvic endometriosis, previous pelvic surgery (for infertility, appendicitis, peritonitis),(Smart et al., 1995, Moll et al., 1997), previous pelvic minimal invasive investigations (HSG, Histeroscopy, dilatation and curettage, IUD applications, past history of abortion) and genital infections with other microorganisms than *Chlamydia trachomatis* : *Neisseria gonorrhoeae*, *Ureaplasma urealyticum*, *Mycoplasma genitalium/hominis* and other germs associated with *bacterial vaginosis*.

To analyze the effects of infection with *Mycoplasma genitalium and hominis* on human fallopian tubes (HFT) and to compare them with the effects of infection with the classical genital pathogens: *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, Baczynsta (2007) used in vitro models in which, pieces of normal HFT were infected with different bacteria and analysed by scanning electron microscopy and confocal microscopy. The conclusion was that tubal infection with *Mycoplasma genitalium and hominis* affected the tubal epithelium resulting in cilia damage but effect was very moderate when compared with the extensive damage of the epithelium caused by *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

As a cause of false negative result for CT antibodies tests, it has been postulated that IgG CT antibodies may decline over time after CT infection, between the primary infection in adolescence and the in fertility investigations in adulthood. But Gijzen (2002) demonstrated that, despite the decrease in the antibody titers over time, they do not completely disappear.

Between the false positive values, it was postulated that there are many cross reactions with other *Chlamydia* species and that the current ELISA method of determining IgG CT antibodies may not be very specific.

According to Ossewaarde (1998) cited by Veenemans (2002), the border values and interpretation "clear indication for an infection in the past", depend mostly on the type of the test used, antibody, conjugation, fluorescence lamp and population. Therefore, comparison of titers from different laboratories and different tests is not possible.

Another question raised was, if testing Ig G antibodies for both *Chlamydia trachomatis* and *Chlamydia pneumoniae* will not enhance the detection rate of adhesions and tubal occlusions in subfertile population.

Gijzen (2001) demonstrated that tubal factor subfertility seem to be more common in subfertile women with IgG antibodies to both CT and CP (49%) versus CT antibodies only (30%), but the difference was not statistically significant.

Another reason for differences in sensitivity and specificity of IgG CT dosages is the threshold point chose by the laboratory for a positive result. If the threshold point is high, sensitivity decrease and specificity increase.

Veenemans(2002) reported a sensitivity of 80% with a specificity of 55% for a threshold of 1/32 and a sensibility of 66% for a specificity of 68% when the threshold was 1/32. In our study, the threshold value was 1/32.

Some research groups were able to correlate prevalence and high serum-titre of immunoglobulin IgG antibodies against *Chlamydia trachomatis* with tubal factor infertility

(Machado et al., 2007; Malik et al., 2009). Hjelholt et al., (2011) confirm an association between tubal factor infertility and antibodies to major outer membrane protein and heat shock protein 60 from *Chlamydia trachomatis*, suggesting antibody testing as a supplement in tubal factor infertility diagnosis. No connection was observed between tubal factor infertility and antibodies to human HSP60, pointing to an infectious rather than an autoimmune inflammation as the cause of tubal factor infertility.

The probability that *Chlamydia trachomatis* produces pelvic and tubal lesions is correlated with the persistence of the untreated uncomplicated genital *chlamydial* infections and with the presence of recurrent infections. In a review study (Geisler, 2010), *chlamydia* resolution occurred in 54% of participants at one year follow up, 83% at two years, 91% at three years and 95% at four years. Clinical and biological factors found to be involved in the resolution of untreated uncomplicated *Chlamydia trachomatis*, were: older age, caucasian race to which the clearance is higher.

Cohen et al., (2005) found that select peripheral blood mononuclear cell lymphoproliferative responses to *Chlamydia* EBS and heat shock protein 60 (cHSP60) correlated with protection against incident *Chlamydia* but not with a reduction in incident *chlamydial* infection. Cervical lymphoproliferative response to cHSP10 were higher in recurrent infection, while lymphoproliferative response to OmpA was higher in primary infection (Agrawal et al., 2007). The presence of OmpA A type E protein favour the persistence of infection at 1 year follow up (Morre et al., 2002), as well as infection load (a quantitative measure of persistence organism burden and presumably a surrogate for *Chlamydia trachomatis* replication. Some other factors were associated with recurrent infections (interferon gamma levels were found higher in cervical wash samples from women with recurrent infections), or protective effects against recurrent *chlamydia* (IL10 gene promoter variant, promoter positions -1082, -819, -592) (Wang et al., 2005).

In our study, it is interesting that the values of sensitivity and specificity are somewhat similar for the presence of adhesions and tubal obstruction. That means that when injuries occur appear equally both tubal adhesions and tubal touching.

Perihepatic adhesions are generally considered pathognomonic for pelvic inflammatory disease. This syndrome, named after the individuals who first described it: Fitz-Hugh (1934) and Curtis (1930) is composed of two phases: acute and chronic.

The acute phase presents as a sharp, pleuritic type pain in the right upper quadrant, worsened with coughing, deep inspiration and movement. A laparoscopy performed in this precise moment could surprise inflammation of the peritoneum, overlying the liver and the anterior abdominal wall.

Usually, in laparoscopy, we can see the chronic phase characterised by typical violin string adhesions between the anterior abdominal wall, inferior surface of the diaphragm and the upper/anterior surface of the liver. It is classically correlated with pelvic inflammatory disease, but Hanjani et al., (1992) and Chatwani et al., (1995) described a subgroup of female patients with perihepatic adhesions and no evidence of acute or chronic PID at the time of laparoscopy. One of our cases can be included in this category

too, the infertility seeming to be related to the male factor. The patient denied previous liver disease, right upper quadrant pain or surgery involving the liver, gallbladder or biliary tree.

Potential causes proposed by the previous authors (Hanjani et al., 1992; Chatwani et al., 1995) include:

- subclinical infections or immunologic process of the liver that would not have been recognised;
- a possible prior episode of perihepatitis, secondary to *Neisseria Gonorrhoeae*, as there is no reliable test to detect prior episodes of gonococcal infection, but there is no clinical evidence of the tubal lesion in this patient;
- another possibility was a mild subclinical non *chlamydial* pelvic infection with no histological or biological evidence of past infection.

5. Conclusion

In countries with low resources without prevention programmes for STD (sexual transmitted diseases), *chlamydial* infection remains on the first place as an etiological factor for pelvic adhesions and tubal obstruction in infertility. In our study Ig G for *Chlamydia trachomatis* didn't correlate well with pelvic adhesions, to be able to indicate a laparoscopy only on its presence. We recommend therefore the HYCOSY or HSG to screen patients who need laparoscopy, but because testing for IgG CT causes minimal inconvenience to patient in contrast with HSG and HYCOSY, it still can be maintained in the infertility work up. We are still looking for other infection or immunological markers to indicate the presence of adhesions and therefore, the laparoscopy from the beginning.

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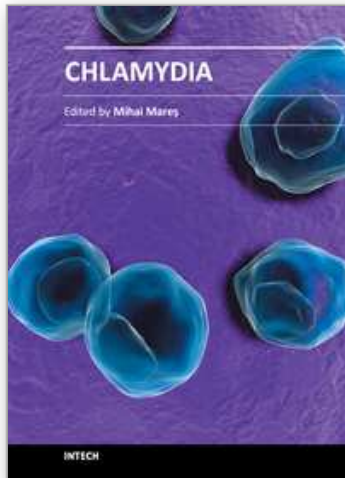
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Nowadays, Chlamydia still represents a redoubtable pathogen. Among its consequences, the blindness in children and severe impairment of reproductive health in adults are the most mutilating. Worldwide, it is estimated that six million of people suffer from post-trachoma blindness and almost 90 million become sexually infected each year. Due to its silent evolution and sexually transmission, the chlamydial infection can occur in anyone. The book "Chlamydia - A Multifaceted Pathogen" contains an updated review of all-important issues concerning the chlamydial infection. It comprises 18 chapters grouped in four major parts dealing with etiology and pathogenicity, clinical aspects, diagnosis and prevention. The new molecular data about the pathogenicity and the exhaustive presentation of clinical findings bring novelty to the book and improve our knowledge about Chlamydia induced diseases.

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