(Fig. 1A). Negative controls were treated with saline only. The nasal swabs were conducted 24 h after the last administration of mupirocin or 40-kDa protein of *L. rubellus*. Swabs were then cultured in phenol red broth at 37 °C. The next day, 10 μL of broth at a concentration of 10^{-1} CFU/mL were inoculated on CHROMagar, incubated at 37 °C for 24 h, and then bacterial numbers were counted. No colonization of MRSA was observed in negative controls.

For quantification of sIgA levels, mice were sacrificed. Nasal mucosa was scrapped, diluted in 5 mL PBS containing a protease inhibitor cocktail (25 μg/mL) and centrifuged at 12,000 rpm (4 °C) for 15 min. Supernatants were purified with 40% ammonium sulfate. Suspensions were then diluted in 1 mL PBS and used for ELISA of sIgA.[12] All experiments were done in duplicate, resulting in similar findings. The numbers of bacterial colonization and sIgA levels were analyzed by one-way ANOVA, followed by Tukey's post hoc test using StatPlus. Significant differences were accepted when $p < 0.05$.

We identified eleven conserved protein bands from *L. rubellus* (Fig. 1B). Among them, low molecular protein bands (50, 40, 27, 25, and 22-kDa) displayed high concentration profiles. Mupirocin and a high dose of the 40-kDa protein of *L. rubellus* significantly inhibited MRSA colonization ($p = 0.03$ and $p = 0.04$, respectively) in the nasal mucosa of mice (Fig. 1C), thereby implying that the 40-kDa protein of *L. rubellus* was able to eradicate MRSA colonization as effectively as mupirocin. No significant antibacterial activity was observed from the other protein bands (data not shown). Although we failed to show that neither mupirocin nor the 40-kDa protein of *L. rubellus* were able to stimulate sIgA secretion ($p = 0.35$) (Fig. 1D), both groups tended to have higher levels of sIgA, suggesting that the level of sIgA would initially be beneficial to protect the nasal mucosa from MRSA invasion. Altogether, the 40-kDa protein of *L. rubellus* exhibits potent activity anti-MRSA *in vivo*.

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Conflict of interest

None to declare.

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References

1. El Aila NA, Al Laham NA, Ayesh BM. Nasal carriage of methicillin resistant *Staphylococcus aureus* among health care workers at Al Shifa hospital in Gaza Strip. BMC Infect Dis. 2017;17:28, http://dx.doi.org/10.1186/s12879-016-2139-1.
2. Kruszewska D, Sahl HG, Bierbaum G, Pag U, Hynes SO, Ljungh A. Mersacidin eradicates methicillin-resistant *Staphylococcus aureus* (MRSA) in a mouse rhinitis model. J Antimicrob Chemother. 2004;54:648–53, http://dx.doi.org/10.1093/jac/dkh387.
3. Laupland KB, Conly JM. Treatment of *Staphylococcus aureus* colonization and prophylaxis for infection with topical intranasal mupirocin: an evidence-based review. Clin Infect Dis. 2003;37:933–8, http://dx.doi.org/10.1086/377735.
4. Mustafa Y, Sahin I, Basak S, Oksuz S, Ozaydin C, Acar S, et al. The investigation of nasal MRSA carriage and colonization of nasopharyngeal pathogens at a primary school in Duzce. Turk J Med Sci. 2007;37:359–65.
5. Lassegues M, Milochau A, Doignon F, Du Pasquier L, Valembois P. Sequence and expression of an *Eisenia fetida*-derived cDNA clone that encodes the 40-kDa fetidin antibacterial protein. Eur J Biochem. 1997;246:756–62, http://dx.doi.org/10.1111/j.1432-1033.1997.00756.x.
6. Kartikaningsih H, Hartati K, Maharani S, Sartika F. Antibacterial activity ethyl acetate extracts of earthworms (*Lumbricus rubellus*, *Eisenia foetida*, *Nereis* spp.) toward *Staphylococcus aureus*, *Enterococcus faecalis Salmonella thyposa* in vitro. El-Hayah. 2019;7:2, http://dx.doi.org/10.18860/elha.v7i2.8248.
7. Ulhaq ZS, Kishida M. Brain aromatase modulates serotonergic neuron by regulating serotonin levels in zebrafish embryos and larvae. Front Endocrinol (Lausanne). 2018;9:230, http://dx.doi.org/10.3389/fendo.2018.00230.
8. Ulhaq ZS. Brain aromatase modulates cardiac functions in embryonic zebrafish. Int J Vet Sci Med. 2019;7:31–4, http://dx.doi.org/10.1080/23144599.2019.1675287.
9. Ulhaq ZS. Dopamine D2 receptor influences eye development and function in zebrafish. Arch Soc Esp Oftalmol. 2020;95:84–9, http://dx.doi.org/10.1016/j.oftal.2019.11.013.
10. Ulhaq ZS, Hendyatama TH, Hameed F, Santosaningsih D. Antibacterial activity of *Citrus hystrix* toward *Salmonella* spp. infection. Enferm Infecc Microbiol Clin. 2020, http://dx.doi.org/10.1016/j.eimc.2020.05.016. S0213-005X(20)30215-9.
11. Jervis-Bardy J, Boase S, Psaltis A, Foreman A, Wormald PJ. A randomized trial of mupirocin sinonasal rinses versus saline in surgically recalcitrant staphylococcal chronic rhinosinusitis. Laryngoscope. 2012;122:2148–53, http://dx.doi.org/10.1002/lary.23486.
12. Wang M, Gong S, Du S, Zhu Y, Rong F, Pan R, et al. The effect of immunoregulation of *Streptococcus lactis* L16 strain upon *Staphylococcus aureus* infection. BMC Microbiol. 2017;17:130, http://dx.doi.org/10.1186/s12866-017-1038-y.

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Pneumococcal osteomyelitis of the rib in a vaccinated infant: An exceptional case☆

Osteomielitis costal por neumococo en lactante vacunado, un caso excepcional

Osteomyelitis mainly affects the long bones of the lower limbs. Rib osteomyelitis is rare (<1%). In the ribs *Staphylococcus aureus* is the cause of the majority of cases, followed by *Mycobacterium tuberculosis*.[1] There are three cases in the literature caused by microorganisms of the genus Streptococcus, and only one by *Streptococcus pneumoniae*.[2]

This was a six-month-old infant who came to the emergency department with a swelling in his left rib cage, with no history of trauma, with a low-grade fever of 37.5 °C 48 h previously, although apyrexial at the time of the hospital visit. No relevant family or personal history. No allergies. Vaccination schedule up to date, including three doses of 13 V pneumococcal vaccine (first dose batch G64205, second G75546), the third dose of pneumococcal vaccine had been administered ten days before (batch G92049). No previous infectious symptoms except for an isolated fever peak of 38.6 °C without other symptoms 24 h after the pneumococcal vac-

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cine. The swelling was located in the 6th left costal arch, and was hard and painful on palpation.

Blood showed only leucocytosis of 20,600 mm^{-3} (40% neutrophils) and CRP 15 mg/l. Admitted for further tests.

Chest X-ray showed a bone lesion with insufflation in the anterior arch of the left 6th rib; rib ultrasound showed insufflation of the 6th rib with interruption of the cortex and hypoechoic content; and bone series had similar findings without additional abnormalities. With suspicion centring on a rib tumour or osteomyelitis, antibiotic treatment was started with cloxacillin 150 mg/kg/day and cefotaxime 200 mg/kg/day.

CT of chest with contrast was performed due to its better visualisation of bone, and its availability, despite the superiority of magnetic resonance imaging in this disease, and an expansive lytic lesion was identified in the anterior 1/3 of the left 6th rib, with destruction of the cortex, compatible with abscessed osteomyelitis. A cancerous tumour was ruled out by Tc-99 scintigraphy. A fine-needle biopsy was taken of the swelling, isolating sensitive *Streptococcus pneumoniae*. Intravenous cefotaxime was continued for eight days, with amoxicillin 100 mg/kg/day at discharge for four more weeks, leading to complete resolution.

Pneumococcal serotyping was performed, finding serotype 1 (vaccine). An immunological study was therefore carried out: immunoglobulins, C3, C4, CH50, AP50, lymphocyte populations and a study of the response to mitogen, burst test, IL-6 response after stimulation as a check of normality of the Toll-like receptor pathway; all within normal limits. Peripheral blood smear without Howell-Jolly bodies. Spleen visualised on ultrasound at admission. At two years of age, response to a polysaccharide vaccine was studied, using purified pneumococcus from Merck®, with the titres of specific IgG against pneumococcus increasing from 8.91 mg/dl pre-vaccination to >27 mg/dl post-vaccination, and specific IgG2 from 0.95 mg/dl to >2.4 mg/dl. The child is currently asymptomatic.

The diagnosis was made from both the symptoms and the radiological images, with culture of the purulent discharge being fundamental, and ruling out other similar diseases such as fibrous dysplasia and histiocytosis. The finding of *S. pneumoniae* type 1 in a child previously immunised with three doses of 13-valent vaccine makes this case even more important, as the pathogenesis is not at all clear.

It was strongly suspected that it could be an immunodeficiency (slight elevation of acute phase reactants, low-grade fever), but no abnormalities were found in the tests performed.

The low pneumococcal antibody titres at two years of age was an interesting finding, perhaps being due to the lack of "immunological memory", considered a variant of normality if the subsequent production of antibodies increases after stimulation; also considering that at two years of age titres are lower than at later ages.[3,4] A three-fold increase in titres is adequate, even more so considering the low pre-vaccination levels in which a greater post-vaccination response is described.[5]

Vaccination failure could be an option, despite being uncommon, and even rarer with serotype 1. With the immunisation received, three doses of 13-valent vaccine, IgG titres are obtained against serotype 1 in 96.1%.[6,7] Vaccination failure for the 13-valent vaccine occurs in 0.66 cases/100,000 vaccinations/year.[8,9] The most common serotypes that appear after vaccination failure are 19F, 19A, 6B and 3.[8,10]

Another hypothetical but plausible option could be bacteraemia prior to the third dose of conjugate vaccine, with haematogenous spread and subsequent rib osteomyelitis.

References

1. Peltola H, Pääkkönen M. Acute osteomyelitis in children. N Engl J Med. 2014;370:352–60.
2. Kalouche I, Ghanem I, Kharrat K, Dagher F. Osteomyelitis of the rib due to *Streptococcus pneumoniae:* a very rare condition in children. J Pediatr Orthop B. 2005;14:55–60.
3. Daly TM, Hill HR. Use and clinical interpretation of pneumococcal antibody measurements in the evaluation of humoral immune function. Clin Vaccine Immunol. 2015;22:148–52.
4. Orange JS, Ballow M, Stiehm ER, Ballas ZK, Chinen J, de la Morena M, et al. Use and interpretation of diagnostic vaccination in primary immunodeficiency: a working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy Asthma & Immunology. J Allergy Clin Immunol. 2012;130 Suppl. 3:S1–24.
5. Perez E, Bonilla FA, Orange JS, Ballow M. Corrigendum: specific antibody deficiency: controversies in diagnosis and management. Front Immunol. 2018;9:450.
6. Public Health-European Commision. Prevenar 13, INN-Pneumococcal polysaccharide conjugate vaccine (13 valent, adsorbed). Annex 1 Summary of product characteristics. Available from: https://www.ema.europa.eu/en/documents/product-information/prevenar-13-epar-product-information_en.pdf [accessed 18.05.20].
7. Picazo J, Ruiz-Contreras J, Casado-Flores J, Negreira S, Baquero F, Hernández-Sampelayo T, et al. Effect of the different 13-valent pneumococcal conjugate vaccination uptakes on the invasive pneumococcal disease in children: analysis of a hospital-based and population-based surveillance study in Madrid Spain, 2007–2015. PLOS ONE. 2017;12:e0172222.
8. Oligbu G, Hsia Y, Folgori L, Collins S, Ladhani S. Pneumococcal conjugate vaccine failure in children: a systematic review of the literature. Vaccine. 2016;34:6126–32.
9. Oligbu G, Collins S, Andrews N, Sheppard CL, Fry NK, Slack MPE, et al. Characteristics and serotype distribution of childhood cases of invasive pneumococcal disease following pneumococcal conjugate vaccination in England and Wales, 2006–2014. Clin Infect Dis. 2017;65:1191–8.
10. Antachopoulos C, Tsolia MN, Tzanakaki G, Xirogianni A, Dedousi O, Markou G, et al. Parapneumonic pleural effusions caused by *Streptococcus pneumoniae* serotype 3 in children immunized with 13-valent conjugated pneumococcal vaccine. Pediatr Infect Dis J. 2014;33:81–3.

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