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Research Article

Molecular Evidences of *Chlamydia trachomatis* and *Urogenital Mycoplasmas* Infections from Birth Evolving into Multisite Infections

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Abstract

Several reports have documented the involvement of *Chlamydia pneumoniae* (Cp) and *Mycoplasma pneumoniae* (Mp) in neonates and children evolving later in asthma, chronic obstructive pulmonary disease (COPD) and chronic bronchitis (CB), depending on individual genetic susceptibility. In adult, either infection can cause, other more severe disease/syndrome on a time depending manner. Nowadays, these critical problems are well noted and the role of their human microbiome is emerging. However, an accurate scientific approach toward these individuals is not routinely carried out by analyzing patients from different department during the neonatal, early and late childhood, putting at risk the health of carriers, genetically susceptible of other latent pathologies. Even worse is the mental attitude of physician/specialist to approach routinely the clinical study of sexual atypical bacteria, such as *Chlamydia trachomatis* (Ct) and *Urogenital Mycoplasmas* [*Ureaplasma urealyticum* (Uu) and *Mycoplasma hominis* (Mh)], at any time, leaving their veiled symptomatology still undervalued and consequently little investigated. An HLA-B_{27} positive 25 years old female, developed at birth, two days after premature delivery, a quite complex respiratory difficulty, occurring with coughing cough and upper respiratory symptoms (non-productive but persistent cough). Time course depending, the patient developed a chronic coughing cough (CCC), laryngopharyngeal reflux (LPR) and/or gastroesophageal reflux symptomatologies (GERD), showing a white line clinical sign and a multisite symptomatology leading to an evolutive inflammaging process, due to sexual atypical bacteria. Anamnesis, serological, hematological, immunological and microbiological analyses, based on cultural and molecular procedures, evidenced the presence of sexual atypical bacteria in all tissues investigated, clarifying the origin of initial oral contamination from atypical sexual *Chlamydiaceae* and *Mycoplasmataceae*, while their spreading into genetically susceptible target tissues was the reason of a precocious "inflammaging" process. The Hp-DNA negative results on oropharyngeal and periorbital sources excluded their involvement into a scenario of GERD and LPR reflux diseases. The Cp-DNA and Hp-DNA negative results on these tissues excluded their involvement in rosacea, remaining attributable only to *Uu* and *Ct*.

Keywords: *Chlamydia trachomatis; Urogenital Mycoplasmas; Chronic Pharyngitis and Multisite Inflammation; Inflammaging*

Abbreviations

pHo: Oesophageal pH; pHg: Gastric pH; TMC: Total Microbic Charge; a-CL: Anti-Cardiolipin; a-PL: Antiphospholipids Antibodies; APPs: Altered Proteins of Acute-Phase; ASOT: Antistreptolysin O Titre; CB: Chronic Bronchitis; CCC: Chronic Coughing Cough; COPD: Chronic Obstructive Pulmonary Disease; Cp: Chlamydia Pneumoniae; CRP: C Reactive Protein; *Ct: Chlamydia trachomatis*; ESR: Erythrocyte Sedimentation Rate; GERD: Gastroesophageal Reflux Disease; LCSS: Lingual Cell and Salivary Secretions; LPNS: Lingual, Pharyngeal, Post

Nasal Drip Mucoid Secretions and Sputum; LPR: Laryngopharyngeal Reflux; NO: Oxide Nitrogen; RF: Rheumatoid Factor; ROS: Free Radicals Oxygen; Spn: *Streptococcus pneumonia*; WAP: Whey Acidic Protein; WBC: White Blood Cells Count

Introduction

The oral and nasal cavities represent the connecting stretches to respiratory and digestive tracts, where their mucosae are continually stressed by the chemical and microorganism agents [1]. On such traits disseminated globet cells, producing mucoproteins, contrast these noxious agents. The healthy human oral cavity microbiome is the consequence of a durable and functional complex interaction arising between microbe distributions and different human cell derived mechanisms, modulating different signal transduction pathways. In addition, we may presume that many physiological stimuli [2], harmonize the coexistence of several factors, evolving from conception to death [3,4]. As widely documented, different mechanisms influence some microbe activity, able to corrupt the physiological embryo development at any time during pregnancy [5,6]. For quite some time, it had been reported the critical damage on trophoblast endocrine functions by *Chlamydia trachomatis* infection [7], together *Urogenital Mycoplasmas*, causing different foetal/neonatal damage [8,9]. Such perturbations may be caused by maternal immune system capacity of neutralizing them and on foetal immune system development, reprogramming the foetal developments towards a good heath status or induction of different diseases. Thus, we are witnessing an increase of new and old genetic syndromes and their genetic variants, still to be revealed. Since a long, it was well known that the *Chlamydiaceae* and *Mycoplasmataceae* infections are able to escape the host immune system and to produce apoptosis, producing free radicals and unbalancing the cellular glutathione potential redox by proinflammatory cytokines [10]. Further, time course depending, these *noxae* can modify the physical and chemical mucin composition and *muc* gene expression, causing an esophageal mucosal injury [11] or contributing to modify their protective action into the contest of respiratory neonatal diseases [12]. Moreover, there are different mechanisms by which the atypical bacteria are able to corrupt the physiological embryo development at any time during pregnancy, acting through several mechanisms that exploit different cell damages depending on maternal immune system capacity of neutralizing them and on foetal immune system maturation. Taken together such mechanisms may direct the foetal develop towards different diseases [7-9]. Thus, a minor dramatic evolutive embryonal *Chlamydiaceae* and *Mycoplasmataceae* contaminations haves been described in asthmatic subjects both in term of ethnogenesis [13], genetic susceptibility [14] and human microbiome knowledges [15], in other minor respiratory diseases [12,16] and, after decades, in juvenile reactive arthritis manifestation [17,18]. Recently, introducing their researches routinely, we reported the presence of these sexual atypical infections into the earliest ophthalmia neonatorum scenery of 3-day-old HLA-B_{27} twins [19]. On the basis of these recent acquisitions of these atypical bacteria, introducing cultural and molecular PCR procedures, we here described a health quality decline of a young HLA-B27 female towards a precocious inflammaging, presenting respiratory difficulties from birth.

Clinical history

A 25 years aged female, suffering from birth of respiratory difficulties with recurrent pharyngitis, was treated in other hospital with antibiotic therapy, based on penicillin and cephalosporin derivatives and oral fluidifying, without any durable benefit. In following years, she periodically received different cycles of antibiotic and anti-inflammatory corticosteroid therapy, without rescuing a stable good health. She spent her prepuberty from some good health moments to pejorative general clinical conditions accompanied with recurrent pharyngitis episodes, respiratory difficulty, high fever (38.5° - 39.5°C) and widespread myalgia. Around fifteen/seventeen, she witnessed a change of salivation from serous to mucoid secretion. After other episodes of recurrent pharyngitis with high fever, the patient manifested a dense and viscous salivation that caused her several bouts of coughing, pain swallowing and breathing difficulties. Her symptomatology showed an alternate trend, characterized by a period of remission followed by recrudescence with increased severity of events. The patient, changing hospital, was referred to our center in ENT for further investigation on a refractory chronic cough and chronic pharyngitis. On admission she showed respiratory difficulties (globe sensation) accompanied with chronic coughing cough (CCC) and laryngopharyngeal reflux (LPR) and/or gastroesophageal reflux disease (GERD). During anamnesis, on demand, her mother detailed us a too premature rupture of membranes during labour, declaring 3 - 5 high feverishness episodes into first year (38° - 39°C), slipping back after antipyretic treatment or naturally "*sine causa*", evolving in the following years with chronic pharyngitis and migrant articular pain manifestations, attributed by pediatrician to child bone increase. About one decade later, bone leg pains and other clinical manifestations (intermittent dysphonia, excessive throat clearing, globus pharyngeus, cough, and dysphagia) began to appear and, time course depending, these were changing into refractory chronic cough and LPR manifestations, that around second decade later, became refractory to common antibiotic, causing dysbiosis, and anti-inflammatory therapies (FANS and corticosteroid-derived). Without receiving any additional applied during the previous 45 days, the patient was submitted to specific clinical evaluation and laboratory investigations. No personal hygiene was made before sampling of microbiological tests. All tissues, clinically involved, were sampled for typical and atypical bacteria. With the

written consent of the patient, the specimens from different inflamed tissues were sent to our laboratory, where blood sample was also collected. After results, the patient was enrolled in a follow-up personal therapeutic program based on different mixed cycles of antibiotic, anti-inflammatory and immunosuppressive therapies.

Clinical results

The lingual and the oropharyngeal features of oral cavity were shown (Figure 1A and 1B); the patient was submitted to a nasopharyngeal endoscopy (Figure 1C and 1D), revealing the white line presence [20]. Using a pharyngeal pH probe, we found positive oesophageal (pHo) and gastric pH (pHg) values, pHo = 3.9 and pHg = 3.7 respectively, confirming the presence of LPR and/or GERD. Considering the clinical ocular and epidermal periorbital features and urogenital symptomatologies, eye and urological consultations were required. She complained of a variable and light ocular flushing with apparent sandy sensation, light redness, burning and dry eyes (Figure 2, panel A and B). A light diffuse bulbar conjunctival redness was detected, the tarsal upper lid conjunctiva was hyperemic without papillae, whilst external lids and margin were initially interested by a skin disorder. Furthermore, from about one year, she also presented a malar dermatological alteration, characterized by multiple small papules, pustules and vesicles of 0.5 - 1.0 mm, localized on the periocular, perioral, nasolabial folds and around nostrils epidermis (Figure 2, panel B), diagnosed like initial acne rosacea. The patient showed a positive Schirmer's I° test with a weak lachrymal functionality (15 mm), Break Up Test (< 12''), ascribable to initial chronic dry eye or reactive conjunctivitis. At the urological visit, the young patient presented two problems, never correctly investigated and solved before. The first one, dating back into five previous years, consisted in a dysuric symptomatology manifesting itself through 8 - 13 urination episodes daily. The previous urinalysis for common cultural tests were always negative and/or were lacking in significant bacterial colony counts, ranging from 1.0 x 10^4 to 5.0 x 10^4 UFC/mL of changeable bacterial flora. The second one, dating back into three previous years, begun with soreness urination and urinary leakage, evolved later into more complex genital problem as vulvar itching, spotting and dyspareunia, receiving, after several gynecological and urological visits in the private polyclinic, anti-fungal first and later oxybutynin treatments, for a long time without any ameliorative persistent health condition. The recrudescence of such pathological events, more and more frequent and the worsening of symptoms severely limited her sexual activity, reducing it almost exclusively to oral intercourse activity. No cardiac murmurs were detected. The next plain X-ray showed a little soft tissue swelling over the proximal interphalangeal joint of second and third fingers of the both hands with flogistic-degenerative arthritic sign on bilateral radial and carpus-metacarpus.

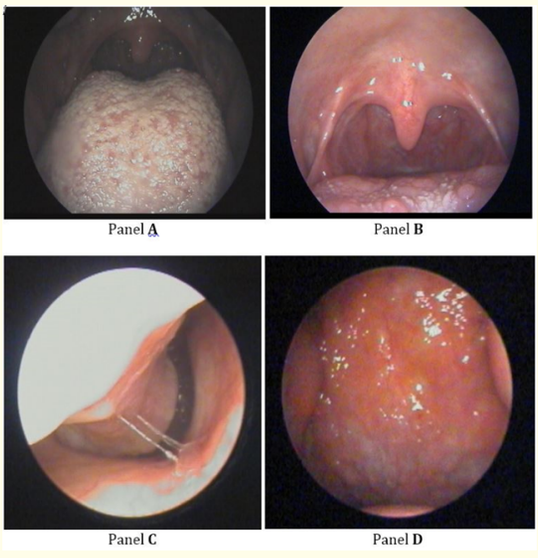

Figure 1: *The oral cavity clinical aspect of the young female showing the altered oropharyngeal ecosystems.*
A panel evidenced the whitish mucoid-caseous patinated tongue, on which was collected biological material by the scraping modality.
B panel shows the inflamed area pharyngeal wall with greyish mucoid secretion.
C panel was the image, obtained during endoscopic examination, showing the mucoid dense secretion examined for mycoplasmas.
D panel was the endoscopic image showing the white line, the clear demarcation between the posterior nasopharyngeal wall and the purplish nasopharyngeal vault.

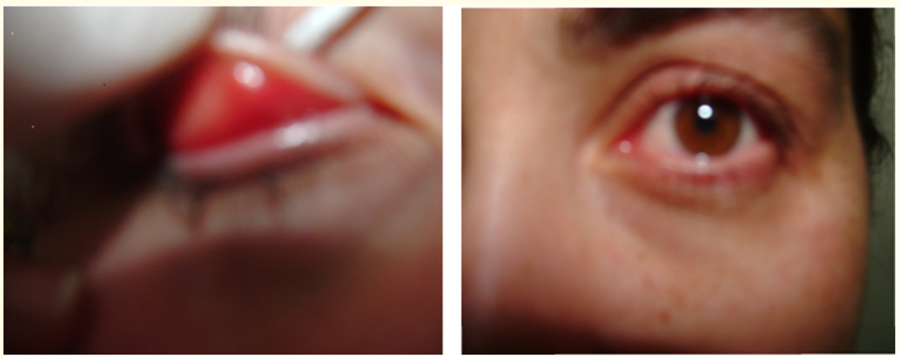

Figure 2: *A panel: The hyperemic conjunctiva of the tarsal upper lid without papillae on which it has been carried out the cellular scraping. B panel: The periorbital region with red-eye; external lids and margin were initially interested in a skin disorder; the periorbital skin inflamed areas of the upper and lower eyelid, of the palpebral fissure and of the epidermal flushed malar region, diagnosed as initial rosacea were scraped for chlamydial (Cp and Ct) researches.*

Materials and Methods

Sample preparations

All tissue, affected by chronic inflammations, were sampled by scraping procedure and pooled into 1.0 mL sterile saline solution to detect Ct-DNA presence [18,19]. 100 µL of each sample was used for pH measure by digital portable pH-meter. lingual cell and salivary secretions (here initialled: LCSS) and Lingual, pharyngeal, post nasal drip mucoid secretions and sputum (here initialled: LPNS) were pooled in three different samples and treated separately. Fecal material, from three different and consecutive daily defecations, was collected and treated for Hp-DNA research [18]. Aliquots (3 x 0.25 ml) were pooled and centrifuged at 12000 rpm for 5' at 4°C; an appropriate amount of pellet was suspended in 180 µL of 0.025 M buffer phosphate pH = 7.0 plus 1.0 mM EDTA, added with 25 µL of proteinase K, incubating at 56°C overnight for molecular analysis.

Molecular section

LPNS and LCSS aliquots (250 µL) for Ct-DNA analysis were centrifuged at 12,000 rpm for 5' at 4°C; each pellet was immediately scattered with 180 µL of 0.025 M buffer phosphate pH = 7.0 plus 1.0 mM EDTA, added with 25 µL of proteinase K, incubated at 56°C overnight; 210 µL of absolute ethanol was added to precipitate the DNA extracted, filtered and eluted from column with 60 µL of elution buffer. 50 µL of this solution was amplified into a Thermocycler of Applied Biosystem (Clemens GmbH), following the manufacturer's instructions [BioAesis srl, Jesi (AN), Italy (Line-20 Chlamydia trachomatis code 04LI20]. Other aliquots (250 µL) of LPNS and LCSS sources were processed for Cp-DNA following the procedure already reported [18,19,21]. Hp-DNA for LPNS and LCSS and Hp-DNA for fecal sources (250 µL) were processed for cagA and ureC templates, using QIAamp DNA mini kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's procedure [22].

Cultural section

Aliquots of 75 µL from LCSS and LPNS was dispersed separately and cultured by Mycoplasma IST 2, following the BioMerieux Manual procedures. 1.0 µL of this remaining suspension was plated onto chromID CPS agar and Columbia CAN agar. Total Microbial Charge (TMC), expressed as the total number of colony-forming units per milliliter (CFU/mL) and the relative presence of each species was reported as percentage of TMC, as previously reported [18,19]. Monoculture was considered significant at ≥ 10^6 CFU/mL. API Coryne galleries (code 20 900) were identified by an API automated system, the confidence ranged between 94.7% and 99.9%, indicating a high level of identification. Cultural section and relative colony characterization were carried out using products purchased from BioMerieux Italia. *Streptococcus pneumoniae* (Spn), grown on Columbia CNA, underwent PCR analysis to confirm the eventual misidentification of *Streptococcus pseudopneumoniae* as true *S. pneumoniae* [19].

Results

Routine clinical biochemistry analyses, from hepatic to renal functionalities, electrolytes and metabolic parameters were all into relative physiological ranges. Antibody titers of most pathogens (bartonella, borrelia, cytomegalovirus, adenovirus, mumps, measles and Parvovirus B19) were negative. Reumatoid factor (RF), antistreptolysin O titre (ASOT), Widal-Wright's reaction and antibodies against cyclic citrullinated peptides [Abs-CCP: 0.9 U/mL (FEIA method)] were negative. The autoimmunity panel [anti-phospholipids (a-PL), anti-cardiolipin (a-CL) and Lupus anti-coagulant (LAC)] was negative, while the VDRL was positive together with TPHA negative results. Significantly altered parameters are shown on table 1. However, the other parameters are detailed as follows: erythrocyte sedimentation rate (ESR): 53 mm after 1h; white blood cells count (WBC): 12.370 PMN/µL (neutrophils: 44.0%, eosinophils: 1.0%, basophils: 0.4%, lymphocytes: 42.9%, monocytes: 11.7%). The unique altered proteins of acute-phase (APPs) were: CRP: 42.7 mg/L (ref. range: 3.0 - 6.0), quantitative fibrinogen: 5.8 g/L (ref. range: 1.8 - 3.5), fibrinogen activity: 759 mg/dL (ref. range: 180 - 400). Serotyping of the human leukocyte antigen (HLA) class I was positive for B_{27}, while cultural and molecular results from different ecosystems were showed on table 2, together with the pH values. The PCR positive analyses for *C. trachomatis* were shown in figure 3, while the negative Cp-DNA and Hp-DNA researches were reported in table 2, together the common bacteria quantification.

ESR	WBC	CRP	Fibrinogen	CD_3	CD_4	CD_8	CD_{56}	CD_{19}	CD_4/CD_8	IL_8	TNF - α
53	1.237 PMN/µl	42.7	5.8	69%	35.6%	34.16%	16.45%	4.56%	1.04	31.6	27.3
Ref. Range: ≤ 15 mm	Ref. Range: 0.5 - 1.0 x 10^4	Ref. Range: 3.0 - 6.0 mg/l	Ref. Range: 1.8 - 3.5 g/l	Ref. Range: 55.0 - 80.0%	Ref. Range: 35.0 - 55.0%	Ref. Range: 20.0 - 30.0%	Ref. Range: 1.0 - 20.0%	Ref. Range: 5.0 - 15.0%	Ref. Range: 1.4 - 2.0	Ref. Range: 5 - 15 pg/dl	Ref. Range: < 8,1 pg/dl

Table 1: *The most prominent inflammatory parameters of the patient.*

LCSS	LPNS	CC	DC	UC	CVC
pH = 8.46 Ref. range: 5.7-6.2	pH = 7.73 Ref. range: 5.7 - 6.2	pH = 7.14 Ref. range: 6.66 +/- 0.24*	pH = 7.31 Ref. range: Variable	pH = 7.78 Ref. range: Variable	pH = 6.56Fertile Ref. range: 3.8 - 4.5
Ct-DNA: (+)	Ct-DNA: (+)	Ct-DNA: (+)	Ct-DNA: (+)	Ct-DNA: (+)	Ct-DNA: (+)
Cp-DNA: (-)	Cp-DNA: (-)	Cp-DNA: (-)	Cp-DNA: (-)	NR	NR
Mh: > 10^5UCC/mL	Mh: 10^4 UCC/mL	Absent	Absent	Mh: > 10^5 UCC/mL	Mh: > 10^5 UCC/mL
Uu: > 10^5 UCC/mL	Uu: 10^4 UCC/mL	Uu: 10^3 UCC/mL	Uu: 10^3 UCC/mL	Uu: > 10^5 UCC/mL	Uu: > 10^5 UCC/mL
Microorganism Cultured					
Cu: > 1.0 x 10^6 UFC/mL	Cu: 3.0 x 10^5 UFC/mL	Cu: 3.0 x 10^5 UFC/mL	Ec: 2.0 x 10^5 UFC/mL	Cu: 5.0 x 10^5 UFC/mL	Cu: 5.0 x 10^5 UFC/mL
Spn: 1.0 x 10^5 UFC/mL	Spn: 1.0 x 10^5 UFC/mL	Spn: 1.0 x 10^4 UFC/mL	Sf: 1.0 x 10^5 UFC/mL	Pv: 8.0 x 10^4 UFC/mL	Kp: 5.0 x 10^5 UFC/mL
Ec: 2.0 x 10^5 UFC/mL	Kp: 3.5 x 10^4UFC/rnL	Se: 4.0 x 10^4 UFC/mL	Pv: 7.0 x 10^4 UFC/mL	Sf: 7.0 x 10^4 UFC/mL	Pv: 3.0 x 10^5 UFC/rnL
Sf: 5.0 x 10^4 UFC/rnL			Cu: 5.0 x 10^4 UFC/mL	Ec: 5.0 x 10^4 UFC/mL	Sf: 1.5 x 10^5 UFC/mL
Kp: 7.0 x 10^4 UFC/rnL					La: 2.0 x 10^5 UFC/rnL

Table 2: *Molecular analyses and cultural bacteria quantification from each surface implicated in inflammatory processes of the young female.*

LCSS: Lingual Cell and Salivary Secretions; LPNS: Pooled Materials from Oral Cavity and Upper Respiratory Tract; CC: Conjunctival Cell; DC: Dermatological Cell; UC: Urethral Cell; CVC: Cervical-Vaginal Cell; Cu: C. ulcerans; Spn: S. pneumoniae; Ec: E. coli; Sf: S. faecalis; Kp: K. pneumoniae; Se: S. epidermidis; Pv: P. vulgaris; La: L. acidophylus. NR: Not Researched.

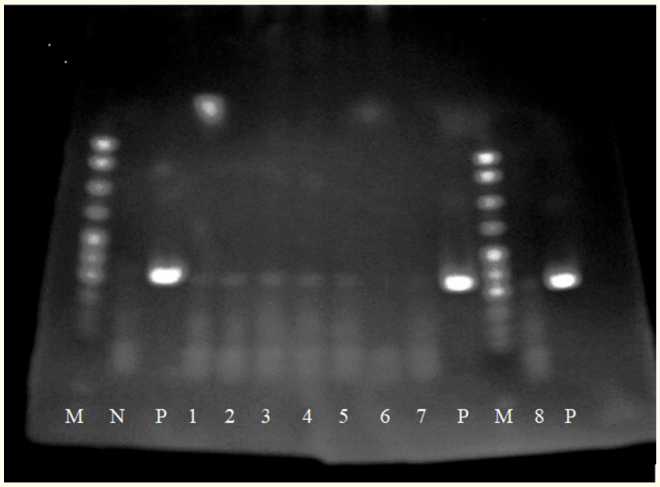

Figure 3: *Ct-DNA results from a multisite sources of HLA-B27 young female. M lines were markers; N and P represented the internal negative and positive controls. All samples showed an electrophoretic fluorescent band of 155 pb migration of C. trachomatis-DNA amplified primers. Line 1, LCSS; Line 2, LPNS; Line 3, conjunctival cell (CC); Line 4, Urethral scraping (UC) Line 5, Cervical scraping (CVC); Line 6, dermatological scraping (DC). Line 7 and 8 were repetitions obtained loading a light major volume of chlamydial amplified.*

Discussion

From several time, the Chlamydiaceae and Mycoplasmataceae, were defined the aetiological agents of chronic inflammatory state, living in human cells as endo- and eso- parasites, respectively. These persistent atypical bacteria trigger, in subjects genetically susceptible, from conception to lifetime, different sequelae of several and severe clinical manifestations. Premising that chlamydial and *Urogenital Mycoplasmas* are atypical bacteria present asymptomatically into 70 - 80% and symptomatically into the 20 - 30% of whole human population, waiting to figure out where to place these atypical sexual bacteria in the scenario of healthy human microbiome, keeping on mind the individual genetic susceptibility and the inflammaging conception [23,24] and combining the serological, immunological and microbiological approaches by cultural and molecular analyses, we discuss a new clinical case. Briefly, if on one side, the ESR, CRP, fibrinogen and C_3c fraction characterized an active non-specific altered clinical setting of the HLA-B_{27} positive young female, from the other side, the monocytes and lymphocytes counting (1447.3 cell/μL) and (5306.7 cell/μL) respectively, represented a renewal of chronic general inflammatory condition, while the high IL8 and TNF-α cytokine levels demonstrated the immunological action in countering mycoplasmal and chlamydial infections [9,25,26]. Altogether, if in a sense these results represented a revival of a chronic inflammatory phase, the concurrent radiological changes, due to a precocious trigger of reactive arthritis [29], confirmed the initial erosive process. Furthermore, the high percentage of monocytes and lymphocytes would represent a suitable time for adequately sampling of the cellular material by scraping procedure in each source clinically manifested due to recrudescence of atypical pathogens. VDRL positivity, into the contest of TPHA negative results [18,27,28], Abs-CCP negative result, in presence of a high fibrinogen levels (mass and activity) [18,27], remained again the unique non-specific parameter, in apparent contrast with recent new guidelines for biomarkers in autoimmune rheumatic diseases [30], indicating precociously an biochemical marker to chronic silent mycoplasmal and chlamydial arthritogenic infective action, also in absence of a more specific a-PL, a-CL and LAC anti-bodies positivity, as already reported [18,27,28]. We found the lymphocyte typing very complex to interpret because of the previous antibiotic and anti-inflammatory therapies and the renewal of atypical infections, documented by the high percentage of lymph-monocytes lines. Into the general contest of a renewed inflammatory scenario, the cytokines, responding to the LPS, MALP-2 and other chlamydial and mycoplasmal derived-molecules from their hydrolytic degradation, increasing the oxidative stress, cause both the apoptosis and the pejorative multisite clinical manifestations [31-34] throughout the unbalance of glutathione potential redox. The "*mucous*" salivary change (pH physiological variability: 5.8 - 6.7 of a "*serous*" salivary secretion) and the higher pH value for LCSS respect to LPNS, confirmed directly the high significance of these parameters in signaling an oral altered microbiota, due to a major alkalizing capacity of silent mycoplasma infection in these two contiguous compartments of the oral cavity and other ecosystems. For this purpose, it was interesting to highlight the key role of high pH value to reduce the protective effect due to whey

acidic protein (WAP) oxidation, bringing on the respiratory disease [35]. Furthermore, it was already reported that the high pH value represents the first step towards the unbalance of human saliva hypothiocyanite ion formation [36], one of the physiological antimicrobial products of the salivary peroxidase system of human microbiome [37]. In our case, the oropharyngeal and upper airways persistent inflammatory scenery, induced by the LPS, MALP-2 and other biomolecules from chlamydial and mycoplasmal debris, were documented by high cytokines levels. Also, it was noted that free radicals oxygen (ROS), oxide nitrogen (NO), endogenous autacoid lipoperoxidation and nitrooleic products, activating the sensory bronchopulmonary C-fibres, induce the chronic cough [38,39], both well documented in COPD [32,33] and tubal infertility [31]. From clinical evidences of oral cavity (Figure 1A-1D), on the basis of unequivocal her mother's clinical history (premature rupture of membranes), for the first time, we reported an interesting clinical case of a young female, suffering from a precocious neonatal breathing difficulty, evolving over the time, in a major CCC, LPR, GERD reflux and other diseases. Cultural analyses showed the growth of polymicrobial saprophytic flora, generally considered belonging to the human health microbiome, while the oral colonization by atypical bacteria might show a different evolution depending on individual tissue susceptibility [40-42]. In detail, the positivity of *C. trachomatis* and *Urogenital Mycoplasmas* (Uu and Mh), together with *C. pneumoniae* negative result from oral sources and with clinical history, permitted us to attribute to them a precocious oropharyngeal and respiratory contamination, happened even before birth [7-9], as reported in an other case [19]. The endoscopic image together with oesophageal and gastric pH results, highlighted an extra-esophageal tissue disorder [20], which together with *H. pylori* negative result from oral and fecal sources, permitted us to exclude its possible co-implication in reflux diseases [43]. The clinical ocular altered evidences and chlamydial presence were characterized for an initial chronic conjunctivitis of more complex belated manifestation of the Reiter's syndrome predisposition. The very low Uu infecting charge was interpreted as a very probable seminal contamination due to her frequent sexual modality although a vertical transmission be ever more possible at any phase of pregnancy/birth [19] and the clinical manifestation might be attributed to individual genetic susceptibility. Thus, this possible invaluable triad (VDRL positivity, iperfibrinogenaemia and Abs-CCP negativity) would represent an early indication to sexual atypical bacteria research at any age in patient genetically susceptible presenting an initial inflammaging process, documented by radiological investigations. Common similar skin disorders, around epidemic ocular, periorbital and malar region, were frequently observed and diagnosed as initial rosacea, but their pathogenesis remain still unclear and controversial [44,45]. Several factor, from the individual genetic susceptibility, the dysregulation of the innate and adaptive immune system to the vascular and neuronal dysfunction, are frequently called into question, but the underlying microbial cause has not yet been identified, existing different approaches to their investigation [45,46]. Thus, it was observed a broad range of common bacteria from Hp [47] to atypical Cp [48]. On the contrary, our microbiological results, culturally and molecularly obtained, revealed Cp-DNA and Hp-DNA negativities, while the sexual atypical researches were positive for Ct-DNA, performed in triplicate with increasing amounts of templates (Figure 3, DC source, Line 6-8) and for low Uu quantification (Table 2, CC column) into the polymorphic quantified microbial setting. All together, these results obliged us to attribute the *rosacea* to sexual atypical pathogens, demonstrating how molecular research and microbial quantification amplify the range of true bacteria involved in this pathology. The physical salivary change ["*serous*" against "*mucous*" (Figure 1 A and1 B)], the high pH value and the presence of *white line* clinical sign (Figure 1C and 1D) would represent an other additional and precocious triad marker indicative of an oropharyngeal altered microbiota, easily, quickly and noninvasively obtainable to evaluate this human microbiome and in other different phenotype diseases, as already reported for ocular microbiota [19]. Although the American Urologic Association and the European Society of Clinical Microbiology and Infectious Diseases had endorsed the guidelines of Infectious Society Disease of America (IDSA) from several time, their applications are still not much applied routinely for sexual atypical bacteria, increasing only the former microbiological knowledge and preventing the resolution or precocious therapeutic cycles in the contest of several and severe clinical scenery. The Ct, Uu and Mh positivities for urogenital sources, into the respective altered ecosystems (typical symptomatology, pH value and *L. acidophilus* low charge), together with all blood altered parameters, demonstrated our correct perceptiveness introducing their researches, well knowing the difficulty to establish for them an adequate and resolutive therapy. The patient was enrolled in a personal follow-up program.

Conclusion

In conclusion, identification of sexual atypical infections in oral cavity, collecting an accurate anamnesis on the slight, but meaningful clinical signs of oral cavity and reproductive organs sexually involved, introducing two simple chemical and physic parameters (pH and salivary viscosity), giving utmost importance to the sampling modality (swabbing vs scraping) for chronic chlamydial infections [18,19,27,28,49], introducing routinely their researches, we ascertained their involvement in oropharyngeal and respiratory diseases, that sometimes could trigger reactive arthritis, and/or CCC, LPR, GERD reflux disease, and/or couple infertility and pelvic inflammatory

disease in genetically susceptible subjects [50-52] and/or could reveal new pathology in other clinical case of rare disease. Nowadays, it remains hard for us to comprise the motivation of their exclusion in clinical studies on pathogens causing upper respiratory tract infections in outpatients, considering the chronicity of these diseases and the necessity of reducing both the health cost and the amount of working hours, providing a good medical assistance, attending the development of effective and durable vaccine against chlamydial and mycoplasmal infections [53].

Conflict of Interest

The authors state that there are no financial interests or conflicts of interest.

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