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June 2024 Vol. 2, No. 2

Pediatric Respiratory Journal

Official Journal of the Italian Pediatric Respiratory Society

Official Journal of the Italian Pediatric Respiratory Society (Società Italiana per le Malattie Respiratorie Infantili – SIMRI)

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PEDIATR RESPIR J Vol. 2(2), 52-54, 2024

EDITORIAL

Women transforming medicine: equity for better healthcare

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Doi
10.56164/PediatrRespirJ.2024.47

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While women have been healers and caregivers throughout history, their formal contributions to medicine have faced systemic obstacles. Despite recent strides in female medical school enrollment, gender disparities persist in leadership, research, and career advancement.

Women have played pivotal roles in medicine since ancient Egypt and Greece where figures like Isis, Hygeia, and Panacea were revered (1, 2). Midwifery and home-based healthcare were primarily managed by women for centuries, though they were rarely recognized as professionals (2).

The mid-19th century witnessed a pivotal moment for women in medicine with the unlikely heroine, Elizabeth Blackwell. Despite her admittance to Geneva College's medical school in New York being initially intended as a prank, it became a watershed event. In 1849, Blackwell defied the odds and shattered barriers, becoming the first woman to receive a medical degree in the United States (3, 4). The late 19th century saw the American Medical Association reform medical schools, implementing more rigorous educational standards, extended training, and increased tuition costs. While well-intended, these changes had the unintended consequence of hindering **women's access to medical education**. As a result, women comprised a mere 6% of US physicians in 1910, a statistic that remained tragically stagnant for the following 50 years. However, a shift has been underway: 2017 marked a historic first, with women outnumbering men in medical school matriculation. This momentum continued, and in 2019, women became the majority of US medical students (4). While the number of women in healthcare is growing, their presence in leadership positions and research output lags behind. Globally, **women represent only around 47% of the healthcare workforce** compared to 72% for men, with a wider gap existing in some countries (5). This disparity extends to leadership roles, where the proportion of women as division and section chiefs has risen from 16% in 2003 to 29% in 2018, reflecting a concerningly slow annual increase of just 1% (5). Furthermore, women in leadership positions are often concentrated in areas perceived as less influential, such as student affairs or diversity initiatives, suggesting a lack of access to key decision-making and budgetary power.

Several factors hinder women's pursuit of academic careers, including age, medical specialty, academic considerations, financial concerns, and work-life balance challenges often centered around pregnancy, maternity, and childcare. Age discrimination is particularly insidious, with reports of **gender-based discrimination reaching 76% in early careers**, 56.7% in mid-career, and still

KEY WORDS
Women; gender wage gap; healthcare; gender bias; leadership; career advancement; inclusive culture.

a troubling 35.8% in late careers. For women physicians, age-based discrimination persists across all career stages. Disturbingly, among older women physicians, 11.9%-18% have experienced bullying or verbal abuse.

Despite achievements and seniority, women physicians continue to encounter limitations in their careers. They are less likely to receive recognition for their accomplishments, such as promotions, awards, or invitations to speak at conferences compared to male colleagues (6). This lack of recognition reflects a persistent **gender bias within the medical field**.

Furthermore, historical, and ongoing inequities exist in certain specialties. For instance, research shows a persistent underrepresentation of women in oncology subspecialties over a 26-year period (7). This underrepresentation highlights the need for a more inclusive environment across all medical disciplines.

However, the contributions of women in medicine are undeniable. Pioneering figures like Dr. Florence Seibert, who isolated the tuberculosis protein molecule, and Dr. Virginia Apgar, who developed the lifesaving Apgar score, stand as testaments to the **impact of women in medical innovation**. A National Institutes of Health study reinforces this, demonstrating that well-represented women physicians achieve comparable productivity to their male counterparts (8-10). Despite these achievements, significant disparities remain in publication rates, editorial board positions, research funding opportunities, and overall career advancement for women physicians compared to men (8- 10).

Women disproportionately adjust their careers and face compromises when navigating the demands of family and work. This is fueled by outdated gender roles, societal expectations of mothers, and the persistent idealization of women as primary homemakers. These pressures often force women into difficult choices. Prioritizing career invites societal judgment, while focusing on family can hinder career advancement and income, further exacerbating the **gender wage gap**.

The lack of support for work-life integration compounds these challenges, despite evidence of the positive impact women physicians have on patient care. Studies suggest they often demonstrate better adherence to guidelines, prioritize preventative care, offer increased psychosocial support, engage in patient-centered communication, and devote more time to patients (11-13).

The consequences are severe: **gender bias, limited recognition, slower advancement, and pay inequity contribute to a lack of belonging and increase burnout risk in women physicians, a pattern supported by research** (14).

Urgent change is needed. Simply increasing women's representation in leadership isn't enough. We must fundamentally reshape the mental image of female physicians, dismantle biases, and create a **genuinely inclusive culture**. It also needs to fight stereotypes; is it really changed something compared to when a journalist asks, «Mrs. Curie, how do you live next to a genius?» assuming that her husband was in charge. The answer did not take long to come: «I do not know, ask my husband», replied Mrs. Curie, making a real revolution (15). While Curie's sharp retort serves as an early challenge to this notion, it is crucial to recognize that women still face underestimation and skepticism. Combating deeply ingrained stereotypes is essential to dismantle barriers and foster a scientific community that fully embraces talent regardless of gender.

In conclusion, addressing the persistent underrepresentation of women in leadership roles and tackling pervasive gender bias within **healthcare requires a multi-pronged approach**. Fostering an environment that empowers women physicians through mentorship and recognition programs is crucial. Furthermore, dismantling stereotypes and promoting a culture of inclusivity are essential steps towards attracting and retaining top female talent. Ultimately, achieving true gender parity within medicine holds the potential to enhance the quality of patient care, create a more diverse workforce, and unlock the full potential of all medical professionals. This necessitates a collaborative effort from institutions, male and female physicians alike, to break down existing barriers and build a truly inclusive future for the field. This is not just a fight for women in medicine; it is a fight for better healthcare for all.

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RESEARCH ARTICLE

Non-typeable *Haemophilus influenzae* directly and through TNF-α production enhances polymorphonuclear leukocytes adherence to bronchial epithelial cells and activation

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ABSTRACT

Non-typeable *Heamophilus influenzae* (NTHi), the leading cause of localized upper respiratory tract infection in children, can be the causative agents of lower respiratory tract disorders and chronic lung disorder exacerbations. Infection of bronchial epithelial cells by NTHi is characterized by a sustained neutrophilic inflammation that is thought to play a key pathogenetic role in lung parenchyma damage. To characterize the mechanisms involved in BEC activation in response to NTHi, a human cell line (BEAS-2B) was stimulated with NTHi lysates. The production of the Tumor Necrosis Factor-α (TNF-α), and the expression of the Toll-like Receptor2 (TLR2), the microbial ligand that recognizes NTHi molecular patterns, and of the Intercellular Adhesion Molecule-1 (ICAM-1), an adhesion molecule required for neutrophil adhesion, were evaluated. The respective role of TNF-α and ICAM-1 in neutrophil adhesion to BEAS-2B cells was then evaluated by inhibition of their activity by specific monoclonal antibodies (mAbs). A time- and dose-dependent induction of TNF-α synthesis and release by BEAS-2B cells was detected after 24-hour exposure to NTHi lysates (0.4 to 1.6 mg/ml). TNF-α, but also directly NTHi lysates, significantly amplified ICAM-1 and TLR2 expression and synthesis by BEAS-2B cells. Stimulation of BEAS-2B cells with NTHi lysates or with TNF-α induced a dose-dependent increase neutrophil adhesion, stronger after NTHi lysates exposure, associated with myeloperoxidase (MPO) production. Finally, the NTHi lysates-induced neutrophil adhesion to BEAS-2B cells was significantly inhibited by anti-TNF-α and anti-ICAM-1 mAbs. Exposure to NTHi lysates induced functional and structural changes in BEAS-2B cells leading to neutrophil recruitment, adhesion, and activation. The observation that all these BEAS-2B cell changes were also induced by TNF-α can at least partially explain the sustained inflammation seen in NTHi infections.

HIGHLIGHTS BOX

What is already known about this topic? Non-typeable *Haemophilus influenzae* (NTHi) airway infection is characterized by a sustained neutrophilic inflammation leading to parenchymal lung damage. Bronchial epithelial cells exposed to NTHi lysate promoted a powerful neutrophil recruitment and activation with a positive feedback loop. **What does this article add to our knowledge**? This *in vitro* study evaluates the mechanisms leading to the powerful neutrophil recruitment and activation and to the promotion, through a feedback loop, of a vicious circle with detriments results. **How does this study impact current management guidelines**? To prevent this vicious circle, in clinical practice, NTHi infections should be treated with a high dosage antibiotic to which NTHi is susceptible and for a suitable period.

ABBREVIATIONS

HOCl: Hypochlorous Acid
ICAM-1: Intercellular Adhesion Molecule-1
MPO: Myeloperoxidase
NETs: Neutrophil Extracellular Traps
NF-kB: Nuclear Factor kappa-light-chain-enhancer of activated B cells
NTHi: Non-typeable Haemophilus influenzae
TLR2: Toll-like Receptor2
TNF-α: Tumor Necrosis Factor-α

KEY WORDS

Respiratory infections; non-typeable Haemophilus influenzae; intercellular adhesion molecule-1; tumor necrosis factor; toll like receptors; neutrophilic inflammation; oxygen burst.

INTRODUCTION

Haemophilus influenzae (*H. influenzae*) is a pleomorphic Gram-negative coccobacillus that frequently colonizes the human mucosa of the upper respiratory tract but is also a common cause of invasive and non-invasive bacterial infections (1, 2). Isolates of *H. influenzae* can be subdivided into encapsulated and non-encapsulated forms. Encapsulated strains express one of their six capsular polysaccharides and are designated "a" through "f" subtypes, based on the capsule type (2, 3). Non-encapsulated strains are referred to as nontypable *H. influenzae* (NTHi) (4). Before the anti-*H. influenzae* type b (Hib) conjugate vaccines became available in 1988, Hib was the leading cause of bacterial meningitis and a major cause of serious invasive diseases among children aged <5 years (5, 6). The anti-Hib vaccination altered the epidemiology of the infection and the striking decrease in Hib incidence was associated with strain replacement with serotype "f" and NTHi strains (7-10). NTHi is present in the nasopharynx in approximately 50% of young healthy children, with colonization rates ranging from 14%, in those aged <6 months, to 32% in those aged 19-26 months (7-11). In young children, the level of carriage varies according to several factors that can have repercussions on resistance to antibiotics and on efficacy of the immune response to infection (11-14). These factors include the child chronological age, the presence, the number, and the age of siblings in the family, the climate of the geographic area and the size and location of the day care centers the children are attending (13). An elevated carriage of strains resistant to antibiotics is a source of a concern because NTHi strains can be highly prevalent and pathogenic in a variety of acute and recurrent/chronic lower respiratory tract conditions (14-17). The pathogenesis of acute infections due to NTHi begins with colonization of the mucosal surface, followed by contiguous spread to adjacent areas, usually consequence of abnormalities in either non-specific, or specific host defenses (18). Airway epithelial cells play a dominant role in innate defenses, being a mechanical barrier to microbial entry, detecting pathogens by pattern recognition receptors, recruiting, and activating leukocytes and directly killing microbes through the up-regulation antimicrobial peptides (19). A damaged airway epithelium facilitates bacteria surface contact thus promoting respiratory tract colonization and a subsequent sustained inflammatory reaction (18-20). Recruitment and activation of high numbers of neutrophils promote the release of toxic oxygen species and of harmful proteases, with further disruption of airway mucosal integrity and increased pathogen growth, creating a dangerous vicious circle (19-22). The pathogenetic mechanisms explaining why NTHi, a common commensal microbe can become an important respiratory mucosal pathogen are complex and not completely understood (23-25). To understand how NTHi can cause acute and/or chronic infection, a critical factor is the assessment of the initial interaction with the airway epithelium. With this background a study was designed to analyze *in vitro* the response of a human bronchial epithelial cell line to NTHi bacterial lysate exposure. The release of cytokines and the expression of surface adhesion molecules involved in neutrophil recruitment, adhesion and activation were evaluated.

MATERIALS AND METHODS

Cell cultures

The human bronchial epithelial cells line (BEAS-2B) was used in all the experiments (26). These cells, which retain electron microscopic features of epithelial cells and show positive staining with antibodies to cytokeratin, were grown as monolayer in a 1:1 mixture of Laboratory of Human Carcinogenesis (or LHC)-9 medium (Invitrogen SRL, Milan, Italy) and RPMI 1640 medium (EuroClone, Milan, Italy).

NTHi bacterial lysate

NTHi strain, originally a clinical isolate from a cystic fibrosis (CF) patient, was used in this study. A NTHi single colony was harvested from a chocolate agar plate, inoculated into 30 ml of brain heart infusion broth (BHI, BD Laboratories, Franklin Lakes, NY, USA) supplemented with hemoglobin B and incubated at 37° C in 5% CO_2 overnight (27). The supernatant was discarded after centrifugation at 10,000 × g for 10 min, the pellet resuspended in 10 ml of phosphate-buffered solution (PBS) and sonicated to lyse the bacteria. The lysate was then centrifuged at 10,000 × g for 10 min and the supernatant collected. The NTHi lysate protein concentration was determined using the Bicinchoninic (BCA) protein assay (Abbexa Ltd. 20 Cambridge, UK) and was

in the range of 0.2 mg/ml. Stock solutions of 3 to 8 mg/ml were aliquoted by 1 mL and stored at -20° C (27).

BEAS-B2 cell cultures

In preliminary experiments, BEAS-B2 cells were cultured in bronchial epithelial cell growth medium (BEGM, Lonza Biologics, Basel, Switzerland), in Petri culture dishes to 90% confluence and then stimulated for 12, 24 or 48 h with different concentrations of NTHi lysates (0.4, 0.8 and 1.6 mg/ml). Cells were counted in a Neubauer chamber, and viable cells detected by trypan blue dye exclusion test (EuroClone) (26). Cell viability in all cultures was >90% after 24 h incubation and decreased after 48 h incubation. Based on this observation, all other experimental cultures were not carried out beyond 24 hours.

TNF-α release

To evaluate TNF-α release, 60,000 cells/well were plated into 24-well plates and treated with 100 ng/mL LPS or with NTHi lysates (0.4-0.8-1.6 mg/ml) for 6, 12 and 24 h. Culture medium supernatants were then collected after each incubation time point and analyzed by enzyme-linked immunosorbent assay (ELISA), according to manufacturer instructions (Biosource, Camarillo, CA, USA) (28). After each incubation time point, the culture medium was collected centrifuged and stored at -20° C until measurement.

Evaluation of ICAM-1 and TLR2 expression on BEAS-B2 cell surface

To evaluate ICAM-1 and TLR2 expression, 100 µl of the cell suspensions were placed into round bottom 96-well plates and stimulated for 24 h with different concentrations of NTHi lysates (0.4, 0.8 and 1.6 mg/ml) or with TNF-*α* (10.0 ng/ml). BEAS-B2 cells were then incubated for 30 min and stained: a) for ICAM-1, with a fluorescein isothiocyanate (FITC) conjugated monoclonal antibody (mAb) anti-human CD54 (Caltag Laboratories, Burlingame, CA) and b) for TLR2, with a FIT conjugated ah-TLR2 Antibody (Santa Cruz Biotechnology Inc, Dallas, Texas, USA.). Cells were then fixed with 0.5% paraformaldehyde and analyzed by fluorescence-activated cell sorting (FACS) (Becton Dickinson, Milan, Italy) (-29). ICAM-1 and TLR2 expression was read on 10,000 acquired events and expressed as mean fluorescence channel (mfc) (29).

Western blot analysis

Analysis of TNF-α, ICAM-1 and TLR-2 expression was performed by Western blot analysis, as previously described (30). Briefly, BEAS-B2 cells were resuspended in lysis buffer and equal amounts of total proteins were loaded to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto a PVDF membrane (Immobilon-P, Millipore, Bedford, MA, USA). The membranes were blocked. The blots incubated with monoclonal antibodies against anti-human TNF-α, ICAM-1 and TLR-2 monoclonal antibodies (Santa Cruz Biotechnology) and visualized using the enhanced chemiluminescence system (Pierce Biotechnology Inc., Rockford, IL, USA). Blots were re-probed with anti-b-actin antibodies (clone C4, Boehringer Mannheim Inc., Mannheim, Germany). The relevant band intensities were quantified using a Versadoc Imaging System model 3000 (Biorad Laboratories Inc., Hercules, CA, USA). Densitometric analysis of the immunoblot was performed, normalized to b-actin, and plotted as means +SEM.

Purification of polymorphonucleated cells

Human polymorphonucleated cells (PMNs) were purified from normal blood donors using density gradient centrifugation (31). Neutrophils were isolated from the resulting cell suspension using Ficol-Histopaque (Healthcare Bio-Sciences AB, Uppsala, Sweden) density centrifugation, and resuspended in RPMI 1640 medium (EuroClone), supplemented with 10% fetal bovine serum (EuroClone), L-glutamine, and penicillin/streptomycin, at a concentration of 10,000,000 cells/ml, and were kept on ice until needed. Neutrophils were counted and stained with Diff-Quick ® (Medion Diagnostics AG, Switzerland) to assess purity (>97%) and with Trypan Blue test (EuroClone) to verify viability (>98%) (31).

Neutrophil adherence to BEAS-2B cells and activation

Isolated neutrophils were resuspended in Hepes/Hanks' balanced salt solution (HBSS), labelled with 1 mM Calcein-AM (Sigma-Aldrich, Gillingham, Dorset, UK) for 30 min in the dark, then washed, resuspended, and added to wells containing BEAS-2B cells stimulated with different concentrations of NTHi bacterial lysate or TNF-α. After incubation for 30 min at 37° C,

non-adherent cells were removed by carefully washing two times with Hepes/HBSS solution (Sigma-Aldrich) (32). Adhesion of neutrophils to BEAS-2B cells was then assayed by measuring the fluorescence of the wells with a fluorescence photometer (485 nm) (32). Purified neutrophil samples were then fixed in 4% paraformaldehyde, permeabilized with 0.2% Triton X-100, blocked with 5% BSA (Sigma-Aldrich), and stained with polyclonal rabbit anti-human myeloperoxidase (MPO) antibodies (DakoCytomation, Glostrup, Denmark) and, after 1 hr incubation, with a secondary antibody (1:200 - Molecular Probe). Slides were cover-slipped using the Vectashield fluoromount (Vector Laboratories Inc., Burlingame, CA, USA) and confocal images were obtained using a confocal fluorescence microscope (TCS SL microscope, Leica, Mannheim, Germany) and 40 × objective lenses (32).To evaluate the functional role of TNF-α and ICAM-1 in inducing neutrophil adherence, anti-human TNF-α or ICAM-1 mAbs (5 μg/ml) or mouse IgG, as isotype control (10 μg/mL), were added to the stimulated BEAS-2B cell cultures for 30 min at 37° C, prior to the adhesion assay.

Statistical analysis

Statistical evaluation was performed using the statistical software package GraphPadPrism3.02 (GraphPad Software, SanDiego, CA, USA). Data are expressed as arithmetic mean ± SEM. Kruskall-Wallis test, followed by post hoc test (Dunn's test), was used for multiple comparisons. The level of statistically significance was set at $p < 0.05$.

RESULTS

NTHi-induced TNF-α release and expression

Enzyme-linked immunosorbent assay analysis of supernatants of BEAS-2B cell cultures, exposed to NTHi lysates supernatants for two time periods (12 and 24 h) showed a significant increase in TNF-α release only after 24 h incubation (**Figure 1A**). The increase was highly significant ($p < 0.001$; each comparison) for all the NTHi lysate concentration tested (0.4, 0.8 and 1.6 mg/ml). Densitometric analysis of the immunoblots, normalized to β-actin, confirmed the remarkable increase of the TNF-α protein production after 24 h stimulation with each the NTHi lysate concentrations tested ($p < 0.001$; each comparison) (**Figure 1B**).

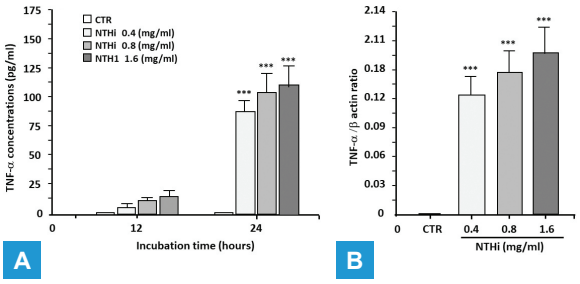

Figure 1. *(**A**)Time-dependent increase in TNF-α release in BEAS-2B cell culture supernatants induced by different NTHi bacterial lysate concentrations (0.4, 0.8 and 1.6 mg/ml); (**B**) increase in TNF-α protein expression by BEAS-2B cells induced by different concentrations of NTHi bacterial lysate (0.4, 0.8 and 1.6 mg/ml). The data are presented as mean+SEM from three independent experiments. *** p <0.001, versus unstimulated cells.*

NTHi lysate and TNF-α-induced ICAM-1 expression

By FACS analysis a significant increase in ICAM-1 expression on BEAS-B2 cell surface was detected after exposure of the cell cultures to NTHi lysates (1.6 mg/ml) for 24 h, the increase after 12 h incubation was not statistically significant (**Figure 2A**). Densitometric analysis of the immunoblots, normalized to β-actin, showed that the exposure of BEAS-2B cells over 24 h to NTHi lysates also induced a significant increase in ICAM-1 protein production, lower but still significant ($p < 0.05$) at the lowest NTHi lysates concentration tested (0.4 mg/ml) (**Figure 2B**). A time-dependent induction of ICAM-1 protein production by TNF-α (10 ng/ml) was also demonstrated, already significant after 12 h incubation ($p < 0.05$) (**Figure 3A**). A highly significant increase in ICAM-1 expression on BEAS-B2 cell surface was also detected when BEAS-B2 cells were co-cultured with TNF-α (10 ng/ml) for 24 h ($p < 0.001$), an increase similar to that found after NTHi lysates (1.6 mg/ml) (**Figure 3B**).

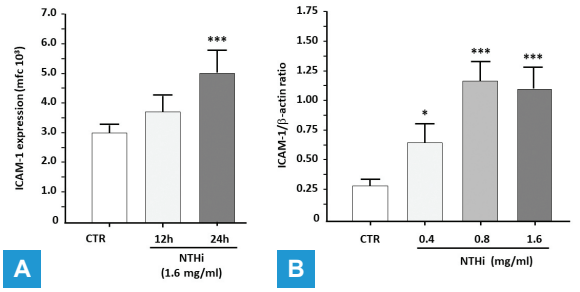

Figure 2. *(**A**) Time-dependent increase in ICAM-1 expression on BEAS-B2 cell surface induced by NTHi bacterial lysate (1.6 mg/ml); (**B**) increased ICAM-1 protein expression by BEAS-2B cells induced by exposure to different concentrations (0.4, 0.8 and 1.6 mg/ml) of NTHi bacterial lysate for 24 h. The data are presented as mean +SEM from three independent experiments. * p <0.05; *** p <0.001, versus unstimulated cells.*

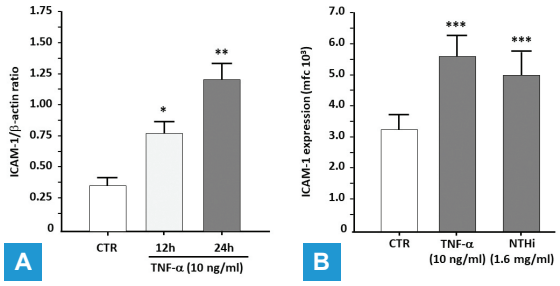

Figure 3. *(**A**) Time-dependent increase in ICAM-1 protein expression induced on BEAS-2B cells by TNF-α (10 ng/ml); (**B**) comparison between the increase in ICAM-1 expression on BEAS-B2 cell surface induced by TNF-α (10 ng/ml) or by NTHi bacterial lysate (1.6 mg/ml) after 24 h incubation. The data are presented as mean +SEM from three independent experiments. * p <0.05; ** p <0.01; *** p <0.001, versus unstimulated cells.*

NTHi- and TNF-α-induced TLR2 expression and production

A significant, but weak, increase in TLR2 expression on BEAS-B2 cell surface was seen after 24 h incubation with TNF-α (10 ng/ml) (p <0.05) (**Figure 4A**). A more significant increase in TLR2 expression was observed when, in the same culture conditions, the cells were exposed to NTHi lysates (1.6 mg/ml) (p <0.001). Densitometric analysis of the immunoblots confirmed the results observed in TLR2 expression, significant production of TLR2 protein, weak after TNF-α exposure (p <0.05) and stronger after NTHi-stimulation (p <0.001) (**Figure 4B**).

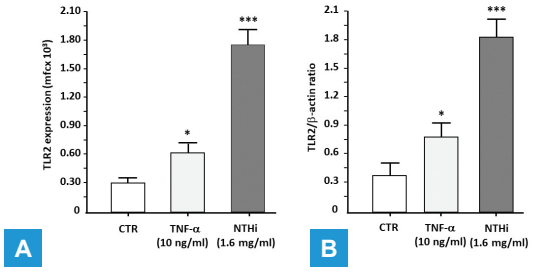

Figure 4. *(**A**) Cell surface TLR2 expression on BEAS-B2 cells after 24 h incubation with TNF-α (10 ng/ml) or NTHi lysates (1.6 mg/ml); (**B**) TLR2 protein expression by BEAS-B2 cells after 24 h incubation with TNF-α (10 ng/ml) or NTHi (1.6 mg/ml) for 24 h. The data are presented as mean +SEM from three independent experiments. * p <0.05; *** p <0.001, versus unstimulated cells.*

Neutrophil adherence to the epithelium and activation

Both pre-exposure of BEAS-2B cells to NHTi (0.4, 0.8 and 1.6 mg/ml) and to TNF-α (0.05, 0.10, 1.00 and 10.00 ng/ml) induced a dose-dependent enhancement of neutrophil adhesion to the BEAS-2B cell surface, significant for all the concentration tested (**Figure 5A**, **B**).

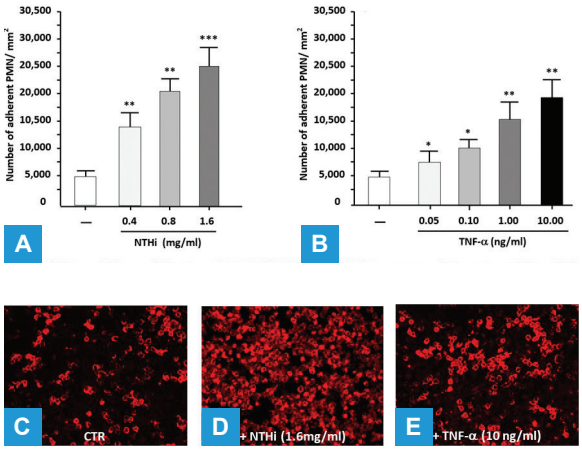

Figure 5. *(**A**) Neutrophil adhesion to the BEAS-2B cell surface after pre-exposure to different concentrations of NHTi lysate (0.4, 0.8 and 1.6 mg/ml); (**B**) neutrophil adhesion to the BEAS-2B cell surface after pre-exposure to different concentrations of TNF-α (0.05, 0.10, 1,00 and 10.00 ng/ml). The data are presented as mean +SEM from three independent experiments. * p <0.05; ** p <0.01; *** p <0.001, versus unstimulated cells; (**C, D, E**) confocal fluorescence microscope images showing neutrophils stained with the anti-human MPO, adherent to BEAS-2B cells pre-exposed to balanced salt solution (CTR), NHTi lysate (1.6 mg/ml) or TNF-α (10 ng/ml).*

However, the magnitude of neutrophil adhesion was more striking higher after NTHi than after TNF-α exposure (p <0.01 and p <0.05, respectively, each comparison). The increase adhesion to the BEAS-2B cell surface was associated with neutrophil activation, as shown by confocal images demonstrating a strong staining with the anti-human MPO antibodies, stronger after NTHi exposure (**Figure 5C**, **D** and **E**).

As expected, the TNF-α-induced enhancement of neutrophil adhesion was completely blocked by anti TNF-α-mAbs (**Figure 6A**), whilst the NTHi lysate-induced neutrophil adhesion was partially inhibited by the anti-TNF-α and the anti-ICAM-1 mAbs, the effect being more effective with the anti-TNF-α than with the anti-ICAM-1 mAbs (p <0.01 and p <0.05, respectively) (**Figure 6B**).

DISCUSSION

NTHi is a Gram-negative human pathogen which accounts for acute, recurrent, and chronic respiratory infections. The host response to NTHi is characterized by airway neutrophil recruitment and activation due to early induction of proinflammatory mediators in airway epithelial cells (33). Using BEAS-B2 cells we have shown that exposure to NTHi lysates induced a time- and concentration-dependent TNF-α production

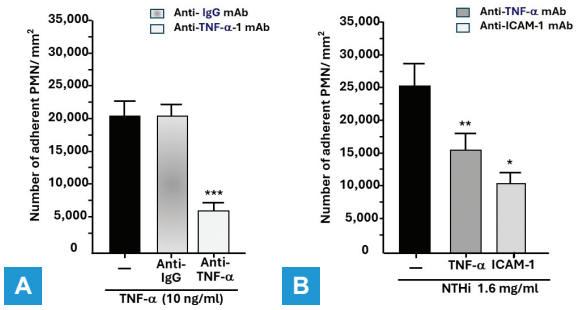

Figure 6. *(**A**) Inhibition of neutrophil adhesion to the BEAS-2B cells stimulated with TNF-α (10 ng/m) by the addition of mouse IgG, as isotype control (10 µg/mL), or of anti-human TNF- α mAbs (5 µg/ml), prior to the adhesion assay; (**B**) inhibition of neutrophil adhesion to BEAS-2B cells stimulated with NTHi (1.6 mg/ml) by the addition of anti-human TNF-α mAbs or anti ICAM-1 mAbs (5 µg/ml), prior to the adhesion assay. The data are presented as mean +SEM from three independent experiments. * p <0.05; ** p <0.01; *** p <0.001 versus unstimulated cells.*

and release, and that exposure to NTHi lysates, but also to TNF-α, significant increased ICAM-1 and TLR2 expression and production.

Moreover, exposure of BEAS-2B cells to NTHi lysate and to TNF-α induced a dose-dependent enhancement of neutrophil adhesion to BEAS-2B cells and that adhesion was associated with neutrophil activation with a sustained MPO production. Airway epithelial cells express TLRs, a class of pattern recognition receptors which, responding to specific microbial ligands, initiate downstream cascades able to activate both the innate and the adaptive immunity (34). TLR2 activation by microbial ligands induces the expression of the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kB), the intracellular signaling factor most strongly associated with NTHi infection. Moghaddam SJ, *et al*., demonstrated that administration of NTHi lysate by aerosol to mice induced a rapid NF-kB activation in airway cells, followed by TNF-α release and neutrophil recruitment (35). The inflammatory response to NHTi can be enhanced by co-secreted cytokines, such as TNF-α. Using human epithelial cell line and primary human bronchial epithelial cell cultures, Watanabe T, *et al*. demonstrated that NTHi and TNF-α synergistically induced a strong NF-kB activation via two distinct signaling pathways, involved in the induction of proinflammatory cytokines (36). TNF-α has been identified as a major cytokine involved in the pathogenesis of inflammatory and autoimmune diseases, characterized by neutrophil activation (37-39). Many of the proinflammatory effects of TNF-α are mediated by the ability to regulate neutrophil-vascular endothelium interactions (40). Neutrophils express adhesive glycoproteins of the CD11/CD18 family, the counter-receptors for ICAM-1expressed by vascular endothelial cells (31, 41). In addition to guide neutrophil trans-endothelial migration, the ICAM-1-CD11/CD18 interaction promotes neutrophil-mediated cytotoxicity through toxic oxygen specie generation and protease release (42, 43). ICAM-1 is also expressed by airway epithelial cells, primarily on the apical surface (31, 42). Given its apical localization, ICAM-1 can promote the retention of the transmigrated neutrophils at the luminal airway surface (44). Neutrophil migration into tissues can function as double-edged swords because, in addition to the first line of defense against invading pathogens, has simultaneously the potential to cause substantial local tissue injury (44, 45). Neutrophil adhesion to the epithelium appears to be prerequisite for a large oxidative burst in response to proinflammatory cytokine (31, 33). Indeed, the results of our study showed that neutrophil adhesion to BEAS was associated with a sustained MPO release (31, 33). MPO is a peroxidase enzyme, catalyzing the oxidation of chloride by H_2O_2 to form the strong oxidant Hypochlorous Acid (HOCl) (46). The primary defensive function of neutrophils is phagocytosis and destruction of microorganisms. A rapid microbicidal effect follows the release of MPO and H_2O_2 into neutrophil phagosomes containing ingested microorganisms. However, MPO and H_2O_2 can also be released outside the cells where HOCl formation can induce extensive damage to adjacent tissues (46). Moreover, a persistent neutrophil activation can contribute to the formation of the Neutrophil Extracellular Traps (NETs). NETs are networks of extracellular fibers, primarily composed by neutrophil DNA and cytosolic and granule proteins assembled on a scaffold of decondensed chromatin (47). NETs can neutralize and kill bacteria, fungi, viruses, and parasites. However, over time NETs have the tendency to become ineffectual in killing and clearing pathogens, but do not lose the ability to incite a vigorous long-lasting proinflammatory stimuli in the airways (48). In NETs, NTHi can persist forming biofilms which protect them from antibiotics and extracellular and phagocytic killing by neutrophils (33, 48, 49).

CONCLUSIONS

The BEAS-2B cells inflammatory response to NTHi lysates can be further promoted by co-secreted proinflammatory cytokines, such TNF-α (**Figure 7**). NTHi lysate- and TNF-α-enhanced neutrophil adhesion to the BEAS-2B cell surface, favored by the increase ICAM1 expression, was associated with neutrophil activation and MPO release, with further enhancement of the inflammatory reaction and the parenchymal tissue damage. The vicious circle, fostered by the increased TLR2 and ICAM-1 expression and by the NET and NTHi biofilm formation, can explains why the sustained neutrophilic inflammation that can become recurrent and chronic. To prevent this vicious circle, NTHi infections should be treated with a high dosage antibiotic to which NTHi is susceptible and for a suitable period (50). As shown for beta-lactam antibiotics in an *in vitro* study, exposure to subinhibitory antibiotic concentrations (*i.e.*, amounts that partially inhibit bacterial growth) can act as a signaling molecule that promotes transformation of NTHi into the biofilm phenotype. Biofilms, act as a reservoir of viable bacteria once antibiotic exposure has ended, favoring antibiotic resistance and increased susceptibility to reinfection after treatment (51).

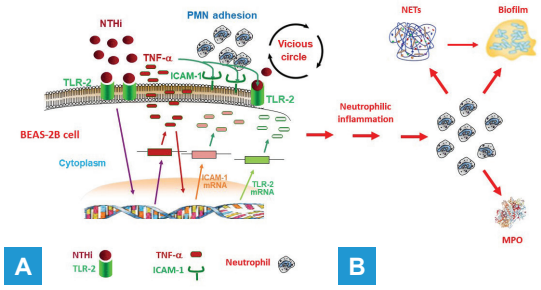

Figure 7. *(**A**) Interacting with TLR2, NTHi lysates induced the release of TNF-α and, together with TNF-α, a significant increase in ICAM-1 and TLR2 expression on BEAS-2B cell surface. Pre-exposure of BEAS-2B cells to NHTi lysate and TNF-α also induced an enhancement of neutrophil (PMN) adhesion to BEAS-2B cells; (**B**) the increased adhesion was associated with PMN activation, myeloperoxidase (MPO) production, which can lead to neutrophil extracellular traps (NETs) formation and biofilm development.*

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

Financial support

There were no institutional or private fundings for this article.

Author contributions

Made substantial contributions to the conception and design of the work: MS and GAR; performed the *in vitro* experiments, and made substantial contributions acquisition, analysis, or interpretation of data for the work: MS and LP; drafted the work: GAR and OS; revised the work critically for important intellectual content: MS, GAR and OS. All the authors provide approval for publication of the content.

Ethical approval

Human studies and subjects

N/A.

Animal studies

N/A.

Data sharing and data accessibility

This is an *in vitro* study and citation of the methodology data are included in the reference list.

Publication ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data correspond to the real.

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PEDIATR RESPIR J Vol. 2(2), 64-71, 2024

RESEARCH ARTICLE

Anxiety and post-traumatic stress disorder in parents of children born with esophageal atresia

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10.56164/PediatrRespirJ.2024.46

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ABSTRACT

Neonates with esophageal atresia (EA) require admission to the neonatal intensive care unit and undergo surgery early after birth. These parents encounter potential stressors that can contribute to mental health manifestations including symptoms of post-traumatic stress disorder (PTSD) and anxiety. The primary objective of this study was to examine the occurrence of PTSD symptoms and anxiety among parents of EA children.

We conducted an ambi-directional cohort study that included parents of EA children followed-up in our unit, between 2021 and 2023. Two separate questionnaires were completed by the parents during the child's visit: the Perinatal post-traumatic stress disorder questionnaire (PPQ), and the State-Trait Anxiety Inventory (STAI).

During the study period, 20 parents completed 28 questionnaires. The mean PPQ score was 3.72, ranging from 0 to 12. Six parents had a PPQ score of 6 or higher indicative of a risk to develop PTSD: 2 with a score of 6 and one each with a score of 9, 10, 11 and 12. The mean State STAI was 46 ± 7, 14% of which had a score of >55 indicating high anxiety levels at the time of visit. Similarly, the mean Trait STAI was 46 ± 5, with only one parent (4%) scoring above 55.

It is important to identify potential symptoms of PTSD and anxiety in parents of EA children early on, allowing for timely referral for counselling and treatment. This approach benefits both the parental mental well-being and the overall adjustment and coping of the entire family.

HIGHLIGHTS BOX

What is known? EA is a chronic disease that can impact the psychological well-being of children born with this disease. EA parents are known to develop PTSD and anxiety. **What does article add to our knowledge?** Family-centered care of children born with EA is becoming more important. We need to include mental health awareness of parents in the long-term follow-up of EA children. **How does this study impact our current management guidelines?** We need to recognize and address the effects of EA on the parents of these children. Emotional well-being and follow-up should be included in the follow-up protocol of EA patients with inclusion of the appropriate members of the multidisciplinary team.

ABBREVIATIONS

EA: Esophageal Atresia

PPQ: Post-Traumatic Stress Disorder Questionnaire

PTSD: Post-Traumatic Stress Disorder

STAI: State-Trait Anxiety Inventory

KEY WORDS

Parental mental well-being; psycho-social follow-up; esophageal atresia.

INTRODUCTION

Esophageal atresia (EA) is a congenital gastro-intestinal abnormality that can be detected during prenatal screening or shortly after birth (1). Neonates with EA typically require admission to the neonatal intensive care unit (NICU) and undergo surgery within the first few days of life. Parents of neonates diagnosed with EA, similar to those whose neonates necessitate early NICU admission, encounter diverse potential stressors that can contribute to subsequent mental health manifestations (2). Furthermore, the care required for a chronically ill child, such as those born with EA, has been demonstrated to demand specialized attention from parents potentially elevating their susceptibility to the development of mental health symptoms (1). Despite extensive literature on the surgical outcomes of EA, little is known about the psychological impact on the parents of neonates born with EA (1, 3).

Mental health symptoms that are known to develop in these parents include parental anxiety, depression and/or post-traumatic stress disorder (PTSD) (1). Validated questionnaires have been used in previous studies to identify parents at risk for developing PTSD and/or anxiety (1). PTSD is a condition that may occur after experiencing a traumatic event, encompassing four characteristic symptom clusters: intrusion, avoidance, negative mood alterations, and changes in arousal and reactivity (3, 4). The Perinatal post-traumatic stress disorder questionnaire (PPQ) is a validated instrument specifically designed for parents of high-risk neonates (1, 5). Originally developed to explore the relationship between specific perinatal stressors (such as gestational age and postnatal complications) and the development of PTSD in mothers, the PPQ has been adapted for the use in various neonatal conditions, including EA (1).

In addition, higher levels of anxiety has been reported in parents of children with chronic diseases (6). Anxiety is defined as feelings of tension and worried thoughts that can lead to physical symptoms such as sweating, increase in blood pressure and palpitations (7). It is not the same as fear but the two are often used interchangeably (7). Parental stress and anxiety have been shown to lead to maladaptive parenting practices which can in turn lead to and predict development of mental health disorders in the children themselves (6). Studies of families with children born with EA have found that child-related factors such as the age of the child and the severity of the illness is directly related to the mental health of the parents (6). The State-Trait Anxiety Inventory (STAI) has been used for parents of children born with EA (1). It is a well-established measurement tool that can be used to evaluate the presence of both transient anxiety caused by a specific situation (State anxiety) and persistent anxiety experienced continuously (Trait anxiety) (8).

The primary objective of this study was to examine the occurrence of PTSD symptoms and anxiety among parents whose children were born with EA in our health care facility.

MATERIALS AND METHODS

An ambi-directional cohort study was conducted at an academic pediatric surgical department. Parents of children born with EA, that were followed-up in our unit, between 2021 and 2023, were invited to participate. Participation was voluntary and parents provided written informed consent. Parents were recruited on a consecutive, convenience basis as their child presented to the pediatric surgical clinic for follow-up or to our unit for hospitalization.

Questionnaires were completed by the parents themselves during their child's follow-up visits. Two separate questionnaires were completed: the "First visit parental psycho-social questionnaire" and the "Follow-up visits parental psycho-social questionnaire "which were scheduled to be completed at least 6 months apart.

First visit parental psycho-social questionnaire

This questionnaire consisted of 3 different sections and was only completed by the parents, during the child's first visit that occurred during the study period. The first section enquired about the socio-demographic information and personal challenges of the parents. Additionally, the child's age and the reason for the visit (admission to the surgical ward *vs*. regular out-patient department visit (OPD)) was recorded.

The second part was the "Perinatal post-traumatic stress disorder questionnaire" (PPQ) (1, 5). This section relied on parental recall, with symptoms considered present if they persisted for more than one

month. The PPQ comprises 14 items, each with a binary response (Yes/No), and a positive response was assigned a score of one. A PPQ score of ≥ 6 is indicative of the presence of PTSD symptoms. The third part was the STAI to evaluate parental anxiety (1, 8, 9). This inventory comprises a 40-item self-report assessment employing a 4-point Likert scale for scoring. Higher scores indicate elevated levels of parental anxiety, with scores exceeding 55 on either the State or the Trait STAI, indicating severe anxiety.

Follow-up visit parental psycho-social questionnaire

For subsequent visits during the study period, parents were requested to complete a condensed version of the first questionnaire that only included basic socio-demographic information of the parents. Details such as the child's age and the purpose of the visit (admission to the surgical ward *vs*. regular OPD visit) were also documented. Parents were allowed to complete this questionnaire multiple times, provided that the visits were spaced at least 6 months apart.

Clinical data of the children born with EA

Clinical data of the children born with EA were collected from hospital records. These included gestational age at birth, type of EA, and details regarding their surgical procedures (specifically whether they underwent esophageal replacement and/or had a gastrostomy at any time and the presence of major peri-operative complications).

Statistical analysis

Descriptive analysis was performed using means, standard deviations, medians, and interquartile range for continuous data, as appropriate. Categorical data were described using number and percentage. For the bivariate analysis, Student T-test were used as appropriate. A p-value of <0.05 was considered statistically significant.

Ethical approval was provided prior to the onset of the study (HREC reference number S20/10/260).

RESULTS

During the study period (2021-2023), a total of 22 children with EA were followed-up in our unit. Twenty-four parents were approached for recruitment. Four parents were excluded: two parents attended only one follow-up visit soon after initial discharge and were subsequently lost to follow-up while two parents declined participation. Twenty parents (18 mothers and 2 fathers) of 18 children completed a total of 28 questionnaires (18 First visit and 10 Follow-up questionnaires). Just less than half (n = 13, 46%) of the visits, during which the parents completed a questionnaire, was documented as regular visits to the pediatric surgery OPD. The remaining 15 (54%) questionnaires were completed during an admission of their child (scheduled or emergency) to the surgical ward.

Six (33%) of the children in this cohort were born prematurely. The mean age of the children at the time of parental questionnaire completion was 63 ± 59 months, of which five (28%) were 6 months or younger. Sixteen (89%) children presented with a typical EA with a distal tracheo-esophageal fistula (TEF) and two (11%) with an isolated EA. Major complication were experienced by four (22%) children post-EA repair, including two significant anastomotic leaks and two with recurrent TEF's. Five (28%) of the children received an esophageal replacement and four (22%) a gastrostomy at some point during their treatment.

Socio-demographic information of the parents

The mean age of parents at time of questionnaire completion was 36 ± 10 years. Questionnaires were mostly completed by mothers (n = 18, 90%) with the majority (n = 15, 75%) of the parents reporting no difficulties in their own personal lives at time of questionnaire completion. Five (25%) parents reported experiencing at least one of the following personal difficulties: health related problems, problems at work, relationship complications, financial difficulties, other problems, or combinations thereof. Parental social demographic data are described in **Table 1**.

Perinatal post-traumatic stress disorder questionnaire (PPQ)

The mean PPQ score was 3.72, ranging from 0 to a maximum score of 12. Six parents (33%) had a PPQ score of six or higher: two parents with a PPQ score of 6 and one each with a PPQ score of 9, 10, 11 and 12. There was no evident correlation between PPQ scores and the children's clinical EA course. One

Table 1. *Parental socio-demographic information.*

	n = 20 **n (%)**
Relationship status	
Married	**6 (30)**
In a relationship	**6 (30)**
Widowed	**1 (5)**
Single	**6 (30)**
Didn't complete this question	**1 (5)**
Number of children in the household	
1	**7 (35)**
2	**4 (20)**
3	**7 (35)**
4	**2 (10)**
Highest level of parental education	
Didn't finish high school	**8 (40)**
Finished high school	**6 (30)**
College diploma	**1 (5)**
Unknown	**5 (25)**
Ever treated by a psychologist or psychiatrist?	**2 (10)**

child of a parent with a high PPQ score (PPQ of ≥ 6) had a straightforward peri-operative course during the neonatal period with no complications. Two children had a colonic interposition as esophageal replacements. Two of the remaining three children had minor post-operative complications (complications that did not require any surgical intervention) and one had a major anastomotic breakdown requiring redo-surgery.

There was no significant association between parents with PPQ scores above or below 6 regarding gestational age at birth, type of EA or the need for additional surgical procedure (*e.g.*, esophageal replacement or gastrostomy) as described in **Table 2**. A significant association was found between PPQ scores and the age of the child at time of questionnaire completion as well as the type of visit. We found a significant difference when we compared parents whose children had major peri-operative complications (defined as those who required surgical intervention) in the neonatal period with those who had minor or no complications (requiring no surgical intervention as part of the treatment). Furthermore, the majority (n = 3, 75%) of parents with children that experienced major complications, had PPQ scores >6.

The State-Trait Anxiety Inventory (STAI)

A total of 20 parents, comprising 18 mothers and 2 fathers, completed 28 STAI's. The mean State STAI (reflecting their current feelings) was 46 ± 7. Four (14%) cases scored above 55 indicating high anxiety levels at the time of visit. The highest State STAI score documented was 59 and was scored by a mother of a premature born infant with an isolated EA and major complications post-operatively requiring a colonic interposition.

The mean score for the Trait STAI (assessing whether parents were generally anxious) was 46 ± 5, with only

Table 2. *Comparison of PPQ scores.*

	n = 18	**Median PPQ (IQR 25 - 75)**	**p-value**
Premature neonates **Term neonates**	6 12	0.5 (0-10) 2.5 (0.5-6)	0.5
Age at visit ≤6 months **Age at visit >6 months**	5 13	0 (0-1) 4 (1-9.5)	0.02
OPD visits **Hospital admission**	5 13	6 (3-10.5) 1 (0-4.5)	0.04
Isolated EA **EA with a distal TEF**	2 16	0 2.5 (0.5-7.5)	0.1
No additional surgeries **Esophageal replacement and/or gastrostomy**	11 7	2 (0-6) 1 (0-11)	0.22
Major complications post-EA repair **Minor or no complications**	4 12 *	10 (4.5-11.5) 2 (0-5)	0.01

PPQ: Perinatal Post-traumatic Stress Disorder Questionnaire; IQR: Interquartile Range; OPD: Out-patient Department; EA: Esophageal Atresia; TEF: Tracheo-esophageal Fistula. * Two children had no neonatal repair done and are not included for comparison in this section.

one parent (4%) scoring above 55 during an emergency admission of the child for a gastroscopy and removal of a foreign body.

There were no statistically significant differences between the State STAI and Trait STAI scores (p = 0.26). The age of children at time of visit, the type of visit, the type of EA, a history of additional surgeries required and major of minor post-operative complications revealed no significant differences in either the State or Trait STAI scores achieved by our parents. The only statistically significant difference was found when we compared the State STAI of parents with children born prematurely to those who were born term. The rest of the results are described in **Tables 3** and **4**.

All parents found to be at risk for developing PTSD (PPQ scores of ≥6) as well as those with scores indicating anxiety were referred for further work-up and counselling.

DISCUSSION

Our study findings indicate that one-third of parents with children born with EA were at risk for developing PTSD, while 14% had scores indication high levels of anxiety at the time of questionnaire completion. The age of the child at the time of hospital visit, major

Table 3. *Comparison of State STAI scores.*

	n = 28	Mean State STAI ± SD	p-value
Premature neonates	9	50 ± 7	0.04
Term neonates	19	44 ± 7	
Age at visit ≤6 months	5	48 ± 6	0.3
Age at visit >6 months	23	46 ± 7	
OPD visits	13	47 ± 7	0.2
Hospital admission	15	45 ± 7	
Isolated EA	4	49 ± 8	0.2
EA with a distal TEF	24	46 ± 7	
No additional surgeries	17	45 ± 7	0.3
Esophageal replacement and/or gastrostomy	11	47 ± 7	
Major complications post-OA repair	8	48 ± 8	0.2
Minor or no complications	18 *	45 ± 6	

STAI: State-Trait Anxiety Inventory; SD: Standard Deviations; OPD: Out-patient Department; EA: Esophageal Atresia; TEF: Tracheoesophageal Fistula. * Two children had no neonatal repair done and are not included for comparison in this section.

Table 4. *Comparison of Trait STAI scores.*

	n = 28	Mean Trait STAI ± SD	p-value
Premature neonates	9	48 ± 2	0.06
Term neonates	19	45 ± 6	
Age at visit ≤6 months	5	46 ± 4	0.5
Age at visit >6 months	23	46 ± 5	
OPD visits	13	47 ± 5	0.2
Hospital admission	15	45 ± 5	
Isolated EA	4	49 ± 3	0.1
EA with a distal TEF	24	46 ± 5	
No additional surgeries	17	46 ± 6	0.2
Esophageal replacement and/or gastrostomy	11	47 ± 4	
Major complications post-OA repair	8	47 ± 5	0.3
Minor or no complications	18 *	46 ± 5	

STAI: State-Trait Anxiety Inventory; SD: Standard Deviations; OPD: Out-patient Department, EA: Esophageal Atresia, TEF: Tracheoesophageal Fistula. * Two children had no neonatal repair done and are not included for comparison in this section.

complications during the peri-operative period as well as type of visit were associated with parental PTSD risk whilst prematurity was significantly associated with increased STAI scores.

Parents of neonates that are admitted to a NICU are exposed to various stressors, contributing to the development of mental health symptoms (2). A systematic review by Malouf *et al*. showed a higher prevalence of PTSD and anxiety in parents with neonates in NICU compared to parents of healthy neonates (2). De Mier *et al*. determined that the severity of neonatal complications, gestational age, and length of hospitalization were significant contributing factors for the development of PTSD in mothers of high-risk neonates (5). Based on these factors, they developed the PPQ as an early PTSD recognition tool for parents (5, 10). The PPQ score encompasses possible symptoms of PTSD as defined by the DSM-IV criteria and include questions about: symptoms of intrusion, avoidance, and changes in arousal (3, 5). Other studies have validated the PPQ's efficacy in identifying PTSD risk in mothers of high-risk neonates (10). In comparison to their studies, we found that major peri-operative complications during the neonatal period were a contributing factor for high PPQ scores in our cohort.

While studies have explored the psychological impact of congenital gastro-intestinal malformations (CGIM) requiring neonatal surgical intervention on parents, specific reports of similar impact on parents of EA patients are scarce (11). It is acknowledged that parents of neonates with CGIM are at higher risk for developing PTSD later in life (11). Roorda *et al*. found that this group of parents were not only vulnerable to psychological distress, due to the birth itself, but also due to contributing factors such as the diagnosis of CGIM, intervention, and potential long-term complications associated with these malformations, as was also seen in our group of parents (11). Although EA was included in the group of malformations studied, they did not specifically report on parents with children born with EA. The prevalence of PTSD in their group of parents was 16.5% with mothers having a higher risk of developing PTSD when compared to a reference group (23% *vs*. 5%). This is slightly lower than the 33% of parents in our cohort, with all the at-risk parents being mothers.

Le Gouez *et al*. investigated the associations between the development of PTSD symptoms, and neonatal disease severity, severe complications in EA, as well as the quality of life and global health status of EA patients (1). They found no association between the presence of PTSD symptoms and neonatal disease severity or the presence of severe complications at 2 years of age (1). We found high PPQ scores in 75% of the parents whose children had major peri-operative complications during the neonatal period and a significant difference in the risk for the development of PTSD when compared with those with minor or no complications. Le Gouez *et al*. reported 59% of their participants had a PPQ score of six or more, which was significantly higher than the 33% in our cohort (1). They found that PTSD was only weakly associated with the age of children in their cohort, which again contrasts with our findings where the age of a child >6 months was associated with a high risk of PTSD (PPQ > 6) (1). These differences in outcomes emphasize the need for larger, more in-depth studies specifically focusing on the correlation between risk factors and the development of PTSD in parents of children born with EA.

The State-Trait Anxiety Inventory (STAI) is a valuable tool to distinguish between State and Trait anxiety in parents of children with EA, allowing differentiation between those who experience anxiety constantly and those with anxiety in specific situations (*e.g*., during an admission *vs*. a routine follow-up visit) (8). In our study more parents had higher State STAI than Trait STAI scores indicating anxiety related to the timing of the questionnaire. Although we were unable to demonstrate a statistically significant correlation between high anxiety scores and the purpose of parent's visit in our cohort, we acknowledge the limitation of our small sample size. The first study examining both PTSD and anxiety in parents of children born with EA, was conducted in 2016 by Le Gouez *et al*. (1). Both our study and the one conducted by Le Gouez *et al*. identified 4 cases with participants having State-STAI scores of above 55. In our study, we followed a similar model as Le Gouez *et al*., with the addition of allowing for multiple completions of the STAI questionnaire for our study parents. The addition of multiple STAI questionnaire completions can possibly be seen as a form of counselling as we observed a decrease in scores in follow-up visits of

parents who initially had scores above 55 in our cohort. This however highlights the need for larger, multicenter studies specifically looking at these different aspects possibly identifying these questionnaires as methods of counselling of parents. Such studies will help shed more light on the psychological aspects and provide valuable insights for better management and support for parents of children born with EA globally.

The small sample size in our study is a limitation that should be acknowledged, as it may restrict the generalizability and applicability of the results. Additional psycho-social determinants, specific to lower-middle income countries were not fully addressed (food insecurity, work insecurity, partner violence, financial worries *etc.*) and should be included in future studies. Only a few parents filled in repeat questionnaires. It may be clinically useful to perform longitudinal assessment to determine an improvement or worsening of at-risk symptoms in these parents with the changing medical state of their children. We also acknowledge that questionnaires identifying parents at risk of developing depression should be included in future studies for a more comprehensive examination of the mental health of parents of EA patients. By addressing the limitation of a small sample size, future research in this domain would enhance our understanding of the psychological aspects and experiences of parents facing the challenges of EA, as well as identifying further potential risk factors. This knowledge could contribute to the development of effective support systems and interventions tailored to meet the specific needs of these parents, ultimately improving the overall well-being of families affected by EA.

CONCLUSIONS

Our study highlights the importance of investigating and identifying potential symptoms of distress, specifically PTSD and anxiety, in the parents of children born with EA at an early stage, allowing for timely referral for counselling and treatment, if necessary.

By addressing the emotional needs of parents and incorporating them into the care process, comprehensive and holistic support for families affected by EA can be provided. This approach not only benefits the parents' mental well-being but also positively impacts the overall adjustment and coping of the entire family in managing the challenges of this disease.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

Financial support

There were no institutional or private fundings for this article.

Author contributions

All the Authors contributed to the study concept and design. The data collection and the analysis have been done by CdV. The first draft has been written by CdV and edited by DS. All the manuscript's versions have been edited and approved by DS, LvW and PG. All the Authors read and approved the final manuscript.

Ethical approval

Human studies and subjects

The protocol for this article conforms to the provisions of the Declaration of Helsinki (1995) and has been approved Ethics approval by the Stellenbosch University Health Research Ethics Committee (S20/10/260).

Animal studies

N/A.

Data sharing and data accessibility

Data are available upon motivated request to the Corresponding Author.

Publication ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data correspond to the real.

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PEDIATR RESPIR J Vol. 2(2), 72-82, 2024

ROSTRUM

How primary immune deficiencies impact the lung in children

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Doi
10.56164/PediatrRespirJ.2024.44

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ABSTRACT

Primary immune deficiencies (PID) encompass a heterogeneous group of genetically determined disorders that affect development and/or function of innate or adaptive immunity. Consequently, patients with PID suffer from recurrent and/or severe infections that frequently involve the lung. Pulmonary complications, which are largely dependent on the components of the immune system that are defective, cause significant morbidity and mortality. Even though the pattern of lung damage and the types of microorganisms involved may provide insights into potential defects in innate, humoral, or cell-mediated immunity, there is a substantial overlap in lung complications among the different types of PID. Consequently, thorough laboratory investigations are necessary to establish a definitive diagnosis and to prompt appropriate treatment. Furthermore, the identification of a large number of PID-causing genes allows early, even presymptomatic diagnosis, thus representing an essential tool for prevention of lung damage.

IMPACT STATEMENT: This article describes the most common forms of PID, their cellular and molecular bases, and the associated lung abnormalities, and reports on available treatment.

INTRODUCTION

Primary immunodeficiency diseases are a heterogeneous group of inherited disorders of the immune system in which one or several immune components are decreased, missing, or of non-appropriate function (1). In the immune system, three primary cell lineages can be identified, all originating from a shared lymphoid precursor cell (LPC). If the LPC migrates to the thymus, it will differentiate into the T cell lineage, characterized by two main subsets: CD3+ CD4+ (associated with helper activity) and CD3+CD8+ (associated with cytotoxic activity). The latter plays a crucial role in the defense against intracellular pathogens, such as viruses and fungi. If the LPC migrates to the bone marrow, it will differentiate, based on the local microenvironment, either into the B cell lineage, a precursor of the immunoglobulin-secreting plasma cells, or into the myeloid lineage, ultimately leading to the differentiation into neutrophils. Immunoglobulins play a crucial role in protecting against extracellular pathogens, whereas neutrophils contribute to protection against both intra and extracellular pathogens. Regardless of the defective cell line, the lungs are a major target in PID, and pulmonary manifestations vary in etiopathogenesis, type, and severity according to the specific branch of the immune system which is compromised.

ABBREVIATIONS

ARA: Autosomal Recessive Agammaglobulinemia
CGD: Chronic Granulomatous Disease
CVID: Common Variable Immune Deficiency
GLILD: Granulomatous Lymphocytic Interstitial Lung Disease
HIES: Hyper IgE Syndromes
ILD: Interstitial Lung Disease
PID: Primary Immune Deficiencies
SCID: Severe Combined Immunodeficiencies
XLA: X-linked Agammaglobulinemia

KEY WORDS

Interstitial lung disease; granulomatous lymphocytic interstitial lung disease; hyper IgE syndromes; chronic granulomatous disease; severe combined immunodeficiencies; X-linked agammaglobulinemia; autosomal recessive agammaglobulinemia; primary immune deficiencies; common variable immune deficiency.

This article will focus on the different patterns of pulmonary involvement in selected forms of PID where one or more immune components are defective.

AGAMMAGLOBULINEMIA

Agammaglobulinemia is a primary immune deficiency characterized by low or absent immunoglobulin serum levels, impaired antibody production, and a severely reduced or absent peripheral B cell population (<2%) due to early arrest of B cell development. Since the T and myeloid cell lineages are normal, agammaglobulinemia can be considered the prototype of primary antibody deficiencies. There are two different forms of agammaglobulinemia: the X-linked (XLA or Bruton disease), affecting males, which is due to mutation in BTK, the gene encoding for Bruton's tyrosine kinase, a cytoplasmic protein essential for early B-cell development, and the autosomal recessive (ARA) form which affects both males and females and is due to mutations in genes encoding for different components of the B cell receptor (BCR), such as μ heavy chain, λ5, Igα, Igβ or for cytoplasmic proteins (*e.g.*, BLNK deficiency, E47 deficiency, p85α deficiency, ZIP7 deficiency), all crucial for B cell development (2).

Typically, agammaglobulinemic patients suffer from recurrent infections, commonly affecting the upper and lower respiratory tracts. In a recent survey of a large cohort of agammaglobulinemic patients (3), respiratory infections were the most frequent clinical symptom at diagnosis, with one or more episodes of pneumonia recorded in 40% of patients. Of note, at the time of diagnosis, 13% of patients were already affected by chronic lung disease (CLD), as indicated by the results of a lung computed tomography scan which showed the presence of bronchiectasis, peri bronchial wall thickening, and atelectasis. The most common pathogens causing pneumonia include encapsulated bacteria such as *Heamophilus influenzae* and *Streptococcus pneumoniae*. Over a mean follow-up period of 8.35 years per patient (range 1-18 years), respiratory infections remained the most common clinical manifestation persisting even with immunoglobulin replacement therapy (IRT). Specifically, episodes of pneumonia and sinusitis were recorded in 34 and 56% of patients, respectively. It is noteworthy that, despite regular IRT, the percentage of patients with CLD increased from 13% to 35% during follow-up, allowing to calculate the cumulative risk of CLD, which is equal to 47% at 50 years of age. The occurrence of CLD already at diagnosis and during follow-up may be partially explained by various factors, such as advanced age, delayed diagnosis, and, in some cases, initial treatment with intramuscular immunoglobulins. It is known that intramuscular immunoglobulins are considerably less effective than intravenous or subcutaneous immunoglobulins in reducing the incidence of respiratory infections. The development of CLD during follow-up suggests that regular IRT may not completely prevent the development of lung complications, likely due to the administered immunoglobulins' inability to reach the mucosal surface. Furthermore, the administered immunoglobulins contain only IgG, which are not selected on antigen specificity and cannot compensate for the lack of IgA at the mucosal surface (3, 4, 5). As pathogens more frequently responsible for pneumonia are encapsulated bacteria, the most common radiological pattern observed in these patients is lobar consolidation, often accompanied by pleural effusion or empyema, depending on the severity of the infectious episode (**Figure 1A**). This radiological pattern is common to all forms of agammaglobulinemia, regardless of the underling genetic defect. This contrasts with what is observed in common variable immunodeficiency (see below). Repeated episodes of pneumonia may result in bronchiectasis during the course of the disease (**Figure 1B**). Early diagnosis and a timely and consistent IRT are crucial for controlling long-term lung complications. Furthermore, considering the impact of CLD on daily life and especially on long-term outcome, each acute infectious episode should be promptly treated with antibiotics. Additionally, a personalized respiratory physio-

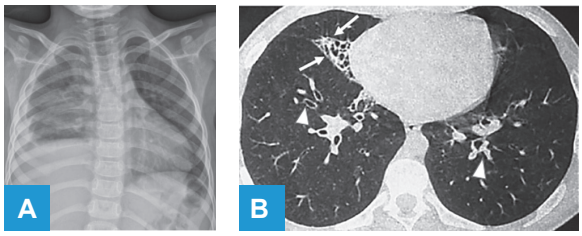

Figure 1. *(**A**) Frontal chest radiograph in a 6-year-old boy with XLA demonstrates extensive parenchymal consolidation in the right lower lobe with pleural effusion caused by Str. Pneumoniae; (**B**) CT scan in a 18-year old boy with XLA shows middle lobe collapse with cylindrical bronchiectasis (arrows); other bronchiectasis are visible in both lower lobes (arrowheads).*

therapy program and/or antibiotic prophylaxis regimen should be considered (5, 6).

COMMON VARIABLE IMMUNODEFICIENCY

Common variable immunodeficiency (CVID) is the most common symptomatic primary immunodeficiency. It is characterized by markedly reduced serum levels of IgG, IgA, and IgM, usually less severe than that observed in agammaglobulinemia. In addition to reduced immunoglobulin levels, individuals with CVID exhibit the inability to mount functional antibody responses to immunization or infection. Peripheral T (CD3) and B (CD20 or 19) cell counts are generally within the normal range, whereas a more in-depth investigation of T and B cell subsets may reveal subtle abnormalities, contributing to the clinical heterogeneity of this disorder (7-9).

In fact, analysis of extensive patient datasets reveals that CVID patients can be broadly categorized in two major clinical groups. The first group predominantly presents infections, with recurrent bacterial infections of the upper and/or lower respiratory tracts being the most common clinical manifestation that often prompts diagnosis of the immune defect. Pneumonia, often caused by *Streptococcus pneumoniae* and *Heamophilus influenzae,* is identified in up to 60% of patients with CVID. The recurrence of pneumonia may lead to the development of structural airway changes, such as bronchiectasis, affecting up to 40-60% of patients (10-13). Therefore, in terms of lung involvement, this patient group shares similarities with those having agammaglobulinemia. Thus, an early diagnosis and a timely and consistent IRT are crucial for controlling long-term lung complications. Furthermore, prompt antibiotic treatment for each acute infectious episode and a personalized respiratory physiotherapy program and/or antibiotic prophylaxis regimen should be considered.

The other group (complex CVID) is characterized by a prevalence of non-infectious complications, including autoimmunity (cytopenia, thyroiditis, enteropathy), inflammatory and/or lymphoproliferation, splenomegaly, lymphoma, nodular hyperplasia, and granulomatous manifestations (9, 14). These complications may occur simultaneously or sequentially over the disease course. They are thought to derive from dysregulated innate and adaptive cellular activation responses due to underlying pathogenetic mechanisms that affect the maintenance of tolerance (14-16). Consequently, lung complications in this group are more severe than those of the first group and encompass, in addition to bronchiectasis, interstitial and parenchymal lung disease (ILD).

These two distinct clinical phenotypes likely result from distinct genetic defects causing CVID, falling broadly into two categories: those that almost exclusively affect B cell development, differentiation, activation, and/or survival, such as genes encoding for the cluster of differentiation 19, 20, 21, or 27 (first group), and those that control/affect crosstalk between B and T cells (*i.e.*, mutations in LRBA, CTL-4, NFKB1, NFKB2, PIK3CD, STAT3), causing immune dysregulation ultimately leading to ILD (second group) (9, 14). CVID-related ILD is presumed to be unrelated to bacterial infections, as it can be observed also in the absence of bronchiectasis, it is not significantly associated with a history of pneumonia and frequently occurs in conjunction with lymphoid hyperplasia in other tissues, such as lymph nodes, spleen, and the mucosal lymphoid tissue of the gastrointestinal tract (17). This phenotypic distinction also has important clinical implications because the risk of death due to lung complications is significantly higher for patients with ILD as compared to those without (9, 14, 18). Lymphoid ILD, found in 8-20% of CVID patients (19), exhibits various pulmonary histological patterns, including follicular bronchiolitis, nodular lymphoid hyperplasia, granulomatous lung disease, lymphocytic interstitial pneumonia, and organizing pneumonia. Since these patterns may coexist in the same patient, the term “Granulomatous and Lymphocytic Interstitial Lung Disease (GLILD)” was coined to identify a distinct clinico-radio-pathological interstitial lung disease characterized by a lymphocytic infiltrate and/or granuloma in the lungs (20, 21).

The main computed tomography (CT) features of ILD/GLILD are nodules, ground glass opacities, reticulations, consolidation, and interstitial fibrosis (**Figure 2A**). Immunohistochemical analysis of pulmonary lymphoid hyperplasia has documented the presence of distinct B-cell follicles and T-cell zones, demonstrating lymphoneogenesis as a feature of lymphoid hyperplasia and implicating B lymphocytes in the pathogenesis of this complication (21). Since ILD is very rare or absent in

X-linked and autosomal recessive agammaglobulinemia, two disorders characterized by absence of B cells, it is tempting to speculate that B cells, by acting as antigen-presenting cells, producing proinflammatory chemokines and cytokines, or both, might perpetuate leukocyte accumulation within the lung, leading to lymphoid hyperplasia (21). This is supported by the adoption of B cell depletive therapy, either alone or in combination with other immunosuppressive drugs, as a fundamental component of CVID-related ILD/GLILD treatment (22, 23) (**Figure 2B**). Since this complication causes significant morbidity and mortality, there is a need for effective treatments. However, currently, there is no standardized treatment protocol. IRT has greatly reduced the number of infections, but it does not appear to prevent or ameliorate most inflammatory and autoimmune conditions. Because the mechanisms underlying CVID-related ILD/GLILD are only partially elucidated, immunosuppressive treatment either as monotherapy or as a combination of different drugs, has been tried with varying results (24). Glucocorticosteroids are considered the first line therapy for ILD/GLILD (20). However, the response may be short-lived or unsatisfactory, and there are significant side effects associated with protracted use. Additionally, a proportion of patients are refractory. Thus, besides glucocorticoids, other immunosuppressive drugs (ciclosporin, mycophenolate mofetil, azathioprine, hydroxychloroquine, infliximab, rituximab, abatacept) have been administered with varying success (24). Finally, HSCT may be an option when other treatments have failed, but the reported mortality rate is still relatively high compared to the overall survival of patients transplanted for other types of PID (24, 25). A better understanding of the pathogenetic mechanisms underlying ILD/GLILD may lead to the development of safer and more effective therapies.

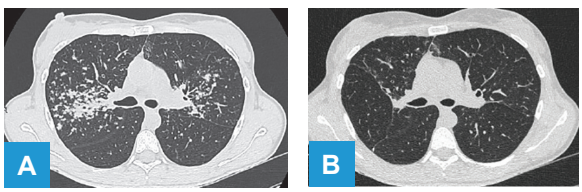

Figure 2. *A 37-year-old female with CVID, admitted to the hospital because of productive cough, mild fever, and dyspnea on exertion. Her clinical history was remarkable for autoimmune thyroiditis, psoriasis, and multiple allergic reactions to drugs. (**A**) CT scan shows multiple nodules in both lungs, more pronounced in the right one; culture of bronchoalveolar lavage fluid excluded bacterial infections, mycobacteria, and pneumocystosis. Lung biopsy revealed numerous non-necrotizing epithelioid cell granulomata and interstitial lymphocytic infiltrates, suggestive of GLILD (data not shown); (**B**) CT scan after treatment with Rituximab shows complete resolution.*

SEVERE COMBINED IMMUNODEFICIENCIES

Severe combined immunodeficiencies (SCID) encompasses a heterogeneous group of genetic disorders characterized by a profound impairment of T cell development and/or function. In some forms of SCID, the numbers of circulating B and/or NK cells is variably affected, but B cell function is impaired due to the lack of adequate helper T cell activity. Based on the number, function, and origin of circulating T cells, SCID may be classified into two categories: typical and atypical forms. The former is characterized by a very low number of circulating T cells (<300 cells/μl) and no or very low T cell function, (<10% of lower limit of normal of T cell proliferation to phytohemagglutinin). Typical SCID also includes the form characterized by the presence of circulating maternal T cells (maternal engraftment). Atypical SCID includes a reduced number (>300 cells/μl) of circulating T cells for age, in the absence of documented maternal engraftment, and <30% of lower limit of normal proliferative response to phytohemagglutinin. Atypical SCID is sometimes referred to as “leaky” SCID (26). The full blood count becomes, therefore, the most accessible and practical first-level diagnostic test. Yet the absolute lymphocyte counts are often overlooked. Lymphocyte counts are higher in infancy than in adulthood, and it is not widely appreciated that an absolute lymphocyte count of less than 2,800 cells/μl is 2SD below the mean. When infants with infections have a count lower than this, it is highly likely they have SCID (27, 28). Following immunophenotyping of blood lymphocytes, SCID patients can be classified, according to the presence or absence of different lymphocyte subsets, into 4 different subgroups: T-B-NK+; T-B-NK-; T-B+NK-; T-B+NK+ (29). A normal lymphocyte count does not preclude a SCID diagnosis. In fact, the presence of B lymphocytes in the absence of T cells, as well as the presence of maternal engraftment and a distinctive phenotype characterized by the presence of autologous, oligoclonal, and activated T cells (Omenn syndrome), may obscure lymphopenia. The identification of the genetic defects underlining different immunophenotypes (29) has helped to clarify

the mechanisms of functional T cell development. Furthermore, precise identification of the gene defect has a relevant impact on treatment, as exemplified using by use of enzyme replacement therapy in Adenosine deaminase deficiency.

Infants with SCID typically appear healthy at birth. However, due to the lack of adequate T cell function, most of them will manifest severe and potentially life-threatening opportunistic infections within the first year of life if not promptly diagnosed and treated. The most frequent reason for seeking medical attention is respiratory tract infections, often associated with other symptoms such as diarrhea, failure to thrive, or thrush (30). Respiratory symptoms in infants with SCID can resemble those of bronchiolitis, including persistent cough, tachypnoea, increased work of breathing, grunting or nasal flaring, and wheezing or rales on chest auscultation. Despite supportive therapy, these symptoms persist beyond the expected duration and can worsen to the point of requiring admission to the pediatric intensive care unit (PICU). In fact, it is widely recognized that patients with SCID are often diagnosed in a PICU, where they were admitted for severe, prolonged, and complicated respiratory infections. Imaging features usually reveal an interstitial pneumonia with varying patterns of consolidation. Opportunistic pathogens, such as *Pneumocystis jirovecii*, cytomegalovirus, adenovirus, parainfluenza virus type 3, and respiratory syncytial virus, are most commonly isolated from bronchoalveolar lavage (**Figure 3A, B, C, D**). Invasive bacterial and fungal infections also occur, with the former mostly resulting from impaired humoral immunity secondary to defects in T-cell immunity.

The definitive cure of SCID involves restoration of the immune system through allogeneic hematopoietic stem cell transplantation (HSCT) or gene therapy. Prophylaxis of infections with antibiotics, immunoglobulin substitution, antiviral and antifungal drugs can, at best, marginally prolong survival (26). The outcome of HSCT is largely dependent on the presence or absence of active infection at the time of HSCT, with increased mortality for patients with an active infection at the time of transplantation compared to those without (31, 32). This raises the question of the need of an early diagnosis of these disorders, just before the occurrence of infections. Early diagnosis can be achieved only through a newborn screening for SCID (SCID-NBS). The efficacy of this approach has been documented in a recent paper showing improved survival of SCID-transplanted patients in countries where SCID-NBS has been implemented (33).

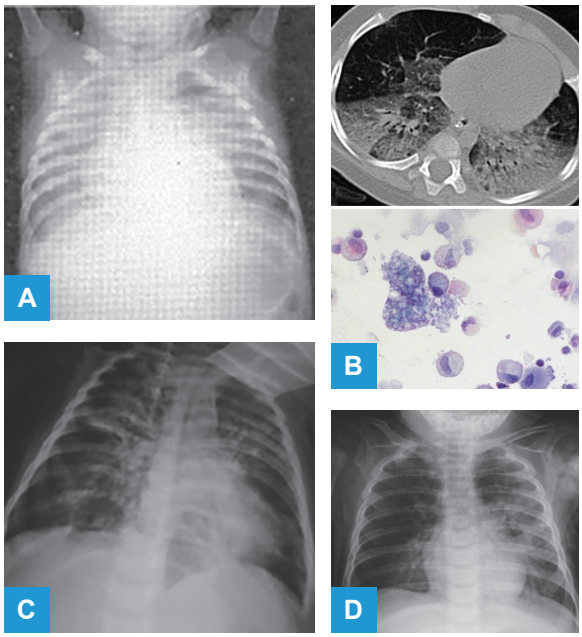

Figure 3. *Lung involvement in SCID patients. (**A**) chest radiograph in a 4-month-old male shows bilateral, perihilar interstitial infiltrates, due to CMV infection; (**B**) lung CT scan in a 6-month-old female showing diffuse interstitial infiltrates in the right lung associated with parenchymal consolidation in the left lung. Pneumocystis jiroveci was isolated from bronchoalveolar lavage fluid; (**C**) chest radiograph of a 6-month-old male showing bilateral parenchymal consolidations in the context of interstitial infiltrates; bronchoalveolar lavage fluid yielded Stenoprophomonas maltofila; (**D**) chest radiograph in a 5-month-old male showing inhomogeneous parenchymal consolidation in the left paracardiac site and another in the right paracardiac site; bronchoalveolar lavage fluid yielded adenovirus. Absence of thymic shadow is documented in c and d cases.*

DEFECTS OF PHAGOCYTIC COMPARTMENT

Neutrophils play a significant role in the clearance of bacterial and fungal infections, making immunodeficiencies due to phagocytic dysfunction notable for infections caused by these organisms. Phagocytic dysfunction, accounting for 10% to 15% of primary immunodeficiencies, impairs neutrophil function in a wide range of inherited disorders. Among these, chronic granulomatous disease, leukocyte adhesion deficiency, congenital neutropenia, and Chediak-Higashi syndrome are some of the most well-known. This section focuses on lung involvement in CGD, offering a key illustration of PID caused by impaired neutrophil function.

Chronic granulomatous disease (CGD) is a genetically heterogeneous disorder characterized by recurrent, life-threatening infections with bacteria and fungi, along with dysregulated granuloma formation. CGD commonly arises from defects in any of the genes encoding the structural subunits of the NADPH oxidase, the enzyme responsible for the phagocyte respiratory burst and the generation of phagocyte superoxide, which is crucial for intracellular killing of ingested microorganisms. NADPH oxidase consists of 2 membrane-bound proteins (gp91^{phox} and p22^{phox} that constitute the cytochrome b_{558}), and 4 cytosolic components (p47^{phox}, p67^{phox}, p40^{phox}, and RAC). CGD caused by mutations in gp91^{phox} is inherited as an X-linked trait (affecting 65% of patients), while mutations in p47^{phox}, p67^{phox}, p40^{phox}, and RAC, lead to autosomal recessive inheritance. The recently recognized EROS (encoded by CYBC1) is required for assembly and transport of cytochrome b_{558} (34, 35).

In CGD, infections typically have an early onset and are mainly caused by catalase-positive microorganisms, such as *Staphylococcus aureus*, *Burkholderia cepacia*, *Serratia marcescens*, *Nocardia*, and *Aspergillus* species. In addition to skin infections, lymphadenitis, and liver abscesses, the lungs are frequently involved (36). This includes recurrent pneumonia, which is the most common infection occurring in 50-80% of CGD patients, hilar lymphadenopathy, empyema, or lung abscesses (37, 38). Radiologically, bacterial pneumonia may manifest as segmental or lobar parenchymal consolidation (**Figures 4A, B, 5A, B**). The presence of pneumatocele is suggestive of staphylococcal pneumonia and usually occurs 10-14 days after the onset of infection, when the patient is clinically improving (39).

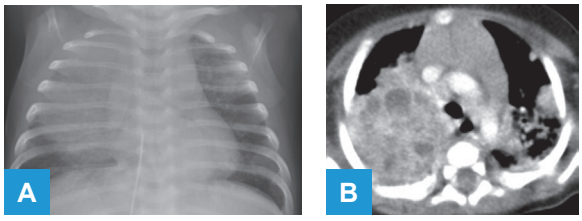

Figure 4. *(**A**) frontal chest radiograph in a 2-month-old male with X-linked CGD demonstrates an extensive parenchymal consolidation in the right lung and another consolidation in the suprabasal area of the left lung; (**B**) CT scan of the same patient shows extensive parenchymal consolidation in the right lung, with multiple hypodense rounded areas, compatible with colliquative necrotic material (abscess lesions). Similar small areas of rounded morphology are also observed in the left lung. Staphylococcus aureus was isolated from bronchoalveolar lavage fluid.*

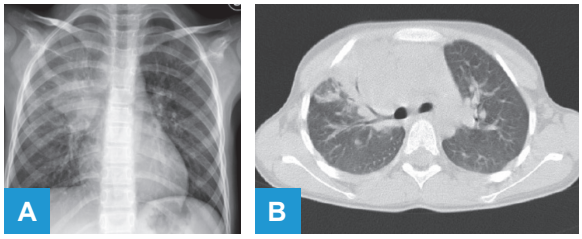

Figure 5. *(**A**) frontal chest radiograph of a 2-year-old girl with CGD showing a parenchymal opacity with irregular profiles in the right perihilar area; (**B**) CT scan of the same patient shows a parenchymal consolidation with a broncogram in the right upper lobe. Burkholderia gladioli was isolated from bronchoalveolar lavage fluid.*

Patients with CGD are particularly susceptible to developing pulmonary aspergillosis. Typically, the focal consolidations characteristic of pulmonary aspergillosis often exhibits a distinct “halo” of ground-glass attenuation (**Figure 6A, B**), indicative of hyphae proliferation into the endobronchial tree, ultimately invading pulmonary vessels and resulting in thrombosis and infarction of the lungs (40). In some cases, especially during the early phase of the disease, *Aspergillus* nodules may be small and lack the characteristic “halo sign”, making it challenging to differentially diagnose between infectious or inflammatory (granulomatous) origins of the nodules (40, 41).

Infections caused by uncommon organisms, such as those mentioned above, should prompt an investigation for CGD in patients of any age who lack other predisposing factors. The diagnosis of CGD is established by assays that rely on superoxide production. These methods include direct measurement of superoxide production, ferricytochrome c reduction, chemiluminescence, NBT reduction, or the flow cytometry-based test dihydrorhodamine oxidation (DHR).

Continuous antimicrobial prophylaxis with co-trimoxazole and azole antifungal drugs has proven effective in reducing the incidence of severe infections in CGD patients. However, the long-term prognosis of CGD remains controversial, and it is advisable to consider HSCT. Allogeneic HSCT is currently the only known cure for CGD. Earlier use of myeloablative regimens led to disease resolution but relatively high risk of mortality (34, 42). More recent application of reduced intensity conditioning regimens has greatly reduced regimen-related toxicity, enabling transplantation

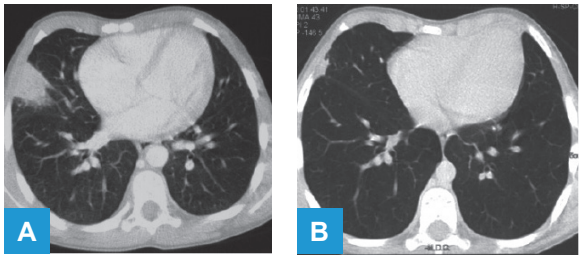

Figure 6. *(**A**) CT scan in an 8-year-old male with X-linked CGD shows focal consolidation in the middle right lobe surrounded by a noticeable «halo sign» at the periphery, suggestive of alveolar hemorrhage. A specimen of the infiltrate obtained with a CT-guided biopsy showed growth of Aspergillus; (**B**) despite long-term antifungal treatment, CT scan performed almost 1 year later shows persistent focal pleural thickening.*

even in the presence of active infection. With these regimens, survival rates range from 80% to 90%, with comparable survival outcomes among patients with matched related, matched unrelated, and umbilical cord donors (34, 43-45). Moreover, promising results have been recently obtained with gene therapy for the X-linked form of CGD (46, 47).

THE HYPER IGE SYNDROMES (HIES): STAT3 DEFICIENCY AS REPRESENTATIVE EXAMPLE OF THE STAT3-RELATED HIES

Hyper-IgE syndromes are primary immune deficiencies characterized by the triad of high serum IgE levels, eczema, and recurrent skin and pulmonary infections. Both autosomal dominant and autosomal recessive forms of the disorder have been described (29, 48, 49). Some types of HIES share common clinical features, including immunological and non-immunological skeletal and connective tissue abnormalities, with a strong involvement of the STAT3 dependent pathway. These conditions, resulting from mutations in genes such as STAT3, IL6ST, IL6R, PGM3, ZFN341, ERBIN and TGFBR, are collectively termed “STAT3-related HIES”. Other forms of non-STAT3-related HIES have been reported (50). The diagnosis of HIES is typically made based on elevated serum IgE levels along with specific clinical symptoms. However, as the symptoms of different forms of HIES overlap to some extent, molecular-genetic testing is required for a definitive diagnosis of HIES.

This section focuses on the lung involvement observed in STAT3 deficiency, commonly known as Job syndrome, as an illustrative example representing the broader category of STAT3-related HIES. STAT3 deficiency, resulting from a dominant negative mutation in the STAT3 gene, is the most frequent form of HIES among the STAT3-related HIES and in the general spectrum of HIES. The lung represents one of the main target organs in STAT3 deficiency, and the prognosis is strongly dependent on the degree of lung disease. Recurrent pyogenic pneumonias, commonly caused by *Staphylococcus aureus, Streptococcus pneumoniae* and *Heamophilus influenzae,* typically manifest in the first few years of life. Most patients have at least one episode of pneumonia, with over 50% having three or more episodes before receiving a diagnosis (51). The systemic signs of infection are often attenuated, leading to delayed diagnosis of pneumonia. For instance, *S. aureus* lobar pneumonia may present with minimal fever, normal peripheral white blood cell count, and normal inflammatory markers.

Aberrant healing, likely resulting from connective tissue abnormalities and the necessity of normal STAT3 signaling for physiological repair of bronchiolar and alveolar epithelium after damage (52), is frequently observed following pulmonary infections. This predisposes individuals to the development of pneumatoceles and/or bronchiectasis, affecting up to 75% of patients (51) (**Figure 7A, B, C, D**). Once the lung

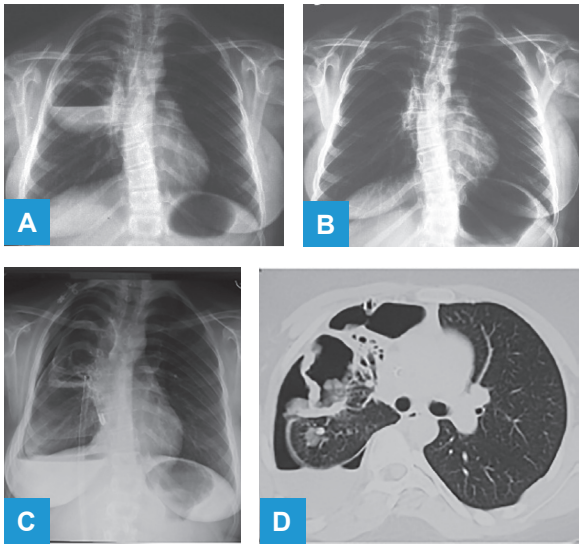

Figure 7. *(**A**) chest X ray in a 12-year-old girl with STAT3 deficiency reveals, in the right lung, a large cavitation (pneumatocele) with air-fluid level, a finding suggestive of an abscess; (**B**) following long-term medical therapy, the air-fluid level resolved; (**C**) one year later, the patient presented with hydropneumothorax, and (**D**) a CT scan showed an aspergilloma inside the pnumatocele. Scoliosis is part of the skeletal and connective tissue abnormalities typical of STAT3 deficiency.*

parenchyma has been altered by pyogenic pneumonias, the spectrum of infecting microbes expands to include non-tuberculous mycobacteria, molds such as *Aspergillus* and *Scedosporium*, and persistent gram-negative bacilli such as *Pseudomonas*. Infections by molds and gram-negative bacilli, often occurring in areas of pre-existing pneumatoceles and bronchiectasis, cause chronic infections which represent the major causes of morbidity and mortality in these patients (53-55).

Treatment of patients with STAT3 deficiency primarily focuses on aggressively treating and preventing bacterial and fungal respiratory tract infections. In some patients, immunoglobulin replacement therapy may also be considered. Due to the absence or limited presence of normal inflammatory signs, even subtle indications of infection must be carefully monitored and investigated, especially when the disease has progressed substantially (42). Antifungals may not always be effective in treating aspergillomas forming inside pneumatoceles (48). Thus, resection is generally accepted as the preferred therapy and offers the possibility of a permanent cure; however, this surgical procedure carries a high rate of complications and recurrence (54, 56, 57). Given that STAT3 expression is ubiquitous, replacing the hematopoietic lineage alone may not be entirely curative. Although HSCT has shown some promising results from an infectious and inflammatory perspective, more studies are needed before a clear pattern can be established (48).

CONCLUSIONS

The lungs are the most affected organ in PID, and associated complications are often the first warning signs of PID. The nature and severity of respiratory symptoms, the specific etiological agents, and the radiological findings play a crucial role in guiding the diagnostic suspicion towards a particular type of immunodeficiency.

Patients with agammaglobulinemia, characterized by a B cell defect, are more susceptible to recurrent pneumonias caused by extracellular pathogens, particularly encapsulated bacteria. This susceptibility can result in the development of structural airway changes, such as bronchiectasis. Similar structural airway changes are observed in patients with CVID presenting only/predominantly with infections. On the contrary, the spectrum of pulmonary complications in CVID patients with symptoms of immune dysregulation (non-infectious complications) is more extensive and encompasses both structural airway disease as well as interstitial or parenchymal lung disease (ILD). Patients with SCID are more prone to respiratory infections by extracellular (encapsulated bacteria) and intracellular pathogens such as viruses and fungi due to the lack of adequate T cell function. In CGD, respiratory infections by catalase-positive microorganisms with suppurative complications are characteristic, whereas the development of pneumatocele is suggestive of hyper-IgE associated with skeletal and connective tissue abnormalities.

Nevertheless, even though the pattern of lung damage and the types of microorganisms involved may provide insights into potential defects in innate, humoral, or cell-mediated immunity, there is a substantial overlap in lung complications among the different types of PID. Consequently, thorough investigations are necessary to establish a definitive diagnosis.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

Financial support

There were no institutional or private fundings for this article.

Author contributions

All the Authors confirmed the contribution to the manuscript's conception and approved its final version.

Ethical approval

Human studies and subjects

N/A.

Animal studies

N/A.

Data sharing and data accessibility

Data are available upon motivated request to the Corresponding Author.

Publication ethics

Plagiarism

All original studies are cited as appropriate.

Data falsification and fabrication

All the data correspond to the real.

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CASE REPORT

An unusual case of juvenile-onset recurrent respiratory papillomatosis with lower airway involvement in a 15-month-old boy

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ABSTRACT

Recurrent respiratory papillomatosis (RRP) is a rare disease caused by human papillomavirus (HPV) infection, especially with types 6 and 11. It is characterised by the presence of multiple airway papillomas located mainly in the larynx; involvement of the distal airways and lungs may also occur. There are two clinical forms of the disease depending on the age of onset, juvenile-onset RRP (JoRRP) and adult-onset RRP (AoRRP). JoRRP is the most common clinical form and usually affects children younger than 5 years of age. It is generally more aggressive with a high recurrence rate and is acquired by vertical transmission during vaginal delivery of infected mothers. There is currently no effective treatment for RRP and surgery remains the main treatment option. However, systemic treatment with bevacizumab, a recombinant humanized monoclonal antibody that binds to vascular endothelial growth factor-A (anti-VEGF-A) and prevents angiogenesis, has been proposed as adjuvant therapy in advanced RRP cases. We present an unusual and aggressive case of JoRRP with distal airway involvement in a 15-month-old boy who showed a complete response to systemic bevacizumab.

IMPACT STATEMENT: Bevacizumab is a promising adjuvant therapy for recurrent respiratory papillomatosis, particularly in cases involving the distal airways that are difficult to treat with standard surgical procedures.

INTRODUCTION

Recurrent respiratory papillomatosis (RRP) is a rare disease characterized by the recurrent growth of papillomas in the airways. Papillomas are benign epithelial tumors usually located in the larynx. However, occasionally, they may become more aggressive and spread distally to the lower airways and, rarely, to the lung parenchyma (1). Despite their benign nature, papillomas can grow rapidly and thus pose a potential risk for airway obstruction. In addition, they tend to relapse and show an increased risk of malignant transformation. More than 90% of all RRP cases are caused by infection with human papillomavirus (HPV) types 6 and 11. Other HPV subtypes, such as 16 and 18 have also been detected, but are much less common (2). Based on their association with malignant transformation HPV subtypes can be divided into low- and high-risk. In particular, HPV

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10.56164/PediatrRespirJ.2023.40

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ABBREVIATIONS

AoRRP: Adult-onset RRP
HPV: Human Papillomavirus
JoRRP: Juvenile-onset RRP
RRP: Recurrent Respiratory Papillomatosis

KEY WORDS

Recurrent respiratory papillomatosis; human papilloma virus; bevacizumab; adjuvant therapy; papillomas.

types 6 and 11 are considered low-risk, while types 16 and 18 are considered high-risk (3).

RRP has a bimodal age distribution with young children and young adults being most affected. There are two clinical forms of the disease depending on the age of onset, with the age limit usually set at 18 years (4). Juvenile-onset RRP (JoRRP) is the most common clinical form with an estimated incidence of 4.3 per 100,000 (5). However, recent data show a significant reduction after the implementation of HPV vaccination (6). In children with JoRRP, HPV infection is usually acquired by vertical transmission during vaginal delivery of infected mothers. It occurs most often in children under 5 years of age with the mean age of onset ranging from 2.8 to 4.6 years in different studies (7). Adult-onset RRP (AoRRP), on the other hand, has an incidence of 1.8 per 100,000 (5). In this case, HPV infection is sexually transmitted and is therefore more common in young adults between 20-40 years of age (8).

The clinical presentation of RRP is variable and depends on the location and size of papillomas. HPV infection usually affects the larynx and thus progressive hoarseness of voice and stridor are the most common manifestations. Patients with RRP may less frequently present with dysphagia, chronic cough, recurrent pneumonia, or respiratory distress (9). The differential diagnosis of JoRRP includes acute laryngitis, congenital airway abnormalities (*e.g.*, laryngomalacia, laryngeal cysts, *etc.*), vocal cord paralysis, airway hemangiomas, vascular malformations, and asthma in case of lower airway involvement (10, 11). The clinical course of the disease is unpredictable. Some patients develop an aggressive disease that requires frequent surgical interventions to maintain airway patency, while others achieve progressive and spontaneous remission (2). JoRRP is generally considered more aggressive than AoRRP and has a high recurrence rate (12).

Management of patients with RRP is difficult and there is currently no effective treatment. Surgical excision of the papillomas with lasers or microdebriders remains the mainstay of treatment. Estimated lifetime surgical procedures at JoRRP range from 6 to 13 (7, 13). The aim is to relieve symptoms and prevent airway obstruction. However, approximately 20% of patients require some type of adjuvant therapy due to the aggressive nature of the disease and its tendency to relapse (14). Adjuvant therapy is considered in cases with a frequent need for surgery (more than 4 to 6 per year) or in cases where papillomas are spread beyond the larynx (15). It includes interferon, antiviral agents (*e.g.*, cidofovir), inhibitors of cyclooxygenase-2 (*e.g.*, celecoxib), monoclonal antibodies (*e.g.*, bevacizumab, pembrolizumab), and HPV vaccine.

Herein we describe an aggressive case of a 15-month-old boy with primary diffuse papillomatosis of the trachea and main bronchi who showed a complete response to systemic treatment with bevacizumab.

CASE REPORT

This is the case of a boy who is now 4 years old and was diagnosed with JoRRP at the age of 15 months. He is the only child in the family and was born at term by cesarean section for non-medical reasons. The perinatal history was unremarkable. His mother had no evidence of HPV infection and had a recent PAP test that was negative. He was breastfed until 12 months of age and his growth was normal. He had no previous medical history and was fully vaccinated. He first presented to our department at 15 months of age with recurrent and progressively worsening episodes of inspiratory stridor over two months. The patient had been treated by his primary care physician with repeated courses of oral dexamethasone but had shown only a partial and transient response. Due to the aggressive and unusual presentation of the disease, the patient was also evaluated for cellular immune deficiency, but no abnormal findings were detected. Therefore, he was referred to a pediatric pulmonology clinic.

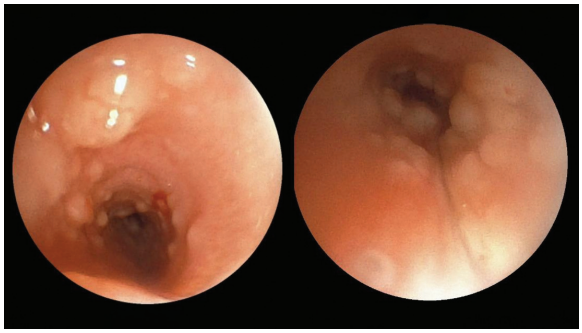

***Figure 1**. Diffuse papillomatosis of the trachea causing significant obstruction.*

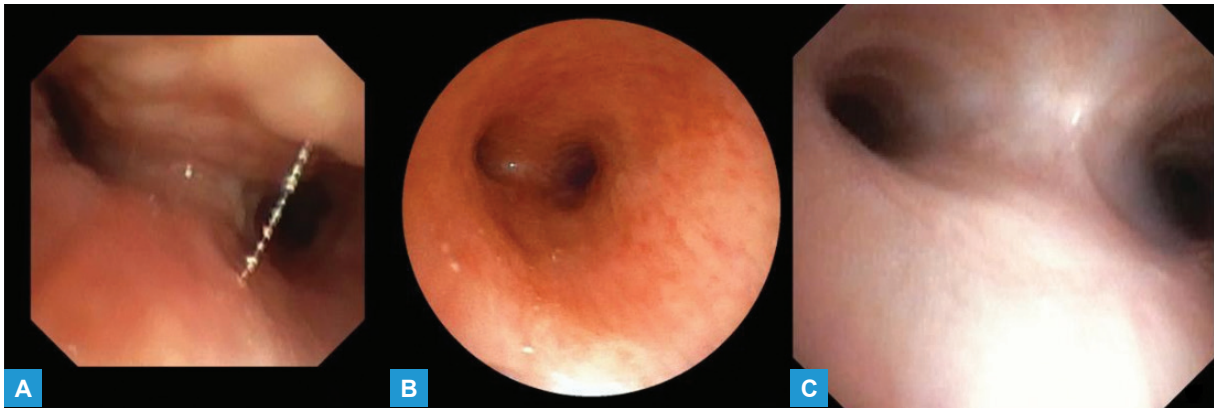

***Figure 2**. (**A**) Diffuse papillomatous lesions in the trachea and main bronchi. (**B**) dramatic regression of papillomas after administration of the third dose of bevacizumab; (**C**) complete response to bevacizumab.*

At the time of referral, the patient was afebrile. He had inspiratory stridor and signs of moderate respiratory distress with normal oxygen saturation levels. Lung auscultation was normal. No choking episode or ingestion of a foreign body was reported by his parents. Blood tests including blood cell count, biochemistry and C-reactive protein were unremarkable. Chest x-ray was also normal. He underwent flexible bronchoscopy, which revealed multiple papillomatous lesions in the trachea and the main bronchi (**Figures 1, 2A)**. These lesions caused significant obstruction, particularly in the middle third of the trachea. No lesions were found in the larynx **(Figure 3)**. Endobronchial biopsies were obtained for histopathological analyses. During flexible bronchoscopy, the patient developed persistent oxygen desaturation and was intubated. Therefore, he was admitted to the intensive care unit for a few days. Histopathological analyses confirmed the diagnosis of benign squamous cell papillomas, and polymerase chain reaction detected HPV type 16. Chest computed tomography (CT) showed no spread of HPV infection to the lung parenchyma.

Surgical excision of the papillomas was not feasible due to multiple lesions and involvement of the distal airways. Adjuvant therapy was necessary and, after discussion with the parents, systemic treatment with bevacizumab, an anti-vascular endothelial growth factor-A (anti-VEGF-A) was initiated. Specifically, he was started on intravenous bevacizumab at a dose of 10 mg/kg every 2 weeks. In addition, he received the 9-valent HPV vaccine (Gardasil 9). A significant clinical improvement was observed immediately after the first dose of bevacizumab. Flexible bronchoscopy was repeated after the administration of the third dose and showed dramatic regression of the papillomas **(Figure 2B)**. After 6 doses, the treatment interval was gradually extended to every 2 months for almost a year. During this period, the patient was closely monitored for relapse symptoms and side effects associated with bevacizumab such as hypertension, epistaxis, and proteinuria. No relapse symptoms or side effects were observed. Follow-up bronchoscopies showed no recurrence of papillomas and HPV DNA was no longer detected in endobronchial biopsies **(Figure 2C)**.

The patient is now 51 months old and has been treated with bevacizumab for 3 years. He has been receiving bevacizumab at 6-month intervals for the past 18 months and remains free of symptoms.

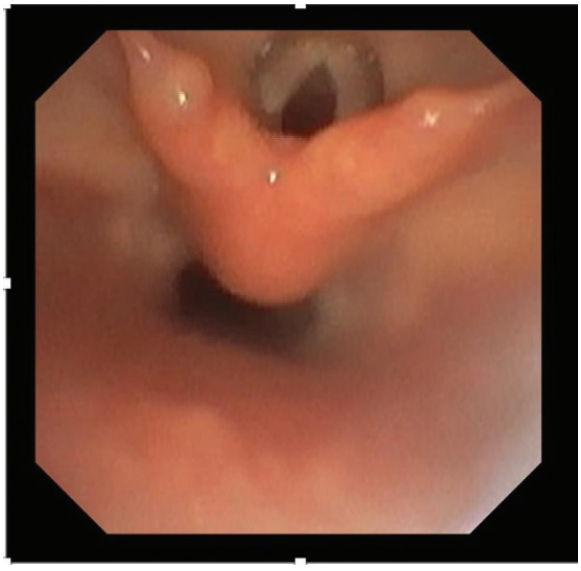

***Figure 3**. No papillomatous lesions were observed in the larynx.*

DISCUSSION

RRP is a relatively rare disease characterized by the development of papillomas in the respiratory tract. It affects both children and adults and is caused by a local infection with HPV. Although there are more than 200 subtypes, HPV types 6 and 11 account for more than 90% of all RRP cases. Other HPV subtypes, such as 16, 18, 31 and 33, can also be detected but at a lower rate (2). There are two clinical forms of the disease depending on the age of onset. JoRRP is the most common and usually occurs before the age of 5 years (8). In this clinical form, HPV infection is usually transmitted at birth, during passage through the birth canals of infected mothers. However, in about 12% of cases, vertical transmission can occur before birth through the placenta (2). Therefore, cesarean section does not eliminate the possibility of vertical transmission of HPV infection to the newborn. Firstborns and children of young mothers are most likely to be infected (7, 16), possibly due to a longer delivery time which implies a prolonged time of exposure to the virus (17). It has also been suggested that newly acquired HPV infections are more likely to be transmitted than chronic infections (18). Notably, our patient was born by cesarean section and although there was no evidence of maternal infection, HPV was probably transmitted through the placenta during pregnancy.

Clinical presentation of the disease is variable and depends on the location and size of papillomas. The larynx, and particularly the vocal cords and surrounding tissues, are the most common sites of infection. Therefore, progressive hoarseness of voice and stridor are the main presenting symptoms. Severe respiratory distress due to airway obstruction may also occur (1). It is estimated that approximately 14% of patients require a tracheostomy to prevent life-threatening airway obstruction (5). Due to its non-specific clinical presentation, JoRRP must be differentiated from other clinical entities such as acute laryngitis, vocal cord paralysis, subglottic stenosis, laryngeal cysts, vascular malformations, laryngomalacia, tracheomalacia, and airway hemangiomas (10, 11). More rarely, papillomas can spread distally to the lower airways and lung parenchyma. In these cases, JoRRP may present with dyspnea, wheezing, chronic cough and recurrent pneumonia, and thus asthma and chronic bronchitis must be excluded (2, 10). Distal airway involvement occurs in 2-5% of patients with laryngeal papillomatosis, while the pulmonary parenchyma is affected in only 1% of cases (1). Involvement of the distal airways in the absence of papillomatous lesions in the larynx, as in the case of our patient, is extremely rare and only a few case reports have been described (19, 20).

Laryngoscopy and/or flexible bronchoscopy are the most reliable methods for the diagnosis of RRP. Endoscopy enables direct visualization of the central airways and collection of biopsy specimens for histopathological analysis. Papillomas usually appear as single or multiple exophytic, pedunculated nodules. Histopathological confirmation and detection of HPV DNA are essential for the diagnosis of RRP (1). The role of chest computed tomography (CT) is complementary and should be considered in patients with a clinical presentation suggestive of pulmonary involvement (21). The typical CT pattern of pulmonary papillomatosis includes multiple multilobular nodular lesions of various sizes, which are often cavitated and distributed throughout the lungs (1). Other less common findings include consolidation, atelectasis, bronchiectasis, air trapping, and pleural effusion (22). The clinical course of the disease is unpredictable. Some patients develop an aggressive disease with distal spread and high recurrence rate, while others achieve progressive and spontaneous remission (2). The severity and aggressiveness of the disease is determined by the number of annual and/or lifetime surgical procedures, distal spread, or a combination of the three (7). Available data suggest that age of onset and HPV subtype may influence the clinical course and severity of the disease. Specifically, age at diagnosis younger than 5 years and infection with HPV type 11 have been associated with more aggressive disease and extralaryngeal spread (7, 23, 24).

Despite the benign nature of papillomas, malignant transformation may occur in 3-5% of RRP cases (25). It mainly affects adults with additional risk factors, such as smoke and radiation exposure, but also children with persistent, advanced disease with distal spread (26). HPV subtype also plays an important role. Infections with HPV types 16 and 18 are considered high-risk and are associated with the potential for malignant

transformation, particularly in squamous cell carcinoma. Within the low-risk types, HPV type 11 has a higher malignant potential compared to HPV type 6 (2). Our patient was diagnosed at a very young age with an unusual and aggressive clinical presentation. Moreover, endobronchial biopsies detected HPV type 16. Therefore, he is a high-risk patient for both papilloma recurrence and malignant transformation.

Long-term management of the disease is challenging due to the frequent relapse of papillomas. Persistence of the viral genome in residual tissue is thought to be the main cause for such relapse (27). Current standard treatment of RRP involves repeated local surgical interventions with lasers or microdebriders, which are associated with a significant risk of complications and chronic morbidity. Therefore, a number of adjuvant therapies have been proposed to enhance surgical outcomes by increasing intervals between procedures or preventing the recurrence of papillomas. It is estimated that adjuvant therapy is required in 20% of patients and is considered in cases with frequent need for surgery (more than 4 to 6 per year) or in cases with lower airway involvement (15). The majority of adjuvant therapies act through immunomodulation, inhibition of HPV replication, control of inflammation, and prevention of angiogenesis. Interferon is one of the first systemic adjuvant therapies used for the management of RRP. Despite some positive evidence, its efficacy remains controversial and is now rarely used due to frequent side effects and the emergence of other adjuvant therapies (14). Such therapies include antiviral agents (*e.g.*, cidofovir), anti-inflammatory drugs such as inhibitors of cyclooxygenase-2 (*e.g.*, celecoxib) and monoclonal antibodies (*e.g.*, bevacizumab, pembrolizumab) (14). These drugs can be administered either systematically or intralesionally. HPV vaccine has also been proposed as adjuvant therapy. There is evidence that HPV vaccination increases the interval between papilloma recurrences and, consequently, the mean duration between surgeries (28). Unfortunately, the majority of these treatments have only been evaluated in small cohorts or case studies, and therefore, more powerful randomized controlled trials are needed to adequately assess their efficacy in the management of RRP.

Our patient presented with primary diffuse papillomatosis of the trachea and main bronchi and, thus, surgical treatment was not appropriate. Systemic adjuvant therapy was required as initial treatment and, after discussion with the parents, intravenous bevacizumab was initiated. The choice of bevacizumab was based on its better safety and efficacy profile compared to other available systemic therapies (14). Bevacizumab is a humanized monoclonal antibody that binds to circulating VEGF-A and prevents receptor activation and subsequent angiogenesis. *In vitro* studies have shown significant expression of VEGF-A in papilloma epithelium and expression of the messenger RNAs of vascular endothelial growth factor receptor 1 and 2 (VEGFR-1 and VEGFR-2) in underlying vascular endothelial cells, suggesting that VEGF activity plays a role in papillomas formation (29). Bevacizumab can be administered either intralesionally or systematically. Recently, a systematic review based on case reports, concluded that systemic bevacizumab is well tolerated and effective in reducing airway and lung lesions and should therefore be considered as adjuvant therapy for severe JoRRP (30). However, clinical trials are lacking and it remains an off-label indication. Based on the available data, the use of systemic bevacizumab is now recommended in cases of progressive and/or severe disease burden and in cases with disease in sites that are difficult to treat with standard surgical procedures (31). To date there is no standard protocol for the dosage regimen and duration of treatment. Bevacizumab is usually administered at a dose of 5-10 mg/kg intravenously at initial mean intervals of 3 weeks (range 2-5 weeks) until maximum response to treatment is achieved. Subsequently, the intervals of maintenance doses are gradually extended to 2-4 months (30). Current data indicate that treatment with systemic bevacizumab is well tolerated in children. Some side effects such as hypertension, proteinuria, epistaxis, joint pain and fatigue have been reported but are mild and usually reversible after discontinuation of treatment (32, 33). A major drawback is that long-term treatment is required for sustained improvement. Indeed, long-term follow-up of patients who received systemic bevacizumab showed that papillomas recurred on average 5.4 months after discontinuation of treatment (34). Our patient showed a complete response to systemic bevacizumab. He has been treated for 3 years and no relapse symptoms or side effects have been reported.

CONCLUSIONS

RRP is a rare disease of the respiratory tract caused by HPV infection. The clinical presentation and course of the disease is variable and depends on several factors including age of onset, HPV subtype and site of infection. Although rare, JoRRP may initially present with multifocal lesions in the distal airways, even in the absence of laryngeal involvement. These cases are difficult to treat with standard surgical procedures and adjuvant therapy is required. Bevacizumab is a promising adjuvant therapy, however, further research is needed to better define its role in the treatment of advanced RRP.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

Financial support

There were no institutional or private fundings for this article.

Author contributions

DM collected the data and wrote the manuscript. KD and KNP supervised the management of the case, carefully reviewed and co-authored the manuscript. SK and VP participated actively to the management of the case. PF performed histopathological analyses. All Authors have read and agreed to the published version of the manuscript.

Ethical approval

Human studies and subjects

The Authors confirm that the patient's parents have given their consent for the anonymous publication of the clinical information.

Animal studies

N/A.

Data sharing and data accessibility

Data are available upon motivated request to the Corresponding Author.

Publication ethics

Plagiarism

The contents of the article are original and any overlaps with other articles are by the Authors themselves and appropriately cited.

Data falsification and fabrication

All the data correspond to the real.

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PEDIATR RESPIR J Vol. 2(2), 90-95, 2024

CASE REPORT

Congenital bronchial stenosis: a case report of an unusual diagnosis and its management

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ABSTRACT

Congenital bronchial stenosis is a rare bronchopulmonary anomaly characterized by significantly narrowing or blindly ending of a segmental or lobar bronchus, typically limited to a few rings, caused by developmental anomalies in the cartilaginous exoskeleton.

We describe a case of a six-month-old male infant with intermittent wheezing present since birth, with no signs of respiratory distress, which seemed to not respond to bronchodilators and to appear more evident with crying and during common colds.

He didn't present respiratory distress, but an expiratory wheezing was heard. A tidal flow-volume loop was recorded and appeared slightly deflected during the expiratory phase, with a normal tidal volume. A bronchoscopy detected a stenosis of the left main bronchus, confirmed by the chest computed tomography (CT). The patient underwent surgical treatment, consisting of resection of the stenotic tract and tracheobronchial anastomosis with a good result.

Congenital bronchial stenosis should be considered in newborns and infants with persistent wheezing that doesn't respond to bronchodilators; a tidal flow-volume loop could suggest the diagnosis, which should be confirmed with chest CT and bronchoscopy.

IMPACT STATEMENT: A case report of congenital bronchial stenosis, with the aim to review its diagnosis and management.

INTRODUCTION

The occurrence of wheezing in infants can be referred to a broad list of differential diagnoses, that include airways or vascular anomalies, cardiac, metabolic, and infectious diseases. A rare cause of persistent wheezing in infants could be congenital bronchial stenosis (CBS). CBS is a bronchial anomaly characterized by a significant narrowing of a segmental or lobar bronchus, usually limited to a few cartilaginous rings, caused by developmental anomalies in the cartilaginous exoskeleton (1) or by compressive vascular anomaly, cardiac anomaly, or congenital pulmonary cyst (2).

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10.56164/PediatrRespirJ.2023.36

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ABBREVIATIONS

CBS: Congenital Bronchial Stenosis
CTS: Congenital Tracheal Stenosis
CT: Computed Tomography

KEY WORDS

Congenital bronchial stenosis; tidal breathing flow volume loop; bronchoplasty; infant; case report.

This report describes the case of a six-month-old boy who presented intermittent wheezing, and who was found to have congenital bronchial stenosis which was surgically corrected.

CASE REPORT

A six-month-old infant was referred to the pediatric respiratory outpatient clinic with a history of expiratory wheezing since birth, with no signs of respiratory distress, which seemed to not respond to bronchodilators and to appear more evident with crying and during common colds.

He was delivered at term; he had clinodactyly of the second finger of the left hand, waiting for surgical correction. His developmental history and his growth were on average; he was bottle-fed with no signs suggestive of aspiration. His family history was unremarkable.

At the examination, he was afebrile, his height was 65 cm (-1 Standard Deviation (SD)), and his weight was 7.4 kg (-1 SD).

Oxygen saturation was 97% in room air. No signs of respiratory distress were present. At chest auscultation, bilateral wheezing was heard. The remaining physical examination was unremarkable, apart from mild pectus excavatum. A tidal flow-volume loop while asleep was recorded (**Figure 1**), which appeared slightly deflected during the expiratory phase with a normal tidal volume (8 mL/kg).

The patient was electively admitted to the ward. A chest x-ray resulted normal; flexible bronchoscopy demonstrated severe stenosis of the left main bronchus, which was later confirmed by CT scan, showing a stenosis of 1.4 mm for a length of 2.2 mm with initial hyperinflation of the left lung. To avoid associated malformations, we performed the echocardiography and

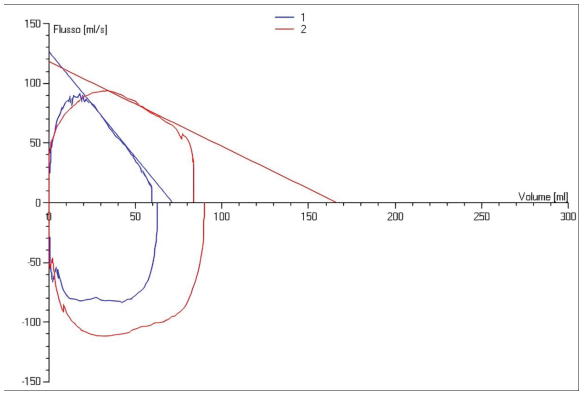

Figure 1. *Tidal flow-volume loop recorded while asleep with slight deflection during the expiratory phase with a normal tidal volume (8 mL/kg); tidal flow-volume loop recorded while asleep after surgery showed a normal morphology with a normal tidal volume (9.8 mL/kg).*

the ultrasound of the abdomen, which were normal. At 8 months of age, the patient was admitted to another tertiary hospital. At admission, sleep studies were performed showing no desaturations or apnea. After a multidisciplinary discussion, a CT scan with contrast medium under general anesthesia was repeated allowing to exclude any vascular malformation that could have determined the stenosis; the 3D volume rendering reconstruction and the virtual bronchoscopy reconstruction (**Figure 2A, B, C**) showed an expiratory collapse of the bronchial lumen, suggestive of associated bronchomalacia.

Rigid bronchoscopy was repeated (**Figure 3A**) and a 2.8 mm flexible endoscope was able to overcome the stenosis and explore the bronchial system beyond the stenosis, which appeared normal.

The case was discussed by a multidisciplinary board with the decision to proceed surgically.

After midline sternotomy, the patient was placed on normothermic cardiopulmonary bypass with the

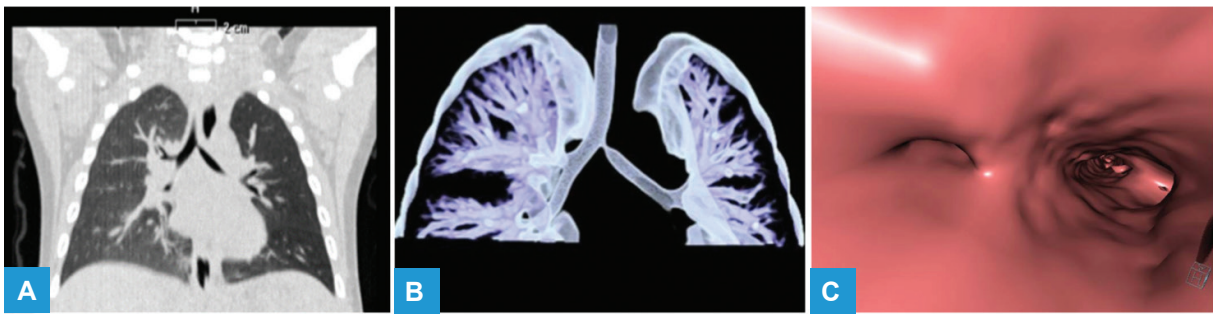

Figure 2. *(**A**) Coronal non-enhanced CT image with lung window showing stenosis of the proximal left main bronchus and hyperinflation of the right lung; (**B**) 3D volume rendering reconstruction of the tracheobronchial tree; (**C**) virtual bronchoscopy reconstruction demonstrating marked narrowing of the left main bronchus lumen.*

beating heart by right atrial and ascending aortic cannulation. The carina and left mainstem bronchus were exposed by dissecting the transverse sinus of the pericardium and removing the subcarinal lymph nodes. Hence a left bronchial transection distal to the site of the stenosis and an oblique section at the origin of the left main bronchus extended to the carina and distal tracheal portion were made. Finally, a terminal-lateral tracheobronchial anastomosis was performed using a running resorbable 6-0 polydioxanone (PDS) suture. An intraoperative fiberoptic control confirmed a satisfactory anastomosis with a wide and patent bronchus (**Figure 3B**), and the cardiac procedure of disconnection from bypass was undertaken. Mediastinal and right pleural drainages were inserted. The patient was extubated after the procedure and remained stable in high-flow-nasal-cannula. On postoperative day 2, he was started on empirical antibiotic therapy with ceftazidime for a right lower lobe pneumonia with effusion. He was discharged on postoperative day 8 with the resolution of the effusion. Audiological assessment and eye examination resulted normal.

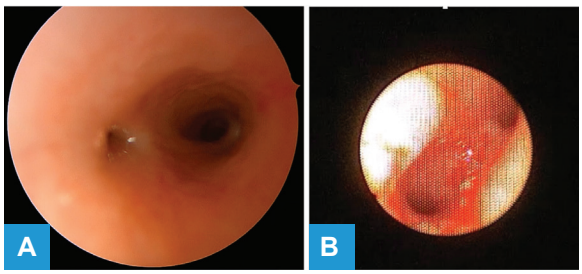

Figure 3. *(**A**) Rigid bronchoscopy showing a significant stenosis of the left main bronchus; (**B**) intraoperative fiberoptic control showing a wide and patent left main bronchus.*

Two months after surgery, he presented intermittent noisy breathing and inconstant inspiratory stridor, with no signs of respiratory distress or infections. A bronchoscopy under general anesthesia was then performed, revealing mild laryngomalacia and raising suspicion of partial left vocal cord paralysis.
Moreover, a tidal flow-volume-loop was recorded, showing no alterations with a normal tidal volume (**Figure 1**).
The patient is undergoing respiratory follow-up with tidal flow-volume loops, and a chest CT will be repeated one year after surgery.

DISCUSSION

Bronchial stenosis could be acquired or congenital. Acquired stenosis in children is usually caused by infection, traumatic granulomas due to chronic intubation, lymph node enlargement, atelectasis (3), tracheobronchomalacia and excessive dynamic airway collapse (4). In particular, in a retrospective study (5) the most common causes of endobronchial obstructions detected in 256 pediatric patients were aspirated foreign bodies (35.9%), endobronchial tuberculosis (31.6%), mucous plugs (16.7%) and granulation scars (6%) and other rare pathologies found were hydatid cysts, hemangiomas, tumors, submucosal nodules, and polyps.
CBS is rare, it can be occasionally diagnosed prenatally (6), but usually it becomes more apparent in the neonatal period (7).
CBS usually involves the left main bronchus (2). Other structural anomalies may also be found including subglottic stenosis, tracheoesophageal fistula, and esophageal atresia (1).
Symptoms can vary, based on the location of the stenosis and the residual lumen (2). In fact, CBS determines a “ball-valve” mechanism with ipsilateral air trapping and lung distention (7) leading to diminished unilateral breath sounds at the physical examination; inspiratory and expiratory stridor can also be present, due to fixed airway obstruction (7) as observed in our patient, which not respond to beta2agonists (7). Other clinical signs include “barking” or brassy cough, “washing machine” airway sounds, cyanosis, and breathing spells (2). In some cases, especially when tracheal stenosis is associated, CBS could present at birth with significant respiratory distress, needing non-invasive or invasive respiratory support (7). Sometimes, patients could present only subtle symptoms of airflow limitation as wheezing and increased work of breathing, especially near the end of the first year of life when physical activity increases, until late childhood or early adolescence, when they tend to develop exercise-associated respiratory difficulties and, consequently, a CBS could be found as an incidental finding during the diagnostic work-up (8).
There is no consensus on the diagnostic approach. Since the same symptoms could be a sign of congenital tracheal stenosis (CTS), it is more cautious to perform a rigid bronchoscopy under general anesthesia

(8) with extreme care, since a mucosal lesion could precipitate edema, leading to a critical obstruction. Even though the stenosis could be detected by a CT scan, the diagnosis is made usually by bronchoscopy, because it provides dynamic information about malacia (1), along with the location, the extension, and the severity (2) of the stenosis.

Other approaches include the virtual endoscopy, which evaluates distal airway anatomy using high-resolution multirow detector computed tomography (CT) scanning to obtain high-resolution 3-dimensional endoluminal images to the level of the segmental bronchi (5) distal to the stenosis which are impassable for flexible bronchoscopy. Virtual endoscopy has the advantage that it can be performed without general anesthesia, and there is no direct invasion of the airways (9). Moreover, it could be used as a complementary technique for the planning of the flexible bronchoscopy, in patients who could not tolerate the bronchoscopy, and for the follow-up of the stenosis (10).

In a retrospective study (11) patients with CBS were found to have similar high comorbidity of cardiovascular anomalies as children with CTS (55.6%), such as pulmonary artery sling, ventricular septum defect, atrial septum defect, patent *ductus arteriosus*. In view of the high proportion of patients with other congenital vascular and cardiac anomalies, echocardiography should be performed to rule out any cardiac defect, along with a contrast CT scan with 3D reconstruction, that will better depict the anatomy of the airways, the parenchyma, and the vessels (2), allowing to characterize any vascular rings as in the case presented. It should be noted that CT frequently underestimates the degree and length of airway narrowing (8). Magnetic resonance imaging enables the assessment of the vascular structures (2), and it has been found to be equally sensitive to CT to detect congenital cardiovascular malformations associated with airway pathologies without any radiation exposure (12).

Furthermore, 3-dimensional printing in case of complex airway anomalies could help in the planning of the surgical approach and educate care providers and family members (13).

Additionally, our patient had *pectus excavatum*. The association between chest deformities and bronchial stenosis is described in the literature (14-16). It has been suggested that the costosternal retraction can be the consequence of the increased intrathoracic pressure during expiration to overcome the airway obstruction due to the CBS (14) and usually the deeper impression corresponds to the side of the CBS. Accordingly to this hypothesis, the *pectus excavatum* of the patient nearly resolved at the 6-month after-surgery follow-up evaluation. However, it is questionable whether *pectus excavatum* is solely due to CBS. CBS, in fact, can be also caused by an abnormal thoracic configuration (17) due to complex mechanisms of vascular compression (15). Based on the clinical presentation, it could be useful to perform a full genotyping as suggested by some Authors (1), along with further diagnostic work-up, especially in the presence of features suggesting skeletal dysplasia (18).

CBS should be managed at major tertiary centers with experience in complex airway malformation, involving a multidisciplinary team including cardiothoracic surgeons, otolaryngologists, cardiologists, pulmonologists, and anesthesiologists (8).

The surgical approaches vary in the literature and are better characterized for CTS rather than CBS. However, they should be tailored to the patient (19) and the presence of other anatomical abnormalities. Surgery includes resection and reconstruction, and bronchoplastic techniques (20, 21); nonetheless, both are complex, especially in infants because of the smaller size of the airways (2). The post-operative bronchoscopy could show granulation tissue, mild restenosis, or formal scarring that could be treated with balloon dilatation (1); in these cases, it would be desirable to avoid laser techniques that could induce thermal damage to the tissue with the consequent risk of restenosis (1). Another postoperative complication is bronchial malacia, which could be addressed with a stent placement (22).

Ultimately, given the lack of international consensus on CBS diagnosis and management, raising awareness of this condition is important to allow a prompt diagnosis.

KEY TAKEAWAYS

- CBS is a rare malformation usually diagnosed during the neonatal period, but even in late childhood.

- Presentation varies from unexplained neonatal respiratory distress to a late presentation with inspiratory-expiratory stridor, wheezing, increased work of breathing, noisy breathing, cyanosis, breath-spells, and apnea.
- Usual findings at chest examination are diminished unilateral breath sounds, wheezing or stridor.
- Chest X-rays usually show ipsilateral lung hyperinflation or could result in normal.
- CBS is usually diagnosed with bronchoscopy, which should be performed carefully to avoid any tissue damage.
- Echocardiography and contrast CT are needed to evaluate the presence of congenital cardiovascular malformations such as vascular rings that are usually associated with CBS; 3D CT reconstruction and virtual bronchoscopy are helpful and complementary to bronchoscopy to assess distal airways and for surgical planning.
- Generally, CBS is managed surgically; the approach should be tailored to the patient and discussed within a multidisciplinary team.
- Close follow-up with monitoring for postoperative complications, and parental education, can impact the outcome of the patient.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

Financial support

There were no institutional or private fundings for this article.

Author contributions

LV: writing the original draft; GF, LT, ANC, FP, VB, ADC: writing, review, and editing; ADC: supervision; RC, GP: conceptualization, and supervision.

Ethical approval

Human studies and subjects

The caregivers gave their informed consent to the submission of this manuscript,that followed the ethical standards established in the Declaration of Helsinki.

Data sharing and data accessibility

N/A.

Publication ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data correspond to the real.

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28th National Congress of Italian Pediatric Respiratory Society

Turin, October 10-12 2024

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