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CONJUNCTIVAL CHRONIC PAIN BY *CHLAMYDIA TRACHOMATIS* SOLVED AFTER ASYMPTOMATIC CHRONIC PROSTATITIS TREATMENT WITH ECHOGUIDED DRUGS INFILTRATION: A CASE REPORT

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Abstract- We present a clinical case report regarding the atypical resolution of a chronic conjunctivitis in young patient with recurrent pharyngitis, asymptomatic chronic prostatitis and infertility of couple, solved after treatment with local, oral and intraprostatic infiltration of massive specific antibiotic therapies. Although several infectious agents are reported causing chronic conjunctivitis, seldom this ocular disease is investigated for Chlamydia trachomatis by molecular PCR approach, in all its whole issue. The chronic conjunctivitis in a young patient represents one of the most common clinical evidences which must be investigated for infectious chlamydial pathology by PCR Chlamydia trachomatis-DNA research together at the other biological sources from different ecosystem sites of the body, but this rarely happens. Further, the couple infertility of the patient was also solved after the massive oral and intraprostatic antibiotic therapies causing a clean-up from several and large intraprostatic parenchymal fibro-calcific areas, condition due to an asymptomatic prostatitis by chronic chlamydial infection. Lastly, the patient developed a precocious reactive arthritis, being a HLA-B27 positive subject.

Key Words- Chronic conjunctivitis, asymptomatic chronic prostatitis, couple infertility, reactive arthritis, HLA-B_{27}, Chlamydia trachomatis, PCR analyses

Case Report

A 30-year-old man, living in a farm in the Pianura Padana, near Bologna, surrounded by several farms with different domestic animals (bovine, ovine, pigs and avians), arrived to our observation for ophthalmic examination after several treatment modalities tried to resolve the chronic conjunctivitis, but with limited success. Despite the use of several short cycle of antibiotic therapy, the patient referred no remission of the specific symptomatology but, on the contrary, he observed subsequent periods of relapsing exacerbation during the wet and windy season [1]. These episodes became always more evident and randomly correlated with recurrent episode of slight whitish urethral discharge and with occasional pelvic pain. This report describe the clinical history of the ambulatory patient with chronic conjunctivitis who underwent five years of semestral controls of unsuccessful therapy. None transrectal prostatic ultrasound scan investigation (TRUS) was required or carried on before.

Occasionally he was suffered from ordinary red throat/pharingitis. Blood and serological analysis showed normal complete blood count, normal erythrocyte sedimentation (7 mm/h), normal C-reactive protein (2.3 mg/L, reference range 0.0 - 6.0 mg/L), VDRL positive but negative IgG and IgM TPHA, TORCH complex negative, negative antinuclear cytoplasmic antineutrophilic, anti-DNA, anti-cardiolipin antibodies and rheumatoid factor, negative Mantoux test. The urine cultural analysis for common germs often resulted negative or positive for saprophytic flora. The research of the antibodies against the Chlamydia trachomatis (IgG and IgA) was negative. The patient has performed several periodical topical steroids (dexamethasone 0.1% QID) and unspecified antibiotic treatments for allergical or microbiological conjunctivitis. Sometimes, the therapies were based on topical different antibiotic treatments alternating a partial recovery with following relapse. In this period he was submitted to several microbiological analyses of conjunctival swabs that were collected from inner canthus and fornix together with seminal liquid coltural analyses for common germs. These microbiological ocular examinations of swabs frequently did not detected growth of any bacteria or fungi, while sometime only a

saprophytic flora was present[2]. The coltural seminal fluid showed a varying and saprophytic flora changing after complete and selective cycles of specific antibiotic therapies in other saprophytic bacteria. The patient underwent 5 years of annual ophthalmologic check-up in other cities Hospitals.

Ophthalmological valuation

The patient was studied according to declaration of Helsinki, obtaining the written informed consent. At the first visit at the Emergency Service of the University Eye Clinic of Chieti, the patient showed relapse with discharge, pain, hyperaemia. He showed a mild redness and slight chemosis of the bulbar conjunctiva and the fibrous scarry tissue of the pathognomonic Arlt's line (similar to the fibrosis for chronic graft versus host disease (GvHD) of the tarsal conjunctiva) in an area of follicles in the upper fornix, due to previous recurrent episodes of periodical irritation (Fig.1). The Schirmer I° test (17 mm) and the Break Up Test (BUT = 8'') showed a weak lachrymal functionality and the BCVA was 7/10.

After one week, the patient was observed a second time to collect an accurate anamnesis and to perform a correct sampling of cellular material by conjunctival scraping on cicatricial lesion after the extroversion of the upper tarsal conjunctiva. We performed also a second ocular swab from inner canthus and fornix to analyse the two different ocular samples in PCR separately, as previously described in detail [2,3]. These biological materials were processed immediately and a sufficient quantity of ophthalmic chloramphenicol ointment was applied afterwards to avoid any possible microbic co-infection and to lubricate the cicatricial conjunctival area exposed to environmental contamination after conjunctival scraping.

Oropharyngeal valuation

At the visit at the Consulting room of the University ENT Clinic of Chieti, the patient signed an informed consent and provided the case history; during the visit he was free from present symptoms, but showed a patinated tongue with viscous salivation; in the last year he had suffered of two pharyngeal episodes. The first was associated with the presence of high fever (38°-39.5°C) accompanied by atypical symptoms like migrant arthralgia. Differently, the second episode exhibited itself with diffused itch, accompanied by slight fever (37°-37.5°C). During these two episodes, the patient received the same antibiotic cycle (amoxicillin-clavulanic acid 1g x 2 times/die for 7 days), obtaining in both cases a temporary recovery.

On demand, the patient declared to have engaged in oral sex frequently. The patient was screened for swab and scraping of the pharynx and both samples were analysed immediately for pathogens, including the Mycoplasmata germs, like Ureaplasma urealyticum (Uu) and Mycoplasma hominis (Mh) (BioMerieux) and Chlamydia trachomatis-DNA research, as previously described [3].

Urological and Prostatic valuation

The patient signed an informed consent and provided the case history. At the initial consultation, the patient referred occasional light urethral disorders (dysuria), slight whitish urethral discharge 8-10 h after sexual activity; he reported on very rare pelvic pain and the failure of repeated cycles of antibiotics (ofloxacin, ciprofloxacin) in the previous two years. Following the recent guideline of National Institutes of Health (*NIH-CPPSI: Chronic Pelvic Pain Symptom Index*)[4], the patient was classified belonging to category IV of asymptomatic inflammatory prostatitis. The TRUS imaging showed different vast disreactive fibrous-calcific parenchymal areas into prostatic gland, probably being due to chronic inflammation from microbiological atypical agents. The patient underwent a) digital rectal examination (DRE), b) transrectal prostatic ultrasound scan (TRUS), c) uroflowmetry, d) coltures for bacteria, yeasts and protozoa and e) molecular researches by PCR for Chlamydia trachomatis (Ct), N. gonorrhoeae (Ng) and Human Papilloma Virus (HPV). 50 mL of each sample of urine were collected during the urological investigations.

Laboratory of Medicine valuation

All routine blood analyses, from hepatic and renal functionalities to all electrolytes, were into leading ranges; the total prostate-specific antigen (PSA_{tot}) level (BioMerieux) was 1.03 ng/mL (95% ile of health population is 0.60 +/- 0.35). The blood of patient, tested for $HLAB_{27}$ by PCR analysis was positive. Microbiological coltures and molecular analyses were processed immediately after the samplings carried out with two different modalities from the same organs like eye and pharynx sources, as already described [2,3]. The different urological samples were collected and immediately processed: 1.0 µL of both the total 50 mL of first voiding and after prostatic massage urine collected was immediately centrifuged at 5.000 rpm for 5 min. The pellet was immediately placed in Eppendorf tube with 1.0 mL of sterile saline solution and vortexed vigorously to disperse cellular material. 50 µL of this suspension were added in mycoplasma medium and put in mycoplasma gallery, covered with paraffin for anaerobic growth (Mycoplasma IST 2), following the BioMerieux instructions. Other 200 µL of cellular suspension previously prepared was taken, centrifuged at 12000 rpm for 15' at 4°C; the pellet was suspended in 180 µL of buffer phosphate 0.025 M pH 7.0, added with 25 µL of proteinase K and incubated at 56°C over-night. 210 µL of absolute ethanol was added to precipitate the DNA. The precipitated DNA was filtered, washed and eluted through the column with 60 µL of elution buffer. The PCR program amplification is already reported[2]). 1.0 µL of seminal fluid for common germs and 50 µL for pathogen Mycoplasmata germs, like Ureaplasma urealyticum (Uu) and Mycoplasma hominis (Mh) (Mycoplasma IST 2) were used. 100 µL of seminal fluid was used for molecular analyses 200 µL of cellular suspension previously prepared was processed following the manufacturer's

instructions [Bio-Aesis srl, Jesi (AN), Italy]. The results are reported in Table I.

Therapy

On the basis of laboratory results, after a total agreement on antibiotic therapies, the patient received a first intraprostatic infiltration of desametazone 12 mg combined with rifampicyn 300 mg and levofloxacine 25 mg, as previously reported [5], adding 5 mM glutathione and 2 mM EDTA[6]. The following cocktails were administered as prostate infiltration and these administrations were repeated after 7 and 14 days, replacing levofloxacine with doxycycline 100mg and rifampicyn with chloramphenicol succinate 1.0g, respectively. The ocular therapy was ophthalmic chloramphenicol/tetracycline ointment, 3 times/day for 7 days together with the oral administration of 500 mg azithromycin, 2 times/day for 3 days. After 4 days from oral completed therapy with azithromycin, the patient began a second short cycle with oral administration of 450 mg rifampicyn, 2 times/day for 5 days. Last cycle of antibiotic therapy was constituted by doxycycline 100mg x 2 times/day for 5 days. The whole treatment was repeated for three months consecutively. During all oral antibiotic therapies, multivitamins and milk enzymes were administered uninterruptedly. Also the sexual partner of the patient received the same oral therapy adding a vaginal lactobacillus to prevent the dismicrobism of vaginal ecosystem.

Comment

Chlamydia trachomatis (Ct) is the most common human infections [7] and it is the major cause for sexually transmitted diseases in European countries. Chlamydial infections are noted for the broad array of clinical distinct manifestations that they produce, ranging from acute self-limiting ocular and genital infections to chronic inflammatory diseases that result in blindness or infertility, passing to chronic prostatitis [7, 8]. The Ct is an intracellular obligate prokaryotic parasite pathogen that is found like elementary bodies (EBs) in rectum, feces and ubiquitary distributed in environment [9], where the flies play a pivotal role as trachoma vectors, as defined in hygiene section of SAFE strategy [10]. The apparent conflicting results for Ct-DNA research obtained from ocular swab and conjunctival scraping are justified from death cellular material from the inner canthus and fornix secretion against living cells from the conjunctival scraping. The presence of Corynebacterium ulcerans in ocular swab has been already fully discussed [2, 11]. The same different modalities of sampling effectuated for the pharyngeal swab and scraping are responsible of apparent conflicting results. More complex seem the results of urological samples. The results of first voiding urine, seminal fluid and urine collected after prostatic massage showed a significant increase for Mycoplasmata germs, varying from absent, 10^3 UCC/mL and 10^4 UCC/mL for Mh and 10^2 UCC/mL, 10^4 UCC/mL and 10^5 UCC/mL for Uu respectively, meaning that the localization of these infections into epithelial cells of terminal urethra are not good sources for their researches, contrary to seminal fluid and urine after prostatic massage. The same behaviour was found for chlamydial infections into several urological samples, where the electrophoretic band of 156 kb for chlamydial cryptic plasmide present into seminal liquid was less evident than that originate from urine collected after prostatic massage, indicating that the parenchymal prostatic tissue was a better container than seminal liquid, while the negative result obtained for the epithelial urethral cells from the first voiding urine cannot always be a good source to research the chlamydial reticulate bodies (RBs), being this late source very poor of infected living cells. It's plausible to think that the RBs, unic forms detectable by PCR, are transformed in EBs, during the final phase of vital cycle and the apoptosis [12]. During the late phase of chlamydial vital cycle, with the decreasing in cellular redox status, the formation of EBs begins with the disulphide-cross-linked proteins formation in the supramolecular structure. This change in the quaternary protein structure confers the EBs rigidity that is missing in RBs [13].

Furthermore, an interesting discussion and suggestions about male infertility induced by Ct infection is reported (14). Although the impact of Chlamydia on semen quality is controversial, our opinion is that it, colonizing the terminal urethral cells, from here could remount up to the parenchymal prostatic tissue where to localize itself, also in approximately 50% of asymptomatic patients, causing a "disreactive fibrous area" around the parenchymal death cells. If we want to speculate about this question, we could suggest that could be the beginning of neoplastic transformation in the population susceptible genetically. In adding, the chlamydial colonization of the epididymal cells and endothelial cells of seminal vesicles, producing a fall of Fructose, ATP and Thiol group's levels, could generate an hostile environmental condition to survival of the nemasperms, reducing the mobility and motility of nemasperms as a consequence of early apoptosis, that could conduce to reduced spermatic function. In support of this, the Mycoplasmas could play a role of impoverishment of the same and the other nutritional molecules. If these new hypothesis of inquiry were proven, this could lead to potentially novel approaches in the treatment of infertile men.

The antibiotic cocktails were administered by transperineal echoguided intraprostatic infiltration either in a random way or inside in any fibrous areas with the aim to dissolve the fibrous-calcific formations and to sterilize them. To reduce the formation of EBs forms, we have added a high intra-prostatic concentration of glutathione that could prevent the incipient conversion of the several chlamydial proteins to the formation of macromolecular complex represented by disulfide-cross-linked homopolymers or heteropolymers[13], stabilizing the RBs forms, susceptible to the antibiotic therapies. In addition, the antibiotic cocktails were supplemented with EDTA to remove Ca^{++} ions from fibrous-calcific areas into the parenchymal gland and to dissolve them.

The patient, before the intraprostatic cocktail antibiotic administration, was screened for blood examinations to evaluate the mineral depletions (Fe^{+2}, Ca^{+2} and Mg^{+2}), creatinine levels and hepatic enzymes activities; these data did show no significant changes, demonstrating that a good renal and hepatic functionality were preserved. Further, some effects including gastrointestinal and musculoskeletal signs, resulting without any clinical aspects were alsao evaluated.

During the first intraprostatic cocktail antibiotic administration, the patient felt a sensation of pleasant prostatic lightly associated two hours after with a copious ocular secretion continuing for the next three days. This clinical manifestation occurred also after the second intraprostatic administration, but in a light manner. After the third intraprostatic administration, no ocular clinical sign was referred by the patient. A light feverishness with night sweats, muscle aches and joint pain appeared 8-10 hours after the first intraprostatic infiltration and in a blurred manner also after the second prostatic infiltration, but they were absent after the third infiltration. These adverse drug reactions (ADR) excluded any allergic phenomenon, while it was classified as a like Jarisch-Herxheimer reaction (JHR) [15]. Five months, later the improved conditions of the patient were confirmed and validated by partner pregnancy: couple infertility indeed was the first reason to start with the urological examination. Afterwards, the patient being HLA-B_{27} positive, hence genetically susceptible [16], developed a reactive arthritis, as already reported [17, 18].

In conclusion, the obtained results confirm that the chlamydial infection involving the whole body needs the correct valuation of all the patients' clinical signs, keeping on mind that they could be asymptomatic in a majority of population and never forgetting that some clinical appearances seem present only in genetically susceptible subjects. Conversely, episodes of chronic relapsing conjunctivitis with papillary nodules and follicular hyperplasia could suggest checking for an undiagnosed prostatitis

We think that only through the recognition of these clinical signs and hypothesizing always the Ct asymptomatic presence, in the future it will be possible to obtain a good chlamydial prevention on Public Health, for which we recommend the complete investigation of all human microbiological ecosystems that can be contaminated by poor hygienic prevention and increasing different sexual practices [19].

Disclosure

Authors have no financial interest or conflict of interest.

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Table 1. The different coltural and molecular analyses obtained from ocular swab and conjunctival scraping, pharyngeal swab and scraping, first void urine, seminal fluid and urine after prostatic massage respectively; the opposite results of the same organs are assignable to different quality of biological sources, resulting in the different sampling modalities.
n.d. = not detected.

Microrganism	Ocular swab	Conjunctival scraping	Pharyngeal swab	Pharyngeal scraping	First voiding urine	Seminal fluid	Urine after prostatic massage
S. pyogenes	n.d.	n.d.	absent	n.d.	n.d.	n.d.	n.d.
S. faecalis	absent	absent	absent	absent	present 40%	present 70%	present 90%
E. coli	absent	absent	absent	absent	present 10%	absent	absent
K. pneumoniae	absent	absent	absent	absent	absent	absent	absent
Staphylo. sp.	absent	absent	absent	absent	present 30%	present 20%	present 5%
G. vaginalis	absent	absent	absent	absent	absent	absent	absent
Corynebacteria	C. ulc. 100%	absent	C. ulc. 100%	C. ulc. 100%	C. ulc. 20%	C. ulcerans 10%	C. ulcerans 5%
Yeast	absent	absent	absent	absent	absent	absent	absent
T. vaginalis	n.d.	n.d.	n.d.	n.d.	absent	absent	absent
M. hominis	n.d.	n.d.	n.d.	n.d.	absent	10^3 UCC/mL	10^4 UCC/mL
U. urealyticum	n.d.	n.d.	n.d.	n.d.	10^2 UCC/mL	10^4 UCC/mL	10^5 UCC/mL
N. gonorrhoeae(*)	absent	absent	n.d.	n.d.	absent	absent	absent
C. trachomatis (*)	absent	**present**	absent	**present**	absent	**present**	**highly present**
HPV (*)	n.d.	absent	n.d.	n.d.	absent	absent	absent

(*) *the molecular analysis is performed in duplicate.*

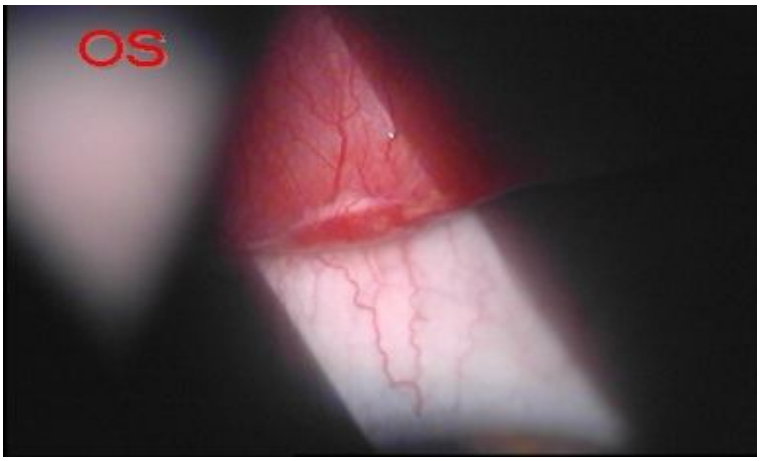

Fig.1- The pathognomonic trachoma sign of a fibrous scarry tissue (Arlt's line) on tarsal conjunctiva in an area of follicles in the upper fornix, where the several samplings have been performed, according to the described modalities. The bulbar conjunctiva shows a mild redness and slight chemosis. A similar Arlt's line is seen in the chronic GvHD.