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Case report

β-Tryptase and quantitative mast-cell increase in a sudden infant death following hexavalent immunization

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Abstract

The association between sudden infant death syndrome and immunization is frequently discussed. Serious adverse events following vaccination have generally been defined as those adverse events that result in permanent disability, hospitalization or prolongation of hospitalization, life threatening illness, congenital anomaly or death. They are generally referred to the inherent properties of the vaccine (vaccine reaction) or some error in the immunization process (programme error).The event could also be totally unrelated but only temporally linked to immunization (coincidental event). A fatal case of a 3-month-old female infant, who died within 24 h of vaccination with hexavalent vaccine is presented. Clinical data, post-mortem findings (acute pulmonary oedema, acute pulmonary emphysema), quali-quantitative data collected from immunohistochemical staining (degranulating mast cells) and laboratory analysis with a high level of β-tryptase in serum, 43.3 μg/l, allows us to conclude that acute respiratory failure likely due to post hexavalent immunization-related shock was the cause of death.

Keywords: Sudden infant death; Hexavalent vaccine; Mast-cell; β-Tryptase

1. Introduction

Combination vaccines for pediatric immunization are being developed to increase the acceptance of and compliance with vaccine recommendations. The reduction in the number of injections simplifies vaccination schedules, reduces pain reactions, exposure to excipients and adjuvants, the number of office visits and stress for parents and children, and will in the future simplify the introduction of new pediatric vaccines. While it is important from a public health perspective to develop combination vaccines, the safety and efficacy of the existent monovalent vaccines has to be maintained in combination vaccines [4]. Adverse events following immunization are defined as medical incidents which take place after an immunization [10]. Serious adverse events after vaccination have generally been defined as those adverse events that result in permanent disability, hospitalization or prolongation of hospitalization, life threatening illness, congenital anomaly or death. They are generally referred to the inherent properties of the vaccine (vaccine reaction) or some error in the immunization process (programme error). The event could also be totally unrelated but only temporally related to immunization (coincidental event) [5].

We present the fatal case of a 3-month-old female infant, who died within 24 h of vaccination with hexavalent vaccine (Infanrix Hexa).

2. Case report

2.1. Clinical history

The 3-month-old baby was a first born child, born at 41st week of gestation by caesarean delivery, with a birth weight of 3.400 g and an Apgar score of 9–10. The child's mother referred no significant family history, unremarkable pregnancy and good health of the baby, who was bottle-fed, until hexavalent immunization. She also referred that, in the morning, a few hours after immunization (at 11:00 a.m.) the baby presented feeding difficulty. Early in the afternoon the clinical conditions of the baby started getting worse with the onset of severe dyspnoea, so she was immediately taken to the

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Emergency Department of the local hospital (7:46 p.m.). A state of shock with critical acute respiratory failure was diagnosed. The baby appeared pale and unresponsive. Inspiratory dyspnoea, triage and inspiration stridor signs were observed; systolic hypotension (50 mmHg) and tachycardia (180 bpm) were also detected (diastolic pressure was unappreciable). Laryngoscopy was unremarkable. Laboratory tests revealed the presence of hypereosinophilia (5%) and metabolic acidosis (pH 7.154) with blood desaturation (pO_2 75.9 mmHg at 8:48 p.m.) and compensatory hypercapnia. Adrenaline was repeatedly administered by aerosol; i.m and e.v. corticosteroids were also administered. The infant died in spite of resuscitation manoeuvres 2 h after hospital admission (10:50 p.m.).

2.2. Autoptic findings

A complete post mortem examination was performed two days after death. No putrefaction phenomena were evident. The autopsy was performed according to the Valdes Dapena method, including careful examination of the cardiac conduction system and of the central and peripheral autonomic nervous structures involved in cardiorespiratory reflexogenesis. The body was that of a 3 month old, well-developed and well-nourished, white infant with a body weight of 4930 g and body length of 55 cm. An immunization puncture mark was observed on the left thigh. Internal examination was unremarkable except for subpleural petechiae and heavy lungs presenting white foam on the main bronchi (Fig. 1).

2.3. Histological studies

The cardiac conduction system was removed in two blocks: The first included the sino-atrial node and the crista terminalis, while the second contained the atrioventricular node, from the His bundle down to the bifurcation and bundle branches. These two blocks were serially cut at intervals of 40 μm (levels) and stained alternately with haematoxylin–eosin and Azan. All tissue specimens were fixed in formalin and embedded in paraffin, then a routine hematoxilin and eosin stain was employed. An immunohistochemical technique was used to estimate mast-cell population, using the anti-tryptase antibody as a mast-cell specific marker on 5 μm thick paraffin sections. Enzyme pretreatment with proteinase K (0.01%; 37 °C) was necessary to facilitate antigen retrieval and to increase membrane permeability to antibodies. The primary anti-tryptase antibody (DAKO) was applied (in) at a 1:100 ratio and incubated overnight at 4 °C. The positive reaction was visualized by 3-diamino-9ethyl-carbazole (AEC) (Sigma). The sections were counterstained with Mayer's hematoxylin and mounted in Aquatex (Merck) and examined under a light microscope. We also proceeded to a quantitative analysis: in each histological section, 10 observations in different fields per slide equivalent to 70 observations were performed. The positive mast-cell count to the tryptase reaction was made at a magnification 10× using a light microscope coupled to a high resolution colour video camera. The video image was inputted to a computer programmed for quantitative morphometry. Afterwards a pulmonary area of 100 mm^2 was analyzed. We examined histological samples from a control group where the cause of death was clearly attributable to traumatic events (10 pediatric cases) [9].

Histological examination revealed polivisceral stasis, mild cerebral oedema. Acute pulmonary oedema mixed with areas of acute pulmonary emphysema were recorded. Myocardial interstitial oedema was also detected. Histological examination of the cardiac conduction system was unremarkable. Small

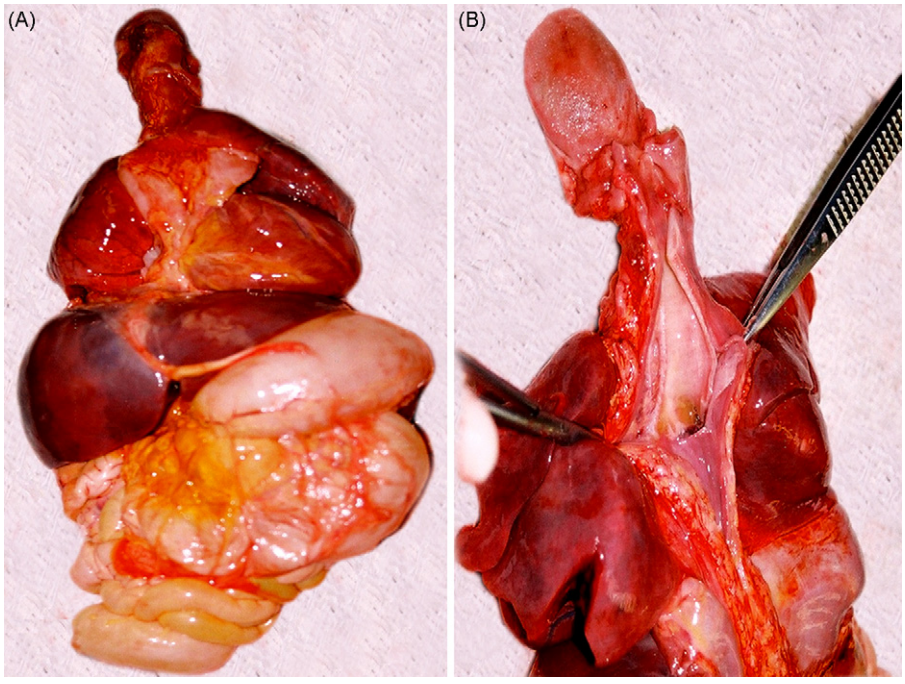

Fig. 1. Post-mortem section according to Valdes Dapena method: (A) sub-pleural petechiae and (B) white foam on the main bronchi.

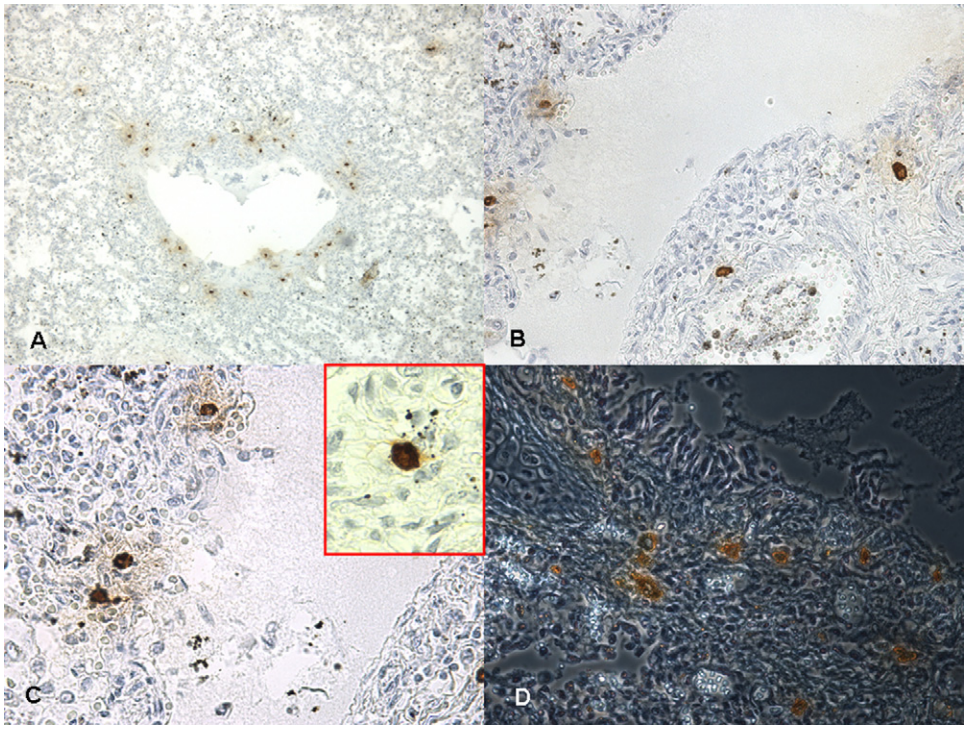

Fig. 2. Lung: degranulating mast cells (starry effect) with tryptase-positive material outside the cells was documented (A–B, Ab anti-tryptase, ×10, ×20). An halo (golden reaction) of tryptase positiveness around the mast cells (insert, ×100) reveals evidence of mast-cell degranulation (C, Ab anti-tryptase, ×40; D phase-contrast, Ab anti-tryptase, ×40).

intraparenchimal haemorrhages on spleen and adrenal glands were observed. Pulmonary mast cells were identified and quantified and a great number of degranulating mast cells with tryptase-positive material outside were observed (Fig. 2). Data resulting from quantitative analysis recorded a numerical increase in pulmonary mast cells in fatal anaphylactic shock (average mast-cell count 12471/100 mm^2) compared with that of the traumatic control group (traumatic death) whose average mast-cell count was 3657/100 mm^2.

2.4. Toxicological analysis

Toxicological analysis on blood and urine specimens for therapeutic and non therapeutic drugs were performed using gas chromatography–mass spectrometry and resulted negative.

2.5. Blood serum dosage

At the dissecting table, 10 cm^3 of femoral blood was extracted and immediately frozen at −20 °C. The tryptase was dosed utilizing an immunoenzymatic technique (UniCAP TRYPTASE Fluoroenzymeimmunoassay Pharmacia, AB Uppsala, Sweden). Tryptase level ≤10 μg/l was considered to indicate that mast cell had not occurred near the time of death. The lower limit of sensitivity for the assay was 10 μg/l. Serum dosage of mast cell β-tryptase from femoral blood detecting serum values of 43.3 μg/l (normal value ≤10 μg/l).

3. Discussion

Clinical data, post-mortem findings (acute pulmonary oedema, acute pulmonary emphysema), quali-quantitative data collected from immunoistochemical staining (degranulating mast cells) and laboratory analysis with a high level of β-tryptase in serum, 43.3 μg/l, lead to the conclusion that acute respiratory failure likely due to post hexavalent immunization-related shock was the cause of death.

The association between sudden infant death (SID) and immunization is frequently discussed. Because of the close temporal association between the first immunizations and the main peak of the SID incidence it has been speculated that immunization could cause SID. Many authors confirmed there was no increase in risk of SID from immunization and temporal association between vaccination and SID is coincidental and not causal [20]. A possible protective effect of immunization was also proposed [8]. Firstly, immunization protects against unrecognised whooping cough, which is a potential cause of sudden and unexpected death in this age group; secondly, there is cross-immunization with other bacteria or viruses, which protects infants at this vulnerable age. Many studies investigated the potential of the anaphylactic mechanism to death in infancy by determining relative levels of α and β tryptase and both total and allergen-specific IgE in sera from groups of infants whose deaths were attributed to SIDS or other causes. The comparison of the results of the specific immunoassay which detected both α and β forms with one which detects predominantly β-tryptase has suggested that

α-tryptase is secreted constitutively by mast cells (as it predominates in the serum of normal subjects and patients with systemic mastocitosis) and that β-like tryptase is released by mast cells upon anaphylaxis degranulation (as high concentrations have been found in the serum of patients with anaphylaxis) [3]. The influence of the post-mortem interval is not clear [6,7]. As artifactual values might occur, it is important to investigate possible confounding factors and to establish an upper cut-off value for tryptase measured post-mortem [6]. So several studies have established the usefulness of tryptase as a forensic marker in these conditions; tryptase levels >10 μg/l were considered to reflect substantial mast-cell activation and levels <10 μg/l were considered to indicate that mast-cell activation had not occurred near the time of death. The lower limit of sensitivity for the assay was 1 μg/l [2,9]. In the current study pulmonary mast cells were also identified and quantified using a immunohistochemical technique. Tryptase-positive mast cells were clearly stained and tryptase-positive material was found outside an otherwise intact-appearing mast cell, suggesting prior degranulation [9].

Vaccine associated anaphylaxis is a rare occurrence with only few cases despite of the million of doses administered, giving a risk of 0.65 cases per million doses. After administration of 7,644,049 vaccine doses only five potential cases of vaccine-associated anaphylaxis have been reported, none of which resulted in death. In addition to antigens vaccine contain several other components, including preservatives, adjuvants and manufacturing residuals; although it is not always clear which component might be responsible for anaphylaxis, gelatin and egg proteins are present in some vaccine in at sufficient level to induce hypersensitivity reactions [1]. Polyvalent vaccines were developed to increase acceptance of vaccinations by decreasing the number of injections necessary. Compared to their pentavalent predecessors, the hexavalent vaccines additionally contain hepatitis B serum. They are used for immunization against diphtheria, pertussis, tetanus, influenza, poliomyelitis and hepatitis B [22]. Hexavalent vaccines have been available on the European market since October 2000. A post marketing study demonstrated the safety and immunogenicity of hexavalent vaccine as an alternative to other licensed vaccines, so until April 2003, approximately 3 million children were vaccinated using this method and about 9 million doses were sold in the European union during this time. Children are to be vaccinated with these vaccines at the age of 2, 4, 6 and 12–14 months [23]. In 2003 the European Agency for the Evaluation of Medical Products (EMEA) through its scientific Committee for Proprietary Medicinal Products (CPMP) reviewed the safety of centrally authorized hexavalent vaccine investigating whether there might be a link between hexavalent vaccines and some cases of sudden infant deaths occurred after immunization and concluded that vaccination offers benefit to the individual child and to the general population and the causes of death of the five children dead within 24 h of vaccination with hexavalent vaccine remain unexplained, and it was impossible to establish a cause and effect association with hexavalent vaccines [17,18]. On the 21 September 2005, EMEA withdraw the marketing authorisation for an hexavalent vaccine, because of the low immunogenicity of the hepatitis B component and the variability in the long-term protection against hepatitis B. The Institute of Medicine accepts a causal relation of anaphylaxis with the hexavalent vaccine but the number of vaccine-associated cases seems to be small. It is not always clear which component of vaccine is involved in anaphylactic reactions: vaccine antigens (tetanus toxoid) [12], animal proteins (gelatine) [15] and antibiotics (neomycin) [11].

Very few cases of sudden unexpected death in infancy after hexavalent immunization have been described in literature and only three cases could be investigated with relation to increased serum levels of mast-cell tryptase, suggesting anaphylaxis as the main cause of death [23,13]. All cases of sudden unexpected death occurring in infancy and perinatal age, suggesting post-immunization-related shock as the cause of death, should always undergo a complete necropsy study [14,16,19,20]; immunohistochemical stainings and quantitative morphometry analysis are to be considered significant for the confirmation of suspects from the pathologists' point of view and toxicological analysis and tryptase serum detection represent important supporting evidence upon which diagnosis can be based [3,6,7,21].

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