Prompt and successful toxin-targeting treatment of three patients with necrotizing pneumonia due to *Staphylococcus aureus* strains carrying the Panton-Valentine leukocidin genes

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Abstract

Three patients with extensive necrotizing pneumonia due to Panton Valentine leukocidin-positive *Staphylococcus aureus* strains and with gravity factors (leukopenia <3 × 10^9/L in all three cases, hemoptysis in two cases) were successfully treated with toxin-suppressing agents introduced rapidly after hospital admission.
CASE REPORT

Patient 1. In December 2007 a 6-month-old boy presented to the emergency department with a 5-day history of a viral-like syndrome including rhinorrhea, fever and diarrhea. On admission he had dry cough, moderate dyspnea, altered general status, and sepsis (39.4°C, heart rate 198 bpm, tachypnea, and marbling). Initial laboratory tests showed a C-reactive protein level of 73.4 mg/L and a total leukocyte count of 7.3 × 10^9/L. Chest radiography revealed a basal left-sided infiltrate without pleural effusion (Fig. 1). Treatment was started with ceftriaxone and supportive measures, but his respiratory status worsened.

Severe hypoxemia was present with paO2 of 3.9 KPa under 3 L/min of nasal oxygenotherapy. Eight hours after admission a second chest radiograph revealed an extensive bilateral infiltrate and pleural effusion (Fig. 1). He was admitted to the pediatric intensive care unit (PICU) with signs of septic shock (oliguria and altered mental status), which improved with fluid resuscitation. Laboratory tests showed lactic acidosis (pH 7.27; lactic acid 5.50 mmol/L), hypoxemia, and leukopenia (1.83 × 10^9/L) (Fig. 2). Pleural puncture yielded purulent fluid (40 mL) that tested negative by Gram staining and pneumococcal antigen detection. Staphylococcal necrotizing pneumonia was suspected in view of the rapid clinical deterioration, leukopenia and negative tests for pneumococci. Vancomycin and clindamycin were added to ceftriaxone 15 hours after admission. S. aureus was detected in pleural fluid 24 hours after admission, and culture yielded a PVL-positive community MRSA strain belonging to European clone ST80. The strain was susceptible to clindamycin and the MIC to vancomycin was 1.5 mg/L. The patient’s status gradually improved, despite the need for pleural drainage because of recurrent pleural effusion. He was discharged from the PICU on day 7. Eight days after PICU admission he remained febrile (39.3°C) and still had respiratory disorders (dyspnea and...
diminished left vesicular murmur), but the leukocyte count had risen to $32 \times 10^9$/L (Fig. 2).

Computed tomography (CT) revealed significant pleural effusion, multiple lung lesions, and pleural abscesses. Pleural decortication was performed. Intra-operative pleural samples were positive for the same strain of PVL-positive MRSA and antibiotic treatment was switched to rifampicin plus clindamycin. The boy was discharged from hospital on day 28, on a three-week course of oral antibiotics. **Serologic tests and PCR were negative for influenza virus.** Follow-up visits showed a healthy child with no clinical or radiographic signs of pulmonary relapse. The father had a history of furuncles but did not appear to carry *S. aureus*.

**Patient 2.** A previously healthy 38-year-old man was admitted to the intensive care unit in February 2008 with a 48-hour history of an influenza-like syndrome. On admission he had dyspnea with hypoxemia (PO2 8.9 kPa; SpO2 86% on room air), tachypnea (40 breaths per min), a heart rate of 130 bpm and mild fever (37.9°C). Initial laboratory tests showed leukopenia ($1.1 \times 10^9$/L) with $0.48 \times 10^9$/L PMN, thrombocytopenia ($120 \times 10^9$/L), an elevated C-reactive protein level (314 mg/L) and a markedly elevated procalcitonin value (10 ng/mL, N < 2 ng/mL). Chest radiography revealed bilateral pneumonia without pleural effusion. Treatment was started with ceftriaxone, amikacin and levofloxacin. Six hours after admission he developed septic shock, with an acute respiratory distress syndrome, hemodynamic failure, and diffuse intravascular coagulation. He was intubated, and tracheal aspiration yielded hemorrhagic fluid containing grape-like clusters of Gram-positive cocci. Treatment was switched to vancomycin plus gentamicin. Eight hours later, methicillin-sensitive *S. aureus* ($10^8$ CFU/mL) was recovered in pure culture from **endotracheal aspiration.** Necrotizing pneumonia due to PVL-positive *S. aureus* was suspected and
antibiotic treatment was again switched, 14 hours after admission, to clindamycin (600 mg every 6 h) and linezolid (600 mg every 12 h), plus IVlg (Tegeline® 1 g/kg/d on two consecutive days). The *S. aureus* isolate was methicillin- and clindamycin-susceptible, with a MIC to vancomycin of 2 mg/L, carried the PVL genes and belonged to ST8. After 48 hours his respiratory status improved and the leukocyte count rose from 1.1 × 10⁹/L to 5.1 × 10⁹/L (Fig. 2); CT scan showed bilateral lung compressions with fluid-air levels suggestive of necrotic lesions, and bilateral pleural effusion. The effusion was drained and found to be sterile. Ten days after admission he developed a biological inflammatory syndrome along with intestinal and pulmonary failure. Clindamycin-associated colitis was suspected and clindamycin was withdrawn. Colitis was not confirmed but *S. aureus* persisted in pulmonary samples. Vancomycin was reintroduced in combination with rifampicin, and linezolid was stopped. Twenty days after admission, vancomycin was replaced by oxacillin for 15 days. Serologic tests were positive for influenza virus on day 13. The patient was discharged from hospital on day 39. Follow-up visits showed a healthy man without relapse after one year.

Patient 3. A previously healthy 35-year-old man from India was admitted to the emergency department in January 2009, with a 72-hour history of fever (38.9°C) and chest pain. On admission he had dyspnea with hypoxemia and tachypnea (PO2 5.9 kPa; heart rate 117 bpm). Initial laboratory tests showed leukopenia (2.2 x 10⁹/L with 1.9 x 10⁹/L PMN), a C-reactive protein level of 193 mg/L and negative urinary tests for pneumococcal antigen. Treatment was started with amoxicillin/clavulanate (1 g every 8 h) and ofloxacin (200 mg every 12 h). He quickly developed an acute respiratory distress syndrome requiring noninvasive ventilation, and lactic acidosis (2.4 mmol/L). Chest radiography showed
bilateral pneumonia without pleural effusion. **CT scan confirmed bilateral pneumonia** associated with disseminated necrotizing lesions (Fig. 3). Bronchoscopy showed hemorrhagic alveolar fluid. **Methicillin- and clindamycin-susceptible S. aureus** was recovered from bronchoalveolar lavage fluid in pure culture (10⁸ CFU/mL); the **MIC to vancomycin was 1.5 mg/L.** On day 2 the isolate was shown to carry the PVL genes. This **MSSA strain was ST217 and belonged to CC22.** Antibiotic treatment was switched to linezolid (600 mg every 12 h), clindamycin (600 mg every 8 h) and ofloxacin (200 mg every 12 h), plus IVIg (Tegeline® 1 g/kg/d on three consecutive days). His status improved and respiratory support was withdrawn. Laboratory values improved at the same time, with an increase in the leukocyte count to 10.2 x 10⁹/L on day 3 and a decline in the C-reactive protein level. He was discharged from the intensive care unit on day 8. Treatment with linezolid and clindamycin was continued for 15 days and the patient was discharged from hospital on day 23. Serologic tests and PCR were negative for influenza virus. PVL was quantified in serial sputum samples with an ELISA method (1); the concentration peaked at 3.6 mg/L on day 2 and fell rapidly when antitoxin treatment was started (0.06 mg/L on day 5) (Fig. 2). **Follow-up consultations showed a healthy man with no clinical sign of pulmonary relapse. Two months later CT scan confirmed a quasi-complete regression of lung lesions.**
Panton-Valentine leukocidin (PVL)-positive *Staphylococcus aureus* strains have been linked to necrotizing pneumonia complicating influenza or other respiratory virus (2, 8, 9, 14, 15, 17). Necrotizing pneumonia mainly affects children and young adults (median age 14 years) and is fatal in one-half to three-quarters of cases (8, 9). Death usually occurs rapidly, after a median of only 4 days.

The risk of superadded infection by PVL-producing *S. aureus* strains may be increased by influenza. Indeed, the incidence of documented *S. aureus* co-infection increased fivefold in the United States during the 2004-2007 influenza seasons compared to the interepidemic periods (5). Kallen identified 51 cases of community-acquired *S. aureus* pneumonia during the 2006-2007 influenza season in 19 American states, of which 79% involved methicillin-resistant strains and 51% were fatal (11). In Hageman's study (10), PVL genes were detected in 85% of community-acquired *S. aureus* strains causing pneumonia during the 2003-2004 influenza season.

Onset of leukopenia and hemoptysis in influenza patients with a rapidly extensive pneumonia syndrome is suggestive of necrotizing pneumonia and is independently associated with poor outcome in multivariate analysis (9). In a previous series of 50 cases of staphylococcal necrotizing pneumonia, the survival rate was less than 10% among patients with leukocyte counts below 3 x 10^9/L (9).

Necrotizing pneumonia being both rare and rapidly lethal, new therapeutic approaches are difficult to evaluate, partly for ethical reasons, thus placing the onus on experimental studies. PVL is overexpressed in the presence of betalactams but it expression can be blocked by combining a toxin-suppressing agent such as clindamycin, linezolid or rifampicin with bactericidal antibiotics acting on the cell wall (3, 4, 16). In addition, intravenous
immunoglobulin (IVIg) blocks the lytic effect of PVL on polymorphonuclear cells (PMN) in vitro (6).

We describe three cases of severe and rapidly progressive necrotizing pneumonia due to PVL-positive strains of *S. aureus*, one of which was methicillin-resistant. The three patients had minimal leukocyte counts below $3 \times 10^9$/L, and two had hemoptysis. PVL was measured in sputum samples of one patient and was found to peak on the second hospital day. Clindamycin was added to the ongoing antibiotic regimen 15 hours after hospital admission in one case; while clindamycin, linezolid and IVIg were administered 14 hours after admission in the second case and within 24 hours in the third case. All three patients survived.

The three cases described here suggest that rapid administration of antitoxicin therapy with clindamycin/linezolid and/or IVIg may improve the outcome of PVL-associated staphylococcal necrotizing pneumonia (13), even when gravity factors are present. Although based on three observations with the quantification of PVL in respiratory samples for one case only, it also suggests that necrotizing pneumonia may evolve through successive phases (Fig. 2). The initial phase, in which the leukocyte count is likely to be normal, is characterized by an influenza-like syndrome that may be followed by the onset of clinical or radiological pulmonary disorders. The following acute “toxicin” phase corresponds to the onset of pulmonary infiltration, hemoptysis and leukopenia that, by analogy to the *in vitro* and animal observations, may be a consequence of an exacerbated inflammation induced by the massive influx of PMN and the subsequent lysis of these cells by PVL (7, 12). PVL production peaked during this phase in our patient #3, reaching a concentration of 3.6 mg/L in sputum samples. Abscess fluid PVL concentrations above 1 mg/L have been linked to larger abscess size (1). In an *in vitro*
system, even very low PVL concentrations are capable of inducing leukopenia (7), and the effect of direct administration of PVL in the mouse pneumonia model recalls the necrotic aspect of lung tissue at autopsy (8, 12). In our three patients, this toxic phase may have been abrogated by rapid administration of clindamycin, linezolid and/or IVlg. The final phase is a more classical suppurative phase with abscess formation, leukocyte counts of 20 to 30 × 10⁹/L (as in our three patients) and no detectable PVL. Dumitrescu et al. (3) showed that β-lactam agents up-regulate PVL release and that the combination of β-lactam with clindamycin, rifampicin or linezolid supressed PVL induction.

It is noteworthy that all three patients showed a marked clinical improvement despite the persistence of *S. aureus* in the lungs. This suggests that the main goal of treatment in the early life-threatening stages of the disease should be to counter the effects of the toxin -- by inhibiting its production or blocking its biological effects -- rather than to obtain bacterial clearance. A re-increase in the leukocyte count would signal that this objective has been reached. However, bacterial clearance is clearly necessary for full recovery, and bactericidal treatment should therefore be given both concomitantly with antitoxicin treatment and after the crisis phase.

In conclusion in the case of severe community-acquired pneumonia, empirical treatment active on the main culprit bacterial species (*Streptococcus pneumoniae*) should be started immediately. If a rapid fall in the leukocyte count is observed in this setting, then PVL-producing *S. aureus* should be suspected and an antibiotic that blocks toxin expression should be rapidly added to any ongoing antibiotic therapy (chosen according to the local epidemiological situation), and the use of IVlg should be considered in the most severe cases. Clindamycin is active on most community MRSA isolates of clonal groups USA300.
Further clinical studies could allow to confirm these recommendations.

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References


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Figure legends

Figure 1. Rapid deterioration of radiographic pulmonary status in patient 1, with bilateral pneumonia and pleural effusion (arrows)

Figure 2. Time course of leukocyte counts and PVL expression during necrotizing pneumonia. Bars represent the leukocyte counts in each patient and the line represents the evolution of PVL expression on patient 3.

Figure 3. CT scan confirmed bilateral pneumonia associated with disseminated necrotizing lesions (arrows) in patient 3.
On admission - Eight hours later

Figure 1.
Figure 2.
Figure 3.