

Pseudomonas aeruginosa severe skin infection in a toddler with X-linked agammaglobulinemia due to a novel BTK mutation

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SUMMARY

Agammaglobulinemia is a congenital deficit of humoral immunity characterized by a decreased level or complete absence of immunoglobulins and profound reduction of B-lymphocytes associated with an increased risk of life-threatening bacterial infection. We report a case of invasive *Pseudomonas aeruginosa* severe skin and soft tissue

infection treated with vacuum-assisted closure and antibiotics in a toddler with a previously unreported mutation of the Bruton tyrosin kinase gene.

Keywords: ecthyma gangrenosum, VAC, X-linked agammaglobulinemia, *Pseudomonas aeruginosa*.

INTRODUCTION

Agammaglobulinemia is a congenital deficit of humoral immunity characterized by a decreased level or complete absence of immunoglobulins [Ig] and profound reduction of B-lymphocytes [1]. Patients with Ig deficit syndromes are more likely to develop life-threatening *Pseudomonas aeruginosa* [Pa] infections in concomitance with varicella [1, 2]. We hereby report a case of invasive Pa severe skin and soft tissue infection treated with vacuum assisted closure [VAC] and

antibiotics in toddler, and that lead to a diagnosis of congenital deficit of humoral immunity due to previously unreported mutation of Bruton tyrosin kinase [BTK] gene.

CASE REPORT

A 11-month-old male, weight of 10 Kg, developed high-grade fever (T 40°C), vomiting and earache. He was treated by his paediatrician with cefixime (8 mg/Kg/day) for three days without clinical improvement. Due to deterioration of his clinical condition and the onset of impaired consciousness and severe respiratory distress, the child was brought to the ER of a peripheral hospital where he was intubated and transferred to our Center.

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At arrival in our Intensive Care Unit (ICU), vesicles surrounded by an erythematous halo on the neck, both arms and legs associated with an ecchymosis-like lesion on the upper-part of the right leg were noticed. Laboratory findings showed: white blood cell count (WBC) $720/\text{mm}^3$ (n.v. $5800\text{-}15300\text{ mm}^3$), neutrophils 160 (n.v. $1690\text{-}7940\text{ mm}^3$), lymphocytes $180/\text{mm}^3$ (n.v. $2030\text{-}8330\text{ mm}^3$) platelets $284000/\text{mm}^3$ (n.v. $150000\text{-}450000\text{ mm}^3$), haemoglobin 10.5 g/dL (n.v.: $11.0\text{-}13.0\text{ g/dL}$), IgG $189\text{ /IgM } 23/\text{ IgA } 23\text{ mg/dL}$ (n.v. 450

- $1350/20\text{ - }145/20\text{ - }100\text{ mg/dL}$ respectively), C-reactive protein 29 mg/dL (n.v. $<0.46\text{ mg/dL}$), procalcitonin $90\text{ }\mu\text{g/mL}$ (n.v. $<0.5\text{ ng/mL}$).

Lumbar puncture and multiple blood cultures were performed, while intravenous treatment with meropenem [120 mg/kg/die in three divided doses] plus acyclovir [60 mg/kg/die in three divided doses] was started.

The lumbar puncture showed clear fluid with $530\text{ cells}/\mu\text{L}$ (n.v. $0\text{-}5\text{ cell}/\mu\text{L}$) (mainly polymorphonucleate leukocytes), slightly increased proteins

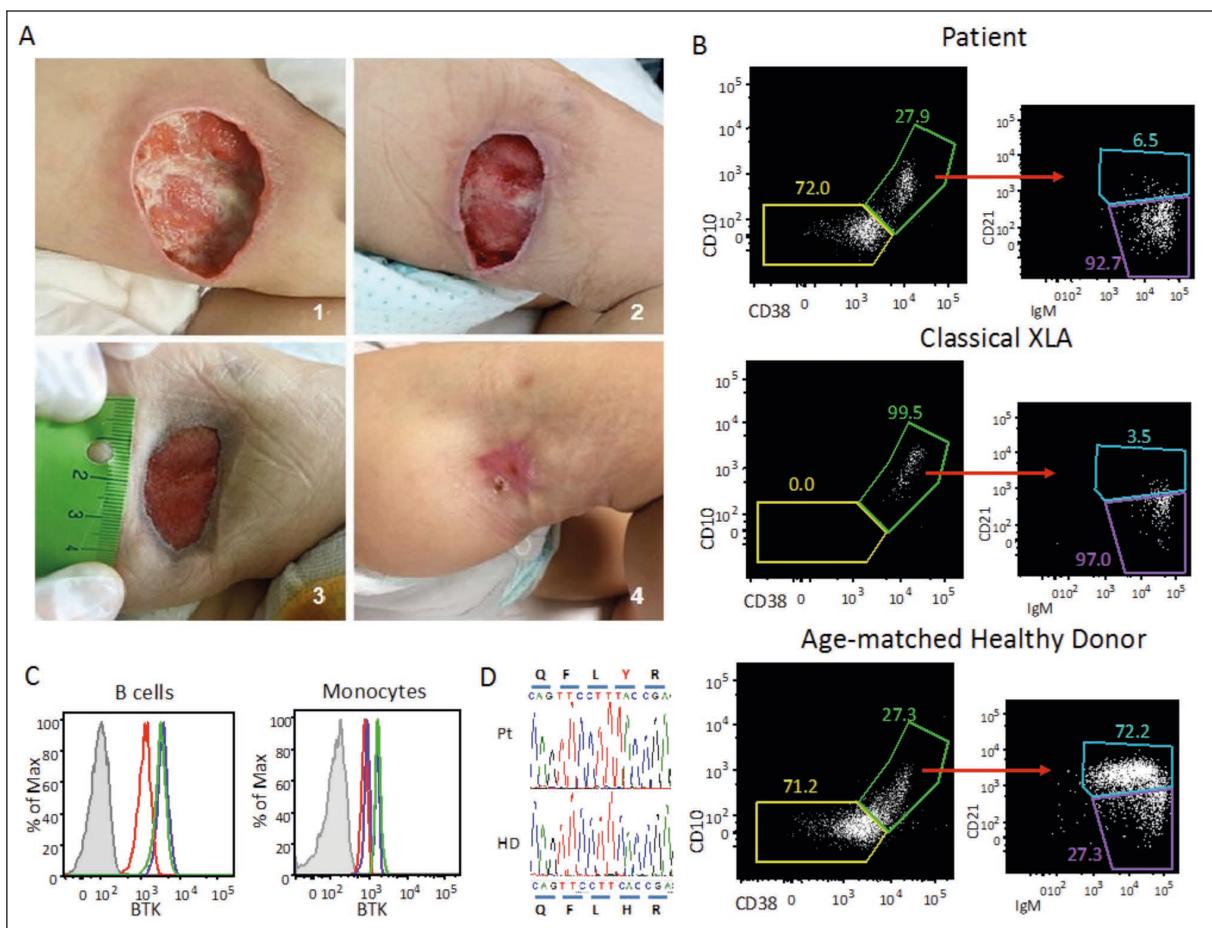


Figure 1 - A) PANEL 1: Major lesion ($8\times 5\text{ cm}$) of the right leg before the beginning of VAC therapy; **PANEL 2:** Reduction in depth of the lesion after 1 week of VAC therapy **PANEL 3:** The same lesion ($3.5\times 1.5\text{ cm}$) after 2 weeks of VAC therapy; **PANEL 4:** Resolution of the lesion after 3 months of VAC therapy. **B) FACS analysis** of peripheral B lymphocyte using the reported membrane markers shows minor abnormalities of B cell maturation in the patient (above) compared to a classical XLA patient (middle) and a age-matched healthy donor (below). B cell subset gating strategy: mature B cells [yellow], recent bone marrow emigrants (green), transitional 1 (purple), transitional 2 (blue). **C) Intracellular FACS analysis** of BTK expression on B cells (left) and monocytes (right): unstained cells (grey), patient (red), carrier mother (purple), normal donor (green). **D) Molecular analysis** of BTK gene revealing the hemizygous c.C1555T mutation.

and normal glucose level. Multiplex polymerase chain reaction (PCR) for bacteria (*Neisseria meningitidis*, *Streptococcus pneumoniae* and *Listeria monocytogenes*), virus (Herpes simplex 1-2, varicella zoster, CMV, EBV, adenovirus, enterovirus) and *Mycobacterium tuberculosis* resulted negative. Brain CT-scan and MRI were both normal.

On day 3, the concomitant presence of both active and healing vesicles confirmed the clinical suspicion of varicella. The major skin lesion in the right leg evolved with ultrasound evidence of soft tissue infiltration and subsequently developed a necrotic, ecthyma gangrenosum-like appearance. Pain at this site was not evaluable because of patient sedation.

Blood, cerebro-spinal fluid and skin lesions cultures yielded a strain of *Pseudomonas aeruginosa*. On the base of antibiotic susceptibility tests that showed full-sensibility of the strain, therapy was changed to piperacillin-tazobactam (MIC <2 mg/L) (administered as piperacillin 400 mg/kg/day) in continuous infusion plus ciprofloxacin (MIC <0.06 mg/L) (40 mg/kg divided in two doses). The patient slowly improved along with negativization of blood cultures, reduction of C-reactive protein and procalcitonin levels.

On day 13, the necrotic lesion of the right leg underwent surgical debridement (Figure 1A, Panel 1) [3].

In order to aid the healing of the lesion, continuous vacuum assisted closure (VAC) therapy was started. Re-evaluation of the VAC therapy was performed every 72 hours with progressive clinical improvement (Figure 1A, Panels 2 and 3).

Because of persistent low Ig levels, a congenital deficit of humoral immunity was suspected. Lymphocyte subpopulations in peripheral blood revealed a severe reduction in B lymphocytes, 1.4% (n.v. 11-45%), with an altered distribution of immature peripheral B cell compartment, represented by a reduced T2 transitional population (CD19+, CD10+, CD38+, IgM+ and CD21+), (Figure 1B). FACS analysis revealed a reduced expression of Bruton tyrosin kinase [BTK] protein in the patient and a double pattern of expression in the mother (as a consequence of random X-chromosome inactivation), Figure 1C. Genetic analysis showed a previously unreported c.1555T p.H519Y mutation in the kinase domain of *BTK* gene (Figure 1D). These results confirmed the diagnosis of X-linked agammaglobulinaemia.

After 55 days of antibiotic treatment and periodic immunoglobulin supplementation the patient was discharged home and continued VAC therapy until the lesion was healed (Figure 1A, Panel 4).

■ DISCUSSION

X-linked agammaglobulinemia is a genetic disorder caused by mutations in the gene for Bruton tyrosine kinase [Btk]. This defect leads to the failure of B cells to develop from pre-B cells. Patients affected are predisposed to encapsulated bacterial infections and their complications such as necrotizing otitis media, pneumonia, severe skin lesions, sepsis and life threatening disseminated infections that need prompt and effective antibiotic treatment. The mutation carried by the patient, p.H519Y, is in a residue that has been shown to be part of a stretch of amino acid residues defined as "regulatory spine", a structure that is assembled only in the active state of kinases and is important for kinase activity, thus strengthening the possible pathogenicity of the variant found. A less severe phenotype of XLA that partially allows B cells to mature beyond the pre-B cell stage, as in this case, have been reported [4]. Finally, despite what appears to be a hypomorphic mutation, the patient presented a typical clinical phenotype with the onset of severe infections before one year of age. Even if not standardized, antibiotic combination therapy against severe Pa infection may be of benefit because of faster bacterial load attenuation as well as less risk of developing resistant subpopulations. The dissemination of the infection to central nervous system, skin and soft tissue led to favor combination therapy in order to maximize tissue penetration and avoid emergence of resistant strain during treatment. However, it remains a controversial choice that must consider the severity of the infection, antimicrobial synergy, PK/PD parameters, drug-drug interactions, hazard of adverse events and antimicrobial stewardship principles.

Large skin lesions with a high risk of infection and significant loss of tissue need secondary wound closure, thus representing a difficult challenge for healthcare providers.

Negative pressure wound therapy (NPWT, also called VAC-therapy) is a well-known strategy to treat complex wounds in Adult Surgical Units but data in the paediatric population are limited [5-7].

NPWT consists in the application of a negative pressure [or vacuum] through a wound dressing. It modifies the wound environment reducing chronic edema, decreasing bacterial populations and enhancing localized blood flow. It has been proposed that VAC-therapy may decrease the production of Pa virulence factors, gene expression and presence of biofilm components [8]. Moreover, the applied forces lead to an increased formation of granulation tissue resulting in a centripetal process of healing. This method, along with antibiotic therapy, allows to minimize pain and risks of further infections, reducing time of hospitalization [9]. In conclusion, we report the efficacy of the combination of antibiotic treatment together with VAC-therapy in a paediatric case of severe Pa infection in a case of XLA due to a novel mutation.

Conflict of interest

The authors declare no conflict of interest

ACKNOWLEDGMENTS

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